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Serum Level of Protein Bound Radioactive Iodine (I^{131}) in the Diagnosis of Hyperthyroidism.*

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(Introduced by Herrman L. Blumgart.)

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It is generally agreed that the blood level of protein bound ("hormonal") iodine is a measure of thyroid activity; in most patients with thyrotoxicosis the plasma protein bound iodine levels are above 8-10 γ %.[1-4] The chemical determination of plasma protein bound iodine is, however, difficult and laborious and despite its importance is not widely used. Recently, Taurog, Chaikoff, and Entenman[5] have used radioactive iodine (I^{131}) to measure the turnover of plasma protein bound iodine in dogs. It seemed possible that thyroid function might be estimated in man by measuring the concentration of serum protein bound radioactive iodine following an oral dose of I^{131}.

Methods. One hundred and fifty microcuries of I^{131}, carrier free, were administered orally to 20 subjects, aged 24 to 65 years; 16 were female. Ten subjects were thyrotoxic; the other 10 were euthyroid. The thyrotoxic subjects had the characteristic symptoms and signs. The basal metabolic rates ranged from +15 to +60, and averaged +34%. The circulation time, blood cholesterol, the body retention of I^{131} as measured by urinary excretion,[6] and the I^{131} uptake in the thyroid measured by external counts[7] were consistent in each instance with the clinical diagnosis of thyrotoxicosis. The other 10 subjects were euthyroid by these criteria. The basal metabolic rate ranged from —10 to +15 and averaged +2%.

The I^{131} was given 3 hours after a light breakfast. Twenty-four hours later, 10 cc of venous blood was obtained from each subject and the serum separated.

Procedure for determination of protein bound radioactive iodine. A. 1.0 cc serum was pipetted into a previously weighed glass boat measuring 25 mm in diameter and 7 mm deep.

B. The protein bound I^{131} was separated by the method of Chaikoff *et al.*[8] modified as follows:

(1) 1.0 cc serum was pipetted into a small centrifuge tube and 1.0 cc 10% trichloracetic acid (cold) added.

(2) After centrifugation (2500 RPM) for 30 minutes, the supernatant was removed and saved.

(3) The precipitate was washed twice with 2-5 cc cold 5% trichloracetic acid. After each addition of trichloracetic acid and centrifugation, the supernatant fluid was removed. The washings were pooled.

(4) The precipitate was dissolved in 1.0 cc 2 N NaOH and transferred to a weighed glass boat.

C. 1.0 cc of the collected supernatant (inorganic fraction) was adjusted to pH 7.5 and

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TABLE I.

M.S., Age 62; Duodenal Ulcer; Euthyroid. Oct. 13, 1948, 10 a.m., 150 μc. I^{131}, carrier free by mouth. Oct. 14, 1948, 10 a.m., 10 cc venous blood drawn.

	cc	Dry wt, mg	Net counts/min., corrected for mass and dilution	Correction for decay, %	Net counts/min./cc
A. Total serum	1.0	110	242	77	315
B. Precipitate	1.0	89	50	77	65
C. Filtrate	1.0	54	185	77	239

Background 15 counts/min.
From nomogram 0.001 microcurie = 450 cts./min.
B. Precipitate (Protein Bound I^{131}) 65 cts./min. = .00015 μc = 15 $\times$ 10^{-5} μc.

transferred to a previously weighed glass boat.

One drop 10% gelatin (2-3 mg) was added to each cup and the samples evaporated slowly (37 to 40°C) to dryness. The cups were reweighed and the radiation determined with an end window Geiger-Mueller tube (3.2 mg cm^2). Corrections for mass absorption, dilution, and decay were made. All determinations were extrapolated to the time the sample was obtained.

Calculations. The total serum activity per cc (Table I) should equal the precipitate (protein bound I^{131}) activity, plus the filtrate (inorganic I^{131}) activity. The conversion to microcuries of net counts per minute per cubic centimeter was made by reference to a nomogram. Under these geometric and physical conditions, .001 microcurie I^{131} gave 450 net counts per minute. The reference standard for I^{131} radiation was Bi 210 (half life 22 years).

The following protocol (Table I) is illustrative.

Results. The results are shown in Fig. 1 and 2. In the hyperthyroid patients the serum protein bound I^{131} ranged from 38 to 146 $\times$ 10^{-5} μc/cc (Fig. 1), averaging 68 $\times$ 10^{-5} μc. In the euthyroid patients the serum protein bound I^{131} ranged from 3 to 28 $\times$ 10^{-5} μc/cc averaging 13 $\times$ 10^{-5} μc. Sixty per cent of the observations in the patients with thyrotoxicosis were above 50 $\times$ 10^{-5} μc, whereas in the euthyroid subjects, 70% were below 20 $\times$ 10^{-5} μc.

There was considerable overlap in the total serum counts (Fig. 2). In the thryotoxic patients the total serum activity ranged from 199 to 650 counts/min./cc and in the euthyroid subjects from 50 to 715 counts/min./cc.

Comment. The tracer technic has been increasingly employed in studies of thyroid function. After an oral dose of I^{131}, urinary excretion accounts for most of the loss from the body;[9-11] the remainder as measured by external counts is mainly in the thyroid gland. A more direct estimation of thyroid function is afforded by the serum level of protein bound radioactive iodine. The higher protein bound I^{131} serum level found in thyrotoxicosis is consistent with increased thyroid activity in these patients. The difference in protein bound radioactive iodine serum levels in euthyroid and thyrotoxic subjects has proved useful diagnostically. Our results indicate, that while the counts cc of whole serum drawn 24 hours after the oral dose are generally lower in euthyroid subjects than in thyrotoxics, the overlap (Fig. 2) precludes differentiation by the relatively simple determination of total serum activity. This overlap may not be present 72 or 96 hours after a tracer dose and is the subject of further studies.

The factors which influenced our choice of a 24-hour period may be summarized. In rats, Chaikoff, Taurog, and Reinhardt[8] have shown that 24 hours after an injection of I^{131}, approximately 90% of the plasma radioactivity is in protein bound form. Preliminary studies[12] on serum obtained from thyrotoxic patients 1, 3, 6, 24, 48 and 72 hours

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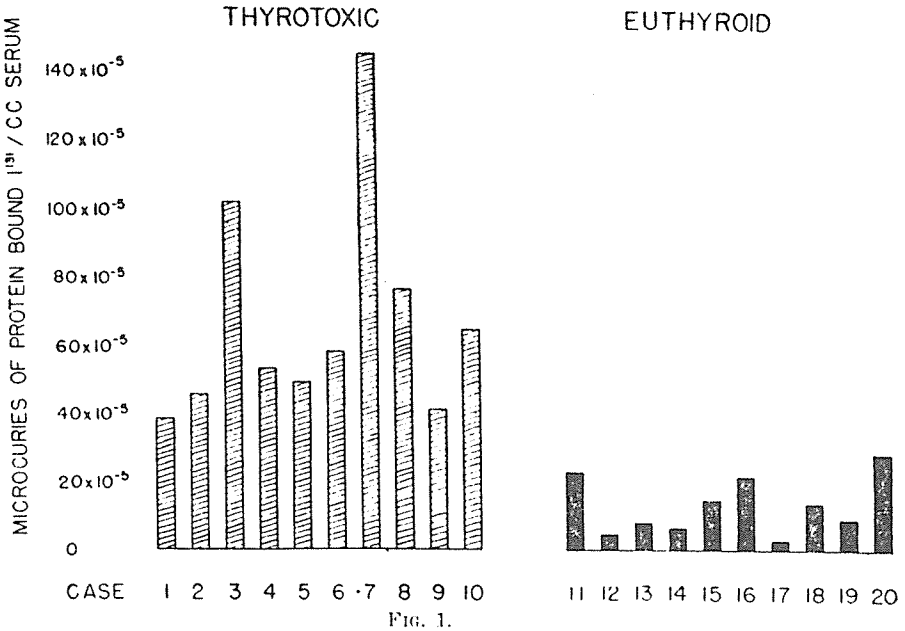

FIG. 1.

Serum Level Protein Bound I^{131}, 24 hours after standard oral dose of 150 μc I^{131}, carrier free, in 10 thyrotoxic and 10 euthyroid subjects.

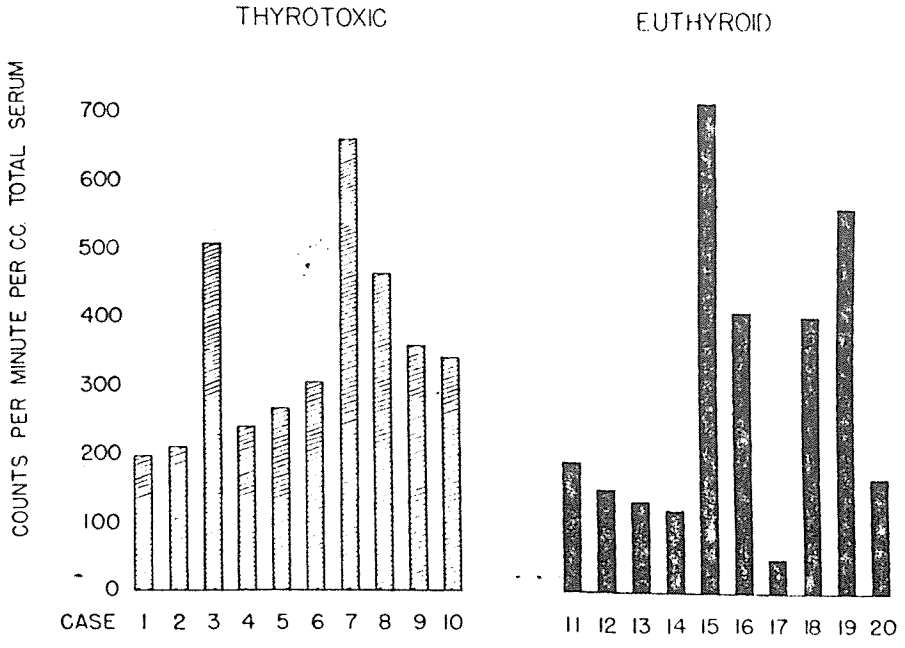

FIG. 2.

Counts/min./cc serum 24 hours after standard oral dose 150 μc I^{131}, carrier free, in 10 thyrotoxic and 10 euthyroid subjects.

after therapeutic doses (4-6 millicuries) showed that the protein bound I^{131} level at 24 hours was 50% higher than at 6 hours. The protein bound I^{131} serum levels at 48 and 72 hours, however, were increased only irregularly over the 24-hour concentration.

Summary. 1. The serum protein bound radioactive iodine (I^{131}) level was determined 24 hours after the oral administration of 150 microcuries I^{131}. Twenty subjects were studied; 10 were thyrotoxic and 10 euthyroid. The protein bound I^{131} was determined by a modification of the method described by Chaikoff *et al.*[8]

2. The serum protein bound I^{131} in thyrotoxic patients was 38 to 146 $\times$ 10^{-5} microcuries/cc averaging 68. In euthyroid subjects, the serum level ranged from 3 to 28 $\times$ 10^{-5} microcuries/cc, averaging 13.

3. It would appear that this test may be of diagnostic value as a measure of thyroid function.

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Inactivity of Bound Plasma Progesterone.*

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Observations have been reported indicating that progesterone in blood is in the plasma and not in the cells and that most of the total progesterone is free while rarely more than 10% is bound to protein or some other substance or substances that render this portion of the progesterone insoluble in acetone and ether.[1] The latter circumstance contrasts with the finding that approximately two-thirds of the estrogen in blood is bound.[2-4] It has been suggested that bound estrogen serves in effect as a reservoir, that the estrogen readily dissociates at the cell membrane, and that the bound fraction is potentially active estrogen.[3] Several observations indicate that a comparable situation does not obtain with respect to bound progesterone. The relative amount of the bound fraction seems too small to serve as a significant source of free progesterone; when introduced directly into the uterus of the mouse the bound fraction had no effect in a test period of 48 hours, presumably sufficient time to permit dissociation if it is to occur; the activity of raw plasma is identical with that of the free progesterone it contains. In short, bound progesterone appeared to be biologically inert.

When progesterone is introduced into the spleen or portal vein and must pass through the liver before reaching the systemic circulation the absorbed progesterone is inactivated in the sense that no effects of the substance are observed in rats,[5] rabbits,[6,7] and mice.[8] If the blood of such animals should contain levels of bound progesterone that would be effective if free, it would constitute further and probably strong evidence for the biological inactivity of the bound fraction. Such an experiment is described here.

Experimental. Young adult mice were used as test animals; the inbred A strain of Strong[9] was chosen to minimize variability. Of a group of 22 animals ovariectomized 13 days earlier, seven were given progesterone pellets subcutaneously, eight were given progesterone

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