Chemical Identification of Defective Thyroid Preparations

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A modification of the Leland and Foster method for estimation of the thyroxine-like fraction in thyroid materials is described. This chemical method can be used to identify commercial thyroid preparations which are defective by clinical evaluation or by biological assay. In contrast, no difference in the content of the thyroxine-like fraction of effective and defective thyroid samples could be shown by our use of Blau's method.

RECENTLY we have found that certain batches and brands of thyroid tablets were relatively ineffective in the treatment of hypothyroidism in agreement with other recent reports (1, 2). Except for a British Pharmacopoeia preparation, all material clinically studied had been standardized in accordance with U.S.P. requirements. Unusually large daily amounts of these defective preparations were needed by patients who nevertheless responded at least as well to the customary small daily doses of other lot numbers or brands of thyroid. Since it was apparent that the defective thyroid tablets contained excessive amounts of nonhormonal iodinated compounds, quantitation of the hormonally active fraction might be useful in the identification of "ineffective" thyroid preparations.

Leland and Foster (3) described a method for the determination of the thyroxine content in an alkaline hydrolysate of large quantities (1 Gm.) of desiccated thyroid. We have modified the Leland and Foster method by using the sealed-tube alkaline hydrolysis procedure of Kennedy (4) for which only small amounts (1/4 to 2 grains) of thyroid material are required. The iodinated compounds in the hydrolysate are extracted into *n*-butanol and a small portion of the butanol extract, after backwashing with alkali, is analyzed directly for iodine content using a procedure modified from Bodansky, *et al.* (5). The iodide-catalyzed decolorization of ceric ion by arsenite is stopped after a predetermined time by adding brucine sulfate as suggested by Grossman and Grossman (6) which produces a stable color for photometric measurement.

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PROCEDURE

Reagents.—Reagents 1–5 were prepared exactly as described by Bodansky, *et al.* (5); all were prepared in glass-distilled water. The reagents used were 1, sodium chromate (0.5%); 2, chloric acid (28%); 3, arsenious acid; 4, ceric ammonium sulfate; 5, potassium iodate standards; 6, brucine sulfate (1%) in water; 7, thiourea (0.2%) in water; 8, 2 *N* sodium hydroxide; and 9, 4 *N* sodium hydroxide saturated with *n*-butanol.

Total Thyroid Iodine.—A single thyroid tablet (1/4 to 2 grains)[1] or a 15 to 60-mg. sample of desiccated thyroid powder in a 25 × 150-mm. Pyrex tube was digested with 0.2 ml. of sodium chromate and 6 ml. of chloric acid. We modified the procedure of Bodansky, *et al.* (5), and digested the sample at 130° for 2 hours in an aluminum heating block with holes 4 cm. deep to accommodate the tubes. Different manufacturers formulate thyroid tablets with various and varying amounts of binders and diluents creating some problems in this first digestion step. Organic diluents in the larger tablets may reduce the chromate resulting in a potential loss of iodine. Additional chloric acid may be required for the complete digestion of such preparations. Certain inorganic binders will form an insoluble residue after digestion but this does not appear to interfere with subsequent steps in the procedure. The digested thyroid sample is quantitatively transferred to a volumetric flask (100 to 500 ml.) and diluted to volume with glass-distilled water. A small aliquot of the diluted sample (0.2 to 1.0 ml.) containing between 0.01 to 0.05 mcg. of iodide is pipetted in duplicate into 25 × 150-mm. test tubes. One milliliter of glass-distilled water (for reagent blanks)

[1] A 1-grain tablet is considered to contain 60 mg. of desiccated thyroid although we are aware that not all 1-grain thyroid tablets contain exactly this amount of thyroid powder.

and 1 ml. of each of the five working standards (0.01 to 0.06 mcg. of iodine per ml.) is pipetted in duplicate into similar tubes. Chloric acid (6 ml.) and sodium chromate (0.2 ml.) are added to the unknown samples, reagent blanks, and standards for digestion at 130° for 2 hours in the aluminum heating block. Glass-distilled water (10 ml.) and arsenious acid (2 ml.) are added to the tubes after digestion as described by Bodansky, *et al.* (5), and the tubes are placed in a 28° water bath. After temperature equilibration, 0.5 ml. of ceric ammonium sulfate is added in sequence at 30-second intervals to each tube, the tube contents rapidly mixed by swirling, and the tubes returned to the water bath. Exactly 25 minutes later, 0.5 ml. of brucine sulfate is added at 30-second intervals to each sample in sequence and mixed. Thus, the iodide-catalyzed ceric ammonium sulfate decolorization reaction is allowed to proceed for exactly 25 minutes in each tube before the reaction is stopped with brucine. The color intensity of each unknown sample, reagent blank, and standard is measured in a Klett photoelectric colorimeter using a No. 42 filter. The instrument is set to zero with water; the reagent blank readings are usually about 300 Klett units. The average reading of each unknown sample, and of each standard, is divided by the average blank reading. Since a linear relationship exists between the logarithm of each standard ratio and its concentration, a standard curve can be plotted on semilogarithmic paper as described by Kontaxis and Pickering (7). The iodide content of each unknown, determined from the standard curve, is multiplied by the appropriate dilution factor to obtain the iodine content in the original digested thyroid sample.

"Thyronine" Iodine.—Although Kennedy (4) used evacuated, sealed reaction tubes for the alkaline hydrolysis of thyroid, we have found it more convenient to place a single thyroid tablet (1/4 to 2 grains) or a 15 to 60-mg. sample of thyroid powder in a 20 × 185-mm. Thunberg tube. After adding 0.1 ml. (0.2 mg.) of thiourea and 12 ml. of 2 *N* sodium hydroxide to the sample, the tube is evacuated at the water pump and sealed (while pumping) by turning the lightly greased stopper. It is heated at 100° for 16 hours in an aluminum heating block. The lower 4 cm. of the Thunberg tube is heated directly by the block while the upper 14 cm. acts as an air-cooled reflux condensor. After hydrolysis, the tubes are cooled and the hydrolysate, with any insoluble residue, is transferred by pipet to 25 × 150-mm. screwcap Pyrex tubes. The Thunberg tube is rinsed with 12 ml. of *n*-butanol which is transferred to the alkaline hydrolysate in the screwcap tube. The tubes are tightly sealed with a small square of Parafilm under the screwcap and vigorously shaken for 1 minute. After phase separation by centrifugation, the butanol layer is removed to a volumetric flask and the aqueous phase is extracted a second time with an equal volume of *n*-butanol. After centrifugation, the second butanol extract is pooled with the first in the volumetric flask and diluted to volume with butanol. The diluted solution should contain about 0.1 to 0.2 mcg. of iodine per ml. Iodotyrosines are removed from a small portion of the diluted butanol extract by shaking with an equal volume of 4 *N* sodium hydroxide saturated with butanol and separating the phases by centrifugation. Aliquots of the alkali backwashed butanol extract (0.1 to 0.3 ml.), reagent blanks and iodide standards are pipetted into 25 × 150-mm. tubes for digestion with sodium chromate and chloric acid as described previously for the determination of total thyroid iodine. It is important to digest no more than 0.3 ml. of the butanol extract with the chloric acid reagent: *larger amounts of butanol react explosively when heated with chloric acid.* After digestion, iodine analysis is done as described previously for total thyroid iodine determination to obtain the butanol-extractable iodine content ("thyronine" iodine) in the alkaline hydrolysate of the thyroid preparation.

Clinical Evaluation and Bioassay of Thyroid Materials.—Many of the thyroid preparations mentioned in this report have been assayed for biological potency.[2] Methods used for bioassay, as well as the clinical evaluation of patient response to these thyroid preparations will be fully described in a forthcoming publication. The methods used for clinical assay purposes included most of the commonly accepted metameters such as the determination of the serum PBI and cholesterol levels, general clinical criteria (including body weight changes and subjective responses), and, in a few instances, the BMR. Primary emphasis was placed on the serum PBI values. Dosage levels were multiple whenever possible for both the standard and unknown thyroid preparations, the latter being given at both high and customary dosages.

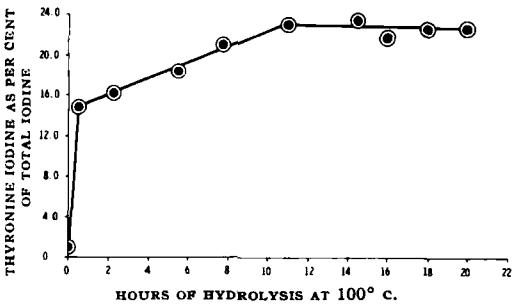

Fig. 1.—"Thyronine" iodine recovery from thyroid tablets subjected to varying periods of sealed tube alkaline hydrolysis. Points on the curve represent the average "thyronine" iodine as per cent of total iodine content for pairs of thyroid samples hydrolyzed for the indicated times.

RESULTS AND DISCUSSION

Identification of Samples.—With reference to the results to be discussed in this report, the thyroid tablets and powders are coded by number as they are received in the laboratory. Each code number refers to the lot number as received by us. A protocol has been filed with Dr. Lloyd C. Miller of the U.S.P. relating each code number to the brand and lot number of the material studied. In some cases, we have tested different lots of tablets or desiccated thyroid powders from the same pharmaceutical company.

Definition of "Thyronine" Iodine.—The term

[2] We wish to acknowledge with appreciation the cooperation of Wilson Laboratories, Chicago, Ill., and Armour Pharmaceutical Co., Kankakee, Ill., for bioassays of a number of thyroid preparations.

TABLE I.—"THYRONINE" IODINE RECOVERY

Sample	"Thyronine" Iodine, %	Total Iodine, %	"Thyronine" Iodine as % of Total Iodine
L-3,5-Diiodotyrosine	0.25	53.8	0.46
L-3,5-Diiodothyronine	21.8	47.8	45.6
L-3,3',5-Triiodothyronine	41.8	50.2	83.3
Sodium L-thyroxine powder	53.0	59.6	88.9
Sodium L-thyroxine powder	55.6	62.8	88.5
Sodium L-thyroxine tablets	43.8	50.0	87.6
Sodium L-thyroxine tablets	54.0	64.0	84.4
Sodium L-thyroxine tablets	44.7	48.0	93.1
Sodium L-thyroxine-I^{131}	9110 c/m[a]	10,240 c/m[b]	89.0
Sodium L-thyroxine-I^{131}	9580 c/m	10,590 c/m	90.5

[a] Total counts per minute recovered in the "Thyronine" iodine fraction. [b] Total counts per minute in the original sample corrected for 3% inorganic iodide-I^{131} counts as judged by filter paper chromatography.

"thyronine" iodine used in this report has special meaning. After alkaline hydrolysis of the thyroid material, the *n*-butanol soluble portion of the hydrolysate is backwashed with 4 *N* sodium hydroxide saturated with *n*-butanol to remove inorganic iodide and iodotyrosines. The alkali backwashed *n*-butanol extract of the hydrolysate contains chiefly thyroxine and triiodothyronine. The iodine content of this *n*-butanol fraction is referred to as "thyronine" iodine in this report although we recognize that this fraction may contain small amounts of other iodinated compounds.

Expression of "Thyronine" and Total Iodine Values.—"Thyronine" iodine and total iodine values are reported as per cent by weight of the material studied. In the case of thyroid tablets, it is assumed that a 1-grain thyroid tablet contains 60 mg. of desiccated thyroid material[1] and this weight is used to calculate per cent "thyronine" iodine or total iodine. In some of the tables, "thyronine" iodine is expressed as per cent of the total iodine content of the material studied. All values reported represent the mean of four or more individual determinations.

Optimal Time for Alkaline Hydrolysis.—The optimal time for alkaline hydrolysis was established by preparing a number of single 1 grain tablets of the same lot number for hydrolysis in sealed tubes. Pairs of tubes were removed at intervals from the heating block for "thyronine" iodine determinations. Points on the curve in Fig. 1 are the average of duplicates and represent "thyronine" iodine as per cent of the total iodine content of the tablet. The "thyronine" iodine recovery curve plateaus after 11 hours of hydrolysis with little change to 20 hours and resembles that reported by Leland and Foster (3). An overnight hydrolysis is convenient and all "thyronine" iodine values in this report are based on a 16 hour hydrolysis period.

Total Iodine Recovery Studies.—To evaluate our method for total iodine analysis of desiccated thyroid powders, thyroglobulin extracts, iodinated casein and L-sodium thyroxine, we compared our total iodine values with the manufacturer's reported total iodine content in 21 different samples. The mean recovery by our method was 98.5 ± 3.3 (s.d.) % of the manufacturer's indicated total iodine value (range 92.3 to 107.7%).

Thyroxine Iodine Recovery Studies.—Sodium L-thyroxine powder and sodium L-thyroxine tablets were analyzed for total and "thyronine" iodine by our method. Theoretically "thyronine" iodine represents thyroxine iodine in these samples and should correspond to the total iodine content. Results are listed in Table I for "thyronine" and total iodine content of thyroxine and other iodinated compounds. Also included in Table I are radioactive thyroxine recovery studies. Mixtures of thyroxine-I^{131} and non-radioactive thyroxine were hydrolyzed in sealed tubes and extracted with *n*-butanol for radioactivity measurement. The total counts per minute in the original thyroxine-I^{131} samples in Table I were corrected for a 3% contamination by inorganic-I^{131} as judged by paper chromatography.

TABLE II.—REPRODUCIBILITY OF REPEATED DETERMINATIONS ON THE SAME SAMPLE

	1 Grain Thyroid Tablets	Undiluted Thyroid Powder
Total Iodine; %		
Mean	0.213	0.747
Std. Dev.	0.005	0.013
No. of Samples	17	14
"Thyronine" Iodine; %		
Mean	0.0491	0.181
Std. Dev.	0.0025	0.006
No. of Samples	18	14

The mean recovery of iodine in the "thyronine" fraction in seven thyroxine samples (Table I) is 88.9 ± 2.7 (s.d.)% of the total iodine content found. This recovery is somewhat better than that reported by Leland and Foster (3) who observed a 25% loss of pure thyroxine in their alkaline hydrolysis method. Blau (8), in a study of the alkaline hydrolysis of thyroid, recovered 97 and 90% of thyroxine iodine when pure samples of thyroxine were boiled in barium hydroxide or in sodium hydroxide, respectively. Kroc, *et al.* (9), in control studies with pure thyroxine, recovered 88 to 90% of the theoretical iodine using Blau's method. In contrast to the recoveries of Leland and Foster, our more satisfactory recoveries may be due to the use of partially evacuated sealed tubes and to the protective effect of the thiourea used during alkaline hydrolysis.

Recovery of iodine in the "thyronine" iodine fraction from samples of diiodotyrosine, diiodothyronine, and triiodothyronine was determined by sealed tube alkaline hydrolysis and *n*-butanol extraction. It is evident from the results in Table I that only a very small fraction of diiodotyrosine iodine remains in the backwashed *n*-butanol extract but about 45% of any diiodothyronine iodine, if present, will be

found in this fraction. Recovery of triiodothyronine iodine (83%) is similar to that reported by Kroc, *et al.* (9).

Reproducibility of the Method.—The "thyronine" and total iodine content of one of our samples, a 1 grain thyroid tablet of known clinical potency, has been determined on many individual tablets in a number of different experiments during the past year. A summary of these results (Table II) shows good reproducibility of the method considering that some individual variation in both total and "thyronine" iodine content in the tablets of this particular lot number may exist. Included in Table II are results for the total and "thyronine" iodine content in fourteen 20-mg. samples of an undiluted thyroid powder analyzed in a single experiment which also show good reproducibility.

Effect of Lactose on "Thyronine" Iodine Recovery.—Lactose, used as a diluent in some desiccated thyroid preparations to reduce the total organic iodine content to U.S.P. or B.P. specifications, was shown by Doery (10) to interfere with the analysis of thyroxine in thyroid. Addition of lactose to desiccated thyroid powder increased the thyroxine iodine value when measured by the 1936 Addendum to the B.P. (1932) method for thyroid. Johnson and Smith (11) using the B.P. (1958) method for thyroid assay confirmed Doery's observations. Doery (10) pointed out that the sodium hydroxide was partially neutralized by organic acids formed from lactose during hydrolysis. Under these less effective hydrolytic conditions, incompletely digested protein (containing non-thyroxine iodine) was adsorbed to the flocculent precipitate which formed after acdification resulting in increased thyroxine iodine values. Doery (10) also measured the recovery of thyroxine from thyroid by the butanol extraction method of Leland and Foster (3) and observed a significant (18 to 29%) loss of thyroxine when thyroid powder was hydrolyzed with sodium hydroxide in the presence of added lactose.

The results of Doery prompted us to study the influence of added lactose on "thyronine" iodine recovery by our method from thyroid powders and from sodium L-thyroxine. The results of our experiments are shown in Table III. In this table, samples 16, 17, and 23 are undiluted, desiccated thyroid powders containing from 0.65 to 0.80% total iodine. Sample 28 is sodium L-thyroxine. The samples were hydrolyzed in the absence of and also in the presence of 20 and 60 mg. of added lactose. The butanol extracts were then analyzed for "thyronine" iodine. The reduction in the "thyronine" iodine value by lactose is relatively small, and the differences we find in the "thyronine" iodine content of effective and defective thyroid preparations cannot be ascribed to the presence of lactose in the defective material. In our procedure, the ratio of sodium hydroxide to thyroid material is greater than that used by Leland and Foster (3). The increased sodium hydroxide content and the use of thiourea in the alkaline hydrolysis step of our procedure may decrease the lactose effect described by others (10, 11).

Evaluation of Blau's Method for Thyroxine Iodine in Thyroid.—Blau (8) described a method for the determination of thyroxine in thyroid materials in which barium hydroxide hydrolysates were acidified prior to extraction of thyroxine into butanol. After backwashing with Blau's reagent, the butanol solu-

TABLE III.—EFFECT OF ADDED LACTOSE ON RECOVERY OF "THYRONINE" IODINE

Sample No.	Sample Wt., mg.	%"Thyronine" iodine in sample with: No Lactose	Lactose, 20 mg.	Lactose, 60 mg.	% Recovery of "Thyronine" iodine in presence of: Lactose, 20 mg.	Lactose, 60 mg.
16	15	0.209	0.196	0.197	93.8	94.3
17	20	0.109	0.104	0.099	95.4	90.8
23	30	0.177	0.163	0.162	92.1	91.5
28[a]	20	53.0	49.6	...	93.6	...

[a] Sodium L-thyroxine powder.

TABLE IV.—COMPARISON OF BLAU'S METHOD WITH THE PRESENT METHOD FOR "THYRONINE" IODINE

Sample No.	Total Iodine, %	"Thyronine" Iodine, % Present Method	Blau's Method	"Thyronine" Iodine as % of Total Iodine: Present Method	Blau's Method	Ratio Blau's Method / Present Method
		Ineffective Thyroid Preparations				
17	0.650	0.109	0.157	16.8	24.2	1.44
9	0.205	0.0295	0.0435	14.4	21.2	1.47
6	0.232	0.0317	0.0480	13.7	20.7	1.51
36	0.208	0.0270	0.0605	13.0	29.1	2.24
57	0.565	0.0725	0.143	12.8	25.3	1.98
42	0.213	0.0270	0.0502	12.7	23.6	1.86
37	0.220	0.0275	0.0390	12.5	17.7	1.42
		Effective Thyroid Preparations				
16	0.800	0.209	0.209	26.1	26.1	1.00
24	0.205	0.0488	0.0522	23.8	25.5	1.07
13	0.242	0.0566	0.0515	23.4	21.3	0.91
44	0.213	0.0491	0.0442	23.1	20.8	0.90
8	0.203	0.0460	0.0605	22.7	29.8	1.31
56	0.795	0.168	0.216	21.1	27.2	1.29
55	0.881	0.187	0.221	21.2	25.1	1.18
58	0.210	0.0443	0.0442	21.1	21.0	0.99

tion was analyzed for iodine. Blau stated that results by his method agreed with those obtained by the B.P. acid precipitation method for samples of commercial thyroid powder although neither his nor the B.P. method gave consistent results with thyroid tablets. He felt that the discrepancies observed in the analysis of thyroid tablets could be attributed to adsorption of iodinated compounds to the excipients used in formulating the tablets. Doery (10) suggested that acidification of a mixture of soluble and insoluble products in the alkaline hydrolysate favored adsorption of non-thyroxine iodine to the thyroxine precipitate.

We determined the "thyronine" iodine content in a number of commercial thyroid powders and tablets using the method of Blau (8) and these results are compared in Table IV with results obtained on the same preparations by our method. The first seven samples in Table IV were defective, and the last eight samples were effective preparations when tested biologically or clinically.

The data in Table IV clearly indicate that, by our method, the results for "thyronine" iodine (calculated as per cent of total iodine) sharply discriminate between effective and ineffective thyroid preparations. In contrast, using Blau's method for the measurement of "thyronine" iodine content of thyroid materials, we were unable to detect any significant difference between the effective and ineffective preparations. It is of particular interest that the "thyronine" iodine content of the effective preparations is comparable by both methods. The ineffective preparations, however, have "thyronine" iodine values by the Blau method which, on the average, exceed by a factor of 1.7 the corresponding results by our method.

Since all preparations in Table IV complied with or exceeded U.S.P. specifications, the high values by Blau's method for "thyronine" iodine in ineffective material are due to nonhormonal iodinated residues in the alkaline hydrolysate, possibly as incompletely hydrolyzed polypeptides. Such residues may be more readily soluble in butanol after acidification of the hydrolysate and are inefficiently removed when the butanol extract is backwashed with sodium hydroxide. The iodine content of the resulting

TABLE V.—SUMMARY OF RESULTS ON EFFECTIVE THYROID TABLETS AND POWDERS

Sample No.	Total Iodine[a], %	"Thyronine" Iodine[a], %	"Thyronine" Iodine as % of Total Iodine	Effective by: Clinical Assay	Effective by: Bioassay
40	0.220	0.0463	21.0	x	x
56	0.795	0.168	21.1	...	x
65	0.227	0.0478	21.1	...	...
58	0.210	0.0443	21.1	...	...
55	0.881	0.187	21.2	...	x
10	0.230	0.0491	21.3	...	...
51	0.192	0.0413	21.5	...	...
61	0.228	0.0496	21.8	x	...
59	0.217	0.0480	22.1	x	...
22	0.212	0.0471	22.2	x	...
33	0.222	0.0496	22.3	x	...
8	0.203	0.0460	22.7	x	...
47	0.855	0.194	22.7	...	...
77	0.233	0.0532	22.8	...	...
44	0.213	0.0491	23.1	x	...
29	0.263	0.0608	23.1	x	x
2	0.242	0.0558	23.1	...	...
35	0.203	0.0471	23.2	...	...
11	0.223	0.0518	23.2	...	...
52	0.237	0.0552	23.3	...	...
13	0.242	0.0566	23.4	x	x
12	0.213	0.0506	23.8	x	...
34	0.202	0.0481	23.8	x	...
24	0.205	0.0488	23.8	...	x
69	0.218	0.0525	24.1	x	x
4	0.208	0.0511	24.6	...	...
65	0.212	0.0521	24.6	x	x
14	0.223	0.0558	25.0	x	x
23	0.708	0.177	25.0	...	...
30	0.218	0.0551	25.3	x	x
5	0.200	0.0510	25.5	...	...
19	0.205	0.0523	25.5	...	...
64	0.218	0.0556	25.5	x	...
49	0.708	0.181	25.6	...	...
18	0.212	0.0550	25.9	...	...
53	0.203	0.0530	26.1	...	...
16	0.800	0.209	26.1	...	x
72	0.585	0.154	26.3	...	...
15	0.218	0.0600	27.5	x	...
32	0.333	0.0930	27.9	x	...
73	0.357	0.100	28.0	...	x
75	0.555	0.161	29.0	...	x
76	0.484	0.142	29.3	...	x
31	0.208	0.0616	29.6	x	x

[a] In the case of thyroid tablets, this per cent is based on an assumed weight of 60 mg. of desiccated thyroid powder per 1-grain tablet. (See footnote 1 in text.)

TABLE VI.—SUMMARY OF RESULTS ON DEFECTIVE THYROID TABLETS AND POWDERS

Sample No.	Total Iodine[a], %	"Thyronine" Iodine[a], %	"Thyronine" Iodine as % of Total Iodine	Defective by: Clinical Assay	Defective by: Bioassay
54	0.245	0.0280	11.4	x	x
37	0.220	0.0275	12.5	x	
39	0.240	0.0303	12.6	x	
42	0.213	0.0270	12.7	x	x
57	0.565	0.0725	12.8		x
36	0.208	0.0270	13.0	x	
62	0.215	0.0282	13.1		
41	0.215	0.0283	13.2	x	x
6	0.232	0.0317	13.7	x	x
3	0.208	0.0293	14.1		
9	0.205	0.0295	14.4	x	x
74	0.247	0.0362	14.6		x
7	0.210	0.0308	14.7	x	x
50	0.595	0.0905	15.2		x
20	0.225	0.0347	15.4	x	
79	0.575	0.0945	16.4		
60	0.203	0.0335	16.5		
17	0.650	0.109	16.8		x

[a] In the case of thyroid tablets, this per cent is based on an assumed weight of 60 mg. of desiccated thyroid powder per 1-grain tablet. (See footnote 1 in text.)

butanol extract, therefore, is due not only to "thyronine" iodine but to an excess of nonhormonal iodinated materials. It is possible that the longer period of sodium hydroxide hydrolysis used in our method and the direct butanol extraction of the alkaline hydrolysate results in an extract which, after backwashing with alkali, has an iodine content more nearly reflecting the true hormonal iodine content of the thyroid material. The effective thyroid materials containing less non-thyroxine iodinated substances would be expected to yield "thyronine" iodine values by Blau's method which are comparable to or only slightly higher than the results by our method.

Results with Present Method.—*Effective Thyroid.* The analytical results, by our method, for all thyroid tablets and desiccated thyroid or thyroglobulin powders which we studied and consider to be effective are presented in Table V. Of the 44 samples in this table, 26 are known to be clinically or biologically effective. The remaining 18 samples in this group, while not tested directly by us, were submitted to our laboratory as "good" thyroid and no unsatisfactory biological or clinical results with any of these preparations have been reported to us. In Table V, the per cent of total and "thyronine" iodine content of each sample identified by our code number is listed. Additionally, a value for the "thyronine" iodine as per cent of the total iodine content of the sample is given along with an indication of which materials studied were clinically or biologically adequate. The mean "thyronine" iodine as per cent of the total iodine content of the effective thyroid preparations in Table V is 24.2 ± 2.35 (s.d.).

Defective Thyroid.—The analytical values for "thyronine" and total iodine in a group of defective thyroid preparations are given in Table VI. Fourteen of the samples in this table have been shown to be either clinically or biologically defective. The remaining four samples, not tested clinically or biologically, are considered as unsatisfactory preparations since "thyronine" iodine expressed as per cent of total iodine content of these samples falls within the range of known defective thyroid material. One of these samples, No. 62, is known to have been formulated with desiccated thyroid powder from the same source used for four other samples in Table VI which were shown to be clinically or biologically defective. The thyroid tablets in Table VI were sold as U.S.P. thyroid and all except one, No. 39 (which had a slight excess of total iodine), met the requirements of the U.S.P. in terms of total organic iodine content. The mean "thyronine" iodine as per cent of total iodine in the defective thyroid group is 14.1 ± 1.55 (s.d.).

SUMMARY AND CONCLUSIONS

A summary of all thyroid preparations studied is given in Table VII. A highly significant difference ($p = < 0.001$) exists between the mean "thyronine"

TABLE VII.—SUMMARY OF ALL THYROID SAMPLES STUDIED

	Effective Samples	Defective Samples
"Thyronine" iodine as per cent of total iodine (Mean):	24.2	14.1
Standard error	±0.35	±0.37
Number of samples tested chemically	44	18
Range of values	21.0–29.6	11.4–16.8
Number of samples tested clinically or biologically or both	26	14
Number effective by clinical testing	19	0
Number effective by biological testing	15	0
Number defective by clinical testing	0	10
Number defective by biological testing	0	10

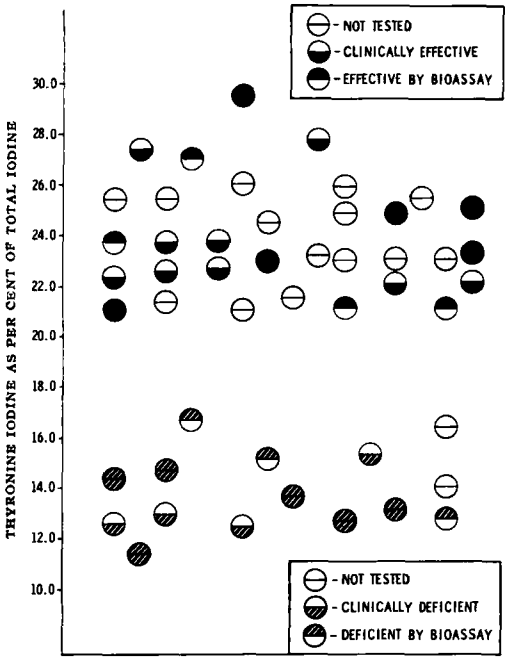

Fig. 2.—Scattergram of the "thyronine" iodine content as per cent of total iodine for individual thyroid preparations. Each point on the graph represents a different thyroid preparation and the symbols used indicate the thyroid preparations which tested clinically or biologically effective or deficient.

iodine per cent of total iodine in the effective and the defective samples with no overlapping of values between the two groups. Twenty-six of the effective and 14 of the defective preparations were biologically or clinically evaluated; some samples in each group were tested by both methods. In the effective group, 19 samples were clinically effective, 15 were biologically effective, and none were defective. In contrast, the defective group contained 10 samples which were clinically and 10 which were biologically defective, but none that were biologically or clinically effective.

In Fig. 2, the results of our chemical evaluation of some of the thyroid preparations studied are presented graphically together with an indication of those particular samples which were examined by clinical or bioassay methods.

In conclusion, we have described a method for the chemical analysis of the "thyronine" iodine content of commercial thyroid material. The "thyronine" iodine content expressed as per cent of the total iodine content of the thyroid samples correlates well with clinical or biological evaluation of the thyroid preparations.

ADDENDUM

The thyroid preparations listed in the table below have been examined for their content of triiodothyronine and thyroxine iodine in the laboratories of the Physiology and Hormones Section of the Canadian Food and Drug Directorate through the kind collaboration of Dr. N. R. Stephenson and Mr. W. F. Devlin using methods previously published by these authors [*J. Pharm. Pharmacol.*, **14**, 597 (1962)]. In the table, the values obtained by Devlin and Stephenson are compared with our "thyronine" iodine values expressed as per cent of total iodine, revealing a substantial degree of correlation.

COMPARISON OF THYROXINE AND TRIIODOTHYRONINE IODINE CONTENT WITH "THYRONINE" IODINE CONTENT OF EFFECTIVE AND DEFECTIVE THYROID PREPARATIONS

Sample No.	Triiodothyronine Iodine Plus Thyroxine Iodine as % of Total Iodine[a]	"Thyronine" Iodine as % of Total Iodine[b]
	Effective Preparations	
16	23.4	26.1
18	25.0	25.9
24	22.6	23.8
73	28.6	28.0
75	24.4	29.0
76	23.5	29.3
	Defective Preparations	
17	10.8	16.8
50	13.4	15.2
54	13.3	11.4

[a] Reported by Devlin and Stephenson (unpublished observations). [b] See Tables V and VI of this paper.

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