

SCIENTIFIC SECTION

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(*To be revised.*)

A STUDY OF THE U. S. P. THYROID ASSAY.*¹

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AUTHORS' NOTE: This paper is a drastic condensation of a lengthy report submitted to the United States Pharmacopœial Committee of Revision and presents only the authors' conclusions. JOURNAL limitations have made it necessary to omit all detail and the comprehensive tables. The senior author will be glad to furnish such details to any interested person.

During the past year an unusual amount of criticism of the U. S. P. X Thyroid assay has been heard, due primarily to a difference of interpretation of method in a case in Federal Court. This led to a brief but critical study of the procedure, covering a period of three months. Sixteen samples of desiccated thyroid or compressed tablets thereof were furnished by producers, who also favored us with criticisms and suggestions regarding the U. S. P. X method. In view of the limited time available, no thought was given to the determination of thyroxine content, but our attention was limited to the determination of the iodine content. Also, in view of the fact that the U. S. P. method was approved in principle by the manufacturer's chemists who dealt most extensively with this drug, no attempt was made to devise a new procedure. We, on the contrary, directed all of our energies to the searching out of sources of error in the method, and the amplification of the directions in order that the pitfalls therein might be avoided.

Practically all of the methods proposed for this assay have been based upon the original work of Baumann (1). Such an assay consists of three stages:

1. Fusion with alkali, destroying the organic matter and binding the iodine as an iodide.
2. Oxidation of the iodide to iodate.
3. Iodometric determination of the iodate.

The Fusion Mixture.—Baumann destroyed organic matter by fusion with sodium hydroxide and nitrate. Kendall (2) used both a solution of and solid potassium hydroxide with potassium nitrate. Kelly and Husband (3) used a modification of Kendall's procedure, while Grutzner (4) used a mixture of sodium hydroxide and peroxide. Kraus (5) and Hunter (6) used a mixture of sodium and potassium carbonates and potassium nitrate for the fusion, while Volhard (7), Hofmeister (8), Neuman and Meinertz (9), Oswald (10) and Middleton (11) have studied the different alkalies and their use in organic fusions. Harden (12) proposed the use of the Eschka fusion mixture of sodium carbonate and magnesium oxide as used for the determination of sulphur in coal, in which atmospheric oxygen serves as the oxidizing ingredient.

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U. S. P. X adopted Hunter's mixture, consisting of 138 parts by weight of anhydrous potassium carbonate, 106 parts of anhydrous sodium carbonate and 75 parts of potassium nitrate. This mixture forms during fusion the eutectic corresponding to potassium sodium carbonate, the fusion point of which is lower than either of its components, and does not have the tendency to creep and spatter that occurs when a caustic fusion mixture is used.

Fusion without an active oxidizing agent may produce apparently complete decomposition, but the sulphur of the tissue protein is not completely oxidized, so that the resulting solution either liberates hydrogen sulphide or precipitates sulphur on further treatment.

Temperature and Time of Fusion.—The first defect in the U. S. P. X direction is: "Heat the crucible over a free Bunsen flame until no further carbonization is observed." Some analysts obeying this may use a strong and rapid heating while others may start with a very low flame and gradually increase the heat to the point of fusion. Results may vary because of this. Hunter recommended rapid fusion because it is safe as well as convenient. He did not observe any loss of iodine when heating thus, but found in controls that when heating was spread, by gradual increments, over one and one-half hours, results were considerably too low. Oswald also believed that rapid oxidation is least likely to cause a loss of iodine.

We find that either too rapid or too slow heating gives results that depart from the truth. Too rapid combustion causes loss of iodine by volatilization, as does a lower but much slower combustion. In our experience, the proper heat will bring the crucible and mass to a dull red in ten minutes, while holding this temperature for an additional ten minutes will yield a white fusion mass, the edges of which will have just begun to melt. No free carbon will be present, so that the solution will not require filtration, and no incompletely oxidized sulphur will be left. The mass, not having fused in its entirety, is friable and easily brought into solution in hot water.

Other advantages of the carbonate-nitrate fusion are apparent here. Carbon always remains after the caustic fusion unless a very large excess of nitrate is used. The Eschka mixture does not form a clear solution until it is acidified. The Eschka mixture also produces decided intumescence during heating.

Oxidation of Iodide to Iodate.—This may be accomplished with such oxidizing agents as bromine, chlorine water, solutions of hypochlorites, and potassium permanganate. Of the halogen-oxidizing agents, bromine is perhaps more widely used, although it has many disadvantages, such as:

- (a) It invariably contains iodine, and only painstaking washing with water will remove this.
- (b) Some of the nitrate forms nitrite during fusion, and this in turn reduces bromine to bromide, which may interfere during the later treatment.
- (c) Excess bromine is removed by boiling with only great difficulty. Therefore an agent such as phenol or salicylic acid must be added to precipitate the bromine.

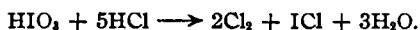
No one of our experiments involving the use of bromine was as successful as the U. S. P. procedure. We are confident that the use of this oxidizing agent requires much more attention than does that of sodium hypochlorite.

Potassium permanganate is used in the U. S. P. thyroxine assay, which is al-

most a micro-determination. An excess of permanganate must always be removed by another agent. Manganese under some conditions may catalyze the atmospheric oxidation of iodine solutions, and has been known to react with iodates.

Chlorine water and solutions of hypochlorites have one disadvantage, they may contain chlorates. Chlorine water is seldom used for the oxidation of iodide to iodate. Sodium hypochlorite, on the other hand, has two distinct advantages; *first*, it oxidizes iodide to iodate in alkaline solution; *second*, it supplies an excess of chlorine which prevents the loss of iodine upon acidification.

The use of sodium hypochlorite introduces considerable quantities of chloride. Later hydrochloric acid is liberated. Iodic acid may react with hydrochloric acid as follows:



This not only requires a large excess of hydrochloric acid but the concentration must be quite high. Such a condition is practically impossible to attain in this assay. Even on addition of sodium chloride no measurable loss of iodine occurred.

The formation of chlorates in hypochlorite solutions depends upon the temperature. We have found that if the temperature of the solution obtained from the fusion exceeded 30° C., our results were higher than when the temperature at addition of the hypochlorite was 15–20° C. This is quite likely due to the formation of chlorate in the hypochlorite solution.

By means of Wilson's (13) method for the determination of hypochlorites by direct titration with sodium thiosulphate in the presence of acetic acid, a method in which chlorates remain unaffected, we have found that the rate of change from hypochlorite to chlorate is somewhat slower in a solution prepared from bleaching powder and sodium carbonate than in one prepared from sodium hydroxide and chlorine. This, we believe, contributes to the sharper end-point in the final titration, for the chlorate may perhaps oxidize iodide at that time. Such an error is minimized if a blank determination is carried through, but it is our opinion that even high blanks should be avoided.

We would say that the greatest differences of opinion among authors have to do with such essential factors as the acidity of the solution, the nature of the oxidizing agent, and the method of removing the excess. Kendall and Kelly (*loc. cit.*) are at absolute variance on the question of acidity if bromine be used as oxidizing agent; Kendall claiming that the p_{H} during bromine removal is of greatest importance, Kelly that it is a matter of little moment. We think that when the U. S. P. procedure of oxidizing with hypochlorite is followed, the degree of acidity during the removal of excess chlorine is of considerable importance, although perhaps not so much so as the duration of or volume during boiling.

U. S. P. X directs:

"Treat the mixture with enough phosphoric acid, diluted with an equal volume of distilled water, to produce an appreciable yellow tint of free chlorine, add about 10 cc. more of phosphoric acid, diluted with an equal volume of distilled water, etc."

Such a procedure is very uncertain since this so-called yellow tint is not easily distinguished as a change from the slight tint of the hypochlorite solution. Guided by tint alone, we have been quite unsuccessful in gaging the acidity of our solution.

On the other hand, too large an excess of acid may cause the liberation of iodine from the iodate on boiling or it may interfere with the proper starch-iodine end-point. The acidity should be controlled by maintaining reasonable precision in the quantities of fusion mixture and of hypochlorite solution used. If one takes altogether 20 Gm. of fusion mixture, as directed, and 50 cc. of freshly prepared chlorinated soda T.S., 60 cc. of dilute phosphoric acid, prepared by diluting U. S. P. acid with an equal volume of water, is ample.

U. S. P. X then directs: "Boil for half an hour or until the volume has been reduced to 150 cc." Hunter observed that after boiling for ten minutes the vapor gave no blue color with starch-iodide test paper, and from this point he heated for fifteen minutes longer. We have evaporated such solutions until the volume was below 150 cc., in some cases losing iodine and in others finding chlorine still in the solution. We believe that the volume before evaporation should be at least 300 cc. and at the end not less than 150 cc., preferably 175 cc. The time of boiling ought to be not less than one and one-half hours, although no blue color is observed with starch-iodide paper held in the neck of the flask after one hour's boiling. To insure complete removal of chlorine, the solution ought to be boiled for at least one-half hour after no blue color is obtained.

Titration of the Iodine.—Obviously the potassium iodide solution added to reduce the iodate must itself be free from iodate. Too little attention, however, is often paid to the starch T.S. According to U. S. P. X, the amount might be regarded as unlimited. Too great an excess will give a reddish color to the solution that will persist after the blue has been discharged. Hence, just enough should be added to give a sharp end-point, and in our experience 3 cc. is ample, to be added just before the conclusion of the titration. Furthermore, starch T.S. prepared from pure arrowroot starch is the most sensitive and gives the sharpest end-point. This has been established by series of comparative titrations in which also potato, corn and soluble starches were used. The use of arrowroot starch T.S. is perhaps the one point on which there is nearly unanimous agreement among workers on thyroid.

Complaints are frequently heard about the fading or slipping of the end-point. It can be discharged and will return, and this may be repeated several times. Some believe that this may be due to the presence of iron or manganese. Hunter has shown that the presence of iron in the thyroid, or added in reasonable amounts before the fusion, does not affect the end-point. If manganese be present, for example, in the hypochlorite, the end-point will not be sharp and the results probably high.

If all of the factors herein discussed are carefully controlled, a sharp end-point should be obtained. We believe that if the solution does not show a return of the blue color until after at least thirty seconds, the true end-point has been reached. We have also found that traces of chlorine, bromine, or nitrous acid in the laboratory atmosphere may influence the slipping of the end-point.

A Blank Test.—U. S. P. X does not require that a control test be made. We believe that even if the purity of the reagents is assured, it is still important to accompany each set of determinations with a control test, and to apply the correction so established. The control test must start with the fusion, and may if desired be performed upon reagent casein to more nearly simulate the actual fusion.

The control will also take cognizance of the bulk of chlorate present. It must be constantly borne in mind that one is performing almost a micro-determination, and that a two-hundredth normal solution is being used in the titration. A blank of from 0.4 to 0.6 cc. of thiosulphate at least will be regularly obtained by the experienced operator, which, on a 1-Gm. sample of thyroid, means over 3 per cent of the total iodine present.

METHOD RECOMMENDED FOR U. S. P. XI.

The method recommended for incorporation in U. S. P. XI agrees in principle with that official in U. S. P. X. The changes that have been made are for the purpose of clarifying the directions and eliminating the uncertainties therein.

Procedure.—Thoroughly mix 1 Gm. of Thyroid, finely powdered and accurately weighed, with 15 Gm. of an intimate mixture of 138 parts by weight of anhydrous potassium carbonate, 106 parts of anhydrous sodium carbonate and 75 parts of powdered potassium nitrate in a nickel crucible of about 125 cc. capacity, and spread an additional 5 Gm. of this mixture evenly over the surface. Heat the crucible with the flame of a Bunsen burner at such a rate as to attain a dull red color in ten minutes, and continue the heating at the same temperature for an additional ten-minute period. At the end of this time the carbonaceous material is completely oxidized and the mixture has just begun to melt around the wall of the crucible. Cool the fusion mixture and place the crucible and contents in a 400-cc. beaker. Add 150 cc. of hot distilled water and stir until the contents of the crucible are completely dissolved. Transfer the solution to a 500-cc. Erlenmeyer flask and rinse the beaker and crucible with four 10-cc. portions of hot water, adding the rinsings to the solution in the flask. Cool the solution to 15° C. and add 50 cc. of freshly prepared chlorinated soda T.S. Cautiously add 60 cc. of diluted phosphoric acid (made by mixing equal volumes of phosphoric acid and distilled water), place the flask on a hot plate and boil the solution until a strip of filter paper moistened with starch-potassium iodide T.S. does not become blue when held in the vapor in the mouth of the flask. The final volume of solution in the flask must be about 175 cc., and distilled water must be added, if necessary, during the boiling to maintain this volume. Cool the solution to about 25° C. and add 10 cc. of a freshly prepared aqueous solution of potassium iodide (1 in 100). Titrate the liberated iodine with two-hundredth-normal sodium thiosulphate, adding 3 cc. of starch T.S. as indicator shortly before the end of the titration. Conduct a blank determination with the same quantities of the same reagents and subtract the volume of sodium thiosulphate consumed from that consumed by the Thyroid. Each cc. of the corrected volume of two-hundredth-normal sodium thiosulphate is equivalent to 0.0001058 Gm. of iodine.

THE ASSAY OF COMPRESSED TABLETS OF THYROID SUBSTANCE.

As a result of our study, it is shown that compressed tablets of thyroid can be assayed by the same method that is used for desiccated thyroid substance. Because of the presence of diluents in the tablets, a larger weight of the sample must be taken for the analysis. The quantity of oxidizing agent (potassium nitrate) in the 20 Gm. of fusion mixture used is sufficient to completely decompose the diluents present, but the total time of heating should be extended to twenty-five minutes. No filtration of the solution is required after the fusion has been taken up in water. Although no monograph for thyroid tablets is included in U. S. P. XI, directions for their assay are presented here.

Procedure.—Select 50 perfect tablets, or enough more than this number to make at least 3.5 Gm. of sample, weigh accurately and calculate the average weight per tablet. Finely powder the tablets in a clean, dry mortar. Mix 1.5 Gm. of the powdered tablets, accurately weighed, with 15 Gm. of an intimate mixture of 138 parts by weight of anhydrous potassium carbonate, 106 parts of anhydrous sodium carbonate and 75 parts of powdered potassium nitrate in a nickel

crucible of about 125 cc. capacity, and spread an additional 5 Gm. of this mixture evenly over the surface. Heat the crucible with the flame of a Bunsen burner at such a rate as to attain a dull red color in ten minutes, and continue the heating at the same temperature for an additional fifteen-minute period. Proceed as directed under the assay of thyroid, beginning with "Cool the fusion mixture." One cc. of two-hundredth-normal sodium thiosulphate is equivalent to 0.0001058 Gm. of iodine. Divide the weight of iodine found by the number of tablets represented by the weight of sample taken to find the weight of iodine per tablet.

Following these procedures as outlined, we have examined sixteen lots of desiccated thyroid powder and compressed, uncoated, tablets from various manufacturers. The results that we have obtained are found in Table I.

TABLE I.—COMPARISON OF IODINE CONTENT OF THYROID.

Sample.	Producer's Claims.	Laboratory Results by Revised Method.
1	0.218 per cent	0.236 per cent
2	0.0155 mg./tab. (Tablet)	0.0154 mg./tab.
3	0.132 mg./tab. (Tablet)	0.129 mg./tab.
4		0.202 per cent
5		0.202 per cent
6	0.40 per cent	0.367 per cent
7	0.0612 per cent (Tablet)	0.061 per cent
		0.128 mg./tab.
8	0.22 per cent	0.176 per cent
9	0.1408 mg./tab. (Tablet)	0.124 mg./tab.
		0.0757 per cent
10	0.0215 per cent (Tablet)	0.090 per cent
		0.0541 mg./tab.
11	0.425-0.422 per cent	0.423 per cent
12	0.2868 mg./tab. (Tablet)	0.240 mg./tab.
		0.117 per cent
13	0.240 per cent	0.223 per cent
14		0.119 mg./tab.
		0.0375 per cent
15	0.2251 per cent	0.237 per cent
16	0.0993 per cent (Tablet)	0.0994 per cent
		0.104 mg./tab.

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Pharmacy Week will be observed during the week of October 21, 1935.
