obtained having the following constants: sp. gr. at 19°, 0.8594; optical rotation $[\alpha]_D^{19°}$ —22.36°. Using a three-bulb Ladenburg flask, the oil distilled as follows: 158 to 160°, 17.0%; 160 to 162°, 46.1%; 162 to 165°, 20.0%; 165 to 175°, 11.5%.

α-Pinene.—After repeated fractionation over sodium the greater portion of the oil distilled between 156 and 158°; its specific gravity at 15° was 0.8629 and optical rotation $[\alpha]_D^{23°}$ —18.96°. The oil gave a good yield of nitrosochloride, m. p. 103°. However, the nitrolpiperidine compound melted at 132.5°. A second preparation from the original oil gave the same result. There was insufficient material available for oxidation to pinonic acid but further proof of the presence of pinene was obtained by formation of the nitrolbenzylamine melting at 122° and of bornyl chloride melting at 128°.

Camphene was not detected.

β-Pinene.—Twenty grams of oil distilling between 161 and 165° having the rotation $[\alpha]_D^{22°}$ —26.2°, gave the characteristic crystals of sodium nopinate on oxidation with alkaline potassium permanganate. The free nopinic acid melted at 125°.

The small amount of oil distilling between 168 and 175° had a pronounced odor of limonene.

Summary.

The oleoresin from the heartwood of Douglas fir contains a volatile oil consisting chiefly of highly rotatory *l*-α-pinene with small amounts of *l*-limonene and *l*-terpineol.

The oil from the oleoresin obtained from the sapwood contained *l*-α-pinene, *l*-β-pinene and probably *l*-limonene.

The "firpene" previously described as a new terpene is evidently highly active *l*-α-pinene.

MADISON, WISCONSIN.

[CONTRIBUTION FROM THE RICHARDSON CHEMICAL LABORATORY OF TULANE UNIVERSITY.]

THE USE OF POTASSIUM PERSULFATE IN THE DETERMINATION OF TOTAL NITROGEN IN URINE.

BY LEONARD C. SCOTT AND ROLLIN G. MYERS.

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The determination of the total nitrogen in urine by methods simpler than the ordinary Kjeldahl, has been the subject of attempts by numerous investigators. Speed and simplicity have been the main points to consider, as well as a relatively high degree of accuracy; and two, at least, of these advantages seem to have been fairly well attained in the microchemical methods of Folin and Farmer,[1] and Folin and Denis.[2] The

[1] O. Folin and C. J. Farmer, *J. Biol. Chem.*, **11**, 493–501 (1912).

[2] O. Folin and W. Denis, *Ibid.*, **26**, 473–489 (1916).

simplicity, however, is a matter of doubt. The numerous manipulations involved in either the one or the other of these methods are not conducive to accuracy, except in the hands of those experienced in their use. Bock and Benedict[1] have pointed out the possibilities of error which according to them vary between two and three per cent., even with extremely careful work, and observe that even greater variations may occur. These errors in a long series of results are of no particular importance and do not detract from the usefulness when speed is a factor to be considered. Where very accurate results are required, the Kjeldahl, or one of its numerous modifications, must be accepted as a standard of choice.

The procedures to be described below are attempts to shorten and simplify the existing macro- and microchemical methods, at the same time to retain such a degree of accuracy, as will make them available for general use. The authors believe that with the use of potassium persulfate as an oxidizing agent accurate results may be quickly obtained. One advantage is, that the high temperatures and probable loss of nitrogen are avoided by the use of a less amount of material and a lowered boiling point resulting in the elimination of a common source of error in the Kjeldahl method. It has been pointed out by Self,[2] that nitrogen is lost unless a certain proportion between the sulfuric acid and the potassium sulfate is maintained. Folin and Denis also believe that prolonged digestion may lead to loss of nitrogen.

The use of potassium persulfate has been questioned, since it has been found to contain nitrogen. Huguet[3] added a 20% solution of sodium persulfate containing the urine directly to the sulfuric acid, then heating to decolorization. His work was criticized by Lemaire[4] later, on the ground that the samples of sodium persulfate, which he was able to obtain, contained varying amounts of nitrogen, and claimed that this rendered the results unreliable. The writers believe that this is not the only source of error, but that it is possible that the action of the persulfate on the chlorides may have produced free chlorine and thus lead to a further loss of nitrogen. The addition of a nitrogen-free persulfate only to the sulfuric acid digestion mixture increases the simplicity as well as the speed, with which the nitrogen in the urine may be determined.

A sample of Merck's potassium persulfate obtained by the writers from E. H. Sargent & Co., proved to contain nitrogen in such small amounts as to be practically negligible. It was noted that the first tests made with the fresh sample showed no appreciable color with Nessler's solution in blank

[1] J. P. Bock and S. R. Benedict, *J. Biol. Chem.*, 20, 47–59 (1915).

[2] P. A. W. Self, *Pharm. J.*, 88, 384–385; cited from *C. A.* 6, 2048 (1912).

[3] Huguet, *Repert. pharm.*, 3, 21, 481; cited from *C. A.*, 4, 934 (1910).

[4] P. Lemaire, *Bull. soc. pharm. Bordeaux*, 50, 306–311; cited from *C. A.*, 4, 3050 (1910).

determinations, using 0.5 g. of the salt; that, however, the longer the sample was in use, often being exposed to the air of the laboratory and to handling, the amount increased considerably.

The urines used for analysis were those commonly received in a hospital laboratory, and were obtained from the Tuoro Infirmary, New Orleans. It has been thought well to state in detail any pathological impurities noted.

As will be seen from the tables the results obtained by the use of the colorimetric do not differ from those of the Arnold-Gunning method to an extent greater than would seem to be warranted by the character of the method.

The Macrochemical Method.

The principle on which the method depends, appears to be a hydrolytic cleavage of the organic constituents followed by a complete oxidation with the persulfate. The action of the sulfuric acid on the persulfate is probably shown by the following equation:

$$K_2S_2O_8 + H_2SO_4 = K_2SO_4 + 2SO_3 + H_2O + O$$

This reaction takes place at a relatively high temperature, so that the persulfate may be thoroughly mixed with the cooled digestion mixture. Then by increasing the temperature the speed of the reaction may be controlled. If the persulfate is thrown directly into the boiling acid, the evolution of oxygen occurs immediately and the maximum effect is not obtained. It then becomes necessary to repeat the addition of the persulfate, using a larger amount than would be necessary otherwise.

The Arnold-Gunning method was used as the standard for comparison.

The following procedure was found, after several trials, to give the best results: Five cc. of the urine are measured into a 500 cc. Kjeldahl flask with an ordinary pipet, 10 cc. of H_2SO_4 C. P. and 10 cc. of a 2.5% $CuSO_4$ solution added and the mixture is boiled until the mass is light brown or yellow. The flask is allowed to cool to the point where it is possible to touch the glass without inconvenience and 1.5–2.0 g. of potassium persulfate, depending on whether the color is yellow or brown, is added. Then heat is applied until the reaction begins. As soon as this occurs, the flask is removed from the flame, the contents rotated until the reaction is complete and the solution is colorless. It is again heated until the evolution of SO_3 fumes has practically ceased. If necessary it may be again cooled and more persulfate added. When satisfied that the color will not change, the flask is placed over the flame and boiled vigorously until the excess of SO_3 is given off and the acid begins to condense on the sides of the flask. The flame is then turned out and the flask allowed to cool. When cold, 150 cc. of distilled water, 40–45 cc. of a 30% NaOH solution, a few pieces of granulated Zn and a small piece of paraffin were added to prevent foaming, and the ammonia distilled into 0.1 *N* H_2SO_4 in the usual manner.

The time necessary for the digestion has been found to be approximately fifteen minutes.

The results as compared to those obtained by the Arnold Gunning method are given in Table I.

It is necessary to mention that in the Arnold-Gunning method it was not possible to digest for 2.5 hours with 20 cc. of H_2SO_4 as recommended, even in 800 cc. Kjeldahl flasks, without loss of nitrogen. It was found necessary to increase this to 25 cc., and after an hour's digestion, 5 cc. more were added. Shorter times for heating were tried without giving the same results as those obtained with the 2.5-hour periods. Frequent checks proved that the method gave uniform results, and was adopted as a standard for comparison.

TABLE I.

Sample No.	Persulfate method. G. per 1000 cc.	Arnold-Gunning method. G. per 1000 cc.	Difference.
47	13.66	13.51	+0.15
48	3.12	3.06	+0.06
49	10.22	10.30	—0.08
50	5.88	5.80	+0.08
51	9.98	9.90	+0.08
52	10.64	10.68	—0.04
53	6.84	6.66	+0.12
54	9.22	9.32	—0.10
55	10.24	10.34	—0.10
56	3.14	3.19	—0.05
57	14.16	14.32	—0.16
58	15.49	15.57	—0.08
59	8.12	8.16	—0.04
60	7.03	7.11	—0.08

The Microchemical Method.

It seemed that the complete oxidation of the organic matter, and the ease with which the oxidation could be carried out, the absence of doubly charged cations and the small quantity of potassium sulfate contained in the digestion mixture, would make possible the determination of the nitrogen by direct nesslerization. Accordingly every effort was made to reduce the quantities of sulfuric acid and persulfate to a minimum, and yet obtain the required results without loss of nitrogen. It was found necessary to dilute the unknown to 250 cc. in order to prevent the undue influence of the electrolytes in solution on the colloidal Nessler compound.

The actual process of direct nesslerization was carried out as follows:

5 cc. of the well-shaken urine were measured into a 50 cc. flask directly, using an an ordinary pipet, holding the latter vertical and allowing one drop to release itself from the tip after the flow had ceased. The flask was then filled to the mark with distilled water and shaken well to

insure uniform distribution of suspended matter. Next, as recommended by Folin and Farmer, amounts equivalent to 0.1–0.2 cc. of the original urine, depending on whether the sp. gr. is above or below 1.018, were removed with a Mohr pipet, graduated in hundredths cc. This is either one or two cc. as the case may be.

The diluted urine was allowed to flow into tubes of Jena glass 200 × 25 mm. which has been lipped previously by heating in the flame and moulding to the desired degree with the end of a file. Even with the small quantity of liquid involved, the large glass tube was better adapted than one of smaller size, and allowed the sulfuric acid to condense on the sides for the most part, without being driven out. Furthermore the bubbles formed broke easily in the larger lumen, and there was no tendency to spurt. Then 0.3 cc. of H_2SO_4 C. P. was added from a similar pipet, and a glass bead or quartz pebble was placed in the tube. In some instances this precaution seemed to be of no especial value, since with the proper mixing of the acid before heating, quiet boiling was insured. The tubes were then clamped to a stand in a slanting position and heat applied with a small burner, the flame of which could be regulated. When the water had been removed and the contents had become black, the sulfuric acid began to condense on the sides of the tube. This part of the process was then considered completed. The tube was allowed to cool thoroughly, and 0.3–0.4 g. potassium persulfate was added. With practice the amount could be accurately enough gauged. The salt might be added with a small platinum spatula or with the blade of a pen knife. The tube held in the hand, was gently heated until the reaction commenced, removed from the flame and the reaction allowed to proceed to completion. The contents rapidly became colorless. Should this not be the case, the addition of a small quantity of persulfate would bring about the desired result. Finally, as in the macro method, the tube was heated until the evolution of SO_3 had practically ceased, and the acid again began to condense on the sides of the tube. It was then set aside to cool, and even when thoroughly cold the contents should not solidify. It was then diluted in the tube with distilled water and washed into a 250 cc. graduated flask. The water with which this dilution was made must not contain a trace of calcium and magnesium, for as has been observed by Whipple,[1] and according to the authors' experience merest traces produced a cloudiness of the nesslerized solution.

The volume was made up to approximately 200 cc., 18–19 cc. of N KOH were added, the contents rapidly rotated, and while in motion the diluted Nessler reagent, 5 cc. to 25 cc. water, was quickly added. The result was a clear solution, perfectly free from cloudiness, which might be readily compared with the standard.

[1] G. C. Whipple, *Tech. Quart.*, 20, 162–169 (1912).

The time required for this procedure varied from five to eight minutes.

The standard for comparison was made by diluting 5 cc. of the standard ammonium sulfate solution to 250 cc. after nesslerizing in a manner described previously. The dilution of the Nessler reagent and the value of the standard have been recommended by Folin and Farmer, and they have been adopted here with the best results.

The standard and the unknown were allowed to stand until the maximum color had been developed. This usually occurred in thirty minutes. They were then compared by means of a Duboscq colorimeter. The authors have been compelled to use the old form, measuring in cc. This has been a drawback in that an error of 1 cc. influences the result to a considerable extent on account of the relatively high nitrogen content of the solution. In making the determinations the unknown was compared with 50 cc. and 25 cc. of the standard. The results obtained by this method are embodied in Table II.

Nessler's Reagent.—Made by dissolving 62.5 g. of KI in 250 cc. of distilled water, reserving a small portion of the solution to be added later. To the main body of the KI solution a cold saturated solution of $HgCl_2$ was added until a faint permanent precipitate forms. Then the KI in reserve was added, and a sufficient amount of $HgCl_2$ was introduced to produce a second faint permanent precipitate. Then a cold solution of 150 g. of KOH in 150 cc. of distilled water was added and enough water to make a liter. This was allowed to stand until clear. The clear liquid was decanted and preserved in dark bottles.

Standard Ammonium Sulfate Solution.—This was prepared according to the Folin procedure. Pure ammonium sulfate was dissolved in a considerable volume of water, made alkaline with NaOH and aerated with a current of air into dilute H_2SO_4. This solution was then treated with alcohol, the ammonium sulfate precipitated, collected on a Büchner funnel and dried over H_2SO_4. 9.4285 g. of the purified salt were dissolved in distilled water and diluted to 1 liter. 100 cc. of this "stock solution" were then diluted to 1 liter for the standard, 5 cc. of which are equivalent to 1 mg. of nitrogen.

TABLE II.

Sample No.	Sp. gr.	Reaction.	Albumen. %	Sugar. %	Indican.	Persulfate method. G. per 1000 cc.	Arnold-Gunning method. G. per 1000 cc.	Difference.
1	1.018	acid	...	....	....	10.18	10.28	—0.10
2	1.017	acid	...	....	....	7.76	7.20	+0.50
3	1.017	acid	...	....	....	7.70	7.49	+0.21
4	1.019	acid	...	....	....	12.20	12.48	—0.28
5	1.020	acid	...	....	....	15.62	15.48	+0.14
6	1.013	acid	...	....	....	5.20	5.10	+0.10
7	1.016	alk.	...	0.25	....	4.90	4.93	—0.03

TABLE II (*continued*).

Sample No.	Sp. gr.	Reaction.	Albumen. %.	Sugar. %.	Indican.	Persulfate method. G. per 1000 cc.	Arnold-Gunning method. G. per 1000 cc.	Difference.
8	1.011	alk.	...	0.25	....	4.54	4.56	—0.02
9	1.022	alk.	0.025	....	....	5.50	5.78	—0.28
10	1.013	acid	trace	....	+	6.71	7.00	+0.29
11	1.026	acid	0.075	1.12	++	14.80	15.30	—0.50
12	1.016	acid	...	....	+	8.56	8.22	+0.34
13	1.027	neut.	0.05	....	+	11.11	11.10	+0.01
14	1.017	acid	...	....	+	2.52	2.65	—0.13
15	1.019	acid	...	....	++	8.70	9.10	—0.40
16	1.010	alk.	...	....	....	4.35	4.80	—0.45
17	1.024	alk.	0.05	....	....	11.40	10.98	+0.42
18	1.014	acid	....	0.87	....	7.05	7.18	—0.13
19	1.010	acid	0.25	....	....	6.58	7.10	—0.52
20	1.023	alk.	trace	....	....	13.15	13.50	—0.35
21	1.009	alk.	0.45	....	....	3.73	3.25	+0.48
22	1.019	neut.	0.045	....	....	5.30	5.32	—0.02
23	1.010	acid	trace	....	....	4.03	4.04	—0.01
24	1.026	alk.	trace	....	++	12.19	12.04	+0.15
25	1.010	alk.	...	....	....	2.50	2.46	—0.04
26	1.010	acid	...	....	....	5.15	4.89	+0.26
27	1.012	acid	...	....	....	4.95	4.94	+0.01
28	1.027	acid	0.025	....	....	9.95	9.73	+0.22
31	1.020	acid	...	....	....	8.80	8.50	+0.30
34	1.014	alk.	...	....	....	6.95	6.76	+0.19
35	1.011	acid	0.05	....	+	4.90	4.60	+0.03
36	1.021	acid	...	0.18	++	13.80	13.50	+0.30
37	1.032	alk.	trace	....	....	14.40	14.44	—0.04
38	1.023	alk.	...	....	....	10.87	10.64	+0.23
39	1.027	acid	...	....	....	18.87	19.38	+0.49
40	1.009	acid	...	0.50	....	4.80	4.40	+0.40
41	1.022	acid	0.05	....	....	10.20	9.98	+0.12
42	1.020	alk.	trace	....	....	8.93	9.00	—0.07
43	1.021	alk.	trace	....	....	8.93	8.46	+0.47
44	1.026	...	...	....	....	12.50	12.54	—0.04
45	1.022	acid	...	....	....	9.09	8.84	+0.25
46	1.023	acid	...	....	++	14.28	14.29	—0.01
47	1.025	acid	trace	....	....	13.51	13.51	—0.00
48	1.010	alk.	...	....	+	3.06	3.33	—0.27
49	1.025	acid	0.025	....	+	10.34	10.64	—0.30
50	1.016	alk.	...	....	....	5.80	5.80	0.0
51	1.023	acid	...	....	....	9.95	9.90	+0.05
52	1.023	alk.	...	....	....	10.68	10.87	—0.19
53	1.021	alk.	0.025	....	....	7.14	6.66	+0.48
54	1.019	acid	trace	....	++	9.61	9.32	+0.29
55	1.018	acid	trace	....	+	10.20	10.34	—0.14
56	1.010	alk.	0.025	....	....	3.24	3.19	+0.05
57	1.029	acid	...	....	....	14.27	14.32	—0.05
58	1.026	acid	...	....	....	15.15	15.57	—0.42

Discussion and Summary.

The following conclusions seem to be justified from the authors' experience with the persulfate methods, as well as from the results obtained:

1. That the process of digestion is shortened considerably in the macro method, thereby decreasing the danger of the loss of nitrogen by prolonged heating.

2. That the oxidation is rapid and complete, so far as the ordinary constituents of the urine are concerned. This is indicated by the agreements with the Arnold-Gunning method.

3. That the entire procedure may be carried out in a small fraction of the time required by the ordinary Kjeldahl process, or one of its modifications.

4. That the accuracy is not inferior to the Arnold-Gunning method, though a closer scrutiny and a greater number of determinations must be made to put this beyond question.

5. That the micro method yields results quickly. These agree well with the standard method employed, considering the inherent possibilities of error.

6. It is necessary that the persulfate salt used be free from nitrogen and that all traces of calcium and magnesium be eliminated from the distilled water used in making the dilutions.

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ON THE OPTIMUM REACTION IN TRYPTIC DIGESTION. I.

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Tryptic activity was long supposed to be possible in a medium of slightly alkaline reaction only. This assumption was in part based on the view commonly held that in man the pancreatic juice is rather markedly alkaline, and that this reaction, along with that from the bile, is imparted to the upper intestinal tract where pancreatic proteolysis takes place. Evidence accumulated, however, to show that the balance between the reaction of the chyme as it passes the pylorus and the several secretions poured into the duodenum is so nicely adjusted that on a mixed diet a nearly neutral condition in the small intestine follows, and indeed throughout its whole extent.[1] Something depends on the character of the food, however, as certain foods, through their digestion products, are potentially acid, while others may be alkaline. Proteins, as amphoteric elec-

[1] *Zentr. Physiol.*, 16, 33, 146 (1902); Cohnheim, "Die Physiologie der Verdauung und Ernaehrung," p. 94 (1908).