

THE CLINICAL DETERMINATION OF PROTEIN-BOUND IODINE ¹

By S. B. BARKER, M. J. HUMPHREY, AND M. H. SOLEY ²

(From the Departments of Physiology and Internal Medicine, State University of Iowa, Iowa City)

(Submitted for publication August 21, 1950; accepted, October 17, 1950)

The value of plasma protein-bound iodine (PBI) measurement in the evaluation of thyroid disease has been well established (1-7), but it has not yet assumed its deserved place in the clinical armamentarium because of the technical difficulties involved in distillation procedures. Modification of an alkaline-incineration technique (8) has resulted in the evolution of a method for PBI determination which is practicable for a clinical laboratory. It must be appreciated that extreme care will always be required in the quantitative measurement of 0.05 μgm . PBI/cc. plasma as well as in avoiding contamination.

SOLUTIONS REQUIRED

All solutions should be made up in double-distilled water. If the amount of iodide in the water is still too high to give a satisfactory blank, it may be necessary to carry out these distillations with such reagents as alkali and permanganate.

All reagents used should be of highest purity obtainable. However, the iodine content in each instance must be judged from the blank values rather than from estimates shown on the labels. Often it is necessary to turn to a new source of supply or a different lot number in case of contamination.

Zinc sulfate. 100 grams of $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ per liter of solution.

Sodium hydroxide. 0.5 N is 20 grams of NaOH per liter of solution. The zinc sulfate and sodium hydroxide reagents should be so related that when 10.0 cc. of the former are diluted with 50-70 cc. of water and titrated with the latter, 10.8-11.2 cc. of the 0.5 N NaOH are required to produce a faint permanent pink color with phenolphthalein.

Hydrochloric acid, 2 N. 200 cc. C.P. concentrated HCl per liter of solution.

Sulfuric acid, 7 N. 196 cc. C.P. concentrated H_2SO_4 per liter of solution.

¹ This work was supported in part by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service and in part by a research grant from the Central Scientific Fund of the College of Medicine, State University of Iowa.

² Deceased June 21, 1949.

Iodide standards. Either NaI or KI of highest purity desiccator-dried may be used. If NaI is chosen, 118.1 mg. are dissolved in water and made to 1 l., if KI, 130.8 mg. These concentrated stock solutions contain 100.0 μgm . I/cc.

A more dilute stock is obtained by diluting 2 cc. of the above solution to 1 l. To prepare the standard of 0.04 μgm . I/cc. solution used in each determination, 10 cc. of this dilute stock are further diluted to 50 cc.

All of these solutions have been found to keep indefinitely at refrigerator temperatures, but it is preferable to make up the most dilute standard fresh every week or two.

Ceric ammonium sulfate, 0.02 N. 12.65 grams of the salt are dissolved in 500 cc. of water plus 230 cc. of 7 N H_2SO_4 . When the solution is clear, it is made to 1 l. with water.

Sodium arsenite, 0.1 N. Dissolve 4.95 grams of As_2O_3 in 25 cc. of 4% (1N) NaOH, warming to hasten solution. This should be diluted with about 300 cc. of water and dilute sulfuric acid added until the solution is slightly acid to litmus paper (if the 7 N is used, about 4 cc. will be required). The solution is then made to 1 l. If sodium arsenite itself is used, 6.50 grams of NaAsO_2 should be dissolved in distilled water and diluted to 1.0 l. No acid or alkali is required with this salt.

ANALYTICAL PROCEDURE

The steps involved can most conveniently be described under four headings: 1) precipitation and washing of plasma proteins, 2) drying and incineration of sample, 3) dissolving iodide from the ash, 4) colorimetric determination of iodide present.

1) **Precipitation (9) and washing of plasma proteins.** The blood sample, about 10 cc., is centrifuged and the plasma or serum drawn off. Duplicate aliquots of 1 cc. each are pipetted into 15 x 125 mm. Pyrex test tubes, diluted with 7 cc. of distilled water and 1.0 cc. of 10% zinc sulfate solution added. The contents of each tube are mixed with a glass stirring rod about 2 mm. in diameter, 1.0 cc. of 0.5 N sodium hydroxide is added and the solutions thoroughly mixed to insure even distribution of the alkali and complete precipitation of the proteins. Any material adhering to the rod can be removed by rubbing it with a rotary movement against the inside wall of the tube.

The tube and contents are centrifuged for 10 minutes to pack the precipitate and the supernatant fluid is poured off. Ten cc. of distilled water are added and the protein resuspended by means of the same stirring rod

originally used. Stirring should be only sufficiently vigorous to distribute the zinc proteinate uniformly throughout the solution; if over-zealously carried out, the precipitate may be so finely divided that it cannot easily be centrifuged down. Centrifugation is again carried out and the supernatant discarded. This washing process is repeated two more times, making a total of three washings (the original precipitation is not included as a washing).

2) *Drying and incineration.* After the last supernatant solution has been poured off, 0.8 cc. of 4.0 N sodium carbonate is added and thoroughly stirred into the precipitate with the same stirring rod used in the washings. Removal of material clinging to the rod can be accomplished by rubbing as described previously, followed by 0.2 cc. of the 4.0 N Na_2CO_3 added dropwise down the rod. This last portion of alkali can easily be mixed with the sludge already present without use of the stirring rod. The tube is then placed in an oven set at 85–95° C. to drive off water. This usually requires 12–18 hours and is usually carried out overnight.

After thorough drying of the alkali and zinc proteinate, ashing is carried out by placing the tube in a muffle furnace for two and one half hours at $600 \pm 25^\circ$ C. At the end of that time, the tube is removed and allowed to cool to room temperature.

3) *Dissolving iodide from the ash.* Two cc. of 2 N hydrochloric acid are added with due caution to avoid excessive effervescence. A glass rod is used to mix any unreacted ash with the acid, and 2.0 cc. of 7 N sulfuric acid and 3.0 cc. of water are added. The contents of the tube are stirred until the reaction appears to be finished, transferred to a clean tube and briefly centrifuged to pack the insoluble material.

4) *Determination of iodide (10).* For each tube a pair of colorimeter tubes is taken. To one of the pair is added 1.0 cc. of distilled water containing 0.04 $\mu\text{gm.}$ of iodine as sodium or potassium iodide and to the other is added 1.0 cc. of water. A 3.0 cc. aliquot of the supernatant in the sample tube is pipetted into each colorimeter tube and 0.5 cc. of the sodium arsenite solution is added to each tube. Mixing should be carried out by some technique which avoids both loss of solution and contamination.

The colorimeter tubes are then placed in a well-stirred constant temperature water bath maintained at $39 \pm 0.1^\circ$ C. for 10 minutes to come to temperature. The ceric ammonium sulfate solution should also be warmed in the bath. Since the actual determination of iodide is accomplished by measuring iodide catalysis of the rate of decolorization of yellow ceric ammonium sulfate by arsenious acid, the times at which colorimeter readings are made must be definitely set up. One cc. of the ceric solution must be added to each tube with adequate time allowance for reading the color change in the previous tube. This is accomplished in this laboratory by leaving a 30-second interval between tubes and by setting up a maximum of only 12 tubes in a single series. Colorimeter readings are then made at six and 12 minutes after adding the ceric sulfate solution to each tube. If 30 seconds is not adequate for making the reading, more time must be allowed, and fewer determinations done in any one series.

Since the measurement depends entirely upon the decrease in amount of color, the pipetting of the 1.0 cc. of ceric solution must be highly accurate, even though done rapidly. Mixing must again be rapid and thorough.

The Klett-Summerson readings (using blue filter No. 42) are plotted on semi-log paper against the time in minutes. Ordinary graph paper is suitable for instruments giving readings in terms of per cent light transmission. The colorimeter setting at zero time is obtained for any given batch of reagents by quickly reading a reagent blank immediately after addition of the ceric sulfate. The lines describing the two reaction rates are drawn using the points for 0, 6 and 12 minutes, as is shown in Figure 1 and then the horizontal line indicating times required to reach equal amounts of decolorization. The amount of iodide in the colorimeter tube without added iodide is calculated from the following equation, obtained from similar triangles (see Figure 1):

$$\mu\text{gm. I in colorimeter tube} = \frac{0.04 \times t_2}{t_1 - t_2}$$

t_1 = time in minutes at intercept of the rate of decolorization in tube containing no extra iodide with the designated horizontal.

t_2 = time in minutes at intercept of the rate of decolorization in tube containing added iodide with the designated horizontal.

The reagents used contain variable amounts of iodide and the total must be determined by blank analyses starting with the zinc sulfate and sodium hydroxide solutions and carried through the entire procedure, including the washings with 10 cc. of distilled water, addition of 1 cc. of sodium carbonate, drying, ashing, treatment with hydrochloric and sulfuric acids and the final colorimetric assay on 3 cc. aliquots just as with an unknown sample. The blank value calculated by the same formula shown previously has been 0.015–0.017 $\mu\text{gm. I/tube}$ in this laboratory.

Since it is customary to express the values in terms of $\mu\text{gm./100 cc. plasma}$, the complete calculation would follow:

$$\begin{aligned} \text{Plasma PBI } \mu\text{gm. I/100 cc.} \\ = 100 \times \frac{7}{3} (\text{I in final aliquot—blank I}) \end{aligned}$$

where the factor of 7/3 has been introduced to correct for the 3.0 cc. aliquot taken for colorimetric evaluation from the 7.0 cc. of acid supernatant from the ashed sample.

If so much iodide is present in the sample that decolorization is nearly complete in the second tube, with 0.04 $\mu\text{gm.}$ of added I, the determination may be repeated using less than the 1.0 cc. of plasma usually called for. Computation of the results must be adjusted as necessary to cover this change.

NOTES AND PRECAUTIONS

It must be emphasized that the various conditions explicitly detailed herein have been evolved after intensive study. Modifications to suit any

other laboratory are quite in order, but they must be rigorously tested before being incorporated into the procedure.

General. Scrupulous care must be exercised to insure clean glassware. A detergent-soap mixture such as "Lakeseal"³ is preferred for routine cleaning. This solution is rinsed off with tap water followed by double-distilled water. Contamination, especially from iodine and mercury, must be guarded against. Since elemental iodine is so volatile and in such prevalent use as an antiseptic, this comprises the greatest hazard. Tincture of iodine must be avoided as an antiseptic on the skin of the patient before the sample is drawn and of any persons carrying out the actual determination. If elemental iodine is being used in any chemical determination or histological procedure in the same laboratory, irregular and unpredictable contamination is almost inevitable. It is thus best to devote an entire small room to the iodine work if dependable results are to be obtained.

In contrast to this trouble which is usually avoidable, once the patient has been administered any form of organic iodide, such as Lipiodol, Priodax, Skiodan, Diodrast or tetraiodophenolphthalein, binding of the iodine compound onto plasma proteins (11-13) renders the determination without value. Within a few hours, the PBI rises, often reaching a peak greater than 350 $\mu\text{gm. \%}$. Depending upon the nature and amount of the particular substance plus the method of its administration, elevated PBI values have been observed for six months to three years in the absence of any genuine metabolic disorder (2, 14). No satisfactory method exists for separating these iodine-containing compounds from the true PBI, even the extraction of "thyroxine iodine" with butyl alcohol (15). It is highly recommended that information as to this type of contamination be widely disseminated so that samples for PBI analysis will be drawn before rather than after a diagnostic procedure using any organic iodide.

1) *Precipitation and washing of plasma proteins.* The blood sample may be collected with an anticoagulant or may be allowed to clot. The advantages of the former are speed in obtaining the plasma and a larger volume of fluid resulting, and of the latter that no foreign material need be added

to the blood. No difference has been found between plasma and serum PBI and the routine in this laboratory has been to use oxalated bottles prepared in advance by evaporating 0.1 cc. of 20% potassium oxalate solution in each. The labels should be distinctive to avoid exchange with bottles from other laboratories. If serum is to be used, a paraffined tube is advantageous to allow easy clot retraction.

The procedure of washing the zinc proteinate precipitate by resuspension in distilled water and centrifugation has been found by both chemical and radioiodine tracer studies⁴ to remove between 99.0 and 99.5% of the inorganic iodine present. At plasma inorganic I levels of 2 $\mu\text{gm. \%}$ (for normal humans) to 100 $\mu\text{gm. \%}$ (on some Lugol's-treated patients), the residual amounts (about 0.01 to 1.00 $\mu\text{gm. \%}$) are not large enough to interfere. However, thyrotoxic patients being given five drops t.i.d. often are up to 300 $\mu\text{gm. \%}$, and one woman receiving 20 drops t.i.d. rose to 1120 $\mu\text{gm. \%}$. Although it is of questionable therapeutic value to use such extremely high iodine doses, the situation occasionally comes up and the laboratory must constantly be on the lookout. If all iodine therapy is stopped at least four days prior to drawing a sample for PBI, the plasma inorganic iodide will return nearly to the normal level, so that three washings are adequate. If I^{131} uptake by the thyroid gland is also to be measured, an even longer period of time will be required. It is felt that this type of precaution is better than increasing the number of washings of the protein precipitate.

2) *Ashing.*⁵ It does not appear necessary to take any elaborate precautions to insure absolutely even distribution of the protein-sodium carbonate suspension on the wall of each tube. However, it is desirable to avoid leaving the entire contents at the bottom of the tube, since this may lead to

⁴ The I^{131} used in this investigation was supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

⁵ For the most consistent results possible, it has been found desirable to have a muffle furnace available for the exclusive use of the iodine laboratory. While awaiting replacement of a defective relay on our furnace, we attempted to share one in the institution's water laboratory. Random contaminations immediately became a serious problem until we could return to our own apparatus, although no source for the added iodide was ever found.

³ Obtainable from the Finger Lakes Chemical Co., Etna, N. Y.

large bubble formation during the drying. Often such a bubble will rise through the entire tube, carrying ahead of it considerable protein material and causing a loss of PBI.

The ashing time and temperature have been found not critical, variations from two to three hours and from 575° to 625° C. causing insignificant differences in results. At temperatures above 650° C., softening of the Pyrex tubes occurs as well as loss of iodine. Although the ash in the recommended temperature range is gray rather than white, the incineration is adequate to put all of the organic iodine into extractable inorganic form. Below 500° C., there are so many products of incomplete ashing that the acid solution is yellow and substances are extracted which give a very high "iodine" value.

Each tube is used three or four times and then discarded in order to avoid excessive etching with possible loss of the sample.

3) *Dissolving iodide from ash.* The effervescence when the hydrochloric acid reacts with the ash is highly desirable in carrying off hydrogen sulfide formed from the sulfur-containing amino acids. If hydrogen sulfide remains when the ceric sulfate is added, insoluble ceric sulfide precipitates. Under the conditions described, this situation has never been observed.

4) *Colorimetric determination of iodide present.* All steps of this part of the procedure must be carried out with great care to reproduce accurately all conditions of volumes, time and temperature, since the measurement is made on a basis of iodide catalysis of a rate reaction. The actual conditions may be varied considerably, but those decided upon must be duplicated carefully from analysis to analysis if good agreement is to be had. This report has described the technique used at present in this laboratory on the experience of two years.

All mixing of solutions in this last phase must be accomplished by some technique which avoids both contamination and loss of solution, such as twirling each tube between the hands, flicking the bottom of each tube with several fingers in succession, or rapidly inverting each tube. The inversion or "flipping" procedure is preferred; this is carried out by grasping the colorimeter tube close to the open end between the thumb and first finger. The tube is inverted by raising it over one's head and then is rapidly re-

turned. With a little practice one can achieve complete mixing of practically all miscible solutions in eight to 10 such inversions without losing a drop. This technique is especially valuable when the ceric sulfate solution must be mixed quickly and completely with the use of only one hand.

The method described earlier for carrying out the calculation was devised in order to rule out any possible effect of substances remaining in the final acid solution from the ash. Many non-volatile compounds could conceivably be present in solution to alter the final colorimetric assay. Any such substance should affect the standard 0.04 μgm. of sodium iodide added to one aliquot as well as the unknown itself. Derivation of the computa-

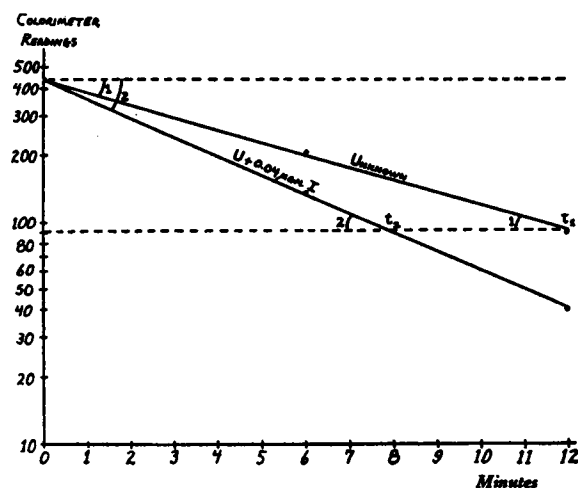


FIG. 1. METHOD OF PLOTTING RESULTS FOR CALCULATION OF PBI

$$\tan < 1 = \frac{\text{Initial colorimeter reading} - \text{final colorimeter reading}}{t_1}$$

This is an expression of the decolorization of the U sample. Since the assumption is being made that the decolorization is due entirely to I,

$$\frac{\text{Difference in colorimeter readings}}{t_1} = U \mu\text{gm. I in sample}$$

$$\text{Difference in colorimeter readings} = U \times t_1$$

In similar fashion:

$$\tan < 2 = \frac{\text{Difference in colorimeter readings}}{t_2}$$

$$= U + 0.04 \mu\text{gm. I}$$

$$\text{Difference in colorimeter readings} = (U + 0.04 \mu\text{gm. I})t_2$$

Since the differences in colorimeter readings are identical,

$$U \times t_1 = (U + 0.04 \mu\text{gm. I})t_2 = Ut_2 + 0.04 \mu\text{gm. I} \times t_2$$

$$Ut_1 - Ut_2 = 0.04 \mu\text{gm. I} \times t_2$$

$$U = \frac{t_2 \times 0.04 \mu\text{gm. I}}{t_1 - t_2}$$

The unknown also includes any iodide present in the reagents and a blank must be carried through as described in order to correct U for the blank.

TABLE I
Comparison of PBI methods on 57 clinical plasmas

Patient	Plasma PBI			Patient	Plasma PBI		
	Incin.	Distill.	Diff.		Incin.	Distill.	Diff.
	(1)	(2)	(1-2)		(1)	(2)	(1-2)
MR	1.0	1.1	-0.1	RS	6.4	6.8	-0.4
MN	1.8	1.6	+0.2	GM	6.8	7.4	-0.6
FMcE	2.2	2.2	0.0	OB	6.9	7.0	-0.1
DD	2.4	2.2	+0.2	EC	7.6	8.4	-0.8
JS	2.8	2.5	+0.3	WM	7.8	7.4	+0.4
LP	3.0	2.2	+0.8	SY	9.1	9.5	-0.4
WM	3.3	2.8	+0.5	EG	9.5	8.8	-0.7
JP	4.2	3.8	+0.4	JW	9.8	9.3	+0.5
KF	4.3	5.6	-1.3	VS	9.8	9.9	-0.1
MB	4.3	4.2	+0.1	AK	9.9	8.1	+1.8
ED	4.4	3.4	+1.0	EB	10.0	13.2	-3.2
HC	4.4	4.2	+0.2	SM	10.5	10.0	+0.5
TH	4.4	4.4	0.0	HB	11.1	12.3	-1.2
LB	4.4	4.6	-0.2	JW	11.4	10.1	+1.3
ID	4.4	4.8	-0.4	AB	12.0	9.0	+3.0
JM	4.6	3.7	+0.9	JZ	12.5	18.9	-6.4
OW	4.6	4.9	-0.3	JT	13.5	12.9	+0.6
CS	4.8	4.0	+0.8	JH	14.9	13.8	+1.1
NP	4.8	4.1	+0.7	JR	15.5	15.7	-0.2
GK	4.9	3.6	+1.3	JR	15.5	16.0	-0.5
EK	5.0	4.8	+0.2	JZ	16.0	17.8	-1.8
CM	5.1	4.6	+0.5	SY	16.2	16.7	-0.5
RM	5.3	6.2	-0.9	DR	19.5	16.4	+3.1
JS	5.5	4.5	+1.0	IB	19.9	17.9	+2.0
LP	5.5	6.0	-0.5	ST	21.3	20.4	+0.9
BT	5.6	4.4	-1.2	FF	21.5	28.0	-6.5
LN	5.7	4.9	-0.8	JE	27.8	26.3	+1.5
ES	5.9	4.7	+1.2	RQ	34.9	36.4	-1.5
MH	5.9	5.6	+0.3				

$$\chi^2 = \frac{\sum (1-2)^2}{2} = 11.096 \text{ for 56 degrees of freedom}$$

Therefore, there is much greater than 99% probability that the results are the same.

tion formula using the 0, 6 and 12 minute readings is contained in the detailed heading accompanying Figure 1. Table I summarizes a series of 57 comparisons which we have carried out on duplicate clinical plasma samples between this PBI procedure and the distillation technique described some time ago (16). A comparison of means would have little significance because of the wide range of PBI values (1 to 35 $\mu\text{gm. } \%$). Chi square analysis of the results was therefore carried out and shows clearly that there is no significant difference between the two series.

That the unorthodox procedure of manipulation of the decolorization readings does not itself introduce a significant error when carefully done is demonstrated by 43 plottings of the identical colorimeter data carried out in the course of communicating the details of this procedure to interested laboratories. The mean of the calculations of the amount of iodine per colorimeter tube was

0.0386 $\mu\text{gm.}$, with a standard deviation of 0.0004. Similarly good agreement has been obtained when different persons have plotted out the same colorimeter readings.

The customary method of calculating by setting up a curve of standards and interpreting colorimeter readings of unknown solutions from this curve is much simpler, but suffers from the possibility of the presence of some substance which would enhance the decolorization or interfere with it. The two methods of computation have been compared on several series of clinical samples; the standard curve technique was consistently higher, by 0.8 $\mu\text{gm. } \%$ using the 6-minute readings and 1.3 $\mu\text{gm. } \%$ using 12-minute readings. Recovery of the added 0.04 $\mu\text{gm.}$ of standard iodide was 99.3% and 100.0%, respectively. No basis for this difference can be offered at present. It is obvious that use of the simpler form of calculation will necessitate upward revision of the values to be considered normal by about 1 $\mu\text{gm. } \%$.

RECOVERY OF ADDED IODINE

As a test of the ability of the procedure to determine known amounts of iodine, standardized amounts of sodium iodide, thyroxine and thyroglobulin were added in triplicate to tubes containing precipitated and washed plasma proteins. Drying, ashing, HCl and H_2SO_4 elution and colorimetric determination of iodide present then followed. The results, shown in Table II, demonstrate adequate recovery of these substances in physiologically important amounts.

TABLE II
Recovery of iodine-containing substances added to precipitated and washed proteins

Substance	Amount I added	Amount I recovered
NaI	$\mu\text{gm. } \%$	$\%$
	2.0	97.5
	4.5	99.0
	5.0	108.9
	10.0	101.6
Thyroxine	20.0	102.3
	3.6	90.6
	7.7	98.0
Thyroglobulin	10.0	96.8
	2.5	90.9
	5.0	107.3
	15.0	94.2

RESULTS WITH THE DETERMINATION

This procedure has been available to the staff of the University Hospitals for nearly two years. On the basis of the first year's experience, the technique was extensively revised during the summer of 1949. In the year since that time, 942 determinations have been carried out on 694 patients; the distribution of these is shown in Figure 2. A

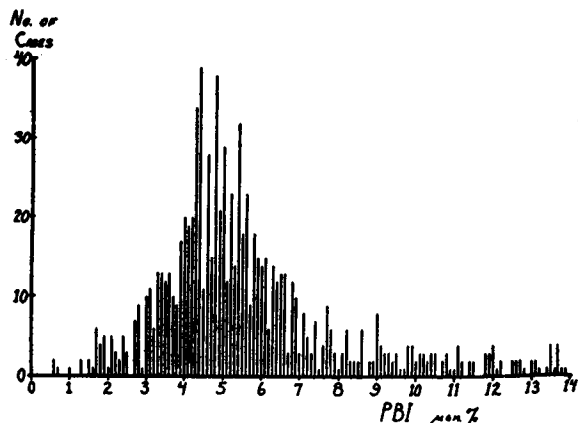


FIG. 2. DISTRIBUTION OF ONE YEAR'S DETERMINATIONS OF PBI ON CLINICAL PLASMA SAMPLES

In all, 942 results were obtained, but 50 values above 14 $\mu\text{gm. } \%$ have been omitted from the chart, since these were scattered up to 45 $\mu\text{gm. } \%$.

clustering of values between 3 and 7 $\mu\text{gm. } \%$ is apparent, but no sharp separations are present between "normal" and either hypo- or hyperthyroid individuals. From a physiological standpoint, such overlapping would be expected on the basis of all studies of normalcy. From the clinical point of view, it is important to establish some limits beyond which thyroid dysfunction can reasonably be expected.

In order to accomplish this, a group of samples has been assembled in Table III from medical

TABLE III
PBI values on 68 euthyroid subjects

Subjects	BMR	PBI		
	Range	Mean $\mu\text{gm. } \%$	Range $\mu\text{gm. } \%$	σ
40 Medical students	+15 to -15 unknown	5.1	3.4-8.0	1.12
28 Miscellaneous		5.1	3.6-6.6	0.79
Entire series of 68		5.1	3.4-8.0	1.00

students, resident staff, technicians and faculty, each person overtly appearing to be euthyroid. The table shows that a range of PBI values from 3.4 to 8.0 $\mu\text{gm. } \%$, with a mean of 5.1, was obtained on 40 medical students who had BMR's between -15 and +15. A smaller group of 27 apparently normal individuals (staff, visitors, etc.) gave an identical mean, although no basal metabolism values were available for standardization. In view of these findings, it is probable that the range of normal, usually considered to be 4.0 to 8.0 $\mu\text{gm. } \%$, must be extended downwards to 3.5 $\mu\text{gm. } \%$. This conclusion is, of course, based partially on acceptance of the validity of the BMR. Since the usual error in this determination is on the plus side, one might argue that this error had operated in the three subjects whose PBI values were below 3.9 $\mu\text{gm. } \%$ and that they actually should be considered myxedematous. No answer to this is possible, except that no genuine symptoms of hypothyroidism were apparent in any of these individuals. It will require a great many carefully controlled determinations in the borderline PBI zone of 3.5-4.0 to settle this physiological as well as chemical problem.

It is highly possible that the upper limit should be 7.5, bringing the entire euthyroid group close to the range of 3.5-7.0 $\mu\text{gm. } \%$ as suggested by Riggs (2). The problems of overlap, as far as the PBI is involved, seem to be intensified in the lower, myxedema, portion, with very few euthyroid individuals above 7.5 or thyrotoxic below 8.5 $\mu\text{gm. } \%$.

The histories on 86 of the patients on whom plasma PBI's had been run also contained results of BMR and I^{131} uptake determinations. The data for PBI and BMR have been plotted in Figure 3, together with the overall clinical evaluation of each patient, proven by surgery, pathology or therapeutic response. The area between a BMR of +15 and -15 and between 3.5 and 8.0 for PBI has been marked off as normal. It can be seen that one value in the myxedema group would be considered normal (at 3.5 $\mu\text{gm. } \%$), one thyrotoxic was well in the normal range, and one very high value (14.3 $\mu\text{gm. } \%$) was obtained on a subject with a non-toxic, diffusely enlarged goiter. In contrast, several BMR determinations in each category were at considerable variance with the general clinical opinion. The greater reliability

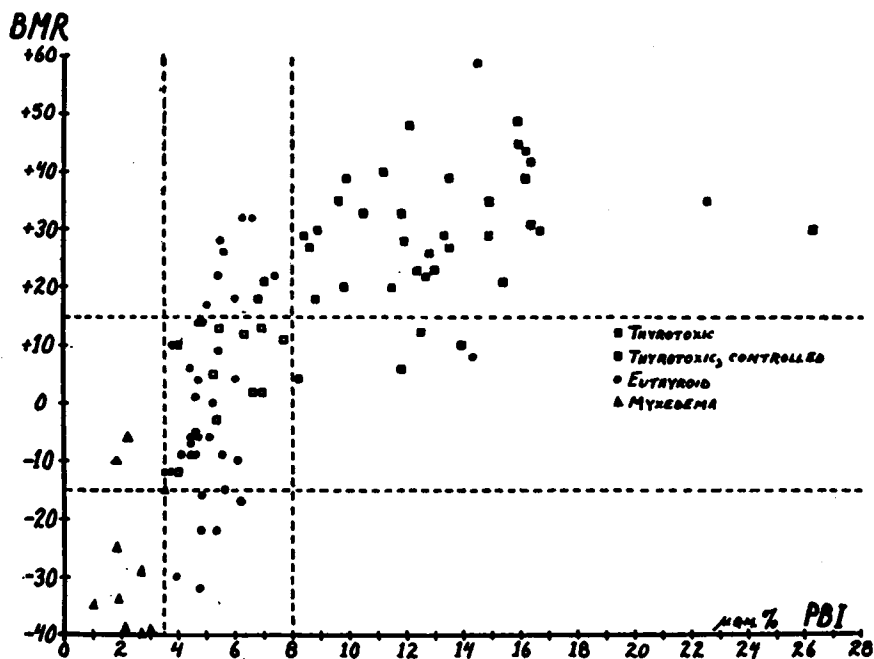


FIG. 3. COMPARISON OF PBI, BMR AND ENTIRE CLINICAL EVALUATION OF 86 PATIENTS

The 12 values represented by hollow squares were performed on previously thyrotoxic subjects, following successful management with surgery, I^{131} , or propylthiouracil. All patients had been on therapy at least one month, which should be an adequate length of time for the metabolic rate to return to normal (17).

of the PBI over the BMR has previously been noted by several investigators (2, 3, 18) and may be attributed, at least in part, to the greater objectivity of the chemical determination.

In discussing the advantages of this procedure, it may simply be stated that, in general, distillation techniques, although having a wider application because of greater flexibility, require such close attention during the digestion and distillation steps as to be poorly suited for routine clinical evaluation. On the other hand, incineration can be automatically regulated, making the final colorimetry the only stage requiring personal concentration. No attempt has been made in this laboratory to determine the maximum number of determinations possible per day, since successful routine analysis depends much more upon consistent performance week after week. It may conservatively be estimated that 15-20 samples can be analyzed in duplicate every two days by a single individual.

SUMMARY

1. A method has been presented for determination of plasma or serum protein-bound iodine

(PBI) using high-temperature incineration in the presence of alkali. The distillation step, requiring close individual attention, has been eliminated. The final determination of inorganic iodide is performed colorimetrically by measuring the catalysis of arsenious acid decolorization of a ceric sulfate solution.

2. Data are given for considering the range of normal to be 3.5 to 8 $\mu\text{gm. \%}$.

3. Examination of two years' experience with this PBI analysis shows it to be an objective test of greater dependability than the BMR.

ACKNOWLEDGMENT

The help of Dr. C. D. Janney of the Radiation Research Laboratory and of Drs. W. H. Ames and J. A. Clifton of the Department of Internal Medicine in carrying out various portions of this work is gratefully acknowledged. Dr. F. W. Schueler aided in the statistical analysis.

REFERENCES

1. Salter, W. T., Euthyroidism and thyroid dysfunction, in *The Chemistry and Physiology of Hormones*, edited by Moulton, F. R. Am. A. Advancement Sc., Washington, D. C., 1944, p. 104.

2. Riggs, D. S., Serum protein-bound iodine as a diagnostic aid. *Tr. Am. A. Study Goiter*, 1947, 137.
3. Rapport, R. L., and Curtis, G. M., The clinical significance of the blood iodine: a review. *J. Clin. Endocrinol.*, 1950, 10, 735.
4. Lowenstein, B. E., Bruger, M., and Hinton, J. W., The protein-bound plasma iodine in patients with thyroid disease. I. Correlation with basal heat production. *J. Clin. Endocrinol.*, 1944, 4, 268.
5. Riggs, D. S., Man, E. B., and Winkler, A. W., Serum iodine of euthyroid subjects treated with desiccated thyroid. *J. Clin. Invest.*, 1945, 24, 722.
6. Curtis, G. M., and Fertman, M. B., Blood iodine studies. VII. The relation of the basal metabolic rate to the blood iodine in thyroid disease. *Ann. Surg.*, 1945, 122, 963.
7. Perry, W. F., and Cosgrove, J. B. R., Protein-bound plasma iodine as an aid in the diagnosis of thyroid disease. *Canad. M. A. J.*, 1949, 60, 602.
8. Salter, W. T., and McKay, E. A., Iodine in blood and thyroid of man and small animals. *Endocrinology*, 1944, 35, 380.
9. Somogyi, M., A method for the preparation of blood filtrates for the determination of sugar. *J. Biol. Chem.*, 1930, 86, 655.
10. Sandell, E. B., and Kolthoff, I. M., Microdetermination of iodine by a catalytic method. *Mikrochimica Acta*, 1937, 1, 9.
11. Silver, S., Nature of blood iodine and its determination. *J. Lab. & Clin. Med.*, 1942, 28, 329.
12. Salter, W. T., The metabolic circuit of the thyroid hormone. *Ann. New York Acad. Sc.*, 1949, 50, 358.
13. Barker, S. B., Plasma protein-bound iodine in the diagnosis of thyroid disease, *in* Soskin, S., *Progress in Clinical Endocrinology*. Grune & Stratton, New York, 1950, p. 61.
14. Hyde, L., and Hyde, B., Effect of retained bronchial lipiodol on blood iodine. *J. Lab. & Clin. Med.*, 1949, 34, 1516.
15. Taurog, A., and Chaikoff, I. L., The nature of the circulating thyroid hormone. *J. Biol. Chem.*, 1948, 176, 639.
16. Barker, S. B., Determination of protein-bound iodine. *J. Biol. Chem.*, 1948, 173, 715.
17. Lowenstein, B. E., Bruger, M., Hinton, J. W., and Lough, W. G., Protein-bound plasma iodine in patients with thyroid disease. II. The effect of thiouracil. *J. Clin. Endocrinol.*, 1945, 5, 181.
18. Salter, W. T., Bassett, A. M., and Sappington, T. S., Protein-bound iodine in blood. II. Its relation to thyroid function in 100 clinical cases. *Am. J. M. Sc.*, 1941, 202, 527.