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# Estimation of the Secretion Rate of Thyrotropin in Man *

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**Summary.** **The plasma concentration of a pituitary hormone is determined by the rate of secretion, degradation, and the volume of distribution of that hormone. Using a radioimmunoassay for human thyrotropin (TSH) and human TSH-$^{131}$I, we have estimated the rates of degradation and distribution of TSH in man and calculated the rate of secretion. Either 0.5 or 5 $\mu$g of TSH-$^{131}$I with specific activities of 1 to 50 $\mu$c per $\mu$g was administered intravenously to 12 euthyroid subjects. Serial determinations were made of TSH-$^{131}$I, and the half-time of disappearance ($t_{\frac{1}{2}}$) was thus estimated. The average $t_{\frac{1}{2}}$ in euthyroid subjects was 53.9 minutes with a volume of distribution averaging 5.8% of body weight. The mean endogenous plasma TSH concentration was 1.8 m$\mu$g per ml (2.7 $\mu$U per ml in terms of the human TSH reference standard A). The mean total TSH pool, excluding the pituitary, was 5.8 $\mu$g (8.7 mU). From these data the mean secretion rate of TSH in euthyroid man was calculated to be 110.1 $\mu$g per day (165.2 mU).**

**Similar data were estimated for 3 mildly hypothyroid patients. The $t_{\frac{1}{2}}$ were 75.1, 97.1, and 83.6 minutes, with a mean of 85.3 minutes (1.6 times normal). The mean TSH pool was 58.1 $\mu$g (10 times normal). The secretion rate was 688.7 $\mu$g per day (1,033.1 mU). In other hypothyroid patients, plasma TSH levels ranging from 6 to 230 m$\mu$g per ml (9 to 345 $\mu$U) have been found. If similar half-times and a normal distribution volume are assumed, the secretion rate of TSH in hypothyroid patients can be estimated to range from about 260 to 15,350 $\mu$g per day (390 to 23,025 mU) or from about 2 to 307 times normal. Therefore, the elevated plasma TSH levels found in hypothyroidism are a result of both slower degradation and increase in rate of secretion.**

## Introduction

Relatively little information is available on the rate of turnover and secretion of thyrotropin in man or laboratory animals. Using bioassays, several workers have estimated the disappearance rate of relatively large amounts of heterologous TSH (bovine) administered to the rat (1–4). In these studies the half-time of disappearance was between 2 and 5 minutes. Similar studies in the rabbit (5) revealed a $t_{\frac{1}{2}}$ of about 35 minutes; the $t_{\frac{1}{2}}$ of TSH in hypothyroid rabbits was longer than that in euthyroid animals. Hendrich and Turner (6) have estimated the secretion rate of TSH in chickens by first labeling the thyroid glands with $^{131}$I and determining the rate of release of this thyroidal $^{131}$I, then suppressing endogenous TSH with thyroxine. The amount of injected bovine TSH required to return the rate of release of thyroidal $^{131}$I to normal was said to be equivalent to the amount of endogenous TSH secreted. By this

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technique the secretion rate was estimated to average about 2.5 USP [1] mU per 100 g body weight per day. In 1962 Bakke, Lawrence, and Roy (7) reported estimates of $t_{\frac{1}{2}}$ and secretion rates of TSH in man. In these studies 7.5 to 40 U of bovine TSH was administered intravenously and the $t_{\frac{1}{2}}$ estimated by following disappearance by bioassay. The mean $t_{\frac{1}{2}}$ in eumetabolic adults was 35.5 minutes, whereas the $t_{\frac{1}{2}}$ in hypothyroid patients averaged 97.9 minutes. The secretion rate in euthyroid adults was estimated to be about 260 USP mU per day. The rate was about 10 times greater in hypothyroid patients. Although these studies were elegantly done, the conclusions might be challenged because of two limitations in the methodology available. First, the amounts of TSH utilized were not tracer amounts. According to our data, more than 10 times the total body pool of TSH was administered. It has been shown previously that the $t_{\frac{1}{2}}$ values for certain hormones (e.g., cortisol) are different when measured after administration of tracer and large doses of hormone (8). Second, bovine TSH was administered to man. In earlier studies (9) we have demonstrated almost complete lack of immunological cross-reaction of bovine and human TSH, suggesting that there are structural differences between human and bovine TSH. One might then question whether the human organism disposes of bovine TSH in an identical fashion to the human hormone.

For these reasons we have estimated the $t_{\frac{1}{2}}$, distribution volume, and the secretion rates of TSH in man using tracer amounts of highly purified human TSH labeled with $^{131}I$. Endogenous plasma TSH levels were determined by radioimmunoassay (10, 11).

## Methods

Highly purified human TSH was utilized in these studies. This material has been studied by several techniques to ascertain its degree of purification; these studies have been previously reported (9, 11). At the time of preparation, it contained 20 USP U per mg. When antisera prepared against this preparation were allowed to react against it in agar gel, a single line of precipitation was observed (9). In these studies, no lines of reaction were observed against bovine TSH even when 200 times more than the human TSH was utilized. In addition, when this TSH was radioiodinated as described later and the iodinated material permitted to react against these antisera, radioautography revealed a similar single precipitation line. The contaminant present in largest amounts was human luteinizing hormone, which accounted for 2 to 5% of the TSH preparation by weight. It is known, however, that this TSH preparation is contaminated with small amounts of other polypeptides, for highly potent antisera prepared against it result in 2 to 3 precipitation lines when reacted against crude human pituitary powder. This TSH was labeled with $^{131}I$ (TSH-$^{131}I$) to yield SA of 1 to 50 $\mu$c per $\mu$g by the method of Greenwood, Hunter, and Glover (12). Lower specific activities than those described in our previous publication (9) were utilized to minimize "damage" to the hormone. Each TSH-$^{131}I$ preparation was sterilized by Millipore filtration, tested for sterility and pyrogenicity, and used as soon as such studies proved negative (within 2 to 3 days). Eighty-five to 95% of the TSH-$^{131}I$ was precipitable by excess anti-TSH, and 95 to 100% was precipitable with 10% trichloroacetic acid (TCA). Adult euthyroid or hypothyroid patients were treated with 4 to 6 drops of a saturated solution of potassium iodide each 6 hours for 24 hours before study. This prevented significant thyroidal trapping of the $^{131}I$ released during degradation of the TSH-$^{131}I$. Either 0.5 or 5.0 $\mu$g of TSH-$^{131}I$ diluted in 0.5 to 1.0 ml of 0.9% NaCl was injected intravenously into each patient, and serial 10-ml heparinized venous blood samples were obtained via an indwelling needle at various time intervals. Patients were given liberal water intake during the period of study, and urine was collected periodically for 24 hours.

The total $^{131}I$ content of 1 ml of the plasma samples was determined in a well-type scintillation counter. TSH-$^{131}I$ content was determined either by reacting the TSH-$^{131}I$ with an excess of the same antihuman TSH serum utilized for radioimmunoassay of TSH and separating antibody bound from free TSH-$^{131}I$ by the double antibody technique of Feinberg (13), as modified by Morgan, Sorenson, and Lazarow (14), or by precipitating the TSH-$^{131}I$ with an equal volume of 10% TCA. All of the samples collected between 20 and 120 minutes from 4 patients were studied by both procedures, and during this time interval no significant differences in the results using the two techniques were found. Endogenous TSH was determined by radioimmunoassay of plasma as previously reported (11).

To determine TSH in plasma from euthyroid patients, we utilized the procedure described by Bates (15) for extraction and prepared 5- to 10-fold concentrates. TSH losses during extraction were quantitated by measuring the recovery of either tracer TSH-$^{131}I$ or bovine TSH added to plasma samples. When the latter technique was used, bovine TSH was measured by specific radioimmunoassay (9). Recovery ranged from 50 to 80%, and the average figure of 65% was utilized to correct for loss of TSH during extraction.

The slope of the disappearance curve of TSH-$^{131}I$ was calculated by the method of least squares, and $t_{\frac{1}{2}}$ was cal-

[1] U. S. Pharmacopoeia Standard, a bovine TSH preparation.

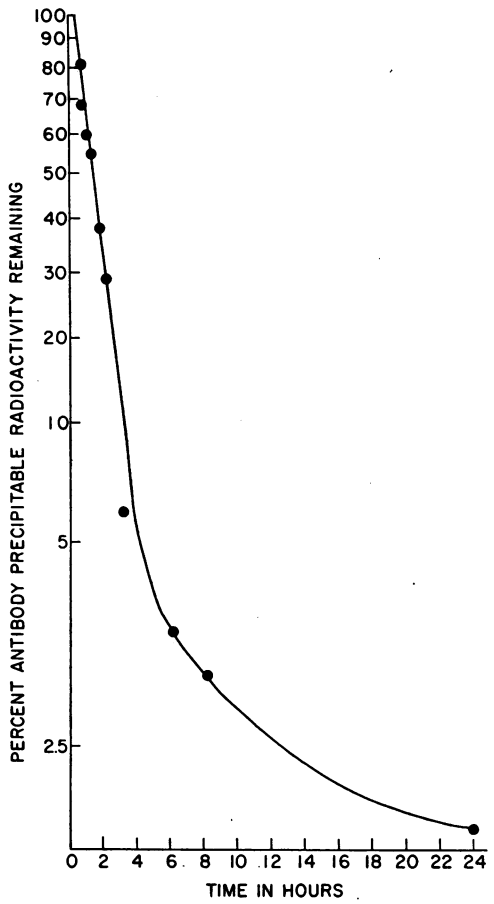


FIG. 1. DISAPPEARANCE OF THYROTROPIN (TSH)-$^{131}$I IN A EUTHYROID MAN. At zero time, 0.5 $\mu$g TSH-$^{131}$I was injected intravenously, and blood samples were taken at times indicated. Plotted values shown represent the amount of radioactivity precipitated after the addition of antihuman TSH serum in excess to each sample.

culated directly from the slope. Volume of distribution was estimated by extrapolation of the disappearance line to zero time. The secretion rate was calculated from the fractional turnover rate and the total body pool of TSH in a fashion similar to that utilized by Sterling (16) in studies of albumin-$^{131}$I metabolism in man. The following assumptions have been made during the course of these studies: *1*) A "steady state" existed during the period of study, that is, the rate of degradation and secretion of TSH remained constant. *2*) The radioactive hormone used to determine disappearance rate and volume of distribution behaved similarly to unlabeled hormone. *3*) The radioactive hormone was rapidly distributed throughout a single compartment.

## Results

After injection into euthyroid subjects, antibody or TCA precipitable radioactivity disap-

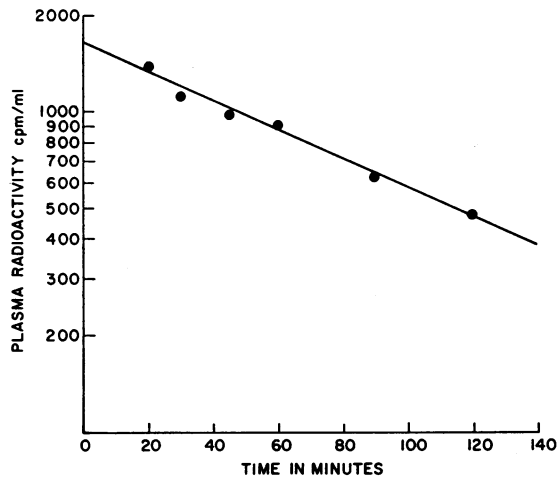


FIG. 2. DETAILED PRESENTATION OF THE DATA OBTAINED BETWEEN 20 AND 120 MINUTES PRESENTED IN FIGURE 1 WITH AN EXPANDED TIME SCALE. Note that plasma disappearance is linear when plotted as a semilogarithmic function.

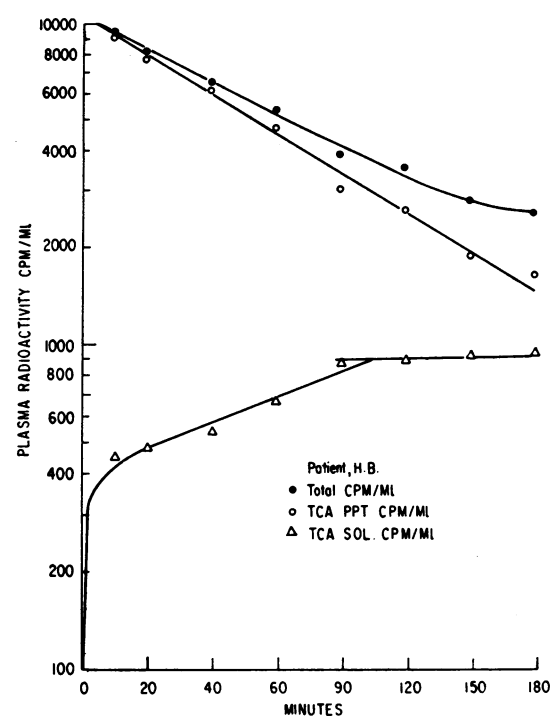


FIG. 3. DISAPPEARANCE OF TSH-$^{131}$I IN A EUTHYROID MAN. At zero time 5.0 $\mu$g TSH-$^{131}$I was injected intravenously, and blood samples were taken at times indicated. TSH-$^{131}$I was determined in this study by trichloroacetic acid (TCA) precipitability. Note the fall in TSH-$^{131}$I and the concomitant rise in free $^{131}$I (TCA soluble).

TABLE I

*Summary of results of human thyrotropin (TSH)-$^{131}I$ disappearance studies*

| | Age | Sex | Weight | $t_{\frac{1}{2}}$ | Distribution volume | % Body weight | TSH pool | Plasma clearance | Secretion rate |
|---|---|---|---|---|---|---|---|---|---|
| | *years* | | *kg* | *min* | | | *μg* | *ml/min* | *μg/day* |
| Euthyroid subjects | | | | | | | | | |
| E.A. | 68 | F | 48.0 | 39.1 | 1,872 | 3.9 | 3.4 | 32.9 | 85.2 |
| D.M. | 26 | F | 70.8 | 40.7 | 2,613 | 3.7 | 4.7 | 44.4 | 115.0 |
| A.D. | 25 | F | 54.1 | 51.5 | 6,492 | 12.0 | 11.7 | 87.0 | 225.0 |
| A.P. | 38 | F | 55.7 | 67.6 | 3,713 | 6.7 | 6.7 | 37.9 | 98.2 |
| A.B. | 17 | M | 45.3 | 48.5 | 3,586 | 6.8 | 5.5 | 50.9 | 131.8 |
| J.W. | 27 | F | 45.7 | 54.2 | 2,419 | 5.3 | 4.4 | 30.7 | 79.5 |
| R.U. | 32 | M | 80.5 | 60.1 | 3,220 | 4.0 | 5.8 | 37.0 | 95.8 |
| G.J. | 58 | F | 58.6 | 50.8 | 3,926 | 6.7 | 7.1 | 53.9 | 139.6 |
| F.W. | 54 | F | 40.6 | 62.8 | 1,745 | 4.3 | 3.1 | 19.2 | 49.7 |
| D.P. | 50 | M | 70.0 | 67.6 | 4,700 | 6.7 | 8.4 | 47.9 | 124.1 |
| D.E. | 66 | M | 47.0 | 50.8 | 1,890 | 4.0 | 3.4 | 25.7 | 66.6 |
| H.H. | 39 | F | 55.7 | 53.2 | 3,278 | 5.9 | 5.9 | 42.6 | 110.3 |
| Mean | | | | 53.9 | | 5.8 | 5.8 | 42.5 | 110.1 |
| Hypothyroid subjects | | | | | | | | | |
| S.A.* | 60 | F | 74.6 | 75.1 | 3,207 | 4.3 | 50.7* | 29.5 | 671.2 |
| A.S.† | 90 | M | 65.8 | 97.1 | 4,755 | 7.2 | 45.2† | 33.8 | 462.4 |
| E.H.‡ | 35 | M | 84.6 | 83.6 | 5,499 | 6.5 | 78.1† | 45.6 | 932.4 |
| Mean | | | | 85.3 | | 6.0 | 58.1 | 36.3 | 688.7 |

* Plasma concentration = 15.8 mμg per ml.
† Plasma concentration = 9.5 mμg per ml.
‡ Plasma concentration = 14.2 mμg per ml.

peared rapidly from the plasma, and free $^{131}I$ appeared in urine and plasma (Figures 1 to 3). When plotted semilogarithmically, the disappearance curve always appeared linear from 20 to 120 minutes, and during this time over 75% of the radioactivity disappeared. After 90 to 95% of the TSH-$^{131}I$ had disappeared, the curve became nonlinear. This was presumed to be due to contamination of the TSH-$^{131}I$ with a polypeptide other than TSH and having a much larger $t_{\frac{1}{2}}$ or to the slow disappearance of damaged TSH-$^{131}I$. Only the data obtained from 20 to 120 minutes were used to estimate $t_{\frac{1}{2}}$. Four to 8 determinations were made on each subject during the interval between 20 and 120 minutes after injection. Four adult men and 8 women euthyroid subjects were studied. Since the disappearance rate of antibody precipitable $^{131}I$ was not significantly different from that of TCA precipitable $^{131}I$ during this time period, the data using both techniques were analyzed together. The $t_{\frac{1}{2}}$ in these 12 patients averaged 53.9 minutes with a range of 39.1 to 67.6 minutes (Table I). As the TSH disappeared from plasma, TCA soluble and nonantibody precipitable radioactivity (presumed to be free iodide) appeared rapidly in the blood and urine. This material increased in urine rapidly, and by 12 to 24 hours all the injected radioactivity had been excreted.

By extrapolation of the disappearance line, the number of theoretical counts per volume of plasma at zero time was calculated, assuming instantaneous mixing. The volume of distribution was calculated by division of the amount of radioactivity injected by the counts per minute per milliliter of plasma at zero time. This volume, expressed as per cent of body weight, ranged from 3.7 to 12.0 with a mean of 5.8% or about $1\frac{1}{3}$ to $1\frac{2}{3}$ of the plasma volume.

The concentration of endogenous TSH in the plasma of 11 other euthyroid adults was measured by radioimmunoassay. A mean of 1.8 mμg per ml (2.7 μU per ml [2]) was found with 95% confidence limits of 0.8 to 2.8 mμg per ml (1.2 to 4.2 μU).

From the volume of distribution and concentra-

[2] In terms of the human TSH reference standard A, obtained from the Medical Research Council, Division of Biological Standards, National Institute for Medical Research, London.

tion of endogenous TSH, total body pool (excluding pituitary) of TSH was estimated. This value was 5.8 μg (8.7 mU [8]) with a range of 3.1 to 11.7 μg. The volume of plasma cleared of TSH was estimated from the total body pool and fractional turnover rate ($0.69/t_{\frac{1}{2}}$) (16). This value averaged 42.5 ml per minute (range 19.2 to 87.0). From these data the secretion rate of TSH in euthyroid subjects was calculated and found to average 75.8 mμg per minute or 110.1 μg (165.2 mU) per 24 hours (range 49.7 to 225.0).

Further studies were done in 1 woman and 2 men hypothyroid subjects. These results are also shown in Table I. The $t_{\frac{1}{2}}$ were 75.1, 83.6, and 97.1 minutes.

The volume of distribution for the 3 patients was similar to that for the euthyroid subjects. The endogenous concentration of TSH averaged 13.2 mμg (19.8 μU) per ml. The total body pool of TSH, excluding the pituitary, was some 5 to 25 times the TSH pool in the euthyroid subjects. The secretion rates of TSH for these 3 hypothyroid patients averaged 688.7 μg (983.1 mU) per 24 hours.

As further support for the similarity between the disappearance of TSH-$^{131}$I and of endogenous TSH, the disappearance of endogenous TSH was studied in 3 other young patients after intravenous injections of 500 μg of *l*-thyroxine or triiodothyronine. One of the patients, initially euthyroid, had plasma TSH elevated to 14 mμg per ml by 12 days' treatment with oral methimazole and presumably had low iodine stores before treatment. The $t_{\frac{1}{2}}$ of TSH in this patient could not be accurately determined but was more than 30 and less than 60 minutes. Two myxedematous patients had $t_{\frac{1}{2}}$ of 90 and 130 minutes, respectively. Data from one of these patients are shown in Figure 4.

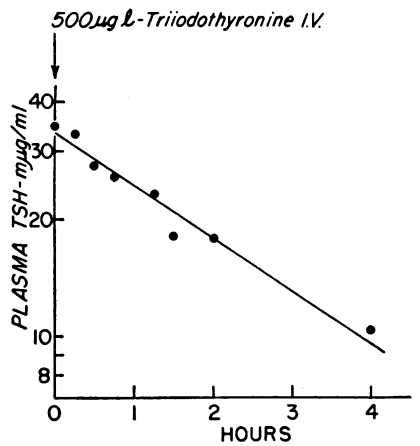


FIG. 4. THE DISAPPEARANCE OF ENDOGENOUS TSH FROM PLASMA IN A HYPOTHYROID CHILD GIVEN 500 μG OF TRIIODOTHYRONINE INTRAVENOUSLY AT TIME ZERO. Note that the disappearance appears linear and that the $t_{\frac{1}{2}}$ in this patient was 130 minutes.

## Discussion

The assumption that a steady state of TSH secretion existed during the course of these studies is supported by earlier studies. Although the present sensitivity of the radioimmunoassay for TSH does not permit detection of TSH in all euthyroid subjects, measurements throughout the day in subjects with slight elevations in plasma TSH have failed to reveal diurnal variations. Plasma TSH levels were in fact found to be remarkably constant from day to day and during different portions of the day in any one individual (11).

The average $t_{\frac{1}{2}}$ of TSH in the euthyroid subjects estimated from these studies was 53.9 minutes. Several studies were performed to ascertain that the TSH-$^{131}$I was actually degraded during this time interval, and not merely distributed in a larger space. *1*) Nonantibody precipitable and non-TCA soluble $^{131}$I appeared in the urine, and 100% of the radioactivity could be recovered in 12 to 24 hours. *2*) The disappearance was linear during disappearance of 90% or more of the hormone, and *3*) endogenous TSH and TSH-$^{131}$I disappeared with a similar $t_{\frac{1}{2}}$. In earlier studies (9) we demonstrated that this TSH-$^{131}$I retained biological activity.

The slower disappearance of TSH in hypothyroidism could be due either to overloading of normally functioning degradative mechanisms or to slower catabolism by a high capacity system. The latter interpretation seems most likely in view of the well–documented reduced rate of metabolism of many substances present in normal or reduced concentrations in the hypothyroid state (e.g., cortisol, thyroxine, digoxin). TSH appears to be more slowly metabolized than other pituitary hormones so far studied. Parker, Utiger, and Daugh-

[8] Milliunits human TSH standard A.

aday (17) have reported the $t_{\frac{1}{2}}$ of labeled and unlabeled exogenous growth hormone (GH) in man to be about 27 minutes. Glick, Roth, and Lonergan (18) have published similar $t_{\frac{1}{2}}$ values for the disappearance of endogenous plasma growth hormone in man. Parker and her colleagues (17) have also previously reported that the disappearance of GH-$^{131}$I was initially rapid with a $t_{\frac{1}{2}}$ of about 27 minutes, during which about 80% of the GH-$^{131}$I disappeared. Later, the GH-$^{131}$I disappeared with a much longer $t_{\frac{1}{2}}$, which was presumed to be due to the slower disappearance of damaged GH-$^{131}$I. The $t_{\frac{1}{2}}$ of disappearance of endogenous GH or administered unlabeled GH was similar to the early disappearance phase that occurred between 10 and 100 minutes. Liddle, Island, and Meador (19) have reported the $t_{\frac{1}{2}}$ of ACTH to be about 20 minutes.

The mean volume of distribution of TSH in our studies was 5.8% of body weight, which is in remarkably good agreement with that calculated by Bakke and co-workers (7) after bovine TSH administration. This volume is also remarkably similar to that estimated for alpha melanocyte–stimulating hormone in the rat using distribution of unlabeled hormone (20). This suggests that TSH is not uniformly distributed throughout the extracellular fluid volume. The total body pool of TSH averaged 6.1 $\mu$g (9.2 mU) in the euthyroid patients and was 7 to 15 times greater in the 3 hypothyroid patients studied. Measurements of plasma TSH by immunoassay in other hypothyroid patients have ranged from 6 to 230 m$\mu$g per ml (9 to 345 $\mu$U) (10, 11, 21). If we assume that the mean euthyroid TSH pool size of 5.8% of body weight applies for all hypothyroid patients (and similar values were found in our 3 hypothyroid patients), the total TSH pool in 70-kg hypothyroid subjects would range from 24 to 920 $\mu$g or from 2 to 290 times the limits of the normal range.

Two reference standards were available to translate these values to units. The USP standard is of bovine origin and does not react in the immunoassay for human TSH. Furthermore the potency ratio of human TSH assayed against this USP standard varies depending on the species of assay animal. However, at the onset of these studies, our human TSH preparation had a potency of 10 USP U per mg in the chick assay of Bates (15). With this value the secretory rate would be 1.09 U per day. If we used the measurements of Bates and Condliffe (also determined in the chick), who found human pituitary tissue to contain from 0.01 to 0.06 U per mg dry weight, and assumed a dry weight of 100 mg (22), from 17 to 100% of the pituitary content of TSH would be turned over each day. If we took the probable USP potency of this human TSH preparation as determined in the bovine slice assay of Bakke and co-workers (7) as 1 U per mg, the secretion rate would be 109 mU per day. This value is about 40% of the estimate made by Bakke and co-workers in their studies using bovine TSH (7). This difference can be largely explained by the observed differences in $t_{\frac{1}{2}}$ values. The recently available human TSH reference standard A may be utilized in the immunoassay. Based upon the unitage per vial assigned by the Medical Research Council, our TSH assayed at 1.5 U per mg. With this potency the secretion rates of euthyroid subjects would average 162 mU per day.

The secretion rate of TSH in the 3 hypothyroid patients studied was about 7 times that of the euthyroid subjects. It is apparent that the elevated TSH levels in circulating blood of hypothyroid patients result from both an increase in secretion rate and a reduction in degradation. If we used the average value for TSH volume of distribution, the average $t_{\frac{1}{2}}$ of 85 minutes, and a range of plasma TSH concentrations in hypothyroidism of 6 to 230 m$\mu$g per ml, the secretion rate would range from about 260 to 15,350 $\mu$g per 24 hours.

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