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## Determination of Iodine in Desiccated Thyroid\*

By Frederick F. Johnson† and Harry A. Nelson‡

In the course of another investigation, the authors have had occasion to determine iodine in various samples of desiccated thyroid and various extracted fractions of thyroid and iodinated protein. For extremely accurate work, and especially when very small amounts of iodine are encountered, a recent modification (1) of Leiper's wet digestion procedure (2) is preferred, but for ordinary purposes we have found that ignition in a simple alkaline fusion mixture free from oxidizing agents will give recoveries of iodine comparable to those obtained by the U. S. P. XI thyroid assay and within three per cent of the calculated theoretical values in the case of known mixtures of low iodine content. Interference by nitrates and nitrites is eliminated and the reagents are simplified.

Using four samples of desiccated thyroid and two standardized mixtures of diiodotyrosine and casein, the proposed procedure was compared with three published procedures: the U. S. P. XI assay, the Burnett and Warkow modification (3) and the Manganese Dioxide Method (4). The latter

method employs a manganese dioxide ignition mixture and is particularly applicable to the determination of iodine in the presence of oxidizing metallic catalysts. In a study involving 106 analyses by four individuals, the proposed procedure when applied to thyroid gave recoveries essentially identical with those obtained by the U. S. P. XI assay. Applied to the standard diiodotyrosine-casein mixtures, recoveries were slightly higher than by the U. S. P. method and varied between 97.2 per cent and 98.9 per cent of theoretical.

In a previous paper (4), the degree of acidity at which iodine should be liberated by iodic acid was reported. At pH 2.6 or above, the recovery was depressed slightly and the proper reagent blank was not obtained. At pH values below 2.0, the rate of return of color after titrating was increased. Burnett and Warkow (3), in their modification of the U. S. P. XI assay, state that the uncertain end-point and the blank are eliminated and accurate results obtained when the pH is adjusted to 2.5-2.7 and the temperature to about 33° before the addition of potassium iodide. In view of this criticism, the recovery of iodine from standard quantities of potassium iodate was again investigated.

*Effect of pH on the Liberation of Iodine.*—The iodine liberated by standard quantities of potassium iodate at different pH values was titrated with sodium thiosulfate. Recovery was depressed when the liberation of iodine was conducted at pH values above 2.55.

A series of standard potassium iodide samples (2.00 mg.) were treated exactly according to the U. S. P. XI thyroid assay, the iodine being liberated at different pH values. At values above pH 2.5, the solution was adjusted to 33° C. before the addition of potassium iodide. The results in Table I indicate that the recovery and the reagent blank are depressed above pH 2.5.

Table I.—Analysis of Potassium Iodide

pH	Blank as Cc.	Per Cent Recovery	Determinations
2.52-2.69	0.0	95.1- 96.9	8
1.92-1.98	0.25	100.2-100.8	5
1.52	0.30	99.74	2

Using 0.40-cc. portions of 0.005*N* potassium iodate as representing a normal blank, it is illustrated in Table II that iodine recovery from the blank is low at pH 2.1-2.2 (the range usually encountered in the U. S. P. XI assay) and that no recovery is obtained

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Table II.—Recovery from Reagent Blank

pH	Cc. 0.005 N	
	KIO <sub>3</sub>	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>
2.18	0.4	0.26
2.22	0.4	0.26
2.36	0.4	0.21
2.54	0.4	0.00
2.62	0.4	0.00
2.20	0.4	0.31
2.20	0.6	0.52
2.20	0.8	0.60
2.20	1.0	0.80
2.16	1.5	1.31
2.60	0.4	0.00
2.60	0.6	0.39
2.60	0.8	0.55
2.60	1.0	0.65
2.60	1.5	1.15

from the blank when the pH is increased above pH 2.5.

*Proposed Method.*—Accurately weigh 1 Gm. of powdered thyroid and place in a 100-cc. crucible of nickel or Corning Vycor glass. Add 5 cc. of saturated sodium hydroxide solution and 5 Gm. of anhydrous sodium carbonate. Mix thoroughly with a glass rod and leave the rod in the crucible. Add 1 to 2 Gm. of shredded filter paper to facilitate drying and to prevent creeping, and dry the crucible and contents over night at 115° C. Place in a heated muffle furnace and maintain the temperature between 500–525° C. for 20 minutes, then cool. Add 60 cc. of distilled water to the sintered mass, heat to boiling, and decant onto a 18½-cm. filter (Whatman No. 2) into a 500-cc. Erlenmeyer flask. Repeat the treatment, wash the char onto the filter and continue washing with hot water until the filtrate measures approximately 250 cc. Add 3 cc. of saturated bromine water and then slowly add 25 cc. of a mixture of equal volumes of phosphoric acid and distilled water. The pH at this step is approximately 3.2 and the bromine color should be evident. Boil until the vapors do not color starch-potassium iodide test paper. (The time required is usually between 5 and 7 minutes.) Add a few crystals of salicylic acid, wash down the sides of the flask, then cool to room temperature in running water. Add 1 cc. of 85 per cent phosphoric acid, 5 cc. of 10 per cent potassium iodide solution, mix, and titrate immediately with 0.005N sodium thiosulfate.

Except for the operation of drying the ignition mixtures over night, a series of eight determinations can be completed within three hours. There are fewer opportunities for serious error than with the U. S. P. XI procedure. Using C.P. reagents, the maximum blank which has been obtained is 0.15 cc. of 0.005N sodium thiosulfate. For an indicator solution which is free from interfering pink at the end-point, the authors prefer to use 5 cc. of a 0.5 per cent solution of arrowroot starch made soluble by the method of Wolff and Fernbach (5) and preserved with 0.01 Gm. mercuric iodide per liter. The bromine water should be prepared fresh at least once a week to prevent a return of color at the end-point.

Twenty-six assays on desiccated thyroid, after titration with sodium thiosulfate stabilized at pH 10, have fallen within the pH range of 2.20–2.55.

*Preparation of Standard Diiodotyrosine Mixtures.*—In order to obtain a thyroid-like material of known iodine content, the authors prepared quantitatively two mixtures of diiodotyrosine and vitamin-free casein. The iodine content of the diiodotyrosine was standardized by the Carius method, the Butler and Burdett method (6), and the Parr bomb method determining the iodine both gravimetrically and volumetrically. The diiodotyrosine was found to contain 54.7 per cent iodine (0.4 per cent variation between the three methods). The quantitative dilutions with casein were milled six hours in a pebble mill. The theoretical iodine contents were corrected for the iodine content of the casein, which was found to be 6 parts per million by the method of Matthews and Curtis (1). Theoretical iodine content: Mixture 1 = 0.1912 per cent; Mixture 2 = 0.2182 per cent.

*Assay of Standard Diiodotyrosine Mixtures.*—The two mixtures were analyzed by four methods, with two or more analysts performing each analysis. Representative results are tabulated in Table III.

*Assay of Thyroid Samples.*—Four samples of desiccated thyroid which had been analyzed five years previously by the U. S. P. XI procedure were analyzed by the four methods, each by four analysts. In Table IV, only the average values and maximum variations are tabulated. The figures in parentheses indicate the number of determinations composing each average.

Table III.—Assay of Standard Mixtures

Cc. Blank	U. S. P. XI	B. and W.	Proposed	MnO <sub>2</sub>
Mixture No. 1. I = 0.1912%				
0.30	0.187			0.183
0.30	0.188			0.183
0.25	0.190			0.184
0.25	0.191			
0.0		0.176		
0.0		0.177		
0.0		0.180		
0.15			0.188	
0.15			0.189	
0.15			0.189	
0.15			0.189	
Average	0.189	0.178	0.189	0.183
Per cent recovery	98.8	93.0	98.8	95.9
Mixture No. 2. I = 0.2182%				
0.30	0.208			0.209
0.30	0.210			0.210
0.30	0.211			0.210
				0.212
0.0		0.199		
0.0		0.199		
0.0		0.202		
0.15			0.212	
0.15			0.212	
0.15			0.213	
Average	0.210	0.200	0.212	0.210
Per cent recovery	96.1	91.7	97.3	96.3

Table IV.—Assay of Desiccated Thyroid

Thyroid	U. S. P. XI	U. S. P. XI, 5 Years Previous	B. and W.	Proposed	MnO <sub>2</sub>
No. 1.	0.237	0.236	0.230	0.237	0.234
Max. var.	0.002 (3)	0.006 (6)	0.007 (4)	0.004 (4)	0.001 (3)
No. 4.	0.191	0.201	0.187	0.196	0.193
Max. var.	0.003 (3)	0.006 (6)	0.002 (2)	0.008 (5)	0.003 (4)
No. 5.	0.199	0.203	0.196	0.199	0.200
Max. var.	0.006 (4)	0.004 (6)	0.001 (2)	0.000 (5)	0.003 (4)
No. 13.	0.217	0.233	0.209	0.219	0.218
Max. var.	0.011 (4)	0.001 (3)	0.006 (4)	0.003 (5)	0.002 (4)

## SUMMARY

A simplified method is presented for the determination of iodine in desiccated thyroid. The procedure involves ignition in an alkaline fusion mixture free from oxidizing agents. Interference by the hypochlorite and nitrate employed in the U. S. P. XI procedure is eliminated. Recovery of 97–99 per cent of the iodine is indicated by analysis of mixtures of standardized diiodotyrosine and casein.

The final titration should be performed at a pH within the range of 2.20–2.50, and the assays of thyroid by the proposed procedure fall within this range.

The stable iodine content of desiccated thyroid, stored in the laboratory for five years with no special precautions having been taken, is worthy of note.

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## New Pyrazolone Derivatives

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Of the hundreds of pyrazolone derivatives that are described in the literature, relatively few contain substituted phenyl groups and none have acetamino or ethoxy radicals on the phenyl. Since acetophenetidine is used in medicine like antipyrine, the introduction of these two radicals into the latter seemed a promising field of investigation. Accordingly, we started out to make some of the ten possible compounds derived by placing (EtO)(AcNH)C<sub>6</sub>H<sub>3</sub> for C<sub>6</sub>H<sub>5</sub> in antipyrine. While none of these has as yet been produced, several new intermediates have been prepared, as well as a nitrophenylpyrazolone.

## EXPERIMENTAL

*4-Acetamino-3-nitrophenetole* (1).—To a cooled solution of acetophenetidine (0.1 mol) in 50 cc. of glacial acetic acid was added gradually a mixture of 6.3 cc. (0.1 mol) of concentrated nitric acid and an equal volume of acetic anhydride. After standing thirty minutes, the whole was poured into 300 cc. of cold water. The lemon-yellow precipitate, after being filtered and dried, had a melting point of 103–104°. Yield 90% of theory.

*4-Amino-3-nitrophenetole.*—The product from the first experiment (0.1 mol) was dissolved in boiling alcohol, and a solution of 12 Gm. of sodium hydroxide in 15 cc. of water was added. The mixture was boiled under reflux for one hour and poured into 500 cc. of cold water. The red precipitate, after recrystallization from alcohol, melted at 109–110°. Yield 91.8% of theory.

*3,4-Dinitrophenetole.*—To 35 Gm. (0.192 mol) of 4-amino-3-nitrophenetole were added 88 cc. of fluoboric acid (2), and to the well-cooled mixture, stirred mechanically, was gradually added a cold solution of 13.2 Gm. of sodium nitrite (0.192 mol). The precipitated diazonium fluoborate, suspended in

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