BUTANOL-EXTRACTABLE IODINE OF SERUM [1]

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It has been demonstrated chemically that the iodine bound to proteins of the serum is predominantly composed of thyroxine (1–4). The concentration of this protein-bound or serum precipitable iodine (SPI) appears to be closely correlated with the activity of the thyroid gland (5–7). It is probably the most precise clinical criterion of such activity that has yet been extensively employed. It is, therefore, generally conceded that SPI ordinarily represents circulating thyroid hormone. In studies of medically treated hyperthyroid patients, however, a number of values for SPI proved unexpectedly high in comparison with other measures of thyroid activity (5, 7). Danowski and associates (8–10) reported that SPI of normal individuals and pregnant women rose after the administration of potassium iodide. It had also been discovered that certain iodine-containing compounds commonly used for diagnostic and therapeutic purposes formed combinations with the proteins of the serum and therefore were measured with SPI (11). For these reasons it seemed desirable to examine more closely the nature of SPI and to find a more specific measure of circulating thyroid hormone.

Taurog and Chaikoff (4) utilized *N*-butanol to extract thyroxine-like compounds from serum. This solvent, originally employed by Leland and Foster (12), can be freed from diiodotyrosine and inorganic iodine by means of an alkaline reagent suggested by Blau (13). Taurog and Chaikoff (4) found that 73 to 93 per cent of the iodine of normal plasma behaved like thyroxine inasmuch as it remained in butanol extracts after these had been washed with Blau's reagent. Danowski and associates (8–10) compared the iodine extracted by butanol with SPI in the sera of normal individuals, pregnant women, and persons taking potassium iodide. The mean value for iodine extraced by butanol in the sera from 23 healthy males and nonpregnant females and 21 pregnant women amounted to 69 per cent of SPI (10). In five normal persons the ratio, butanol-extractable iodine (BEI) : SPI varied from 0.65 to 0.82 (8). BEI remained unchanged after the administration of potassium iodide, but both SPI and inorganic iodine increased (8, 9).

By a simplification of the procedure of Taurog and Chaikoff (4), BEI of a series of subjects of varied categories has been measured and compared with SPI.

METHOD FOR DETERMINATION OF BUTANOL-EXTRACTABLE IODINE

Apparatus

Pyrex centrifuge tubes with heavy glass walls containing 25 cc. below a ground glass stopper. Holes in both the stopper and tube serve as an outlet for compressed butanol vapor.

Apparatus for digestion, distillation and colorimeter recordings of micro SPI procedure (6).

Reagents

Sulfuric acid 10 per cent by volume.

N-*Butanol,* analytical reagent.

Sodium hydroxide 4 *N.*, to each liter of which are added 50 gm. of anhydrous sodium carbonate (4, 13).

Reagents for digestion, distillation and colorimetry of micro SPI procedure (6).

Procedure

To 1 cc. of serum, mixed with 0.2 cc. of 10 per cent H_2SO_4 in the special centrifuge tubes, are added rapidly, in a fine stream, 5 cc. of butanol during continuous shaking. The mixture is shaken without the stopper to prevent loss around the ground glass joint. The tube is centrifuged at 2,000 r.p.m. for exactly eight minutes to insure a firmly packed precipitate which can be completely dispersed in subsequent portions of butanol.

After centrifugation the butanol is siphoned into a second centrifuge tube through a glass tube of such short length and small diameter as to prevent loss of butanol. To the precipitate in the original tube are added 4 cc. of butanol. Before tightening the stopper, excess vapor in the tube escapes through the vent. After vigorous shaking the stopper is removed cautiously to prevent loss of butanol. When the mixture has been centrifuged for five

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minutes the second portion of butanol is siphoned into the tube containing the original butanol extract. A third portion of 3 cc. of butanol is added to the precipitate. Shaking, centrifugation for 10 minutes and siphoning are repeated as for the second extract.

To the combined three portions of butanol is added an approximately equal volume of 4 *N.* sodium hydroxide containing 5 per cent sodium carbonate. After vigorous shaking the stopper is removed cautiously to prevent loss of butanol. The mixture is centrifuged at 2,000 r.p.m. for at least 12 minutes.

The lower layer of alkali is then removed by suction or siphoning through a tube of small diameter equipped with a stopcock which permits the tube to be inserted through the butanol layer for the removal of the alkali without admitting any butanol in the tube. Two additional extractions with the alkaline reagent are performed in a similar manner. Finally, a fourth portion of the reagent is added. After shaking, the layers are either contrifuged or allowed to separate overnight.

The butanol is drawn, with suction, into an iodine digestion flask (6). When all but a one-quarter inch layer of butanol has been removed, the ground glass stopper is held above the centrifuge tube and washed with 3 to 4 cc. of butanol. This final portion of butanol is then added to the flask containing the original extract. These final 3 to 4 cc. of butanol permit the removal of most of the thin butanol layer remaining after the first suction without admission of any of the alkali and also wash the original butanol extract from the inside of the siphon tube.

The butanol is then evaporated to dryness in a boiling water bath with suction applied through wide glass tubes inserted in the necks of the flasks. The dried material is freed from the inside of the flask by adding 5 to 8 cc. of double distilled water. Eight cc. of 2 *N.* potassium permanganate and 18 cc. of 27 *N.* sulfuric acid are added and determination of iodine is conducted as in the steps for digestion, distillation and measurement of SPI (6).

The reagent blank is determined by extracting a mixture of 12 cc. of butanol and 0.2 cc. of 10 per cent sulfuric acid with the alkaline reagent in the same manner used in treating the butanol extract of serum. The butanol is extracted four times. After evaporation of the butanol, the visible residue of alkali is loosened by adding 5 to 8 cc. of water before the addition of a definite quantity of potassium iodide or iodate. The necessity of adding a known quantity of potassium iodide for the determination of an accurate blank has been discussed (6). The blank with the reagents employed has varied from 0.4 to 1.3 gamma per cent of iodine. It appears to arise from both the butanol and the alkali.

Notes

Considerable difficulty was encountered in extracting serum with butanol until a pH of 3.5 to 4.0 was obtained by adding sulfuric acid, using liquid brom phenol blue as an indicator. This pH is the same as that used by Taurog and Chaikoff (14, 15) and Rall (3) to extract thyroxine from an alkaline extract of thyroid gland. In the actual determination no brom phenol blue is used since it has been found that addition of 0.2 cc. of 10 per cent sulfuric acid gives the correct pH with 1 cc. portions of serum.

In Taurog and Chaikoff's (4) method the 45 cc. of butanol extracts of 3 cc. of plasma were washed with 50 cc. of alkaline reagent in separatory funnels. Decrease of the volume of 1 cc. of serum permits centrifugation and reduces the time required to layer the solutions. Preliminary separation of the organic from the inorganic iodine in serum by washing the precipitated proteins and using the washed precipitate is not necessary in this method. Values obtained by extracting untreated serum were the same as those obtained by extracting the washed zinc hydroxide precipitate of the same serum. For example, BEI's obtained from untreated serum were 7.2 and 7.3 gamma per cent, whereas BEI's obtained from the washed protein precipitate of the same serum were 7.8 and 6.9 gamma per cent (average 7.3 gamma per cent).

RESULTS

Recovery of thyroxine. Thyroxine, diiodotyrosine and inorganic iodide were added to butanol or to sera. The quantities of thyroxine added were of the magnitude that might be expected in sera from normal subjects or patients. The mixtures were then analyzed for BEI by the method described above. The sera were also analyzed by the same method before the additions. The results of these experiments appear in Table I. Multiple measurements were made to ascertain the extent of analytical variation.

Of thyroxine added to butanol in aqueous solution (Experiments 1 to 4) 93 to 102 per cent was recovered. When thyroxine was added to sera (Experiments 5 to 8), the increment recovered amounted to 88 to 103 per cent of the thyroxine added. The addition of large amounts of diiodotyrosine (Experiments 4 and 10) or a massive quantity of inorganic iodine (Experiment 9) did not affect these recoveries. Both diiodotyrosine and inorganic iodine appeared to be quantitatively removed by the washing with alkali. It had been shown earlier (16) that a fraction of diiodotyrosine adheres to protein, which is not removed by the method of washing employed in the measurement of SPI.

Although recoveries have been expressed in terms of per cent, there is nothing in these experiments to indicate that any precise fraction of thyroxine is recovered or lost. The differences between theoretical and analytical values in no instance exceed analytical errors; nor are they pro-

TABLE I

*Recovery of thyroxine iodine by butanol extraction**

Calculated iodine†			No. determinations	Determined BEI			Iodine recovered
Serum‡	Thyroxine	Total		Min.	Max.	Aver.	
γ %	γ %	γ %		γ %	γ %	γ %	%
0	5.3	5.3	3	5.0	5.1	5.1	96
0	8.5	8.5	7	7.3	8.3	7.9	93
0	10.6	10.6	2	10.2	10.6	10.4	98
0	10.4	10.4§	2	10.5	10.7	10.6	102
3.5	7.6	11.1	2	9.4	10.2	9.8	88
4.6	4.8	9.4	3	8.2	9.0	8.6	91
4.0	7.5	11.5	3	10.5	10.5	10.5	91
3.8	8.0	11.8	3	11.5	12.5	12.2	103
4.2	0	4.2‖	3	3.8	3.9	3.9	93
3.8	0	3.8§	3	2.9	4.0	3.6	95

* The thyroxine was given by Squibb Co. for investigational purposes. The iodine content by Squibb's analytical report was 64.4 per cent.
† 1 cc. samples were used for analyses.
‡ Calculated from BEI of original serum.
§ 32.0 gamma per cent diiodotyrosine iodine added.
‖ 22.4 gamma per cent diiodotyrosine iodine and 1,000 gamma per cent inorganic iodine added to serum with an SPI of 4.2 gamma per cent. Since the patient was receiving no iodine nor thio drug, the original serum was not subjected to butanol extraction.

portional to the amounts of iodine in the samples. It would be improper to conclude from these experiments that recovery was not complete. It may be that a small quantity, of the order of 0.4 gamma per cent, is lost in the course of the procedure.

BEI and SPI of normal serum. Duplicate or triplicate measurements of BEI and SPI in the sera of six normal individuals are presented in Table II. The difference, SPI − BEI, varied from 0 to 1.3 gamma per cent, averaging 0.6 gamma per cent. These differences again are of doubtful significance and not correlated with the amount of iodine present. The consistency with which SPI exceeds BEI in these and other experiments suggests, however, that the extractable iodine retained by butanol is actually slightly smaller than the SPI.

BEI and SPI of untreated patients. In Table III BEI and SPI of 17 patients with a variety of conditions are compared. None of the subjects had been given thyroid or antithyroid preparations, with the exception of Du, who had had no treatment for six months. There is reasonable doubt about B9489, who was extremely unreliable and given to self-medication. The first four were normal pregnant women. Cases 5 to 8 inclusive had large goiters. With the possible exception of B9489 all seemed to be in a euthyroid state. Cases 9 to 12 were suffering from obvious hyperthyroid-

TABLE II

SPI and BEI in normal individuals

Subject	Sex	SPI	BEI	SPI−BEI
		γ %	γ %	γ %
H	F	6.2	4.9	1.3
C	F	4.1	4.1	0
F	F	5.3	4.0	1.3
A	M	6.7	6.4	0.3
E	M	5.0	4.8	0.2
C	M	4.5	4.0	0.5
Average		5.3	4.7	0.6

TABLE III

SPI and BEI of untreated patients

Subject	SPI	BEI	SPI−BEI
	γ %	γ %	γ %
C45201	7.3	5.7	1.6
B53121	7.3	7.6	−0.3
	8.4	7.0	1.4
Du	8.4	8.2	0.2
Wa	7.7	5.7	2.0
C43177	5.0	4.0	1.0
C7178	6.9	5.8	1.1
C41885	7.0	6.0	1.0
B9489	13.7	7.8	5.9
Na	10.5	9.2	1.3
B86525	10.6	10.9	−0.3
A81700	11.5	10.8	0.7
B7958	16.6	15.1	1.5
Be	4.1	4.3	−0.2
Ma	5.8	4.7	1.1
C44978	4.6	5.1	−0.5
C32352	4.1	4.1	0
C44776	6.6	7.2	−0.6
Average			0.6

TABLE IV

SPI and BEI of hypothyroid patients taking thyroid

Subject	Thyroid	Lugol's solution	SPI	BEI	SPI−BEI
	gm./day	*gtts./day*	*γ %*	*γ %*	*γ %*
A79490	0.06		3.3	2.4	0.9
C9886	0.13		6.0	5.4	0.6
48533	0.13		4.3	3.9	0.4
B7257	0.13		4.5	5.0	−0.5
La	0.13		3.6	4.1	−0.5
B9007	0.13		4.2	3.2	1.0
18730	0.13		6.9	6.6	0.3
C45900	0.40		6.0	5.4	0.6
	0		3.3	3.2	0.1
	0.20		3.0	3.6	−0.6
B44180	0.33		11.0	8.9	2.1
C40160	0.06	15	9.9	6.2	3.7
A7885	0.17	14	10.8	5.1	5.7
	0.17	14	13.4	6.6	6.8

ism. The last group of five contains, in order, two young males with obesity and a woman who were suspected of hypothyroidism, a woman five or six months after thyroidectomy, and an elderly male with cerebral arteriosclerosis. The analyses in every case were made to exclude hypothyroidism. There was not satisfactory evidence in any one of them to establish this diagnosis. With one exception, B9489, mentioned above, BEI and SPI differed by no more than 2.0 gamma per cent. Again the differences are not correlated with the concentrations of iodine. BEI is usually lower than SPI, the average value of SPI − BEI being 0.6 gamma per cent.

BEI and SPI in hypothyroidism. From Table IV it can be seen that BEI and SPI of patients with hypothyroidism receiving thyroid, with one exception, differed by no more than 1.0 gamma per cent; while in three patients who were also receiving inorganic iodine the differences were 3.7, 5.7 and 6.8 gamma per cent. SPI always exceeded BEI.

BEI and SPI of patients with hyperthyroidism on medical treatment. In contrast to the data al-

TABLE V

SPI and BEI of patients with hyperthyroidism on medical treatment

Subject	Dosage for six weeks prior to study		SPI	BEI	SPI−BEI	Pulse	Serum cholesterol	Clinical impression
	Lugol's solution	Propylthiouracil						
	gtts./day	*mg./day*	*γ %*	*γ %*	*γ %*		*mg. %*	
Hyperthyroid								
A81700	14	200	15.9	14.1	1.8	76		Sweating. Skin fine. Tremor 2+
B20089	10*	300*	17.1	12.3	4.8	98		Sweating. Tremor 2+
Sh	10	0	10.0	7.8	2.2	88		Loss of weight
B37523	14	300	11.9	8.0	3.9	98	223	Sweating. Tremor 2+
B86525	14	300	10.9	6.4	4.5			Cardiac irregularity
C26245	14	300	12.5	8.2	4.3	96		Skin fine
Pe	15	150	13.5	8.0	5.5			
Na	14	200	12.0	8.9	3.1			
B74809	15	250	13.6	8.2	5.4			Tense
68952	0	150	6.5	7.5	−1.0			? Hyper.
A83086	0	100	8.8	8.2	0.6	92		? Hyper. Sl. weight loss
Hypothyroid								
C36063	14	300	12.4	2.2	10.2		584	Puffy dry skin. Hoarse voice
	14	0 (4 wks.)	11.8	2.3	9.5		419	Looked less hypothyroid
B59207	14	200	7.7	2.1	5.6			Puffy skin. Hoarse voice
	14	0 (2.5 wks.)	10.1	0.9	9.2		409	Cold dry skin
			12.6	0.9	11.7		398	Looked less hypothyroid
An	14	0 (2 wks.)	5.9	3.1	2.8	66		Sleepy. Wt. gain. Sl. increase in exophthalmos
C38870	14	200*	6.5	3.2	3.3		217	
	14	0 (2 wks.)	5.6	3.3	2.3			
B57397	0	300	2.5	2.3	0.2	72	265	
B92499	16	0	9.8	6.0	3.8	72	214	? Hypo. Hair normal. Skin sl. coarse. Enlarged goiter
De	15	0	7.4	2.1	5.3			? Hypo. Large goiter

TABLE V—*Continued*

Subject	Dosage for six weeks prior to study		SPI	BEI	SPI − BEI	Pulse	Serum cholesterol	Clinical impression
	Lugol's solution	Propylthiouracil						
	gtts./day	*mg./day*	*γ %*	*γ %*	*γ %*		*mg. %*	
Euthyroid								
C38870	14	0 (4 wks.)	6.6	3.4	3.2		249	
			9.4	4.6	4.8		297	
12966	14	150	11.4	4.3	7.1	80		Sl. weight gain
	14	150	5.7	1.5	4.2			
Bar	15	100	9.2	4.5	4.7			
A59918	10	400	12.3	7.1	5.2	96		Sweating. Tremor 2+
B37523	15	300	7.8	3.1	4.7	88	280	Feels better than ever before
31298	14	300	10.0	6.5	3.5			
	14	300	8.5	4.0	4.5			Constant weight
Bo	15	50	11.5	4.8	6.7			
B86525	14	300	7.2	5.9	1.3			
	14	0 (3 da.)	7.8	4.5	3.3			3 days after thyroidectomy
	0 (4 da.)	0 (1 wk.)	4.9	3.5	1.4			1 week after thyroidectomy
C20773	14	0	7.2	4.0	3.2			
51251	15	50	9.2	4.4	4.8			Asthmatic heart disease
A91311	10	150	10.2	3.7	6.5			
	10	150	13.7	4.7	9.0	72		Constant weight
A37787	5	150	14.8	8.6	6.2	80		Enormous goiter
	5	150	17.8	7.7	10.1	68		Sl. weight gain
B82863	14	100	7.5	3.7	3.8	72		Skin normal
Sc	7	100	10.6	3.6	7.0	80		
	7	200	10.6	6.0	4.6	78		Working without fatigue Adenoma seems smaller
Sco	8	150	6.2	3.2	3.0			
Mi	15	150	10.0	6.0	4.0		140	Lipiodol 2 yrs. previously
C40379	14	0	7.2	4.6	2.6	72		euthyroid
	0	0	7.1	7.4	−0.3	85		? Sl. hyper. Fine tremor
A44295	0	200	10.7	8.0	2.7	78		Weight constant
C1807	0	100	7.3	6.6	0.7			Hypertensive CV disease ? Pernicious anemia
C2869	0	100	5.8	4.8	1.0			
Sh	10	100	5.8	5.7	0.1			
Pregnant patients with hyperthyroidism								
A2544	15*	300*	15.1	9.5	5.6			Vomiting. Euthyroid
	15	300	11.2	6.7	4.5			Euthyroid. No sweating
			8.6	6.1	2.5			
B94697	15	25 (3 wks.) s-urea	10.5	7.7	2.8			Euthyroid
	15	25	11.1	6.8	4.3			Euthyroid
C24644	8	75	9.1	7.6	1.5	84		Euthyroid
	8	100	8.9	7.2	1.7			Euthyroid
98175	15	450	9.4	6.9	2.5			Euthyroid
	15	450	14.5	13.7	0.8			Actively hyperthyroid
	14	500 (2 wks.)	8.0	5.1	2.9			Euthyroid
	14	300	8.6	6.4	2.2			Euthyroid
	14	150 (2 wks.)	14.9	12.9	2.0			Hyperthyroid

* Taken irregularly.

ready shown are wide discrepancies between 64 simultaneous measurements of SPI and BEI on 40 patients receiving antithyroid drugs for hyperthyroidism (Table V). The difference between SPI and BEI may amount to 10 gamma per cent. In only 11 of the 64 instances was the difference as little as 2.0 gamma per cent, the maximum difference in subjects not on Lugol's solution and thio drugs. The normal range of SPI is between 3.8 and 7.8 gamma per cent (6). Often SPI is well above this when BEI lies in the euthyroid range or at lower values suggestive of hypothyroid-

ism. The euthyroid range for BEI has not really been established but has been considered to be 3.2 to 7.2 gamma per cent, 0.6 lower than the SPI range, because the average difference between the SPI and BEI of the six euthyroid subjects and of the 16 patients not on medication was 0.6 gamma per cent.

The clinical status of the patients in Table V was evaluated by a clinician when the blood was drawn. These evaluations have been used to classify the condition of these patients who were receiving medication for hyperthyroidism into the hyper-, hypo-, and euthyroid groups. In many instances only the final clinical impression has been included in the patient's history so that it is impossible to include notes on pulse, tremor, texture of skin and hair for every determination. After classification of clinical status the values for SPI and BEI were entered in the table.

All three patients who were still hyperthyroid according to pulse, tremor, etc., had SPI's and BEI's above the euthyroid range. Eight values on patients with mild hyperthyroid symptoms were above the normal range for SPI and BEI with one exception in each type of iodine determination. Eleven measurements on hypothyroid and questionable hypothyroid subjects were above or within the normal range for SPI, except for B57397, but below or at the lower limit of the range for BEI with one exception, B92499. The hypothyroid symptoms of B92499 were not classical and her serum cholesterol of 214 mg. per cent is compatible with euthyroidism. Elevation of serum cholesterol to values between 398 and 584 mg. per cent confirmed the diagnosis of the patients classified as definitely hypothyroid. Of the 30 measurements when patients appeared euthyroid, 25 BEI's were between 3.1 and 7.1 gamma per cent, but only 14 SPI's were between 3.8 and 7.8 gamma per cent. The BEI, therefore, appears to be a more accurate measure of thyroid activity than the SPI.

This conclusion is verified but is not as obvious in the values of the last group of Table V where are listed 11 values on four hyperthyroid patients who were pregnant and one value on B94697 two days postpartum. Except on two occasions of thyroid overactivity (98175) these patients appeared to be euthyroid. In euthyroid pregnancy the SPI is elevated to 6.0 to 10.0 gamma per cent (17). The SPI correlated closely with the clinical state of the patient except in the first value of 15.1 gamma per cent when A2544 seemed to be euthyroid. The BEI at the time of the 10 measurements, when patients appeared euthyroid, ranged between 5.1 and 9.5 gamma per cent. The range for BEI in euthyroid pregnancy appears reasonable because the SPI of normal pregnant women not on antithyroid medication was 6.0 to 10.0 gamma per cent and the BEI was only 0.6 gamma per cent lower than the SPI of euthyroid and untreated patients. When 98175 exhibited signs of thyroid overactivity, the BEI rose to 13.7 and 12.9 gamma per cent, concentrations indicative of hyperthyroidism even during pregnancy.

DISCUSSION

The reasons for the false high values of SPI are still being investigated.

The amount of propylthiouracil taken does not seem to be the single factor associated with the discrepancy between the SPI and BEI. For example, four of the largest discrepancies of 9.0 to 10.1 gamma per cent were observed in two patients, C36063 and B59207, who had stopped taking propylthiouracil two to four weeks earlier, and in two patients who were taking only 100 mg. of propylthiouracil daily. In contrast to these high differences are three below 2.9 of 98175 taking 450 or 500 mg. and four differences between 0.2 and 4.5 of four patients taking 300 mg. of propylthiouracil daily (B57397, C26245, 31298, B86525).

Differences between the SPI and BEI are obvious in patients taking Lugol's solution. This is especially apparent from the results on the 12 hypothyroid patients. The SPI and BEI agreed within 1.0 gamma per cent in nine determinations on eight patients taking either 1, 2, 3 or 6 grains of desiccated thyroid daily. But two patients who were taking Lugol's as well as desiccated thyroid had SPI's 3.7, 5.7 and 6.8 gamma per cent higher than their BEI. Seven of 10 patients on Lugol's but no propyl had SPI higher than BEI in amounts varying between 2.2 and 4.8 gamma per cent (Sh, B92499, An, C38870, C20773, C40379, B86525, C36063, B59207, De).

As yet we have only six values on patients receiving propylthiouracil but no Lugol's solution. The SPI's were 0.2, 0.6, 0.7, 1.0 and 2.7 higher and 1.0 gamma per cent lower than the BEI (A44295, B57397, 68952, A83086, C2869, C1807).

The dosage of Lugol's solution does not seem to be the factor which affects the increment between the SPI and BEI. Two patients on only 5 drops of Lugol's daily had differences between the SPI and BEI of 3.8 and 6.2 gamma per cent, while two patients on the large amount of 30 drops daily had differences of 3.8 and 7.4 gamma per cent. In 40 comparisons of SPI and BEI of patients taking 14 or 16 drops of Lugol's daily, the differences ranged from 0.8 to 10.2 gamma per cent.

The difference between the values for SPI and BEI is not the result of deterioration of serum since the BEI of two hyperthyroid patients on Lugol's and propylthiouracil was the same immediately after the blood was drawn, two, and four or five days later. In one serum the SPI was 15.9, the BEI 14.1, 14.0 and 13.5 gamma per cent. In the other blood the SPI was 11.2 and the BEI 6.7, 6.2 and 6.9 gamma per cent immediately, two and five days after the blood was drawn.

The differences between the SPI and BEI of patients on Lugol's solution can not be ascribed merely to inclusion of inorganic iodine with the precipitated serum protein. SPI has been measured before and after the addition of potassium iodide to three different sera from patients not taking inorganic iodine. Two of these fresh sera were incubated at 37° for 24 hours with 200 gamma per cent of potassium iodide. In the third experiment a similar quantity of iodide was added immediately prior to precipitation with zinc hydroxide. The concentration of 200 gamma per cent of potassium iodide had been selected because this amount or less has been observed in sera of patients on 15 to 30 drops of Lugol's daily. In each instance the SPI of the serum alone was lower than that of the serum plus iodide but the differences were 0.8, 0.8, and 1.1 gamma per cent, amounts far smaller than the differences between the SPI and BEI of the patients on Lugol's solution. For example the SPI of a patient taking 30 drops of Lugol's solution (serum inorganic iodine 225 gamma per cent) was measured in two samples of serum centrifuged four times with double distilled water and on two samples centrifuged five times. The average of those washed four times was 11.8, of those washed five times 11.3 gamma per cent in spite of a BEI of 4.4 gamma per cent. No inorganic iodine could be measured in the fifth washing and only 0.3 gamma per cent in the fourth. Danowski's observations (8, 9) are in agreement with our conclusion that the presence of inorganic iodine in serum would not in itself cause the elevation of SPI above the BEI of patients taking inorganic iodine.

It seems rather that when inorganic iodine is taken, some iodine-containing compounds in addition to thyroxine are included in the SPI. This hypothesis depends on the observation that the metabolic status of patients correlated more closely with the value for butanol-extractable iodine than with that of the SPI. Our data give no evidence of the exact chemical composition of the noncalorigenic iodine compounds included with the SPI. Diiodotyrosine is a suspect since it has been demonstrated that this compound is not entirely removed from serum proteins in the procedure for the determination of SPI (16). Monoiodotyrosine and other organic compounds containing iodine may have similar affinities for the proteins (15, 18–27).

Obviously SPI may give falsely high values for circulating thyroid hormone in patients who are receiving inorganic iodine. The conclusion has been drawn that a SPI as high as 8.0 gamma per cent is compatible with euthyroidism, although the average value for normal subjects is 5.4 gamma per cent. Engstrom and coworkers (7), in a critical analysis of the SPI of 139 hyperthyroid patients under medical treatment, found that SPI agreed with clinical criteria in 119, or 86 per cent. Discrepancies were encountered most frequently when patients became slightly or frankly hypothyroid (5). In these cases SPI did not fall below 4 gamma per cent.

Investigations are continuing to ascertain whether, as seems probable, BEI is a more accurate measure than SPI of circulating thyroid hormone.

The artifactual high values of SPI produced by priodax are also found in BEI.

CONCLUSIONS

SPI and butanol-extractable iodine (BEI) differ by no more than 2.0 gamma per cent in normal persons, pregnant women, and persons with thyroid disorders who are untreated or receiving thyroid. SPI usually exceeds BEI by an average of 0.6 gamma per cent.

In patients who have received inorganic iodine, SPI frequently exceeds BEI. The BEI seems to be more consistent than SPI with other criteria of the clinical status of such patients.

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