

## Radioimmunoassay of Human Plasma Thyrotropin

Robert D. Utiger

*J Clin Invest.* 1965;44(8):1277-1286. <https://doi.org/10.1172/JCI105234>.

Research Article

**Find the latest version:**

<https://jci.me/105234/pdf>



## Radioimmunoassay of Human Plasma Thyrotropin \*

ROBERT D. UTIGER †

(From the Metabolism Division, Department of Medicine, Washington University School of Medicine, St. Louis, Mo.)

Many pituitary hormones possess partial or complete immunological species specificity. Earlier reports suggested that thyrotropin (TSH) did not have such specificity since the biological activity of human pituitary or plasma TSH could be neutralized by antisera to bovine TSH in doses similar to that which blocked the biological activity of bovine TSH (2, 3). However, recent studies have shown that much smaller doses of antisera to bovine or human TSH were able to neutralize the biological activity of homologous TSH preparations than the activity of the other hormone (4, 5). Furthermore, in studies using both human and bovine TSH and antisera to each, Arquilla, Catz, and Finn (6) found no immunological cross-reactivity *in vitro* using hemagglutination and hemagglutination-inhibition techniques. More recently data were presented that antisera to highly purified human and bovine TSH reacted *in vitro* and, in separate experiments, blocked the biological effect *in vivo* only of the homologous antigen (7). Thus, considerable immunological species specificity of TSH now seems well established.

Previous attempts to measure endogenous plasma TSH in man by immunoassay utilizing the hemagglutination-inhibition technique were unsuccessful due to the presence of nonspecific inhibitory substances in plasma and to the use of anti-bovine TSH sera (8, 9). A radioimmuno-

precipitation technique for human TSH assay was described by Utiger, Odell, and Condliffe (7). This procedure, in which free human TSH- $I^{131}$  and that bound to rabbit antihuman TSH were separated by precipitation of the antibody-bound TSH- $I^{131}$  by antirabbit  $\gamma$ -globulin serum, proved useful in demonstrating the species and hormonal immunological specificity of antihuman TSH serum. However, the presence of nonspecific inhibitory factors made it unsuitable for assay of plasma TSH. These plasma effects were reduced but not eliminated by use (10) of the conditions employed by Schalch and Parker (11), which have eliminated the nonspecific effects of plasma in the double antibody method for the immunoassay of human growth hormone. In a preliminary report, Odell, Wilber, and Paul (12) have described a radioimmunoassay for plasma TSH utilizing an ethanol-saline precipitation technique to separate free and antibody-bound TSH- $I^{131}$ .

The following communication describes the development of a sensitive TSH radioimmunoassay employing chromatoelectrophoretic separation of free and antibody-bound TSH- $I^{131}$ . This technique permits the measurement of TSH in unextracted plasma. Results of plasma TSH assays in patients with various thyroid disorders and studies of plasma TSH concentration following several physiological stimuli and treatment with various thyroid hormones are reported herein.

### Methods

*Human thyrotropin (HTSH) and anti-HTSH serum preparations.* The HTSH (preparation PC16-140B3) used in these studies was prepared (13, 14) and kindly donated.<sup>1</sup> The biological activity of this material after purification was 20 IU per mg as measured in the chick thyroidal- $I^{131}$  depletion assay of Bates and Cornfield (15) using U.S.P. thyrotropin reference standard (bovine) as bioassay standard (1 U.S.P. U = 1 IU). It has since declined to 10 IU per mg during storage in the

<sup>1</sup> By Dr. Peter Condliffe, National Institute of Arthritis and Metabolic Diseases.

\* Submitted for publication February 5, 1965; accepted April 8, 1965.

Supported by research grant AM-08630-01 from the National Institute of Arthritis and Metabolic Diseases. Clinical Studies were carried out on the Barnard Hospital-Washington University School of Medicine Clinical Research Center, under support of grant FR-36, Division of Research Facilities and Resources, National Institutes of Health.

Presented in part at the Thirty-seventh Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., November 6, 1964 (1).

† Address requests for reprints to Dr. Robert D. Utiger, Dept. of Internal Medicine, Washington University School of Medicine, St. Louis, Mo. 63110.

dry state. Stock solutions (50  $\mu$ g per ml) for immunoassay standards were prepared in 0.25% bovine serum albumin (BSA) in 0.01 M phosphate-0.15 M NaCl, pH 7.5, and kept at  $-20^{\circ}$  C. Under these conditions this HTSH preparation was immunologically stable for at least 18 months since it has consistently produced inhibition of the HTSH- $I^{131}$ -anti-HTSH reaction to the same degree as when originally prepared. Furthermore, no change in the amount required to produce a single precipitin band in agar plates with anti-HTSH sera was observed. Purified bovine TSH (20 IU per mg)<sup>1</sup> and less purified bovine (3.5 IU per mg) and ovine (3.8 IU per mg) TSH preparations<sup>2</sup> were also donated.

The preparation and specificity of the anti-HTSH sera have previously been described (7), and those sera were used in these studies.

**HTSH- $I^{131}$ .** The  $I^{131}$ -labeled HTSH preparation procedure, based on the method of Hunter and Greenwood (16), was reported previously (7). Routinely, 5  $\mu$ g HTSH and 5 mc  $I^{131}$  were used. Attempts to use less HTSH in order to obtain higher specific activity HTSH- $I^{131}$  preparations have generally yielded moderately damaged and poorly immunoreactive preparations. Since different  $I^{131}$  shipments<sup>3</sup> have had a pH of 7 to 11, careful control of the pH of the iodination mixture (to pH 7.5 to 8.0) has proved helpful in obtaining reproducibly highly labeled and immunoreactive HTSH- $I^{131}$  preparations. The HTSH- $I^{131}$  preparations used in this study had estimated specific activities of 40 to 150 mc per mg and were 75 to 90% reactive with anti-HTSH under the conditions described below. From 7 to 15% of the HTSH- $I^{131}$  migrated in the separation system in the absence of antibody. This fraction presumably represented damaged HTSH- $I^{131}$  nonspecifically bound to plasma proteins.

**Method of immunoassay.** The immunoassay depended on the ability of unlabeled HTSH or endogenous plasma HTSH to inhibit competitively the reaction of HTSH- $I^{131}$  with antibody. The amount of hormone in unknown solution was found by comparison with known quantities of purified HTSH. Separation of free HTSH- $I^{131}$  from antibody-bound HTSH- $I^{131}$  was accomplished by the chromatoelectrophoretic method first described by Yalow and Berson (17) for the immunoassay of insulin. HTSH- $I^{131}$ , as other protein hormones, was found to adsorb to paper at the site of application, whereas HTSH- $I^{131}$  bound to antibody migrated anodally with the serum proteins under the proper conditions.

In the assay, 50  $\mu$ l HTSH- $I^{131}$ , 100  $\mu$ l unlabeled HTSH or diluted plasma (at least 2 dilutions of 1:2 and 1:4 or greater), and 50  $\mu$ l anti-HTSH serum (in a dilution such that 45 to 65% of HTSH- $I^{131}$  present was bound in the absence of added HTSH) were mixed together and reacted for 2 to 3 days at  $5^{\circ}$  C. All reagent dilutions were prepared in 0.25% BSA-0.05 M barbital, pH 8.6. With some plasma samples, an increase in the

amount of radioactivity migrating in the absence of antibody was noted. Consequently, control tubes containing each plasma sample and HTSH- $I^{131}$  without antibody were routinely run and a correction introduced for the amount of radioactivity migrating in the absence of antibody.

Free and antibody-bound HTSH- $I^{131}$  were separated by chromatoelectrophoresis on 2.5  $\times$  23-cm strips of Whatman DE81 DEAE cellulose paper (0.1 M barbital, pH 8.6, constant current 6.25 ma per strip, 50 to 70 minutes, room temperature) with a hydrostatic gradient of 13 cm. The protein band, containing the antibody-bound HTSH- $I^{131}$ , moved 6 to 8 cm under these conditions. To prevent adsorption of antibody-bound radioactivity to the paper, 25 to 50  $\mu$ l of normal human serum was applied to each strip before application of the assay mixture. After drying, the strips were cut into bound and free segments (usually 3 to 4 cm on the anodal side of the origin), based on prior experience with strip scanning or cutting of strips into multiple small segments, and counted in a well scintillation counter. To calculate the percentage of free HTSH- $I^{131}$ , the procedure described by Parker, Mariz, and Daughaday (18) was employed to correct for the radioactivity that migrated in the absence of antibody. The percentage of radioactivity bound to antibody was then calculated.

**Plasma samples.** Specimens were stored at  $-10^{\circ}$  C. Under these conditions no deterioration of activity occurred in up to 12 months. Similar results were obtained with plasma and serum samples. When physiologic changes in plasma HTSH in a single subject were studied, assays were performed in the same run.

## Results

**Effects of various TSH preparations.** The addition of unlabeled HTSH in increasing amounts to the HTSH- $I^{131}$ -anti-HTSH system led to a progressive decrease in the percentage of HTSH- $I^{131}$  bound to antibody. When the percentage of HTSH- $I^{131}$  bound to antibody was plotted as a function of the logarithm of the unlabeled HTSH content, a straight line relationship was obtained (Figure 1). Only the linear portion of the standard curve was utilized in computing the HTSH content of unknown samples. The results reported below are the mean of the values obtained with plasma dilutions that fell on that portion of the standard curve. As little as 0.075 m $\mu$ g unlabeled HTSH was detected (1.5 m $\mu$ g per ml plasma). Less sensitive assays were obtained with lower specific activity HTSH- $I^{131}$  preparations. Standard errors of the mean of these points ranged from 0.1 to 2.6%.

As in the double antibody system previously reported (7), either partially or highly purified bovine TSH did not reduce the percentage of HTSH-

<sup>2</sup> Gift of the Endocrinology Study Section, National Institutes of Health.

<sup>3</sup> Union Carbide Nuclear Co., Oak Ridge, Tenn.

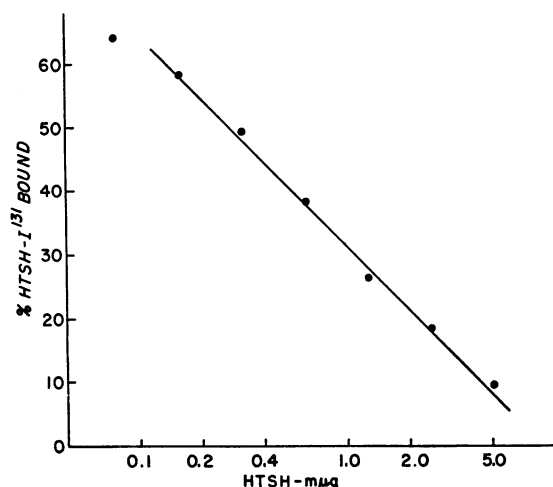


FIG. 1. STANDARD CURVE SHOWING THE RELATIONSHIP BETWEEN UNLABELED HUMAN THYROTROPIN (HTSH) AND THE PERCENTAGE OF RADIOACTIVITY BOUND BY ANTI-HTSH SERUM.

I<sup>131</sup> bound, even when added in microgram doses. Ovine TSH behaved similarly. Saline extracts of human pituitary tissue produced a dose response curve parallel to the linear portion of that obtained with unlabeled HTSH.

Plasma samples containing large amounts of endogenous TSH and therefore assayable in multiple dilutions also produced a dose response line paral-

lel to that of the standard (Figure 2). In contrast, the lack of effect of plasma from a hypopituitary patient in reducing the percentage of HTSH-I<sup>131</sup> bound is also shown. Plasma or serum from beef, sheep, rabbits, rats, and a TSH-tumor bearing mouse did not inhibit the binding of HTSH-I<sup>131</sup> to anti-HTSH.

**Recovery and removal of HTSH.** HTSH added to plasma *in vitro* was detected by the assay almost quantitatively. The results of two such experiments are shown in Table I. Over 85% of the immunologically detectable HTSH of hypothyroid plasma was removed by absorption with anti-HTSH serum, followed by removal of the HTSH-anti-HTSH complexes by precipitation with goat antirabbit  $\gamma$ -globulin serum (Figure 3). Absorption of such plasma samples with antihuman  $\gamma$ -globulin serum did not alter its HTSH content.

Gel filtration of HTSH-rich plasma on Sephadex G-200 columns, as described by Flodin and Killander (19), separated the plasma proteins into three major components (Figure 4). Endogenous or exogenous HTSH was recovered after such fractionation in the albumin peak or, in larger amount, from the effluent slightly more retarded than albumin. HTSH recovery from such col-

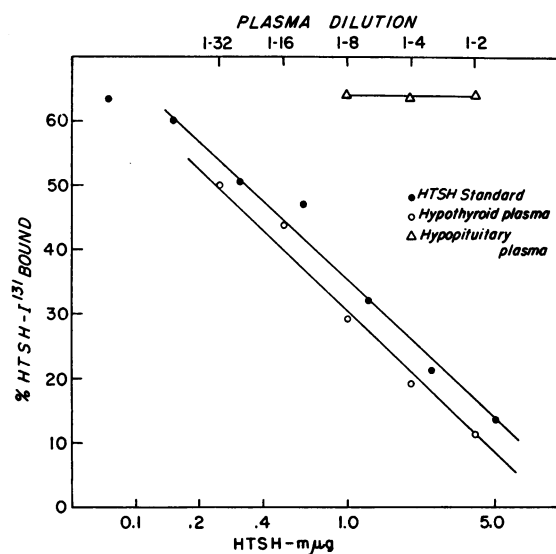


FIG. 2. COMPARISON OF THE EFFECTS OF MULTIPLE DILUTIONS OF HYPOTHYROID PLASMA (○) AND HYPOPITUITARY PLASMA (Δ) WITH THAT OF PURIFIED HTSH PREPARATION (●).

TABLE I  
Recovery of human thyrotropin (HTSH)  
added to plasma *in vitro*

HTSH added	Plasma assayed	Plasma HTSH	% recovery
$\mu\text{g/ml}$	$\mu\text{l}$	$\mu\text{g/ml}$	%
	50	<3.0	
	25	<3.0	
12.5	25	12.0	96
	12.5	12.0	96
25	25	22.0	88
	12.5	27.0	108
50	25	44	88
	12.5	31	62
	50	<3.0	
	25	<3.0	
10	50	10.0	100
	25	10.8	108
20	50	17	85
	25	17.6	88
40	50	38	95
	25	40	100
	12.5	30.4	76

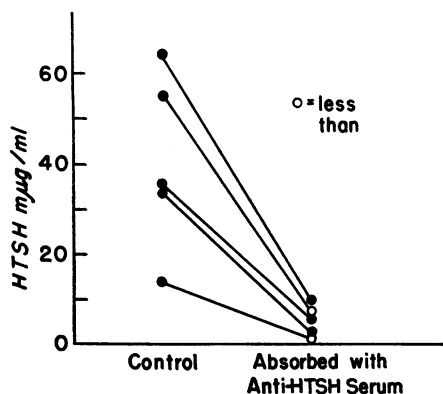


FIG. 3. RESULTS OF PLASMA HTSH ASSAYS BEFORE (LEFT COLUMN) AND AFTER (RIGHT) ABSORPTION WITH ANTI-HTSH SERUM. Plasma was reacted with 5 to 10  $\mu$ l anti-HTSH for 24 to 48 hours. Goat antirabbit  $\gamma$ -globulin (200 to 400  $\mu$ l) was then added and the precipitate removed 24 hours later. To eliminate reaction between the goat serum and anti-HTSH in the immunoassay mixtures, 100  $\mu$ l normal rabbit serum was then added and any further precipitate removed before immunoassay.

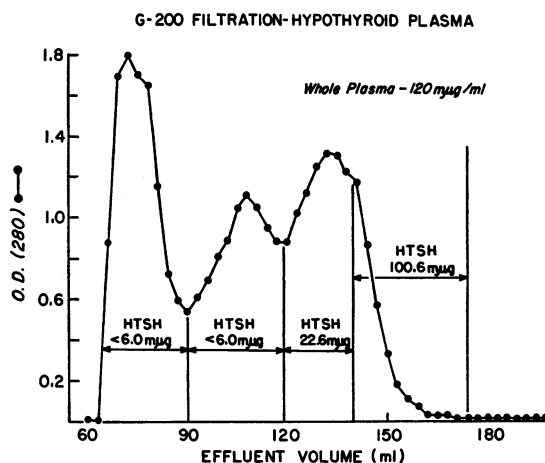


FIG. 4. GEL FILTRATION OF HYPOTHYROID PLASMA. One ml of plasma was applied to a 2.5- $\times$ 60-cm column of Sephadex G-200. The column was prepared and eluted with 0.1 M  $\text{NH}_4\text{HCO}_3$ . The three optical activity peaks represent (from left to right) macroglobulins, 7 S gamma globulins, and albumin.

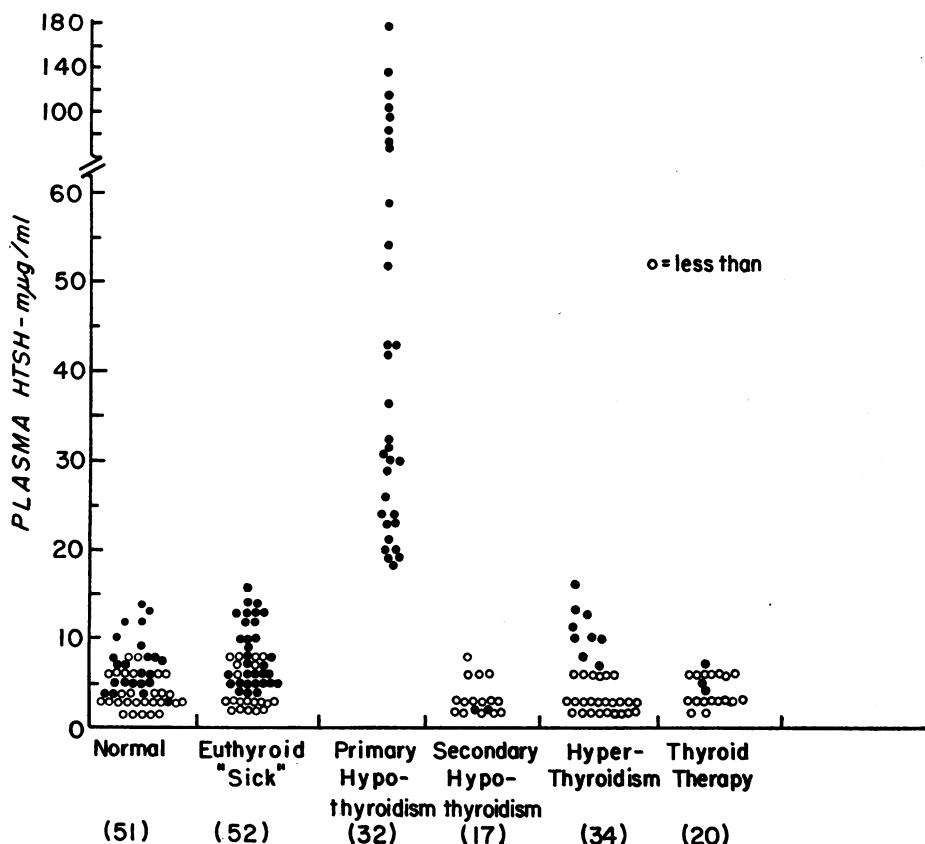


FIG. 5. RESULTS OF PLASMA HTSH ASSAYS. The open circle (○) refers to results less than the sensitivity of the assay at that time.

umns ranged from 75 to 110%. These data are in agreement with the known size characteristics of pituitary TSH and would suggest that endogenous plasma HTSH is not bound to plasma protein. Furthermore, McKenzie (20) has reported recovery of HTSH biological activity from hypothyroid plasma in similar fractions.

**Plasma HTSH assays.** Plasma HTSH concentrations were measured in 103 euthyroid subjects (Figure 5). Of these, 51 normal adolescent or adult subjects had plasma HTSH values ranging from undetectable to 15  $\mu\text{g}$  per ml. Activity was found in 50%. The other 52 euthyroid patients had a variety of thyroidal or other disorders (single or multinodular goiter, thyroid cancer, obesity, diabetes, or acromegaly). In them, plasma HTSH was in the same range, activity being detectable in 61%. No differences dependent on age or sex were found. All 32 patients with primary hypothyroidism had plasma HTSH values above 18  $\mu\text{g}$  per ml (range, 18 to 180  $\mu\text{g}$  per ml). No activity was detected in plasma from patients with secondary hypothyroidism occurring spontaneously or after hypophysectomy. In untreated hyperthyroidism, no HTSH was detectable in 25 of 34 cases. In the remaining 9 patients, plasma HTSH was within the normal range. In 20 patients studied while receiving 120 mg or more of desiccated thyroid daily or its equivalent, plasma HTSH concentrations were within the normal range or undetectable. In the few patients with moderate or severe exophthalmos, no consistent alterations in plasma HTSH were found, and the results are therefore included in the appropriate groups shown in Figure 5.

A number of these samples have been repeatedly assayed, and some of these results are shown in

TABLE II  
Between-assay reproducibility of  
plasma HTSH assays

Sample	Number of assays	Range	Mean	SD
		$\mu\text{g}/\text{ml}$	$\mu\text{g}/\text{ml}$	
Hypopituitary	4	<1.5, <1.5, <1.5, <3.0		
Normal	5	3-7	6	1.8
Normal	4	4-8	6	2.4
Normal	3	12-15	13	1.6
Hypothyroid	4	37-49	44	6.0
Hypothyroid	3	39-57	46	9.0
Hypothyroid	3	58-78	68	10.0

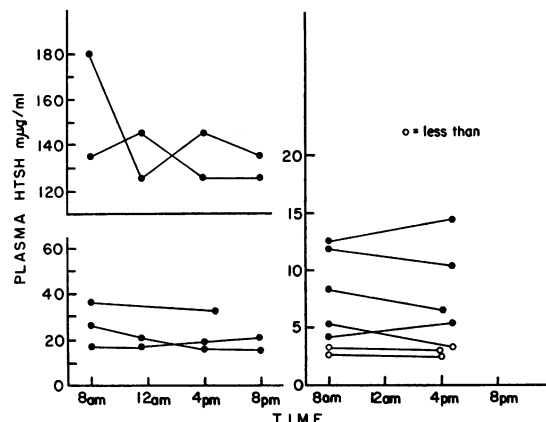


FIG. 6. LACK OF DIURNAL VARIATION IN PLASMA HTSH CONCENTRATION IN HYPOTHYROID (LEFT) AND NORMAL SUBJECTS (RIGHT).

Table II. Assay variation was no greater than  $\pm 15\%$ . Therefore, the reliability of single measurement was satisfactory.

**Diurnal variation in plasma HTSH.** Several plasma samples from within a single 24-hour period from seven normal and five hypothyroid subjects have been assayed (Figure 6). No evidence of diurnal variation was found in either group.

**Effect of hypoglycemia and hyperglycemia.** No change in plasma HTSH activity was found in either eight normal or five hypothyroid subjects during the induction or recovery from insulin hypoglycemia. Typical responses of plasma glucose and HTSH are shown in Figure 7. Also shown is the normal plasma growth hormone re-

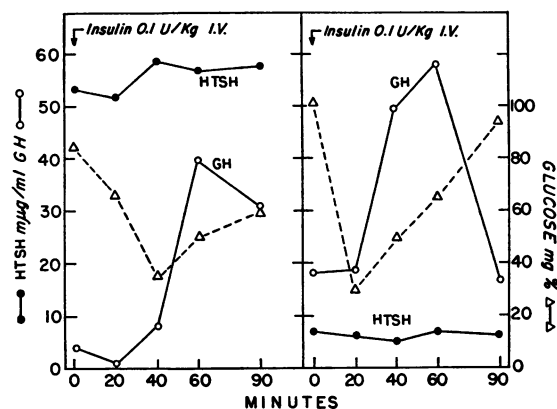


FIG. 7. LACK OF EFFECT OF INSULIN HYPOGLYCEMIA ON PLASMA HTSH CONCENTRATION IN A HYPOTHYROID PATIENT (LEFT) AND A NORMAL SUBJECT (RIGHT). Plasma HTSH ( $\bullet$ ), growth hormone ( $\circ$ ), and glucose ( $\Delta$ ) are all shown.

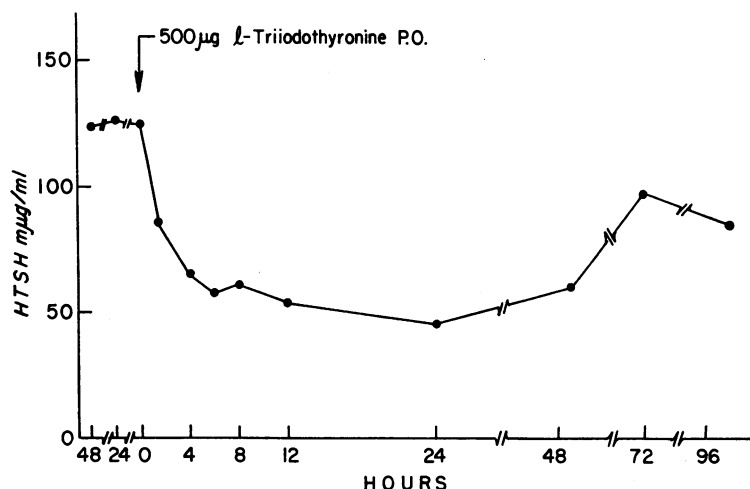


FIG. 8. CHANGE IN PLASMA HTSH CONTENT AFTER ADMINISTRATION OF 500  $\mu$ g L-TRIIODOTHYRONINE ORALLY TO A HYPOTHYROID PATIENT.

sponse to hypoglycemia found in these patients. The growth hormone assays were kindly performed by Dr. Mary L. Parker (11). In the hypothyroid patients, two of the five failed to show the usual growth hormone rise consequent to hypoglycemia (21). Assays performed during the course of 5-hour glucose tolerance tests in four normal subjects showed no change in plasma HTSH.

**Effect of thyroid hormones.** Plasma HTSH measurements were performed after the acute and chronic administration of various thyroid hormones to both hypothyroid and normal subjects. The administration of a single oral dose of 500  $\mu$ g L-triiodothyronine to one hypothyroid patient was followed by a rapid fall in plasma HTSH (Figure 8); the lowest HTSH concentration occurred 24 hours after hormone administration, and thereafter plasma HTSH returned toward the initial level. Assays performed with similar frequency after the administration of single oral doses of 12.5 or 100  $\mu$ g L-triiodothyronine to four other hypothyroid patients revealed no change in plasma HTSH over a 3-day study period. An intravenous dose of 100  $\mu$ g L-thyroxine was also ineffective in reducing plasma HTSH in another hypothyroid patient.

Normal subjects were given 100  $\mu$ g L-triiodothyronine orally for 4 days. The initially detectable plasma HTSH concentrations fell in five of the six subjects, becoming undetectable in four. Daily

replacement therapy with L-triiodothyronine and L-thyroxine resulted in a progressive fall in plasma HTSH content in several hypothyroid subjects. In the L-triiodothyronine-treated patient, plasma HTSH was normal by day 9 of therapy (Figure 9). The response was slower in a patient receiving 200  $\mu$ g L-thyroxine per day (Figure 10); 18 days of therapy were required before the plasma HTSH became normal although there was some initial fall. A similar slow response was found in another thyroxine-treated case. Equally elevated plasma HTSH values became normal in 7 to 10 days in two other hypothyroid patients who re-

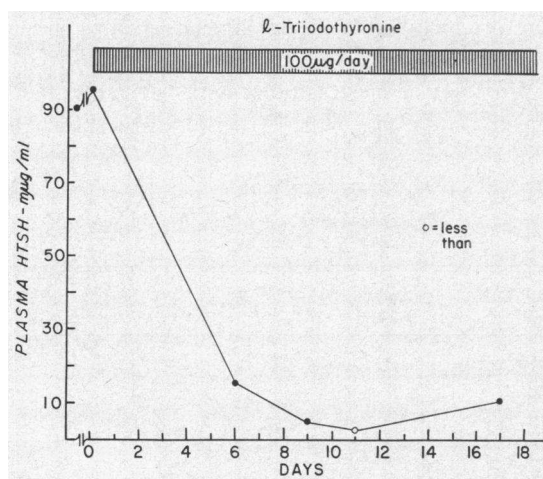


FIG. 9. EFFECT OF L-TRIIODOTHYRONINE (100  $\mu$ g PER DAY) ON PLASMA HTSH IN HYPOTHYROIDISM.

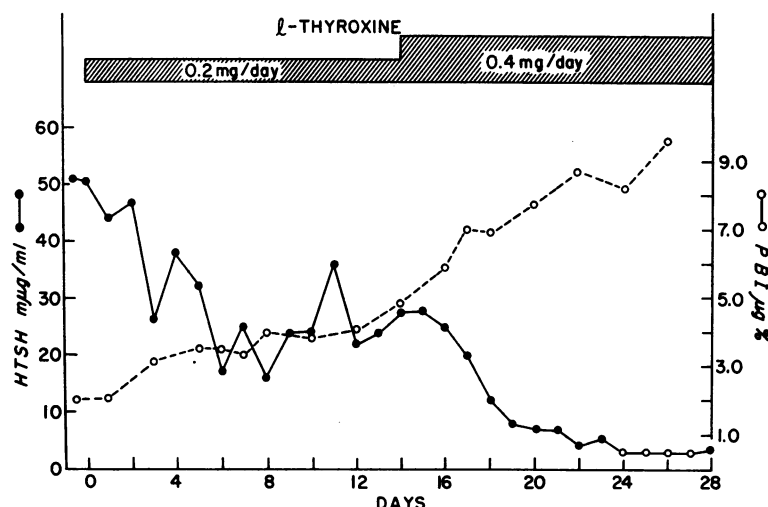


FIG. 10. EFFECT OF L-THYROXINE (200  $\mu$ G, THEN 400  $\mu$ G PER DAY) ON PLASMA HTSH (●) AND PROTEIN-BOUND IODINE (PBI) (○) IN A HYPOTHYROID PATIENT.

ceived 120 mg desiccated thyroid per day. A study performed on a patient who had been receiving 100 mg methimazole for 6 to 7 months was of special interest (Figure 11). This patient entered the hospital complaining only of an enlarging goiter; the plasma HTSH was 54  $\mu$ g per ml and the protein-bound iodine (PBI) 1.1  $\mu$ g per 100 ml. After discontinuance of the methimazole, the PBI value rapidly rose to the normal range, and plasma HTSH fell within 1 week with the recovery of endogenous thyroid hormone production.

Eight additional hypothyroid patients were studied before and 3 or more weeks after initiation of

maintenance desiccated thyroid therapy. Plasma HTSH values, initially elevated, had fallen to within the normal range in all (Table III).

### Discussion

The results obtained with this radioimmunoassay of HTSH attest to its sensitivity and specificity. With the HTSH- $I^{131}$  preparations available, as little as 1.5  $m\mu$ g HTSH per ml plasma was detected. Further increases in sensitivity may be possible by increasing the specific activity of HTSH- $I^{131}$  preparations, extraction and concentration of plasma HTSH, or the preparation of anti-HTSH sera of higher binding affinity. With the present assay conditions there was

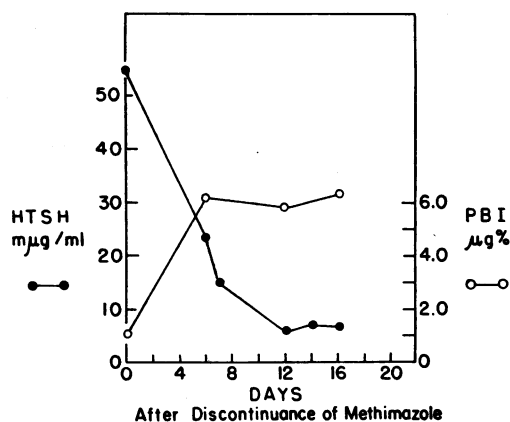


FIG. 11. RECOVERY OF ENDOGENOUS THYROID FUNCTION AND FALL IN PLASMA HTSH AFTER DISCONTINUATION OF PROLONGED THERAPY WITH 100 MG METHIMAZOLE PER DAY.

TABLE III

*Effect of thyroid hormone therapy on plasma HTSH in hypothyroidism*

Patient	Plasma HTSH		Duration of therapy wks	Desiccated thyroid mg/day
	Initial $m\mu$ g/ml	After therapy $m\mu$ g/ml		
S. K.	25	15	6	15
		13	9	15
R. S.	108	10	12	180
V. C.	58	6	8	120
H. O.	32	6	4	120
A. B.	124	5	4	120
G. G.	30	3	3	120
B. G.	42	6	4	180
S. M.	93	4	3	180



no evidence of nonspecific interference since plasma from nonprimates, from hypopituitary and hypophysectomized subjects, and from many of the patients receiving exogenous thyroid hormones had no detectable HTSH activity. HTSH activity, when present, produced dose response curves parallel to that of the purified HTSH preparation. In addition, the constancy of the results during the day or during a glucose or insulin tolerance test, the disappearance of activity after thyroid hormone administration in hypothyroid patients, and the success in removing activity by absorption with anti-HTSH further support the specificity of the system. Thus it is reasonable to conclude that the technique is actually measuring plasma HTSH.

Heretofore, TSH measurements have been made only by biological assay procedures. The existing bioassay techniques have a number of shortcomings when applied to human plasma. These are: *a*) frequent lack of sufficient sensitivity; *b*) inconsistencies in the reported concentration of plasma HTSH in various thyroid disorders; *c*) the presence of nonspecific factors in plasma that affect the assay; *d*) interference by the long-acting thyroid stimulator (LATS) found in the plasma of many patients with hyperthyroidism; and *e*) the lack of agreement of results when the same materials have been assayed by different techniques (22). Several recent reviews have thoroughly covered these problems (9, 23, 24). When detectable, results of plasma HTSH determinations in normal subjects have varied from as little as 0.001 mU per ml to 1.3 mU per ml in various bioassay systems (24).

With radioimmunoassay and the same purified HTSH preparation utilized in the present study, Odell, Wilber, and Paul, in a preliminary report (12), have described values of  $< 3.0$  m $\mu$ g per ml ( $< .03$  mU per ml) in normal subjects. Plasma HTSH concentrations reported herein in normal subjects ranged from undetectable to 15 m $\mu$ g per ml; in biological units using a potency of 10 IU per mg the upper limit would be 0.15 mU per ml with most values less than 0.1 mU per ml. This is somewhat less than the values found in most of the bioassays. These values are of the order predicted by Greer and Shull (25) on the basis of thyroidal responsiveness to exogenous TSH in humans. Since it is known that the potency of different species TSH may vary depending on the species utilized for assay purposes (26), compari-

sons of assay results of human plasma and bovine TSH standard preparations in various test systems may be misleading. For these reasons, we prefer to report results in weight units, recognizing that the absolute numbers may be in error due to impurities present in the HTSH preparation.

Whatever the assay system, elevated plasma HTSH concentrations usually have been found in primary hypothyroidism. This is true for immunoassay results as well. There was no apparent correlation between the plasma HTSH content and the duration or severity of the hypothyroidism. Reduction of elevated plasma HTSH concentrations to normal levels occurred within 7 to 10 days in those patients given moderate daily doses of L-triiodothyronine or desiccated thyroid. Therapy with L-thyroxine required somewhat longer, possibly due to its higher affinity for plasma thyroid-binding proteins and therefore reduced tissue penetration. The fall in plasma HTSH within 4 hours to half the initial level in the hypothyroid patient given 500  $\mu$ g L-triiodothyronine orally suggests that the plasma half-life of endogenous HTSH is less than 1 to 2 hours. This result may be compared with the finding of an HTSH- $I^{131}$  plasma half-life of 39 to 53 minutes after intravenous administration in six normal subjects (27).

Reduction of plasma HTSH concentration to undetectable levels was found in most normal subjects studied following the administration of 100  $\mu$ g L-triiodothyronine per day for 4 days. These observations confirm studies that have suggested that pituitary HTSH release, as measured indirectly by reduction of thyroidal  $I^{131}$  uptake, could usually be suppressed by the administration of 75 to 150  $\mu$ g L-triiodothyronine per day for 4 to 7 days (28, 29). The effectiveness of considerably smaller doses and the ineffectiveness of larger doses of L-triiodothyronine in suppressing thyroidal  $I^{131}$  uptake have also been reported (29, 30).

The finding of undetectable levels of HTSH in most cases of hyperthyroidism is in agreement with current concepts of the etiology of Graves' disease. A long-acting thyroid stimulator (LATS) unresponsive to thyroid hormonal control is frequently found in the plasma of hyperthyroid patients. Many authorities consider this factor to be responsible for the disorder. Attempts to neutralize the biological activity of LATS with antbovine TSH sera have yielded variable re-

sults (2, 3). Using anti-HTSH serum, Adams, Kennedy, Purves, and Sirett (5) were unable to block the biological activity of LATS. More recent immunological (31) as well as a number of chromatographic, electrophoretic, and biological studies (20, 32-34) strongly suggest that TSH and LATS are different substances. The results of plasma HTSH assays in hyperthyroidism reported herein would further support the concept of dissimilarity between HTSH and LATS.

### Summary

A method for the radioimmunoassay of human plasma thyrotropin (HTSH) has been developed. Highly purified HTSH (Condliffe, 10 IU per mg) was used for immunization, radioiodination, and immunoassay standard. The assay depended on the binding of HTSH- $I^{131}$  by rabbit anti-HTSH serum. This binding was competitively inhibited by unlabeled HTSH. The method was sensitive to as little as .075  $\mu$ g unlabeled HTSH, permitting detection of plasma HTSH concentrations as low as 1.5  $\mu$ g per ml (0.015 mU per ml) under the conditions employed. Separation of free and antibody bound HTSH- $I^{131}$  was effected by chromatoelectrophoresis.

Plasma from hypothyroid patients and human pituitary extracts produced dose responses identical to that of the purified HTSH preparation. No HTSH was detectable in plasma from various animals, in plasma from hypopituitary patients, in most patients receiving exogenous thyroid therapy, or in bovine or ovine pituitary TSH preparations. HTSH added to plasma could be recovered quantitatively. Plasma HTSH ranged from undetectable to 15  $\mu$ g per ml (0.15 mU per ml) in euthyroid subjects, measurable activity being found in 55%. Most values were lower than 10  $\mu$ g per ml (0.1 mU per ml). In 32 patients with primary hypothyroidism, plasma HTSH concentrations ranged from 18 to 180  $\mu$ g per ml (0.18 to 1.8 mU per ml). In hyperthyroid patients, plasma HTSH was undetectable in 25 of 34 patients studied.

No change in plasma HTSH followed insulin hypoglycemia or hyperglycemia, and no diurnal variation was found. In all hypothyroid patients studied, the initially elevated plasma HTSH concentrations fell to within the normal range during

the course of replacement therapy. In patients in whom assays were performed at frequent intervals, plasma HTSH was reduced to normal after 7 to 10 days of maintenance L-triiodothyronine or desiccated thyroid therapy. The response to L-thyroxine was slower. Oral administration of 500  $\mu$ g L-triiodothyronine to a hypothyroid patient produced a 50% fall in plasma HTSH concentration in 4 hours and a maximal reduction in 24 hours.

### Acknowledgments

The author wishes to acknowledge the advice and encouragement of Dr. William H. Daughaday, the generosity of Dr. Peter G. Condliffe for the supply of highly purified human TSH, and the able technical assistance of Miss Carmen Scheidt during these studies.

### References

1. Utiger, R. D. The radioimmunoassay of plasma thyrotropin in hyperthyroidism and hypothyroidism (abstract). *J. Lab. clin. Med.* 1964, **64**, 1013.
2. McKenzie, J. M., and J. Fishman. Effects of antiserum in bioassay of thyrotropin and thyroid activator of hyperthyroidism. *Proc. Soc. exp. Biol. (N. Y.)* 1960, **105**, 126.
3. Werner, S. C., and J. Tierney. Neutralization of "long-acting thyroid stimulator" in Graves' disease by antisera to bovine pituitary thyrotropin. *Proc. Soc. exp. Biol. (N. Y.)* 1961, **108**, 780.
4. Levy, R. P., W. L. McGuire, and M. L. Heideman, Jr. Quantitative studies of the reaction of bovine thyrotropin antiserum with human and bovine thyrotropin preparations. *Proc. Soc. exp. Biol. (N. Y.)* 1962, **110**, 598.
5. Adams, D. D., T. H. Kennedy, H. D. Purves, and N. E. Sirett. Failure of TSH antisera to neutralize long-acting thyroid stimulator. *Endocrinology* 1962, **70**, 801.
6. Arquilla, E. R., B. Catz, and J. Finn. Immunological studies with partially purified bovine and human TSH. *J. Immunol.* 1963, **90**, 843.
7. Utiger, R. D., W. D. Odell, and P. G. Condliffe. Immunologic studies of purified human and bovine thyrotropin. *Endocrinology* 1963, **73**, 359.
8. Pascasio, F. M., and H. A. Selenkow. Immunologic and biologic properties of thyrotropin antisera. *Endocrinology* 1962, **71**, 254.
9. Werner, S. C. Immune method of thyrotropin assay in *Thyrotropin*, S. C. Werner, Ed. Springfield, Ill., Charles C Thomas, 1963, pp. 297-300.
10. Utiger, R. D. Unpublished observations.
11. Schalch, D. S., and M. L. Parker. A sensitive double antibody immunoassay for human growth hormone in plasma. *Nature (Lond.)* 1964, **203**, 1141.
12. Odell, W. D., J. F. Wilber, and W. E. Paul. Radioimmunoassays for human and bovine thyrotropic hormones (abstract). *Clin. Res.* 1964, **12**, 461.

13. Condliffe, P. G. Purification of human thyrotropin. *Endocrinology* 1963, 72, 893.
14. Parlow, A. F., P. G. Condliffe, L. E. Reichert, Jr., and A. E. Wilhelmi. Recovery and partial purification of FSH and LH during the purification of TSH from human pituitary glands. *Endocrinology* 1965, 76, 27.
15. Bates, R. W., and J. Cornfield. An improved assay method for thyrotropin using depletion of  $I^{131}$  from the thyroid of day-old chicks. *Endocrinology* 1957, 60, 225.
16. Hunter, W. M., and F. C. Greenwood. Preparation of iodine- $I^{131}$  labelled human growth hormone of high specific activity. *Nature (Lond.)* 1962, 194, 495.
17. Yalow, R. S., and S. A. Berson. Immunoassay of endogenous plasma insulin in man. *J. clin. Invest.* 1960, 39, 1157.
18. Parker, M. L., I. K. Mariz, and W. H. Daughaday. Resistance to human growth hormone in pituitary dwarfism: clinical and immunologic studies. *J. clin. Endocr.* 1964, 24, 997.
19. Flodin, P., and J. Killander. Fractionation of human-serum proteins by gel filtration. *Biochim. biophys. Acta (Amst.)* 1962, 63, 403.
20. McKenzie, J. M. Gel filtration of thyrotropin and the long acting thyroid stimulator (abstract). *Endocrine Society 45th Annual Meeting, Atlantic City, N. J., 1963*, p. 13.
21. Roth, J., S. M. Glick, R. S. Yalow, and S. A. Berson. Hypoglycemia: a potent stimulus to secretion of growth hormone. *Science* 1963, 140, 987.
22. Werner, S. C. Comparison of results of assay of thyrotropin by various methods *in Thyrotropin*, S. C. Werner, Ed. Springfield, Ill., Charles C Thomas, 1963, pp. 195-199.
23. McKenzie, J. M. Bio-assay of thyrotropin in man. *Physiol. Rev.* 1960, 40, 398.
24. Robbins, J., J. E. Rall, and P. G. Condliffe. The thyroid-stimulating hormone and the iodine-containing hormones *in Hormones in Blood*, C. H. Grey and A. L. Bacharach, Eds. New York, Academic Press, 1961, pp. 55-77.
25. Greer, M. A., and H. F. Shull. A quantitative study of the effect of thyrotropin upon the thyroidal secretion rate in euthyroid and thyrotoxic subjects. *J. clin. Endocr.* 1957, 17, 1030.
26. Dodd, J. M., K. M. Ferguson, M. H. I. Dodd, and R. B. Hunter. The comparative biology of thyrotropin secretion *in Thyrotropin*, S. C. Werner, Ed. Springfield, Ill., Charles C Thomas, 1963, pp. 22-27.
27. Odell, W. D., and R. D. Utiger. Unpublished observations.
28. Werner, S. C., and M. Spooner. A new and simple test for hyperthyroidism employing L-triiodothyronine and the 24-hour  $I^{131}$  uptake method. *Bull. N. Y. Acad. Med.* 1955, 31, 137.
29. McConahey, W. M., and C. A. Owen, Jr. Studies of the inhibitory effect of L-triiodothyronine on thyroidal  $I^{131}$  uptake in euthyroid persons and patients with exophthalmic goiter. *J. clin. Endocr.* 1956, 16, 1480.
30. Hales, I. B., J. Myhill, T. H. Oddie, and M. Croydon. Quantitative observations with the triiodothyronine suppression test of thyroid function. *J. clin. Endocr.* 1961, 21, 189.
31. Dorrington, K. J., and D. S. Munro. Immunological studies on the long-acting thyroid stimulator. *Clin. Sci.* 1965, 28, 165.
32. Monro, D. S. Observations on the discharge of radioiodine from the thyroid glands of mice injected with human sera. *J. Endocr.* 1959, 19, 64.
33. McKenzie, J. M. Studies on the thyroid activator of hyperthyroidism. *J. clin. Endocr.* 1961, 21, 635.
34. Adams, D. D. A comparison of the rates at which thyrotropin and the human abnormal thyroid stimulator disappear from the circulating blood of the rat. *Endocrinology* 1960, 66, 658.

## ERRATUM

In the paper entitled, "Modification of the Antidiuretic Effect of Vasopressin by Acid and Alkaline Loads," by T. D. Ullmann, W. J. Czaczkes, and J. Menczel, published in the May issue (p. 754), the following correction should be noted: On page 759 in the last column of Table I, there should be a minus sign before the numbers in  $NH_4Cl$  experiments no. 4, 5, and 6. The numbers should be -0.6, -3.8, and -0.5, respectively, as correctly printed in Table III.