

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%.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 Entenman⁵ have used radioactive iodine (¹³¹I) 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 ¹³¹I.

Methods. One hundred and fifty microcuries of ¹³¹I, carrier free, were administered orally to 20 subjects, aged 24 to 65 years; 10 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 work was carried out under a contract of the Office of Naval Research Atomic Energy Commission and the President and Fellows of Harvard College.

¹ Bassett, A. M., Coons, A. H., and Satter, W. T., *J. Am. Med. Soc.*, 1941, **202**, 516.
² Satter, W. T., Bassett, A. M., and Sappington, T. S., *J. Am. Med. Soc.*, 1941, **202**, 527.
³ Mann, E. B., Smitnow, A. R., Gildner, R. F., and Peters, J. P., *J. Clin. Invest.*, 1942, **21**, 773.
⁴ Riggs, D. S., *Trans. Am. Assn. Study of Goiter*, 1947, pp. 137-144.
⁵ Taurog, A., Chaikoff, I. L., and Entenman, C., *Endocrinology*, 1947, **40**, 86.
⁶ Freedberg, A. S., Baka, R., and McManus, M. J., *J. Clin. Endoc.*, in press.

W. O., *Endocrinology*, 1947, **40**, 47.
 s Chaikoff, I. L., Taurog, A., and Reinhardt, M., *Fed. Proc.*, 1949, **8**, 50.
 7 Freedberg, A. S., Trelles, A., and Van Dilla, organic fraction) was adjusted to pH 7.5 and C. 1.0 cc of the collected supernatant (in glass boat.

(4) The precipitate was dissolved in 1.0 cc 2 N NaOH and transferred to a weighed moved. The washings were pooled.

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

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

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

B. The protein bound ¹³¹I was separated by the method of Chaikoff *et al.*,⁵ modified as follows:

The protein bound ¹³¹I was separated deep.

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

of venous blood was obtained from each sub-breakfast. Twenty-four hours later, 10 cc The ¹³¹I was given 3 hours after a light averaged +2%.

metabolic rate ranged from -10 to +15 and euthyroid by these criteria. The basal of thyrotoxicosis. The other 10 subjects were measured by external counts⁶ were consistent in each instance with the clinical diagnosis body retention of ¹³¹I as measured by urinary excretion,⁶ and the ¹³¹I uptake in the thyroid

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

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TABLE I
M.S., Age 62; Duodenal Tumor; Euthyroid. Oct. 13, 1948, 10 a.m., 150 μ c. I¹³¹, carrier free by mouth. Oct. 14, 1948, 10 a.m., 10 cc venous blood drawn.

Net counts/min.		Net counts/min.		Net counts/min.	
A. Total serum	B. Precipitate	C. Filtrate	Background 15 counts/min.	From nomogram 0.001 microcurie = 450 cts./min.	B. Precipitate (Protein Bound I ¹³¹) 65 cts./min. = $15 \times 10^{-5} \mu$ c.
1.0	1.0	1.0	15	450	1500
10	89	54			
242	20	185			
77	77	77			
315	65	239			

Comment. The tracer technique has been increasingly employed in studies of thyroid function. After an oral dose of I¹³¹, 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 I¹³¹ 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 thyrotoxic, 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

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 (Kiger-Mueller tube (3.2 mg cm²)). 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 I¹³¹) activity, plus the filtrate (inorganic I¹³¹) 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, 0.01 microcurie I¹³¹ gave 450 net counts per minute. The reference standard for I¹³¹ radiation was Bi 210 (half life 22 years).

The following protocol (Table I) is illustrative. The results are shown in Fig. 1 and 2. In the hyperthyroid patients the serum protein bound I¹³¹ ranged from 38 to 146 $\times 10^{-5}$ μ c/cc (Fig. 1), averaging 68 $\times 10^{-5}$ μ c. In the euthyroid patients the serum protein bound I¹³¹ ranged from 3 to 28 $\times 10^{-5}$ μ c/cc averaging 13 $\times 10^{-5}$ μ c. Sixty per cent of the observations in the patients with thyrotoxicosis were above 50 $\times 10^{-5}$ μ c, whereas in the euthyroid subjects, 70% were below 20 $\times 10^{-5}$ μ c.

There was considerable overlap in the total serum counts (Fig. 2). In the thyrotoxic 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.

The factors which influenced our choice of a 24-hour period may be summarized. In rats, Chaikoff, Taurag, and Reinhardt¹² have shown that 24 hours after an injection of I¹³¹, approximately 90% of the plasma radioactivity is in protein bound form. Preliminary studies¹² on serum obtained from thyrotoxic patients 1, 3, 6, 24, 48 and 72 hours after Hamilton, T. G., and Soley, M. H., *Ann. N.Y. Acad. Sci.*, 1939, **127**, 557.

¹⁰ Hertz, S., Roberts, A., and Sailer, W. T., *J. Clin. Invest.*, 1942, **21**, 57.

¹¹ Keating, R. G., Power, M. H., Berkson, J., Haines, S. F., *Trans. Am. Assn. Study of Govt.* 1947, pp. 201-215.

¹² Unpublished data.

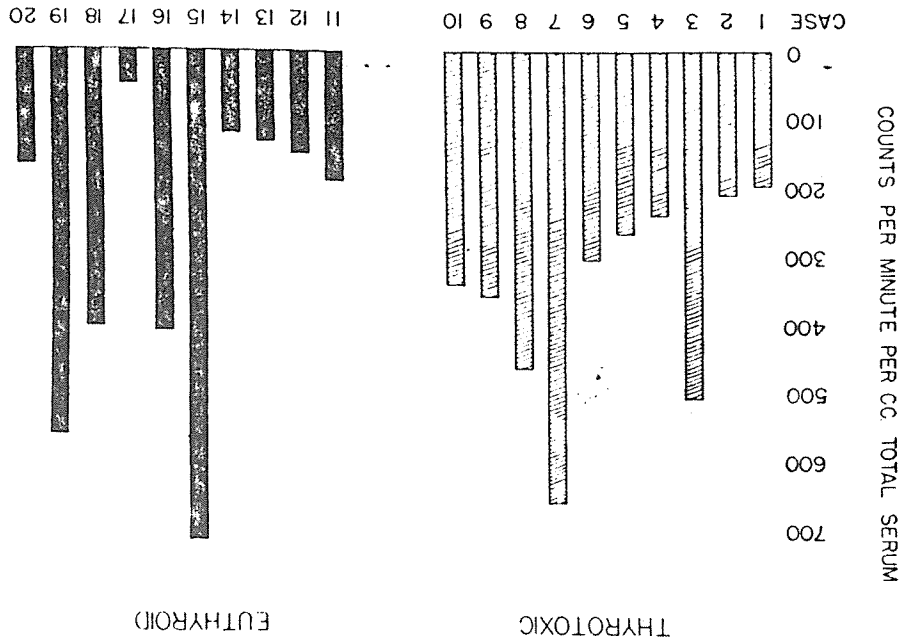
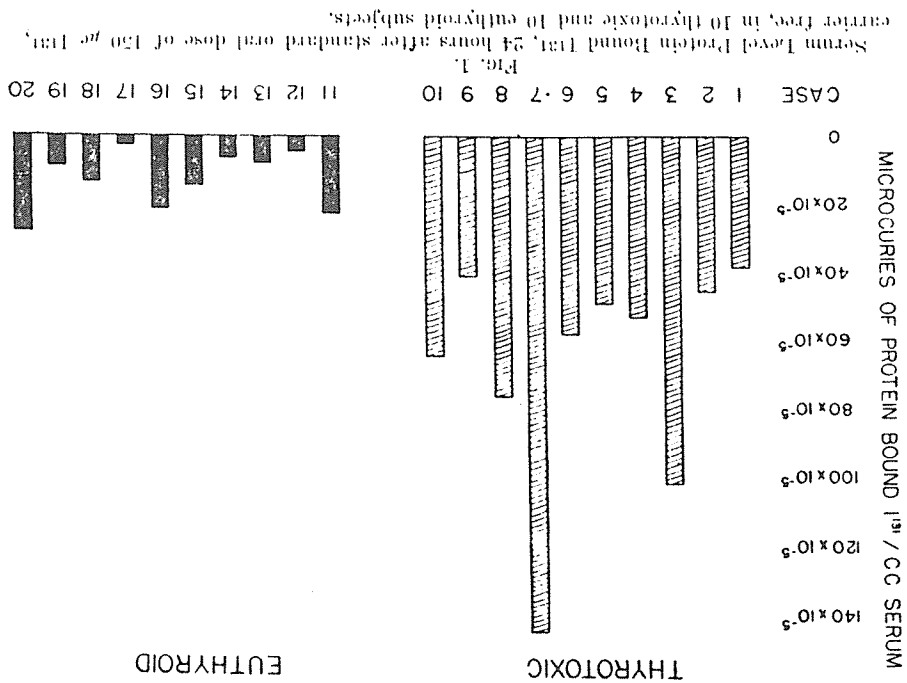


Fig. 1. Serum Level Protein Bound ¹³¹I, 24 hours after standard oral dose of 150 μ Ci ¹³¹I, carrier free, in 10 thyrotoxic and 10 euthyroid subjects.

Fig. 2. Counts/min/cc serum 24 hours after standard oral dose 150 μ Ci ¹³¹I, carrier free, in 10 thyrotoxic and 10 euthyroid subjects.

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