SERUM IODINE FRACTIONS IN HYPERTHYROIDISM [1, 2]

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(Received for publication June 15, 1942)

In untreated hyperthyroid patients, blood or serum iodine has been found to be above the normal range (1). Whether this iodine occurs in a protein-like or an inorganic form has been investigated by a number of workers, employing a variety of methods (2). Previously reported experiments from this laboratory indicated that most of the iodine was contained in the serum in the organic or protein-like form (3). It was only after iodine salts had been ingested that readily demonstrable amounts of inorganic iodine were found in the blood serum of either euthyroid or hyperthyroid people. The present investigation deals with the behavior of the serum protein-bound iodine, during iodine treatment of 15 hyperthyroid patients.

In comparison with other methods, Somogyi's zinc sulfate precipitation (4) has proved a simple and effective technique for the separation of the iodine fractions, in 6 cc. aliquots of serum of patients receiving inorganic iodine. A study has been made of the effect on the serum protein-bound iodine of giving Lugol's solution to euthyroid subjects. The kinds of iodine containing compounds precipitated by zinc sulfate and sodium hydroxide have also been investigated.

REVIEW OF METHODS

Trevorrow (5), Alpert (6), and Lein (7) have all used Somogyi's zinc sulfate precipitation method for the separation of iodine fractions, but their investigations have not dealt with protein-bound iodine in serum of hyper- or hypothyroid patients. Trevorrow worked with blood after the *in vitro* addition of thyroxin or potassium iodide (5). Alpert has used the filtrate from precipitated plasma for the estimation of diodrast iodine (6). Lein has used filtrate for evaluation of ionic iodide after the intravenous injection of potassium iodide solution in iodide tolerances (7).

The zinc sulfate precipitation method was selected because previously described methods for differentiating iodine fractions, such as precipitation with organic solvents, dialysis and precipitation of proteins with heat and acetic acid, were either inaccurate or time consuming. Trevorrow, after repeated and prolonged extraction of blood with ethyl alcohol, was able to remove completely all of the iodine from the insoluble protein fraction (with ethanol "30 to 50 per cent being removed in the first fraction, 30 to 45 per cent in the four hour continuous extraction and the remainder in the next 24 hours") (5). Boyd has recently confirmed these observations, that in blood the amount of iodine dissolved by alcohol depends on the efficiency of extraction (8). Trevorrow also found that after 4 acetone extractions, each 1 to 12 hours in length, all of the blood iodine was soluble in acetone (5). On the other hand Davison, Zollinger, and Curtis, who did not make such prolonged extractions with acetone, have tried to use acetone to fractionate the iodine in blood (9, 10). McClendon and Foster (11) employed methanol precipitation of blood iodine. It is probable, however, that with prolonged extractions methanol might be found to dissolve as much blood iodine as the ethanol and acetone employed by Trevorrow. From her work, it has been concluded that organic solvents do not separate potassium iodide or thyroxine from the iodine normally occurring in blood.

Bassett, Coons, and Salter precipitated proteins with heat and acetic acid to separate inorganic from "protein-bound" iodine. However, when the serum inorganic iodine was elevated, as after the administration of iodine to hyperthyroid subjects, there was a "spurious elevation" of protein iodine. They thought that this elevation might "be due to occlusion or adsorption and thus

[1] This investigation was aided by a grant from the Knight Fund and Fluid Research Funds of the Yale University School of Medicine.

[2] Presented in part before the Federation of American Societies for Experimental Biology, Boston, Massachusetts. April, 1942.

might be regarded as a chemical artefact" (12, 13).

Riggs, Lavietes and Man (3) dialyzed blood serum and were able to separate potassium iodide iodine from serum iodine. However, this method was time consuming and would not be practicable for clinical purposes.

PRECIPITATION METHOD

Serum is precipitated with the reagents used by Somogyi (4) to prepare filtrate for blood sugar determinations. The precipitate is washed with distilled water. Iodine in precipitate is determined by the Riggs and Man permanganate acid ashing method (14).

Reagents

Solution I—"12.5 grams of $ZnSO_4 \cdot 7H_2O$ are dissolved in water, 125 cc. of 0.25 N H_2SO_4 are added, and the mixture is diluted with water to 1 liter."

Solution II—0.75 N sodium hydroxide. "The two solutions must be so related that when 50 cc. of Solution I are titrated with Solution II, 6.70 to 6.80 cc. are required to produce a permanent pink color with phenolphthalein. The titration must be carried out by slow addition of the sodium hydroxide with continuous shaking" (4).

Procedure

One volume of serum, 8 volumes of zinc sulfate solution, and 1 volume of sodium hydroxide are shaken thoroughly. For usual sera, 6 cc. of serum, 48 cc. of zinc sulfate solution, and 6 cc. of sodium hydroxide are convenient aliquots. The mixture is then filtered through 11 cm. Whatman number 42 filter paper. The precipitate is washed 8 to 12 times with iodine-free distilled water. Stirring the precipitate with a blunt stirring rod facilitates and accelerates the washing. (The washings may be tested with dilute silver nitrate solution acidified with nitric acid. A white precipitate indicates that some chloride is still mixed with the protein precipitate. As serum contains much more chloride than inorganic iodide, the latter substance is undoubtedly completely removed when silver chloride precipitate does not form.)

The iodine determination is made by the Riggs and Man permanganate acid ashing method (14). This method has recently been shortened because only 1 heating was found necessary for complete digestion of the organic iodine compounds in serum.

The precipitate and filter paper are transferred to a 1200 cc. iodine distilling flask and the iodine determination is continued as if serum had been used. If 6 cc. of serum were precipitated, 15 grams of potassium permanganate and 210 cc. of 18 N sulfuric acid are used to digest the filter paper and precipitate. When the flasks are heated to about 100° C., excessive foaming may occur. Therefore, the flames should be low until the mixture begins to boil. If foaming is excessive, heating should be stopped and distilled water may be added to the contents of the flask. However, if the first 80 cc. of 18 N sulfuric acid are added in 2 portions so that a moderate reaction occurs, subsequent application of heat does not cause excessive foaming. It is on account of this foaming that a 1200 cc. rather than a 900 cc. iodine distilling flask should be used. An oscillating machine which shakes all the flasks back and forth during digestion has proved efficient in preventing excessive bumping during digestion.

After heating the flask and contents to 195 degrees centigrade, 80 cc. of distilled water are used to dilute the mixture before distillation.

Iodine in the filtrate may also be determined. The aliquot used depends on the probable inorganic iodine content of the serum. If the patient has been taking iodides, 10 to 20 cc. of filtrate should be sufficient. If he has had no inorganic iodine, as much filtrate as possible (40 to 45 cc.) should be used.

Protein bound iodine in serum of euthyroid subjects given Lugol's solution

In Table I are the data on 6 euthyroid subjects who were given 10 to 45 drops of Lugol's solution per day during lengths of time ranging from 1 to 7 days. The table gives the dosage of Lugol's and the number of days that elapsed between the time that administration was discontinued and the final determination of total iodine. Duplicate and average values for the total and precipitable iodines are given in order to show the agreement between the duplicates of precipitable iodine.

During Lugol's administration, the total iodines were enormously increased, ranging from 29 to 522 gamma per cent. In the first experiment, the serum total iodines before and several days after omission of Lugol's were 5.1 gamma per cent; the precipitable iodines were 5.6 and 5.9 when the patient had taken sufficient Lugol's to elevate the total iodine to 50 and 29 gamma per cent. In the second experiment, there was just as satisfactory agreement between the total iodines of 5.0 and 5.5 gamma per cent before and after Lugol's, and precipitable iodines of 5.7 and 5.9 gamma per cent when total iodines were above 100 gamma per cent. In the last 4 experiments, with the exception of one aliquot of precipitable iodine of patient 2831, the serum total iodines after Lugol's agreed within 1.6 gamma per cent with the serum precipitable iodines during Lugol's administration. That the precipitable iodine of a euthyroid individual on Lugol's solution is the same as the total iodine when not taking Lugol's

TABLE I

Serum iodines of patients with normal thyroids who were given Lugol's solution

Patient number	Date	Lugol's			Serum iodine gamma per cent					
					Total			Precipitable		
		Drops daily	Days given	Days since Lugol's	Duplicates		Average	Duplicates		Average
2782	October 14, 1941		0		4.9	5.3	5.1			
	October 16, 1941	30	2		49.0	50.6	49.8	5.8	5.3	5.6
	October 21, 1941	10	7		29.2	28.8	29.0	5.4	6.3	5.9
	October 24, 1941			2	4.9	5.4	5.1			
2798	October 22, 1941		0		4.6	5.4	5.0			
	October 27, 1941	30	4		108.0	110.0	109.0	5.7	5.7	5.7
	October 30, 1941	30	7		119.0	124.0	121.0	5.8	6.0	5.9
	November 6, 1941			2	4.2	6.8	5.5			
2814	November 14, 1941	45	1		298.0	308.0	303.0	5.2	6.2	5.7
	November 25, 1942			10	6.2	5.5	5.9			
2801	November 21, 1941	45	3		341.0	342.0	342.0	7.5	7.8	7.7
	November 25, 1941			4	5.9	6.3	6.1	5.6	5.1	5.4
2831	December 9, 1941	45	3		520.0	524.0	522.0	10.1	5.8	
	December 13, 1941			4	19.9	24.6	22.3			
	December 18, 1941			10	6.3	6.7	6.5			
2877	March 5, 1942	45	3		286.0	290.0	288.0	5.9	6.8	6.4
	March 10, 1942			5	7.4	7.4	7.4			

illustrates that this method for determining bound iodine is reasonably reliable.

Nature of iodine compounds precipitated by zinc sulfate and sodium hydroxide

In Figure 1 are illustrated certain experiments with serum precipitable iodine and thyroxin, diiodotyrosine, and after giving desiccated thyroid by mouth. Values represent the iodine in the actual aliquots used, and are the average of duplicate determinations.

In the first 2 experiments, the precipitating agents were added to thyroxin solution in tenth normal alkali. Barred columns at the left represent thyroxin iodine; cross barred columns, filtrate and washings iodine. In the first experiment, only 0.88 of a gamma of iodine was recovered in the precipitable and filtrate iodines, although the original sample contained 1.22 gamma. In the second experiment the water washings, shown at the top of the right column, were found to contain the iodine not recovered in precipitate and filtrate. It is apparent that in the absence of blood serum, prolonged washing with distilled water removed iodine from the precipitate.

In the third and fourth experiments, thyroxin solution was added to duplicate pairs of 2 different sera before precipitation. The amount of iodine in the serum is represented by the open column above the barred thyroxin column. In these 2 experiments, in spite of prolonged washing, total precipitable iodines were 93 and 94 per cent of the sum of the thyroxin and serum iodine.

In the fifth and sixth experiments, an aqueous solution of diiodotyrosine was added to serum before precipitation. Equal aliquots of serum alone were precipitated simultaneously. The iodine was determined in the precipitate and in the combined filtrate and washings of the serum alone and of the serum to which diiodotyrosine was added. The determined diiodotyrosine iodine was only 94.8 per cent of the iodine which should have occurred in the amount of diiodotyrosine (Roche) used. However, the diiodotyrosine had not been repurified or dried. Two different solutions of another sample of diiodotyrosine (Eastman Kodak Company) also gave only 91.5 and 93.5 per cent of the calculated iodine content. In calculating percentage recoveries, it was assumed that the determined iodine was more accurate than the value calculated, on the basis that

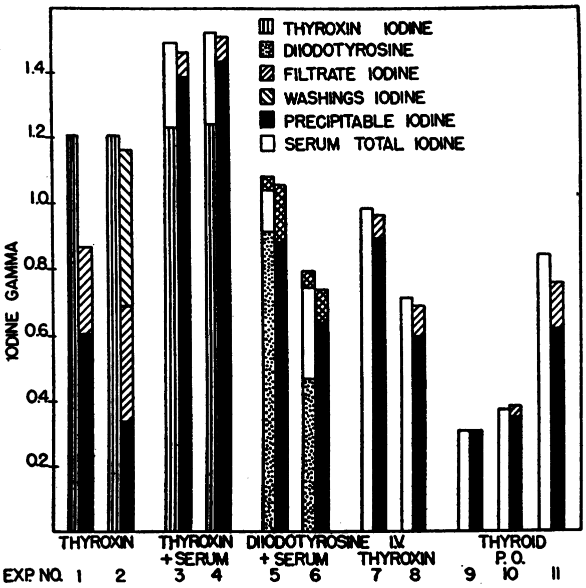

FIG. 1. PRECIPITABLE IODINE RECOVERIES IN *in vitro* AND *in vivo* EXPERIMENTS

Iodines in combined filtrate and washing solutions are represented by superimposing the cross barred symbol for filtrate iodine over the cross barred symbol for washings iodine. In Experiments 5 and 6, the open sections on the left are serum preciptable iodine, and not serum total iodine as in other Experiments.

exactly 58.6 per cent of the weighed diiodotyrosine was iodine.

In experiments 5 and 6, the iodine recovered from precipitate, filtrate, and washings of the serum to which diiodotyrosine had been added, amounted to 95.9 and 93.8 per cent of the sum of the iodine originally in precipitate, filtrate, and washings of the serum, plus the diiodotyrosine which had been added. Analysis of the diagrams in Figure 1 reveals that the major portion of the diiodotyrosine was precipitated with the proteins; but precipitation was not as complete as that of thyroxin. In the precipitates were found only 83.3 and 85.2 per cent of the iodine that should have been expected if all the diiodotyrosine had come down with the precipitable iodine of the serum. In keeping with this, there was more iodine in the filtrate and washings of the treated than of the original serum. Since the quantity of diiodotyrosine added in the 2 experiments differed greatly, while the proportions of bound iodine were the same, the failure of the diiodotyrosine to combine with protein cannot be attributed to the excessive amounts of the compound used. In addition, it may be seen that far larger amounts of iodine were bound when thyroxin was added. It is impossible to reduce further the quantity of diiodotyrosine and have theoretical recoveries of significant accuracy. What physical or chemical factors enter into this reaction, so that only 83 per cent of the diiodotyrosine iodine was precipitated, are not known.

In the seventh and eight experiments, serum total, precipitable, and filtrate iodines were determined 2 and 24 hours after intravenous injection of thyroxin solution into a normal male. Precipitable iodines were 94 and 90 per cent of the serum total iodine. In the third, fourth, seventh, and eighth experiments, the filtrate iodines were all within the range found in sera of normal subjects.

In the last 3 experiments, total and precipitable iodines were determined in the sera of 3 different subjects who had been receiving 3, 5, and 15 grains of desiccated thyroid per day by mouth. In the ninth and tenth experiments, after 3 and 5 grains of thyroid, the serum total iodines were still within the normal range and the precipitable iodines were 100 and 92 per cent of the total iodine. In the last experiment, after 15 grains of thyroid, serum total iodine rose to 14 gamma per cent, although prior to thyroid administration the serum total iodine had been 5.6 gamma per cent. When the serum iodine was elevated, the precipitable iodine was only 74 per cent of the total iodine. Whether inorganic iodine rises when large doses of thyroid are given orally cannot be confirmed until another patient can be given large doses of thyroid.

The data in Table I demonstrate that precipitable iodine is not contaminated by inorganic iodine. The third, fourth, seventh, and eighth experiments demonstrate that, in the presence of serum, thyroxin iodine would be included in the precipitable iodine. The fifth and sixth experiments show that at least 83 per cent of diiodotyrosine iodine would also be included in the precipitable iodine.

Precipitable iodine in the serum of hyperthyroid patients

In Table II are the serum precipitable iodines, basal metabolic rates, and treatment of 15 hyperthyroid patients. The term thyroidectomy indicates a one stage, and hemithyroidectomy a two stage, operation. The clinical diagnosis of hyperthyroidism was confirmed in all these patients by pathological study of glands and subsequent course after thyroidectomy.

TABLE II

Serum precipitable iodines of hyperthyroid patients

Number, age in years, sex, duration of symptoms in months	Date	Basal metabolic rate	Treatment	Serum iodine		
				Total	Precipitable	Filtrate
		per cent		*gamma per cent*		
B3065	November 14, 1941	+38			20.2	1.6
20 M						
24 mon.						
A92514	November 8, 1941	+79		17.3	15.5	2.2
54 F	November 10, 1941		Lugol's 15 drops daily			
	November 17, 1941	+27				
	November 21, 1941					
	November 22, 1941	0	Thyroidectomy		4.4	
	December 2, 1941				4.8	0
	February 27, 1942	+ 8		2.0		
B21559	November 8, 1941	+78			16.0	1.1
36 F	November 12, 1941		Lugol's 15 drops daily			
12 mon.	November 17, 1941	+37				
	November 21, 1941				9.6	
	November 25, 1941		Hemithyroidectomy			
	December 2, 1941		Hemithyroidectomy			
	December 11, 1941	− 7		9.6	8.3	0
B23831	January 22, 1942	+46		11.8	11.5	1.1
62 F	January 23, 1942		Lugol's 15 drops daily			
12 mon.	January 28, 1942	+31			9.4	
	February 3, 1942	+27		128.7	7.0	
	February 7, 1942		Thyroidectomy			
	February 15, 1942					
	February 16, 1942				5.6	
B26309	March 31, 1942	+46			10.7	1.1
53 F	April 2, 1942		Lugol's 15 drops daily			
36 mon.	April 4, 1942	+42				
	April 10, 1942	+30				
	April 16, 1942	+29			7.7	
	April 18, 1942		Thyroidectomy			
	April 25, 1942	+19				
	May 15, 1942			4.5		
B29589	October 25, 1941	+20		10.3		
19 F	October 26, 1941		Lugol's 7 drops daily			
1 mon.	November 26, 1941	+ 6			8.0	
	April 3, 1942					
	April 17, 1942			33.7	30.1	
	April 18, 1942		Lugol's 15 drops daily			
	April 21, 1942	+42				
	April 23, 1942				15.3	
	April 27, 1942				16.5	
	April 28, 1942	+25				
	May 1, 1942				15.6	
	May 4, 1942		Hemithyroidectomy			
	May 5, 1942					
	May 6, 1942		Lugol's 30 drops daily			
	May 12, 1942				12.4	
	May 14, 1942		Hemithyroidectomy			
	May 23, 1942				7.5	
A53613	April 10, 1942	+26		11.6		
32 F	April 11, 1942					
4 mon.	April 13, 1942	+18	Lugol's 15 drops daily			
	April 20, 1942	+ 4				
	April 22, 1942	+ 3			4.7	
	April 23, 1942		Thyroidectomy			
	April 30, 1942					
	May 1, 1942				4.8	
B21125	October 16, 1941			16.6		
36 F	October 17, 1941	+34	Lugol's 15 drops daily			
12 mon.	October 21, 1941	+16				
	October 23, 1941			116.6	8.6	109.3
	October 24, 1941	+11				
	October 27, 1941		Thyroidectomy			
B21360	October 24, 1941	+30		11.3		
33 M	October 25, 1941		Lugol's 15 drops daily			
12 mon.	October 27, 1941	+24				
	October 29, 1941	+26				
	November 5, 1941		Thyroidectomy			
	November 11, 1941					
	November 12, 1941	+ 9			4.0	
B22239	November 26, 1941	+36				
12 F	December 1, 1941			16.3		
1 mon.	December 2, 1941	+16				
	December 5, 1941	+22				
	December 9, 1941	+19		17.2		
	December 12, 1941					
	December 18, 1941	+14	Lugol's 3 drops daily			
	December 22, 1941	+11				
	December 29, 1941	+17		40.8	5.9	
	January 4, 1942					
	January 5, 1942		Thyroidectomy			
	January 6, 1942		Lugol's 10 drops daily			
	January 14, 1942				8.3	
	January 17, 1942	−17				
	February 12, 1942			6.2		
B22457	December 4, 1941			18.6		
35 M	December 8, 1941	+50*				
18 mon.	December 10, 1941					
	December 18, 1941		Lugol's 15 drops daily		8.5	
	December 19, 1941	+45*				
	December 24, 1941	+20				
	December 27, 1941		Hemithyroidectomy			
	December 29, 1941		Potassium iodide 5 drops daily			
	January 1, 1942					
	January 3, 1942		Hemithyroidectomy			
B23562	January 31, 1942	+35				
48 F	February 6, 1942			10.1		
3 mon.	February 18, 1942			9.2		
	March 6, 1942		Lugol's 15 drops daily			
	April 17, 1942	+22			6.1	
	April 27, 1942					
	April 30, 1942		Thyroidectomy			
B23799	January 27, 1942	+50				
62 M	January 28, 1942			19.4		
3 mon.	January 29, 1942		Lugol's 15 drops daily			
	February 2, 1942	+21		246	9.8	
	February 9, 1942	+15		99	7.3	
	February 10, 1942		Thyroidectomy			
	February 11, 1942		Syrup HI 12 cc. daily			
	February 15, 1942					
	February 16, 1942				6.9	
Pi	November 10, 1941			9.4		
35 M	November 11, 1941		Lugol's 5 drops daily			
2 mon.	January 5, 1942			28	8.9	
B25107	February 25, 1942	+25		11.7		
53 F	March 2, 1942	+31			9.8	
18 mon.	March 3, 1942					
	March 10, 1942	+42				
	March 13, 1942		Lugol's 15 drops daily		8.9	
	March 16, 1942	+18				
	March 20, 1942	+26			8.3	
	April 18, 1942				10.6	
	April 21, 1942	+30			11.8	
	April 24, 1942		Thyroidectomy			
	May 4, 1942				8.3	
	May 8, 1942	− 4				
	May 12, 1942				6.8	

* Unsatisfactory.

RESULTS

Before the administration of Lugol's solution, measurement of the precipitable, and either the total or filtrate, iodine was made in the serum of the first 6 patients in Table II. All 3 types of iodine were determined in the serum of only

A92514 and B23831. In these 2 instances, the total iodines exceeded the precipitable iodines by 1.8 and 0.3 gamma per cent, while the filtrate iodines were 2.2 and 1.1 gamma per cent respectively. The precipitable iodine of B29589 was 3.6 gamma per cent lower than her total iodine, but the serum in small aliquots was precipitated several days after the blood was taken. Usually serum has been precipitated immediately. However, in this instance contamination from inorganic iodine was feared, and the remainder of the serum was precipitated to rule out this contingency. Since the filtrate iodines were between 1.1 and 2.2 gamma per cent in 5 cases, it has been assumed that, before iodine administration, total iodines would not exceed precipitable iodines by more than about 2 gamma per cent, and therefore in the other cases total iodines alone have been determined before iodine therapy.

Total iodines during Lugol's administration were determined in the sera of only 5 patients (B23831, B21125, B22239, B23799, and Pi). These total iodines were between 28 and 246 gamma per cent. Total iodines of the other patients were undoubtedly similarly elevated.

In all patients, except B29589, Pi, and B25107 who were studied before and after Lugol's, before thyroidectomy, precipitable iodines fell to levels below or just above the maximum normal serum iodine, 8.0 gamma per cent. B29589 had mild symptoms of hyperthyroidism, a basal metabolic rate of plus 20 per cent, and a serum total iodine of 10.3 gamma per cent, in October, 1941. After 31 days, on 7 drops of Lugol's per day, her basal metabolic rate fell to plus 6 per cent, and the precipitable iodine was 8.0 gamma per cent. She was maintained on small doses of Lugol's until 2 weeks before admission to the New Haven Hospital in April, 1942. At the time of admission, her precipitable iodine was 30.1 gamma per cent. After 2 weeks of bed rest and administration of 15 drops of Lugol's daily, she showed some clinical improvement, although the precipitable iodine was 15.6 gamma per cent. A right hemithyroidectomy was followed by a stormy postoperative period. Eight days after removal of the right lobe, the precipitable iodine was still elevated, 12.4 gamma per cent. Two days later, a left hemithyroidectomy was performed. Nine days after this second stage operation, the precipitable iodine had fallen to 7.5 gamma per cent.

The precipitable iodine of Pi fell only to 8.9 gamma per cent after 49 days of treatment with 5 drops of Lugol's solution daily. At that time, he consulted Dr. Frank Lahey and Dr. Lewis M. Hurxthal. They decided to take him off iodine, but 2½ months later he showed definite clinical evidence of hyperthyroidism. At operation, each lobe of the gland was enlarged to about 3 times normal size. B25107, whose hyperthyroid symptoms increased somewhat and who did not improve even after prolonged Lugol's therapy, had no diminution but a gradual elevation in precipitable iodine. At operation, a large, substernal thyroid was excised.

In contrast with the precipitable iodines of B29589 and B25107, which have just been discussed, are those of B23831 and B23799. After 6 days on Lugol's, the precipitable iodine of B23831 had fallen to 9.4, and after 11 days, to 7.0 gamma per cent. The precipitable iodine of B23799 was 9.8 after 4 days, and 7.3, after 11 days on iodine therapy. It is possible that the precipitable iodines of B21559, B21125 and B22457, which fell only to 9.6, 8.6, and 8.5 gamma per cent, decreased more before thyroidectomy because these precipitable iodines were determined 4, 4, and 9 days before operation. These data indicate that a fall to the normal range of precipitable iodine demonstrated a good response to iodine therapy. Failure of the precipitable iodine to fall after Lugol's administration corresponded with failure of improvement in patient's symptoms. If it is difficult to obtain satisfactory basal metabolisms, the behavior of the precipitable iodine is of diagnostic significance.

Six to 97 days after thyroidectomy, the total or precipitable iodines of 10 patients were determined. The serum total or precipitable iodines of B23831, B26309, B29589, A53613, B21360 and B23799 were within the normal range. A92514, 97 days after thyroidectomy, had a total iodine of 2.0 gamma per cent which was below the normal range, but there were no evident symptoms of thyroid deficiency. B21559, B22239 and B25107, 9, 9, and 10 days after thyroidectomy, had serum precipitable iodines slightly above the normal range. However, subsequent serum total or precipitable iodines in the sera of B22239 and B25107 were within the normal range. While these postoperative studies have not been made at frequent or prolonged intervals in any patient except A92514, the serum precipitable iodine seems to reach normal concentration about 2 weeks after thyroidectomy.

DISCUSSION

How long after the administration of inorganic iodine the serum total iodine is elevated is a question which determines whether a total or precipitable iodine should be determined. In Table I, 2 euthyroid subjects, 2782, 2798, 2 days after Lugol's solution was stopped, had total iodines of the same magnitude as before iodine was given. One subject, 2831, who had the highest serum total iodine, 522 gamma per cent, has an elevated serum total iodine 4 days after Lugol's was stopped. Unfortunately, her total iodine was not determined again until 6 more days had elapsed. At this time, her total iodine was normal, 6.5 gamma per cent. Riggs, Lavietes and Man have already reported that a hyperthyroid patient, 4 days after omission of 15 drops of Lugol's solution, had a total iodine equivalent to the serum undialyzable iodine while on Lugol's solution (3). These data indicate that, while 2 to 4 days may suffice for the elimination of inorganic iodine, after large doses more than 4 days but less than 10 days may be required.

The serum iodine values, in Table II, of hyperthyroid patients before treatment are in good agreement with blood iodine levels of untreated hyperthyroid patients, enumerated in an earlier article (1). In blood, the normal range of iodine was found to be 2.4 to 4.2 gamma per cent (1). Determination of the iodine in blood and serum demonstrated that, in the absence of previous iodine therapy, the erythrocytes contained practically no iodine (3, 15). Assuming a normal red cell volume, the normal blood values of 2.4 and 4.2 gamma per cent would be equivalent to serum iodines of 4 to 8 gamma per cent. The blood iodines of 6.4 to 21.9 gamma per cent in the 31 hyperthyroid patients previously described (1) would correspond to serum iodines of 10.7 to 36.0 gamma per cent. The serum iodines of the 15 hyperthyroid patients in Table II, before treatment range from 9.4 to 33.7 gamma per cent.

That all of the 15 hyperthyroid patients in Table II had serum iodines above the normal range confirms the observation in the earlier paper that blood iodine levels are increased in hyperthyroidism. Before treatment, no definite relation existed between the elevation in serum iodine and the severity of exophthalmos. Six patients had no noticeable lid lag or prominence of eyes; exophthalmos was a definite symptom of B21559, B23831, B29589, A53613, B21125, B21360, Pi, and B25107. The average of the initial serum iodines of the patients without exophthalmos was 15.9 and that of the patients with exophthalmos 14.7 gamma per cent. It was reported previously that, even after prolonged hyperthyroidism, the blood iodine was elevated (1). The serum iodine of B26309 is in agreement with this statement. Though this patient had had symptoms of hyperthyroidism for 3 years, her serum iodine was elevated to 10.7 gamma per cent.

SUMMARY AND CONCLUSIONS

A method for separating protein-bound from inorganic serum iodine by precipitation with zinc sulfate and sodium hydroxide has been described. The advantages of this method over previously published techniques have been discussed. The measurement of iodine in the precipitate by a modification of the permanganate acid ashing technique has been described.

The precipitable iodines of 6 euthyroid subjects, who had taken 10 to 45 drops of Lugol's solution per day for 1 to 7 days, agreed within 1.6 gamma per cent with the serum total iodines after Lugol's administration was stopped. Total iodines, before Lugol's, of 2 of these 6 euthyroid subjects differed by only 0.9 gamma per 100 cc. of serum from the precipitable iodines when the patients were on Lugol's. In euthyroid individuals, 2 days after cessation of iodine administration may suffice for the elimination of excess inorganic iodine from the serum, but in 1 subject, between 4 and 10 days were necessary.

In two experiments, after the *in vitro* addition of thyroxin to serum, the precipitate contained 93 and 94 per cent of the thyroxin iodine. After intravenous injection of thyroxin, the precipitable iodines were 94 and 90 per cent of the serum total iodine. Only 83 and 85 per cent of diiodotyrosine iodine were recovered in the precipitate when diiodotyrosine solution was added to serum. After oral administration of 3 and 5 grains of thyroid daily, the precipitable iodines were 100 and 92 per cent of the serum total iodine, but after 15 grains daily the precipitable iodine was 74 per cent of the total iodine. It is concluded that this precipitate of serum contains at least 80 per cent of diiodotyrosine iodine and virtually all of the iodine in thyroxin or larger organic compounds of iodine.

Precipitable iodines of 15 hyperthyroid patients have been studied. The clinical diagnosis was confirmed in all these patients by pathological study of the glands. Before treatment with iodine, the serum total or precipitable iodines were between 9.4 and 33.7 gamma per 100 cc., distinctly above the normal range of 4 to 8 gamma per cent. In the first 5 patients, before administration of iodine, the total and precipitable iodines agreed within 2.0 gamma per cent. After Lugol's, before thyroidectomy, precipitable iodines of 11 of 14 patients decreased to concentrations below or just above the maximum normal serum iodine, 8.0 gamma per cent. In 3 patients, whose precipitable iodines did not decrease noticeably, the clinical response to iodine administration was poor. If it is difficult to obtain satisfactory basal metabolisms, the behavior of the precipitable iodine is of diagnostic significance.

Serum total or precipitable iodines of 10 of the 15 patients were within or just above the normal range about 2 weeks after thyroidectomy.

In this investigation we acknowledge with deep gratitude the aid of Dr. Paul H. Lavieties, Dr. Alexander W. Winkler, Dr. Jack D. Rosenbaum, and Dr. Branch Craige, Jr.

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