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Method of Estimating Thyroid Hormone Secretion Rate of Rats and Factors Affecting It

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ACKNOWLEDGMENT

While the primary aim of this project has been the extension of our knowledge of the role of the thyroid gland and its hormones in relation to milk secretion, it has made possible, also, the training of graduate students and research fellows in the field of animal physiology and endocrinology. Many of these men now have fine positions in colleges, experiment stations, and commercial research laboratories both in the United States and abroad where their training and experience in course work and research at the University of Missouri is having an ever-widening influence upon graduate education in endocrinology and the advancement of knowledge.

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INTRODUCTION

Interest in the thyroid gland and its hormones was aroused in dairy physiologists by the observation that thyroidectomy of dairy cattle depressed the rate of milk secretion and that upon replacement therapy with desiccated thyroid glands, milk yield was not only returned to normal but in many cows was increased above the preoperative yield (Graham, 1934a). It was then shown that the feeding or injection of thyroxine stimulated increased lactation in many normal cows (Graham, 1934b).

The fact that exogenous thyroxine stimulated increased milk and fat production in many dairy cows suggested that this was because the cow's endogenous secretion was less than optimal and upon increasing the level of thyroxine in the blood, increased milk secretion was stimulated. The role of the thyroid hormones in milk secretion was thus established.

While supplemental therapy indicated the cows deficient in thyroxine secretion, the development of a method by which thyroxine secretion rate could be determined and used as a selective index in the improvement of dairy cattle was indicated. Steps in the development of such methods will be discussed.

The story of the role of the thyroid glands in milk secretion has been presented (Turner, 1968).

Regulation of Thyroid Hormone Secretion Rate

Thyroid hormone secretion rate (TSR) is regulated by the thyroid stimulating hormone (TSH) of the anterior pituitary and it, in turn, is regulated by the anterior hypothalamus, which secretes a factor that stimulates the secretion and release of TSH by the pituitary (TRF).

The role of the hypothalamus in the regulation of TSH secretion has been shown recently by the effect of lesions upon the uptake and release of I^{131} by the thyroid, by lower protein-bound iodine (PBI) levels in the blood, and by changes in the TSH levels in the pituitary and blood. A second type of evidence is concerned with the extraction and purification of a factor which stimulates the release of TSH from the pituitary (TRF).

The thyroid stimulating hormone (TSH) secreted by the pituitary has three important functions in relation to the activity of the thyroid gland:

1) It stimulates the uptake (trapping) of iodine by the thyroid gland;

2) It stimulates the secretion of thyroglobulin into the lumen of the alveoli and the formation of thyroxine ($L-T_4$) and triiodothyronine ($L-T_3$) as an integral part of the thyroglobulin molecule;

3) It stimulates the hydrolysis of thyroglobulin and the release of $L-T_4$ and $L-T_3$ from the thyroid into the blood.

Hypothalamic Control of TSH Secretion

The first evidence of the role of the hypothalamus in the regulation of TSH secretion was presented by Greer (1951), who produced bilateral symmetrical electrolytic lesions in the hypothalamus of rats. These animals failed to show the goitrogenic response to thiouracil feeding. However, the iodide-concentrating capacity of the thyroid was about normal.

Bogdanove and Halmi (1953) repeated Greer's work using propyl-thiouracil and noted impairment of the hyperplastic response of the thyroid but no interference of the thyroid/serum iodide concentration ratio.

Florsheim (1959) reported that rats with anterior hypothalamic lesions showed lower TSH levels after goitrogen feeding than control animals as measured by bioassays based on iodine discharge from the chick thyroids. The discharge of labeled hormone from the thyroid of lesioned and control rats was inhibited by equal doses of exogenous thyroid hormone. This was taken to indicate that the thyroid-pituitary feedback system controlling TSH release was independent of the hypothalamus.

Moll *et al* (1961) produced electrolytic lesions of the hypothalamic area between the fornix columns at the level of the paraventricular nuclei which inhibited thyroid function as judged by thyroid weight, uptake and release of I^{131} and response to methylthiouracil.

Averill *et al* (1961) observed a significant and permanent depression of thyroid secretion effective within 6 hours, following anterior ventral hypothalamic lesions in the rat.

Van Beugen and van der Werff ten Bosch (1961) studied the effect of lesions in the basal midline of the anterior hypothalamus on thyroid activity of rats exposed to temperatures of 24°C and 4°C. The index of thyroid function was the biological half-life (t½) of thyroidal-I^{131}. The t½ values were 7.5 days at 24°C and 4 days of 4°C in rats with hypothalamic lesions. This part of the hypothalamus does not seem essential for the response of the pituitarythyroid axis to a low environmental temperature.

Van der Werfften Bosch and Swanson (1963) compared the thyroid enlargement caused by 0.15 percent of propyl-thiouracil in the diet on normal and lesioned rats after 14 and 28 days. It was found that the lesions reduced the thyroid weight to about 75 percent of the intact rat on either diet. It was concluded that lesions caused a lowered steady state of the pituitary-thyroid feedback system.

De Jong and Moll (1965) made lesions in midline or bilaterally in the hypothalamus of rats. Single midline lesions which destroyed the area between the fornix bundles at the level of the paraventricular nuclei (PVN) resulted in a reduced rate of thyroidal-I^{131} release in rats at room temperature and blocked the thyroidal response at 4°C. Single midline lesions just inferior to the PVN caused inhibition of the response to cold but were less effective at room temperature. Lesions made anterior to the PVN caused partial blocking of the cold response but had no effect at room temperature. No effect was produced by midline lesions either superior or posterior to the PVN or bilaterally places lesions laterally to the PVN.

Panda and Turner (1967c) determined the changes in the TSH control

of the plasma and pituitary of mature female rats after placing lesions in the supraoptic area of the hypothalamus of normal and thyroidectomized animals kept at normal and low environmental temperatures.

The unilateral lesions after 8 and 48 hr caused a temporary decrease (not significant) in the percentage of circulating TSH in relation to the total pituitary TSH, followed by a restoration to normal. This observation suggests that the unilateral lesion temporarily reduced the total amount of TSH-releasing factor, but that the reduction was quickly compensated for by an increased secretion and release of TSH-releasing factor on the normal side.

When bilateral symmetrical lesions were placed in the hypothalamus, the percentage of circulating TSH was 0.41 of the pituitary content 8 hr after operation, followed by a slight rise at 48 hr. Thereafter there was a continuous decline up to 10 days. On the other hand, the pituitary content of TSH increased markedly to 99.50 mU /mg of wet gland after 8 hr, followed by a slight decrease to 93.90 mU /mg at 48 hr. Thereafter there was a gradual increase to 121.9 mU /mg at 10 days. These data suggest that the area of the hypothalamus in which the lesions were placed is directly involved with the release of TSH from the pituitary, and the releasing function was depressed by the lesions. These observations indicate that bilateral lesions depressed the plasma TSH level by about 65%. That they did not completely depress TSH discharge may be due in part to the lack of complete destruction of all areas of the hypothalamus secreting TSH-releasing factor. It is possible also that the 35% normal level of TSH represents the autonomous capability of the pituitary to discharge TSH, independent of hypothalamic control.

TSH secretion and release increases in animals exposed to cold. In thyroidectomized animals the circulating TSH increases markedly with a concomitant reduction in pituitary TSH; the combination of thyroidectomy and cold exposure presents a similar picture of increased TSH secretion (Panda and Turner, 1967b). From the present data, it was observed that animals having bilateral supraoptic lesions and exposed to a cold environment (4°C) showed reduced TSH content of blood with a concomitant increase in pituitary TSH as compared with their controls, but the difference in pituitary TSH levels was not statistically significant $(0.10 > P > 0.05)$. The circulating TSH in these animals was 0.73% of their pituitaries. Although this was markedly lower than that of two control groups (Table 1), it was higher than that of the animals having similar lesions and kept at 26°C (Table 2).

This picture reveals two aspects. First, the release of increased amounts of TSH from the pituitary is markedly influenced by the supraoptic region of the hypothalamus without which the concentration of TSH/ml of plasma in cold exposed experimental animals (0.96 mU/ml) is almost the same as that of the intact rats at 26°C; and, second, the pituitary maintains a limited autonomy to release more TSH in the cold environment, thus increasing the circulating level.

When thyroidectomy and bilateral symmetrical supraoptic lesions were

Group No.	No. of Animals	Experimental Conditions	Final Body Wt. (g) Mean	TSH/ml Plasma (mU) Mean±S.E.	Pituitary Wt. (wet) (mg) Mean±S.E.	TSH/Pituitary Gland (mU) Mean±S.E.	TSH/mg Pituitary (mU) Mean±S.E.
1	9	Control, unilateral sham lesions	235	0.94±0.10[1]	9.3±0.26	652.±5.80	69.35±5.80
2	7	8 hr after unilateral lesions	249	0.87±0.03[2]	9.7±0.24	678.03±19.23	69.90±1.50
3	4	48 hr after unilateral lesions	240	0.77±0.02[3]	9.6±0.22	781.40±62.30	81.40±6.90
4	7	120 hr after unilateral lesions	236	0.89±0.12	9.6±0.15	586.60±13.31	61.10±3.50
5	5	240 hr after unilateral lesions	247	0.92±0.08	9.5±0.42	670.70±37.46	70.60±6.40
6	9	Control, bilateral sham lesions	235	0.98±0.13[4]	9.4±0.25	680.10±57.50	72.35±5.10
7	7	8 hr after bilateral lesions	234	0.48±0.02[5]	9.7±0.42	965.20±74.80	99.50±3.90
8	6	48 hr after bilateral lesions	240	0.65±0.01[6]	9.8±0.18	915.30±33.14	93.90±7.50
9	5	120 hr after bilateral lesions	245	0.51±0.02	10.9±0.04	1269.80±28.24	116.50±5.60
10	5	240 hr after bilateral lesions	242	0.57±0.12	9.7±0.14	1182.40±35.99	121.90±3.80

S.E. = Standard error

TSH = Thyrotrophic hormone

All calculations made from mean of duplicate assays Student's t
test used for test of significance.

1 vs. 2 not significant; 2 vs. 3 0.20 P)0.10 not significant; 4 vs. 5
0.001)P highly significant; 4 vs. 6 0.001)P highly significant.

Table 2. Effect of Thyroidectomy and Bilateral Electrolytic Supraoptic Lesions in the
Hypothalamus of Rats Exposed to 26°C (78±1°F) and 4°C (40±1°F) on the TSH Content of Blood and Pituitary

Group No.	No. of Animals	Experimental Conditions	Final Body Wt. (g) Mean	TSH/ml Plasma (mU) Mean±S.E.	Pituitary Wt. (wet) (mg) Mean±S.E.	TSH/Pituitary Gland (mU) Mean±S.E.	TSH/mg Pituitary (mU) Mean±S.E.
1	6	Control, bilateral sham lesions 3 days, 26°C	235	0.98±0.13	9.40±0.25	680.09±53.63	72.35±5.10[1]
2	5	Controls, bilateral sham lesions, 3 days, 4°C	226	1.24±0.27	9.60±0.36	950.69±37.08	99.93±7.36[2]
3	5	Bilateral supraoptic lesions, 3 days, 4°C	223	0.96±0.10	9.60±0.12	1104.00±56.81	115.90±4.61[3]
4	5	Thyroidetomized, bilateral lesions 3 days, 26°C	231	0.59±0.03	9.08±0.36	718.04±43.51	79.80±7.20[4]
5	5	Thyroidectomized, bilateral lesions 3 days, 4°C	206	1.15±0.32	9.60±0.43	923.90±34.61	96.24±5.87[5]

S.E. = Standard error

TSH = Thyrotrophic hormone

All calculations made from mean of duplicate assays Student's t
test used for test of significance

1 vs. 2 0.100 P)0.050 not significant; 1 vs. 3 0.001)P highly significant; 1 vs 5 0.025)P)0.010 significant; 2 vs. 3 0.100 P)0.050 not significant; 4 vs. 5 0.200)P)0.100 not significant.

combined and the animals were kept at 26°C, there was a marked reduction in plasma TSH concentration and an increase in pituitary content, suggesting that increased release of TSH in thyroidectomized animals is controlled by the hypothalamus and that circulating thyroxine maintains its negative feedback through the hypothalamus. When such animals were exposed to cold (4°C), plasma concentration increased slightly, with a significant (0.025)P)0.010) increase in pituitary TSH content compared with intact rats at 26°C. Again, these data suggest some degree of autonomy of the pituitary in releasing its trophic hormones.

Effect of Thermal Lesions

Averill *et al.* (1961) placed thermal bilateral lesions in the anterior ventral hypothalamus of female rats. There followed a significant and permanent depression of thyroid function as measured by the rate of loss of I^{131}. This depression was effective less than 6 hr after the lesions had been made. Rats retained a significant depression of both 24 hr uptake of I^{131} and rate of release of incorporated I^{131} when measured 2, 4, and 8 weeks post-operatively. Rats with lesions required only 1.0 ug of L-thyroxine/100 g body weight/day to prevent thyroid secretion, compared to 3-4 ug daily.

Electrical Stimulation of Hypothalamus

Harris and Woods (1958) and Campball *et al,* (1961) reported a change in TSH release caused by hypothalamic stimulation of the rostral portion of the median eminence.

D'Angelo and Snyder (1963) reported that electrical stimulation of the anterior hypothalamus and rostral portions of the median eminence effected significant increases in serum TSH to levels which were 3 to 4 times greater than in sham-stimulated controls. These effects were not observed in animals with lesions.

D'Angelo *et al* (1964) simulated electrically the preoptic, suprachiasmatic and anterior hypothalamic area for 10 minutes daily for 4 to 9 days. There was a marked reduction in AP-TSH stores, increased plasma TSH concentration (4. 5x) and histological stimulation of the thyroid gland. Excitation of the posterior median eminence and arcuate nuclear regions of the tubular hypothalamus produced effects of similar magnitude. They suggest that the portions of the hypothalamus involved in the regulation of TSH and ACTH are overlapping and diffuse rather than discrete.

Thyroxine Feedback on Regulation of TSH Secretion

It is well established that the circulating level of thyroid hormones plays an important role in regulating the normal secretion of TSH through a feedback mechanism. It was formerly considered that this effect was upon the pituitary. With the discovery of the role of the hypothalamus and the secretion and release of a TSH-releasing factor (TRF), it is equally possible that the thyroid hormones may act upon the hypothalamus as well as the pituitary.

In rats, Yamada and Greer (1959) suggested that L-T_4 acted on the

Table 3. Plasma and Pituitary TSH Levels of Rats Under Different Experimental
Conditions Exposed to 26°C for 10 Days

Group No.	No. of Animals	Treatment	Final Body Wt. (g) Mean	Thyroid Weight (mg) Mean±S.E.	Pituitary Weight (mg) Mean±S.E.	TSH/ml Plasma (mU) Mean±S.E.	TSH/Pituitary Gland (mU) Mean±S.E.	TSH/mg Pituitary (mU) Mean±S.E.
1	6	Control; bilateral thyroidectomy	230	--	8.5±0.19	1.56±0.08[1]	418.03±11.00	49.60±4.26
2	6	Bilateral thyroidectomy; transplanted into brain	239	--	10.0±0.14	1.64±0.05[2]	387.87±22.00	38.78±3.74
3	5	Control; unilateral thyroidectomy	255	10.2±0.22	8.6±0.16	0.98±0.11[3]	454.10±24.09	52.28±4.67
4	8	Unilateral thyroidectomy; transplanted into brain	241	9.3±0.48	10.5±0.15	1.11±0.05[4]	468.85±27.88	44.65±2.86
5	5	Unilateral thyroidectomy; transplanted into muscle	265	7.5±0.59	8.1±0.08	0.88±0.06	538.34±12.04	66.17±4.67
6	4	Unilateral thyroidectomy; drilled at pituitary	231	9.1±0.08	7.7±0.26	0.89±0.06[5]	443.00±10.20	57.53±3.30
7	8	Unilateral thyroidectomy; transplanted into pituitary	216	6.3±0.41	11.2±0.86	0.62±0.06[6]	--	--
8	7	Unilateral thyroidectomy; transplanted into pituitary	218	--	10.1±0.69	0.74±0.06[7]	--	--

TSH = Thyrotrophin
S.E. = Standard error of the mean
All calculations were made from mean of duplicate assays.

Student's t test used for test of significance. 1 vs. 2 not significant;
3 vs. 4 not significant; 3 vs. 5 not significant; 5 vs. 6 (P⟨0.005) sig-
nificant; 1 vs. 7 (P⟨0.001) significant.

Table 4. Plasma and Pituitary TSH Levels of Rats under Different Experimental
Conditions Exposed to 4°C for 10 Days

Group No	No. of Animals	Treatment	Final Body Wt. (g) Mean	Thyroid Weight (mg) Mean±S.E.	Pituitary Weight (mg) Mean±S.E.	TSH/ml Plasma (mU) Mean±S.E.	TSH/Pituitary Gland (mU) Mean±S.E.	TSH/mg Pituitary (mU) Mean±S.E.
1	6	Control; bilateral thyroidectomy	232	--	9.10±0.22	2.95±0.25[1]	468.44±22.00	51.47±2.19
2	7	Bilateral thyroidectomy; transplanted into brain	207	--	10.40±0.29	1.02±0.10[2]	1510.54±29.80	146.45±4.66
3	4	Control; unilateral thyroidectomy	204	17.9±0.09	8.70±0.26	2.57±0.17[3]	743.32±20.00	85.44±17.52
4	6	Unilateral thyroidectomy; transplanted into brain	221	10.4±0.71	12.10±0.)8	0.90±0.05[4]	1257.96±13.42	103.89±4.39
5	5	Unilateral thyroidectomy; thyroid in muscle & drilled at pituitary	176	9.9±0.91	7.40±0.19	1.83±0.05[5]	708.00±48.24	95.53±6.44
6	5	Unilateral thyroidectomy; drilled at pituitary	222	13.3±1.28	7.40±0.25	2.41±0.22[6]	627.00±00.00	84.73±3.67
7	10	Unilateral thyroidectomy; transplanted into pituitary	222	15.9±0.53	15.3±0.50	2.28±0.18[7]	--	--
8	7	Bilateral thyroidectomy; transplanted into pituitary	209	--	12.9±0.89	2.79±0.18[8]	--	--

TSH = Thyrotrophic hormone
S.E. = Standard error of mean
All calculations made from mean of duplicate assays
Student's t test used for test of significance.

1 vs. 2 (P(0.001) significant; 3 vs. 4 (P(0.001) significant; 3 vs. 6 not
significant; 4 vs. 5 (P(0.001) significant; 6 vs. 7 not significant; 1 vs.
8 not significant.

pituitary and on the hypothalamus to prevent release of TSH. Kendall (1962) showed that L-T_4 injected into the rat pituitary blocked thyroidal-I^{131} release.

Panda and Turner (1968a) estimated the plasma and pituitary TSH levels following intrapituitary and intrahypothalamic thyroid autotransplants.

Totally thyroidectomized female rats bearing thyroid autotransplants in the pituitary had a significantly lower plasma TSH than that of controls at 26°C but not at 4°C. (Tables 3 and 4)

Thyroidectomized rats bearing thyroid autotransplants in the supraoptic area of the hypothalamus showed a significantly lower level of plasma TSH and a higher pituitary TSH at 4°C but not at 26°C.

Study with both unilaterally and bilaterally thyroidectomized rats bearing thyroid autotransplants either in the pituitary or in the hypothalamus revealed that L-T_4 feedback operates at the pituitary level in a normal environment but at the hypothalamus level via TRF in cold exposure.

Vale *et al* (1967) reported that when increasing amounts of L-T_4 are administered to rats given a constant dose of TRF, a dose is found to completely inhibit the TSH-releasing activity of that dose. Conversely, when increasing amounts of TRF are administered to rats pretreated with a constant dose of L-T_4, a dose of TRF is reached which will overcome the inhibitory effect of the L-T_4.

TSH-stimulating Hypothalamic Peptide (TRF)

Guillemin *et al* (1962) reported the purification of a hypothalamic extract fraction active in stimulating release of TSH in *in vivo* preparations.

Justisz *et al* (1963) showed that the TRF fraction was a peptide and produced a linear response as a function of the log of the dose injected. It was found to be inhibited by a large dose of L-T_4 and to have no TSH-like activity when injected into hypophysectomized animals.

Schreiber (1964) extracted a peptide type substance from the rat hypothalamus which stimulated TSH release both *in vitro* and *in vivo*. It was shown that this TRF was not a neurohypophyseal hormone, that it did not have TSH-like activity and that it could be blocked with either L-T_4 or L-T_3. He suggested that this factor caused both the synthesis and discharge of thyrotropin.

Factors Influencing the TSH of the Pituitary and Plasma of the Rat

Since the thyroid-stimulating hormone (TSH) secreted by the pituitary and its level in the circulating plasma plays an important role in regulating the thyroid hormone secretion rate (TSR), a series of studies has been conducted to investigate the factors influencing the amount of pituitary and plasma TSH. These studies were made possible by the development of assay methods for TSH.

D'Angelo (1958) determined the TSH of the pituitaries of 12 rats weighing a mean of 290 g. The mean TSH/mg was 70 mU U.S.P. units (range 60-90) and 875 mU/gland.

D'Angelo (1961) determined the TSH of the AP of rats 4 to 8 months

of age. The mean was 71 ± 17 mU/mg and 690 ± 40 U.S.P. mU/gland. In old rats, the TSH fell to less than 10 percent of these values. D'Angelo (1966) reported a gradual decline in AP-TSH in normal aging female rats. TSH of 15-month-old animals was about one-third that of the young adults and dropped to very low levels in 22- 24-month-old animals.

Effect of Advancing Age

It has been shown that the TSR of female and male rats gradually declines with advancing age. Since the secretion and discharge of TSH is involved in TSR, it was decided to determine the changes in the pituitary and blood content of TSH (Panda and Turner, 1967a).

Table 5 shows that the TSH content of the pituitary and plasma of female rats was low at weaning time and gradually increased until 80 or 95 days of age, then declined slightly (Panda and Turner, 1967a). To explain the decline in TSR associated with an increase in plasma TSH, it was suggested that

(1) the sensitivity of the thyroid gland to TSH declines with age,

(2) the t½ of TSH may decrease with increasing age, and

(3) the secretion of L-T_3 in the immature rat may be high and decline with age.

Effect of Thyroidectomy and Replacement Therapy on TSH.

The effect of thyroidectomy and replacement therapy with L-T_4 upon the TSH content of the mature female rat pituitary and plasma was determined by Panda and Turner (1967b). The rats were maintained at a temperature of $78°F$ ($26°C$).

The two groups of control rats maintained at $26°C$ for 10 and 20 days had the same plasma TSH levels, 0.94 mU/ml (Table 6). The pituitary TSI levels were 69.35 and 67.18 mU/mg, respectively. Based on a plasma volume of 3.79 ml/100 g body weight, the total circulating TSH was 8.6 mU, or 1.3 and 1.2 percent of the pituitary TSH.

After 10 days of thyroidectomy, the plasma TSH showed a significant increase of 229 percent, or 2.15 mU/ml and a slight further increase to 299 percent or 2.81 mU/ml after 20 days. The TSH/mg of the pituitary gland was reduced to 40.4 mU after 10 days and to 35.0 mU after 20 days. They represent decreases of 58 and 50 percent compared to the controls. The total circulating TSH increased to 19.4 and 24.4 mU/ml. This represents 4.8 and 6.6 percent of the pituitary TSH.

In three groups of rats thyroidectomized for 25 days, the effect of the daily injection of 2.5 ug of L-thyroxine (T-T_4)/100 g body weight for 1, 3 and 5 days was observed. The plasma TSH level was reduced to 1.59 ± 0.16 mU/ml after 1 day of L-T_4, to 1.48 ± 0.10 mU/ml after 3 days, and to 1.06 ± 0.16 mU/ml after 5 days. The pituitary TSH was 37.5 ± 2.95 mU/ml after 1 day, 38.57 ± 1.99 mU/mg after 3 days, and 77.95 ± 12.9 after 5 days. Thus in 5 days the relation of the plasma TSH to the pituitary gland returned to normal, 1.2 percent, by L-T_4 injections.

Table 5. TSH Content of Pituitary and Blood of Rats of Increasing Age

Group No.	No. of Animals	Age (days)	Mean Body Weight (g)	TSH/ml Plasma (mU) Mean±S.E.	Pituitary Wt. (wet) (mg) Mean±S.E.	TSH/Pituitary gland (mU) Mean±S.E.
1	10	24	43	0.316±0.04	2.81±0.160	127.2± 4.80
2	10	40	83	0.463±0.22	5.10±0.080	300.9± 7.57
3	10	50	150	0.573±0.04	6.80±0.220	478.2±24.10
4	10	65	171	0.797±0.01	7.81±0.120	693.4± 7.82
5	10	80	197	0.912±0.01	9.00±0.003	834.0±17.90
6	8	95	236	1.178±0.01	9.75±0.610	637.1±53.92
7	9	110	245	1.046±0.10	10.60±0.370	673.4±34.80

Effect of Low Environmental Temperature (40°F, 4.4°C) and Thyroidectomy On TSH.

When rats previously held at 26°C were exposed to 4.4°C for 10 and 20 days, the plasma TSH increased to 2.15 mU/ml and 2.69 mU/ml, respectively (Panda and Turner, 1967b). These increases are quite comparable to the effect of thyroidectomy (Tables 6 and 7).

The pituitary TSH also increased to 108.04 and to 240.9 mU/mg, respectively. The circulating plasma TSH was 1.7 and 1.0 percent of the pituitary TSH.

Under the combined effects of thyroidectomy and 4.4°C temperature for 10 and 20 days, the plasma TSH increased to 4.37 mU/ml after 10 days, then declined to 2.40 mU/ml after 20 days. The corresponding pituitary TSH declined to 43.49 mU/mg and to 37.58 mU/mg. The total circulating plasma TSH increased to 7.3 percent of the pituitary TSH at 10 days, then declined to 4.8 percent after 20 days.

When a group of thyroidectomized rats held at 4.4°C for 20 days was returned to 26°C for 4 days, the plasma TSH declined to 1.44 mU/ml, but the pituitary TSH remained low, 34.90 mU/ml.

Diurnal Variation of Plasma and Pituitary TSH in Adult Female Rats.

The diurnal variation in the plasma and pituitary TSH of adult female rats exposed to 14 hours of light and 10 hours of darkness was studied by Singh et al (1967).

The mean plasma TSH content of the rats showed a gradual rise from 3 A.M. to 3 P.M. followed by a gradual reduction. The total diurnal increase in TSH was 40.9 percent. The highest mean TSH/pituitary was observed at 3 A.M., then declined gradually until 3 P.M., a decrease of 9.8 percent. The change which followed was irregularly higher. From 3 A.M. to 3 P.M., there was an inverse relation between pituitary and plasma TSH, thus as the plasma TSH increased diurnally, the pituitary TSH declined (Table 8).

Effect of Goitrogens on TSH of the Rat

D'Angelo (1961) reported that rats on propyl-thiouracil showed decreased TSH levels with depletion almost complete after one month. The hormone level was less than 5 percent of normal. After goitrogen withdrawal, the TSH level remained low for 3 days, but by 7 days TSH stores had increased 500 percent of normal and were still above normal on day 20.

Bakke and Lawrence (1964) gave 0.05 percent propyl-thiouracil in the drinking water. Since the rats drank about 30 ml/day, they received about 15 mg/day. This treatment produced a progressive and parallel increase in serum TSH over a year period. Pituitary TSH declined markedly for 4 weeks, returned to control levels at 10 weeks, then rose until a level 5 times the control was achieved after 32 weeks.

Effect of Castration on TSH

Van Rees et al (1965) noted decreases in both AP and serum TSH lev-

Table 6. TSH Content of Plasma and Pituitary Gland of Intact and Thyroidectomized Rats
at 26°C

Group No.	No. of Animals	Rats Kept at 26°C for Different Intervals	Final Body Wt. (g) Mean	TSH/ml Plasma (mU) Mean±S.E.	Pituitary Wt. (wet) (mg) Mean±S.E.	TSH/Pituitary Gland (mU) Mean±S.E.	TSH/mg Pituitary (wet) (mU) Mean±S.E.
1	9	Intact - 10 days	235	0.94±0.10	9.3±0.26	652.0±62.5	69.35±5.80
2	8	Thyroidectomized - 10 days	237	2.15±0.27	10.9±0.71	414.7±21.1	40.40±5.53
3	9	Intact - 20 days	240	0.94±0.09	10.3±1.20	692.0±63.0	67.18±5.80
4	9	Thyroidectomized - 20 days	229	2.81±0.24	10.53±0.74	367.6±30.8	35.00±3.10
5	8	Thyroidectomized - after 25 days given 2.5 ug L-T_4/100 g b.w. Blood and pituitary gland after 24 hours	236	1.59±0.16	9.70±0.37	364.0±19.3	37.50±2.95
6.	5	Thyroidectomized - after 25 days given 2.5 ug L-T_4/100 g b.w. for 3 days	232	1.48±0.10	9.80±0.38	378.0±35.4	38.57±1.99
7	6	Thyroidectomized - after 25 days given 2.5 ug L-T_4/100 g b.w. for 5 days	235	1.06±0.16	9.80±0.08	764.0±116.9	77.95±12.90

TSH = Thyrotrophic hormone
S.E. = Standard error
All calculations were made from duplicate assays.

Table 7. TSH Content of Plasma and Pituitary Gland of Intact and Thyroidectomized Rats
at 4.4°C

Group No.	No. of Animals	Rats kept at 4.4°C for different intervals	Final Body Wt. (g) Mean	TSH/ml Plasma (mU) Mean±S.E.	Pituitary Wt. (wet) (mg) Mean±S.E.	TSH/Pitui- tary Gland (mU) Mean±S.E.	TSH/mg Pituitary (wet) (mU) Mean±S.E.
1	8	Intact - 10 days	223	2.15±0.17	9.70±0.91	1048.0±86.63	108.04±15.96
2	9	Thyroidectomized - 10 days	217	4.37±0.66	11.38±0.47	495.0±48.00	43.49± 3.83
3	9	Intact - 20 days	229	2.69±0.10	9.54±0.20	2270.0±44.66	240.90±20.30
4	9	Thyroidectomized - 20 days	215	2.40±0.40	10.80±0.24	405.9±47.00	37.58± 4.20
5	6	Thyroidectomized - 20 days; 26°C - 4 days	215	1.44±0.29	9.50±0.78	327.6±15.50	34.90± 7.59

TSH = Thyrotrophic hormone
S.E. = Standard error
All calculations were made from duplicate assays.

els of castrate male rats. Testosterone propionate (TP) increased serum TSH of castrate males, but on the AP the TP prevented the decrease induced by castration.

Table 8. Diurnal Variation in TSH Content of the Pituitary
and Blood of the Female Rat

Group No.	Time	No. of Animals	Mean Body Wt. (g)	TSH/ml Plasma, mean ± SE (mU)	Pituitary Wt. mean ± SE (mg)	TSH/Pituitary Gland, mean ± SE (mU)
1	7 A.M.	5	241	.754±0.040	9.4±0.63	655.7±34.9
2	11 A.M.	5	244	.885±0.084	9.7±0.69	623.0±58.7
3	3 P.M.	5	232	1.016±0.061	7.2±1.22	603.3±48.2
4	7 P.M.	5	250	.951±0.061	10.3±1.02	655.7±42.3
5	11 P.M.	5	249	.787±0.069	11.5±0.99	616.3±16.05
6	3 A.M.	5	255	.721±0.066	9.4±1.71	668.8±8.0
7	7 A.M.	5	242	.820±0.067	8.3±0.98	629.5±16.1

Anatomy of the Thyroid Gland of the Rat

The thyroid gland of the rat comprises a right and left lobe connected by an isthmus which partly surrounds the upper end of the trachea (Fig. 1). The isthmus crosses the front of the trachea. Small isolated masses of thyroid tissue may be present in the neighborhood of the gland or above the isthmus. Because these tissues may not be removed by thyroidectomy, the effects of thyroidectomy may not always be fully demonstrated.

The thyroid is a typical type of gland with spherical *follicles* (alveoli or acini) with storage space in the central *lumina* containing *colloid* or *thyroglobulin,* the thyroid hormone as it is secreted.

The inner lining of the follicles consists of a single layer of epithelial cells which may be columnar, cuboidal or flat, depending upon whether small, moderate, or large amounts of colloid are present.

The arteries of the thyroid gland branch over the surface and throughout the gland and form a capillary network around each follicle. The thyroid gland normally receives a rich blood supply which is regulated by the functional activity of the gland as stimulated by TSH.

Development of the Fetal Rat Thyroid Gland

In the fetal rat thyroid gland, typical follicular structure appears about the 18th day (Carpenter and Randon-Tardetti, 1957). Radioactive iodine (I^{131}) was injected into pregnant rats by Gorbman and Evans (1943). Thyroid glands of 19-day fetuses were the first to show iodine accumulation, indicating the beginning of functional activity. Earliest activity was noted in peripheral follicles.

At this same time, Geloso (1956) and Sfez and Nataf (1960) reported the presence of circulating thyroxine. It is interesting to note that at this stage of development a functioning thyroid-pituitary feedback system appears (Hwang and Wells, 1959). However, Florsheim *et al* (1966) are of the
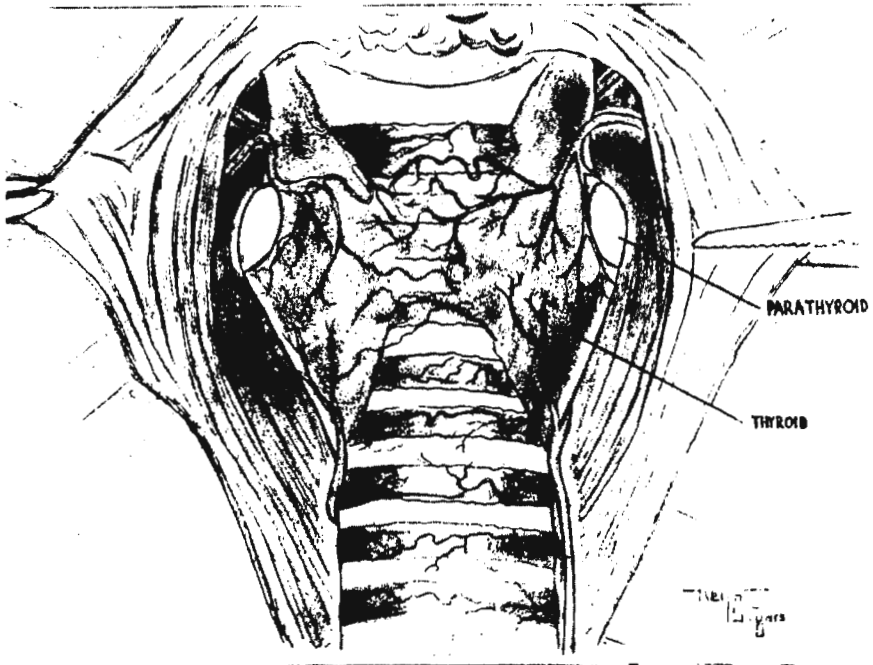
FIGURE 1. Location of the thyroid and parathyroid glands of the rat.

Table 9. Factors Influencing Thyroid Gland Weight in Rats
(Wistar Strain)

Sex and Condition	No. of Animals	Mean Body Wt. (gm)	Mean Thyroid Wt. (mg)	Thyroid Wt. 100 g b.w. (mg)
Males: growing	21	82.2	7.1	8.6
	21	126.7	11.1	8.8
	17	173.0	15.8	9.1
	8	216.1	16.8	7.8
	8	274.4	22.9	8.3
Females: growing -	17	81.5	6.9	8.5
	18	122.7	13.8	11.2
	11	178.3	15.0	8.4
	20	223.0	19.7	8.8
	16	259.9	17.6	6.8
pregnant -	10	207.0	16.5	8.0
lactating -	15	203.1	14.9	7.3
Males & females: suckling, 16 days old	119	17.1	3.1	18.1

opinion that hypothalamic influences upon the thyroid-pituitary axis appear to be of little importance in prenatal life.

That the fetal pituitary is beginning to secrete TSH has been shown by the production of a goiter *in utero* in response to goitrogens fed to the mother (Jost, 1957) and it was shown by Knobil and Josimovich (1959) that this occurs even in hypophysectomized mothers.

Function Activity of the Thyroid Gland of Newborn Rat

Florsheim *et al* (1966) reported that I[131] uptake showed a marked peak immediately after birth, then declined by the 7th day and remained low to the 22nd day. Monoiodotyrosine in the glands dropped sharply from a level of 29.9 ± 2.0 percent two days before birth to 23.1 ± 1.9 percent on the first day of life but returned within 2 days to a level of 31.7 ± 1.0 percent, which was maintained. At the same time, diiodotyrosine levels appeared to rise slightly toward a plateau which was reached by the 3rd day. Triiodothyronine dropped at the time of birth from 4.5 ± 0.6 percent to a plateau value of 1.3 ± 0.2 percent on the 4th day. Thyroxine maintained a fairly stable level of 10 to 15 percent from birth to day 22.

Mitochondrial-glycerophosphate dehydrogenase activity in the liver declined to a minimum at 3 days before birth, then increased steeply for 5 days, followed by a more gradual rise until the 19th day.

## THE THYROID GLAND WEIGHT OF RATS

Growing Rats

Since the thyroid hormones play an important role in growth as well as the regulation of energy metabolism and heat production, it would be expected that thyroid weight during growth might be related to the body weight.It has been shown in a wide range of animals with varying body weights that energy metabolism varies with the 0.73 power of body weight. This means that for each increasing unit of body weight, energy metabolism increases about ¾ as fast. This follows the concept that as body size increases, the surface area per unit height decreases and the energy required to maintain constant body temperature decreases.

In a study by Monroe and Turner (1946) of growing male and female rats of the Wistar strain, it was shown that the increase in body and thyroid weight of male rats could be represented by the equation: $y = 0.136X^{0.90}$, where y = thyroid weight and X = body weight. The comparable equation for females was: $y = 0.230X^{0.87}$. Since the exponents of the males and females are approximately 0.90, it appears that the rat thyroid gland increases about 90 percent as rapidly as body weight (Table 9 and Figure 2).

FACTORS INFLUENCING THYROID GLAND WEIGHT

In connection with studies on the effect of certain treatments on TSR, data on the effect of the treatment on thyroid gland weight was reported. The rats were of the Sprague-Dawley-Rolfsmeyer strain.

FIGURE 2. Relation of thyroid weight to body weight in growing rats.
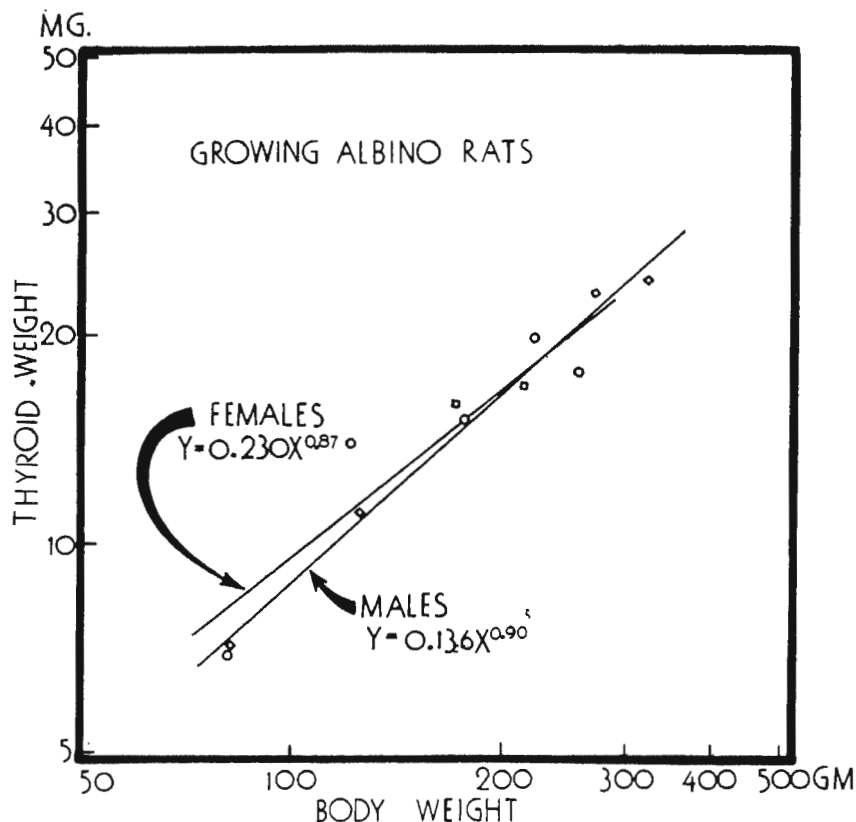
Estrogen

Grosvenor and Turner (1959) injected estradiol benzoate (EB) at levels of 1.0, 3.6, 15.0, and 50.0 ug/day for 5 days into a group of 18 female rats weighing a mean of 254 g. The weight of the thyroids of the controls was 4.68 mg/100 g body weight. The increasing levels of EB influenced thyroid weight as follows: 4.50 mg, 4.61 mg, 5.55 mg, and 5.93 mg. Injection of 1.0 or 3.6 ug/day did not affect thyroid weight but significant increases of 18.6 and 26.7 percent occurred following EB levels of 15 and 50 ug for 5 days.

Testosterone Propionate

The thyroid gland weights of 20 normal mature male rats weighing 351 g was observed by Kumaresan and Turner (1967a) to be 18.6 mg. After the injection of 2 mg testosterone propionate (TP), the thyroid glands of 21 rats weighing 330 g was 24.9 mg. After castration, the thyroid glands of 18 rats weighing 321 g declined to 16.6 g and when 17 such rats weighing 309 g were injected with 2 mg TP daily, the thyroid weight increased to 19.2 mg.

Neonatal Administration of Testosterone Propionate

Kumaresan and Turner (1966b) injected 2 mg of testosterone propionate (TP) into female rats when 2-days old. This treatment resulted in an anovulatory sterile condition associated with a condition of constant estrus. The female rats were sacrificed at 100 days of age, at which time the controls weighed 197 g, with thyroids weighing 10.8 mg, or 5.5 mg/100 g body weight. The TP injected animals weighed 238 g with thyroids weighing 14.4 mg, or 6.1 mg/100 g body weight.

A second group was ovariectomized at 110 days and sacrificed at 120 days. The mean body weight of this group of 12 animals was 252 g with thyroids weighing 14.4 mg or 5.7 mg/100 g body weight. The body weight of TP injected controls sacrificed at 120 days was 254 g, the thyroids 16.02 mg, and 6.3 mg/100 g body weight.

The thyroid glands were slightly, but not significantly, increased in intact and ovariectomized animals and compared with controls on a body weight basis.

Effect of Light and Darkness on Thyroid Gland Weight.

Singh and Turner (1969a) exposed groups of female rats to continuous light and darkness for 56 days. The control group of 25 rats weighing 226 g had thyroids weighing a mean of 10.4 ± 0.24 mg or 4.59 ± 0.11 per 100 g body weight. The group of 15 rats maintained in total darkness, weighing 227 g, had thyroid glands weighing a mean of 10.2 ± 0.69 mg, or 4.49 $g\pm0.12$ mg per 100 g body weight. The group of 22 rats maintained in continuous light weighed 205 g, had thyroid glands weighing a mean of 10.3 ± 0.28 mg, or 5.0 ± 0.43 mg per 100 g body weight. It was concluded that continuous light or darkness had no effect upon thyroid weight.

Effect of Methimazole on Thyroid Gland Weight

The goitrogen, methimazole, causes enlargement of the thyroid gland due to its effect on reducing iodine uptake and thus preventing thyroid hormone secretion. When methimazole was injected daily into female rats at a level of 400 ug/100 g body weight for 30 days, the control group of 18 rats with a mean body weight of 215 g had a mean thyroid weight of 10.96 mg, or 5.10 mg/100 g body weight. The goitrogen increased the thyroid weight to 21.78 mg, or 9.43 mg/100 g body weight, an increase of 98.72 percent. After a withdrawal period of 7 days, the thyroid weight was reduced to 16.73 mg, or 6.95 mg/100 g body weight or a decrease of 23.19 percent (Hendrich and Turner, 1965).

Histological study of thyroid glands after goitrogen treatment indicates that there is a depletion of the gland follicles of all stored colloid but greatly increased height of the follicular epithelium. To determine whether the doubling of the gland weight noted above is primarily due to an increase in cell multiplication or to an increase in cell size, Panda and Turner (1966b) determined the DNA content of the rat thyroid gland. The mean thyroid gland weight of a control group weighing 225 g was 9.73 mg or 4.32 mg/100 g body weight. After 20 days of the goitrogen, the thyroid glands of a group of 14 rats increased to 16.04 mg, or 6.94 mg/100 g body weight

an increase of 64.85 percent and after 30 days a group of 13 rats showed an increase in thyroid weight to 20.53 mg, or 9.08 mg/100 g body weight, an increase of 110. percent.

From these data it was calculated that the increase in thyroid gland weight due to the goitrogen for 30 days represented a 54 percent increase in cell growth (multiplication) and a 46 percent increase in cell size.

Effect of Melatonin on Thyroid Gland Weight

Recently it was discovered that the pineal gland secretes melatonin, which has been reported to play a role in the reproduction of seasonal breeding animals. It was shown by Narang et al. (1967) that melatonin depressed TSR of immature rats.

The effect of 100 ug of melatonin/day on the thyroid glands of immature female rats was determined by Panda and Turner (1968b).

The thyroid weights of the melatonin injected animals of each age group are seen to be higher than those of the controls. It was suggested that melatonin acts as a goitrogen and directly inhibits thyroid hormone synthesis or release (Table 10).

Table 10. Effect of Melatonin on Thyroid Gland Weight of
Immature Female Rats

No. of Animals	Age (days)	Body Wt. (g)	Thyroid Wt. (mg)	Thyroid Wt./ 100 g b.w. (mg)	Body Wt. (g)	Thyroid Wt. (mg)	Thyroid Wt./ 100 g b.w. (mg)
		Controls (Saline)			Melatonin (100 ug/day)		
10	35	92	6.57	7.14	92	7.22	7.85
10	45	133	9.56	7.19	131	11.63	8.88
10	55	144	9.78	6.79	156	12.64	8.10
10	65	160	11.16	6.97	180	13.08	7.27

Biochemistry of Thyroid Hormone Secretion

The thyroid gland has the capacity to take up the circulating iodine to a very great extent. The epithelial cells take up iodine and secrete thyroglobulin into the lumen of the follicle. The critical amino acid in thyroglobulin is L-tyrosine, which has the ability of exchanging hydrogen for iodine. Thus the tyrosine is converted to L, 3-monoiodotyrosine and to 3-5-diiodotyrosine, still an integral part of the protein molecule (thyroglobulin).

In the thyroid follicle, two molecules of diiodotyrosine are oxidatively coupled with the elimination of one alanine side chain to form L-thyroxine $(L-T_4)$. In addition, one molecule of monoiodotyrosine and one molecule of diiodotyrosine are oxidatively coupled to form triiodothyronine $(L-T_3)$. These two substances are still part of the thyroglobulin molecule. When the thyroid gland is desiccated for clinical use, the two active thyroid hormones are still an integral part of the thyroid colloid or thyroglobulin.

Before these hormones are secreted into the blood, the thyroglobulin is hydrolyzed to the constituent amino acids of the protein and L-T$_3$ and L-T$_4$ pass from the thyroid follicle into the blood.

To determine the daily rate at which the thyroid hormones leave the thyroid gland and enter the blood is the problem. The solution of this problem is of great importance to determine the many factors which influence the secretion rate. Over recent years, several methods have been suggested, and will be described.

METHODS OF DETERMINING THYROID
HORMONE SECRETION RATE

Goitrogen Method

The discovery of certain chemical substances, now called goitrogens, which prevented the formation of the thyroid hormones and caused increased size of the thyroid glands due to the increased secretion of TSH, formed the basis for the early determination of the rate of thyroid hormone secretion. Thus, Dempsey and Astwood (1943) suggested that the thyroid hormone secretion rate could be determined by the administration of a goitrogen to block the endogenous secretion of the thyroid hormones and by simultaneously administering graded doses of thyroxine. By removing the thyroid glands at the end of the experimental period and by plotting their mean weight against the dosages of thyroxine administered, the thyroid hormone secretion rate would be considered the amount of thyroxine required for the maintenance of the thyroid glands at a normal level.

The general concept of thyroid gland regulation and size was based on the balance between the rate of secretion of TSH and the secretion of thyroid hormones.

It was reported that 5.2 ug of D, L-thyroxine were required daily to maintain a thyroid of normal weight in young male rats of the Long-Evans and Wistar strains at 25°C (77°F). At a temperature of 1°C (33.8°F), the thyroxine was increased to 9.5 ug/day. The body weights of the animals were not indicated (Dempsey and Astwood, 1943).

This method was widely used for the estimation of thyroid hormone secretion in terms of thyroxine.

The goitrogen method was of great value in the estimation of the thyroxine secretion rate of groups of animals and of environmental conditions. However, it required the sacrifice of the animals and it did not permit the estimation of the secretion rate of individual animals or of repeated estimations in the same animals.

This method was used extensively in the early study of the thyroid hormone secretion rate at this Station in fowls and dairy goats by Schultze and Turner (1945), in rats by Monroe and Turner (1946) and in the mouse by Hurst and Turner (1948).

Radioactive I¹³¹ Method

When I¹³¹ became available, a method of estimating the thyroid secretion rate (TSR) was gradually evolved in this laboratory.

In some respects, the method is based upon the same principle as the

goitrogen method, namely, the interplay between the circulating thyroid hormones and the secretion of TSH. When increasing amounts of thyroxine are administered to a normal animal, the release of TSH is reduced correspondingly and the secretion of thyroxine is reduced. When the amount of exogenous thyroxine in the circulation equals the amount of thyroxine being secreted, the release of TSH and of thyroid hormones is blocked and the animal becomes dependent upon the exogenous supply.

By the use of I^{131}, the uptake of iodine by the thyroid gland can be determined and correspondingly, the release rate of the thyroidal I^{131} can be observed by repeated counts of the I^{131} still remaining in the thyroid gland.

When graded amounts of thyroxine or of triiodothyronine are administered to such animals, the exogenous hormone gradually blocks the release of TSH and in turn gradually depresses the release of thyroidal I^{131}. When the amount of thyroxine administered totally blocks the release of thyroidal I^{131}, this amount of thyroxine is believed to be the equivalent of the hormone secreted by the thyroid gland and is said to be the *thyroid hormone secretion rate* (TSR).

Since the release rate of thyroidal I^{131} is based upon repeated counts of radioactivity of the thyroid glands, a false estimate of the release rate results because the thyroxine I^{131} and triiodothyronine I^{131} which are released from the thyroid gland are gradually metabolized in the animal body to thyronine and free I^{131}. The free I^{131} returned to the blood may then be taken up again by the thyroid gland. This iodine is called *reutilized* or *recycled*. Since it cannot be distinguished from the I^{131} originally taken up by the thyroid gland, it causes the apparent thyroid release rate to be slower by the extent of I^{131} recycling.

To prevent the uptake of I^{131} resulting from the metabolism of thyroidal I^{131}, it is possible to administer a goitrogen. Then, when thyroidal I^{131} is metabolized, the returning I^{131} is not taken up by the thyroid gland, and the release rate of thyroidal I^{131} represents the true rate of release of the original I^{131} taken up.

It is highly desirable to block the recycling of I^{131} by the use of a goitrogen and thus observe the true rate of release of thyroidal I^{131}. This is due to the fact that the slope of the curve of the true release rate is greater. The end point of the estimation of thyroxine secretion rate is the smallest amount of thyroxine which will block thyroidal I^{131} release. The end point is more apparent when the slope of the curve is greater. When the release rate curve is flat, there is a tendency to overestimate the TSR because the approach to a plateau or complete blockage of thyroidal I^{131} release is more difficult to estimate.

Goitrogens. In the earlier studies in our Laboratory, two goitrogens were used, thiouracil and 6-methyl-thiouracil. In the studies on rats reported here, the compound 1-methyl-2-mercaptomidazole (called methimazole or tapazole) has been used. The term goitrogens has been applied to these compounds since they block thyroxine synthesis in the gland. With reduced circulating thyroxine, the secretion of TSH is increased, which in turn causes an enlargement of the thyroid gland (a goiter).

With the cessation of L-T₄ secretion, the use of I by the gland is greatly reduced and the uptake or trapping of I^{131} is prevented.

In the rat, the daily injection of 400 ug/100 g body weight of methimazole was shown by Hendrich and Turner (1965) to reduce the I^{131} uptake from 28.08 percent to 2.43 percent after 14 days and to 1.36 percent after 24 days of treatment. Feed consumption was reduced from 6 to 10 percent by this treatment during a period of 30 days.

The rapid metabolism of methimazole is indicated by an I^{131} uptake of 8.93 percent 4 days after withdrawal and 23.97 percent after 7 days.

Development of the Extrapolation Method

In the early study of Wolff (1951) using I^{131} in the rat, the release rate of thyroidal I^{131} was measured in normal and propylthiouracil-treated rats. These data showed the value of a goitrogen as the t½ was reduced from 3.3 to 1.6 days. It was stated that the decrease in I^{131} in the thyroid may therefore be considered to reflect secretion of I^{131}-labeled thyroxine (to this would now be added I^{131}-labeled triiodothyronine). The effect of thyroxine on the release of thyroidal I^{131} was also demonstrated. The injection of 15 ug D,L-thyroxine as early as 24 hours reduced the release of I^{131} from 40 to 3 percent.

Perry (1951) showed that graded levels of thyroxine progressively reduced the thyroidal I^{131} release and proposed it as a rapid and simple procedure for the bioassay of thyroidal preparations.

Reineke and Singh (1955) showed that both individuals and groups of rats showed progressive decline in thyroidal I^{131} release measured as percentage of previous count or as a percentage of the initial thyroidal count by progressively increasing doses of thyroxine. By plotting such data it is possible to extrapolate to 100 percent of the previous count, i.e., complete blockage of thyroidal I^{131} release to estimate the thyroidal hormone secretion rate. Their estimates by this technique were quite comparable to estimates of the same strain of rats by the goitrogen technique.

Pipes and Turner (1956) reported on an extensive study of the effect of thyroxine function in a number of species in which concurrent studies were conducted on the effect of graded levels of thyroxine on thyroid function as shown by I^{131} blood changes and by *in vivo* measurements of the thyroid gland. It was shown that as the thyroxine dosage was increased, the blood I^{131} decreased and the thyroidal I^{131} release rate gradually decreased. The two methods gave similar results in the estimation of thyroid hormone secretion rate.

A "Direct-output" Method.

Reineke (1964) suggested a method of determining TSR by measuring the thyroidal-I^{131} output rate by four daily counts. The thyroids were then removed, analyzed for total I^{127}, and the TSR estimated as the product of daily fractional output rate X thyroidal iodine content. The estimate by this method was low, 1.08±0.13 as compared to the previous method of 1.88±0.11 ug L-T₄/100 g body weight. This difference may be due in part to the fact that the L-T₃ was not measured by this method.

Later, Reineke and Lorscheider (1967) presented their method in more detail and suggested a correction for the secretion of L-T$_3$. (They suggested that L-T$_3$ was 4.6 times as active as L-T$_4$.) They reported that in rats receiving an adequate iodine supply, TSR values determined by the substitution method and the "direct-output" method were similar. However, on iodine deficient diets, the direct-output method was superior. In a study of low iodine diets in this laboratory, no change in TSR was noted.

FACTORS INFLUENCING THYROID HORMONE SECRETION RATE IN THE RAT

Due to the great effect of the thyroid hormones upon all phases of the metabolism of mammals, it seemed of great importance to study all the possible factors influencing the thyroid hormone secretion rate. Extensive studies have been conducted using the rat as the experimental animal and the thyroxine replacement method for the estimation of the secretion rate (TSR). By this method, repeated estimations of the TSR of the same animals may be made. Estimation of the TSR of animals may be made prior to the experimental treatment so that each animal serves as its own control.

Early in our own studies, the great diversity in the TSR of individual animals and even in groups of rats of the same strain was observed. This diversity in TSR is believed to be caused by genetic or inherited differences in TSR. In the studies to be reported, it is hoped that the use of large groups of rats and further by the use of rats as their own controls will minimize the genetic differences.

It should be pointed out that studies on the mode of inheritance of TSR would contribute greatly to our knowledge of the ability of breeding animals of optimal TSR.

This would be a very complicated study. It would involve not only the end result, namely, the TSR, but the role of the pituitary in secreting TSH and in the hypothalamus in secreting TRF. It is possible, also, that the rates of secretion of other hormones may influence TSR, as indicated by the studies to be reported concerning the effect of various hormones on TSR.

The factors influencing TSR, other than genetic, may be divided into several categories. It is of great interest to determine the effect of physiological processes such as growth, maturity and senescence, and the effects of pregnancy, lactation and involution and related processes.

Second, the effect of the environment was considered. This included the environmental temperature (heat and cold), the effect of nutrition including underfeeding, the role of protein and iodine and of various vitamins.

Third, the effect of the removal of various endocrine glands and replacement therapy; and also the effect of various hormones.

Finally, the effect of various factors which cannot be classified in the above groupings.

Environmental Factors Influencing TSR

The rats included in these studies were maintained at a uniform temperature of $78 \pm 1°F$ (26°C), unless indicated. The room was artificially illu-

Table 11. Effect of Age on TSR of Female and Male Rats

No. of Animals	Age (days)	Mean Body Weight (g)	Mean TSR (ug L-T₄ 100 g b.w.)	Reduction with Age from Previous Month %
		Females (Narang & Turner, 1966)		
27	25	58	1.52	
	55	166	1.22	19.7
	85	203	1.10	9.8
	115	223	0.88	20.0
				42.0%
		Females (Kumaresan & Turner, 1967b)		
29	30	117	1.47	
	60	193	1.55	
	90	223	1.24	
	120	242	1.12	
	150	258	1.12	
	180	258	1.16	
	210	269	0.92	
	240	271	0.87	
				41.0%
		Males (Kumaresan & Turner, 1967b)		
36	30	151	1.40	
	60	284	1.22	
	90	381	1.15	
	120	430	1.03	
				26.0%

minated during normal daylight hours during most of the experiment. Recently the lighting system was changed to provide 14 hours of light and 10 hours of darkness.

The animals were fed Purina Lab Chow with an energy value of 4.4 calories per gram and 23.4 percent protein. It was reported to contain 1.18 ppm of iodine. The feed and water were provided *ad libitum*.

In the studies on the effect of low environmental temperature on TSR, the rats were maintained in a room held at a temperature of 40°F (4.5°C).

Effect of Advancing Age on TSR

The effect of advancing age on the TSR of the male and female rat is of considerable interest in experiments using animals of varying ages. If TSR changes with age, then animals of the same age must be compared. Thyroid function has been reported to decline with advancing age (Narang and Turner, 1966) in a number of domestic and experimental animals.

In this laboratory, the TSR of the same group of 27 females was determined starting on day 25 and repeated at 30-day intervals up to the time of sexual maturity (115 days). During this period the mean TSR declined from 1.52 ug/100 g body weight to 0.88 ug, a decline of 42 percent (Narang and Turner, 1966), shown in Table 11.

In a second group of 29 females, the TSR was started at 30 days and continued at 30-day intervals until 240 days. In this group, the TSR increased from 1.47 ug at 30 days to 1.55 ug at 60 days, then declined to 1.12 ug at 120 days, a decline of 28 percent, and to 0.87 ug at 240 days, a total decline of 41 percent (Kumaresan and Turner, 1967b), as in Table 11.

Singh and Turner (1969a) determined the TSR of 62 female rats at 30 days of age as 1.24 ug L-T$_4$/100 g body weight. When 56 days of age, the mean TSR of 25 rats was reduced to 1.11 ug and 56 days later when 112 days of age, the mean TSR had declined to 0.81 ug/100 g body weight, a total decline of 34.68 percent.

Effect of Senescence on TSR

Grad and Hoffman (1955) determined the TSR in rats by the goiter prevention method, reporting that the TSR was less in senescent rats than in younger mature animals. In a later study, Wilansky *et al* (1957) compared the functional status of the thyroid gland in 4-to 5-month old with 24- to 25-month old female rats. The actual thyroid gland weight of the younger rats weighing 186 g was 18.27 mg, while the older rats weighed 267 g and had thyroid glands weighing 23.43 mg. However, on the basis of the thyroid gland weight to 100 g body weight⅔, the glands were of equal size.

The 24 and 40 hour uptake of I^{131} and the biological decay of thyroidal-I^{131} were significantly diminished in the older group, the biological half life for the older animals was 4.0 days and for the young, 3.1 days. The TSR of the older animals was estimated as 1.75 ug L-T$_4$/100 g body weight⅔, and for the younger animals, 2.25 ug.

TSR of Mature Female Rats

In the study of TSR of female rats, groups of normal control animals were used. A summary of these observations indicates the variation in TSR of groups of rats (Table 12).

In some experiments, various carrier solutions have been administered to compare with the hormones which were administered to the experimental groups (Table 13).

The Normal TSR of Growing, Pregnant, and Lactating Rats

In connection with studies concerning the effect of various factors upon TSR, it is important to understand the effect of changes with age, of pregnancy, lactation, involution and old age.

Effect of Lactation upon TSR

The average TSR of a group of 20 non-lactating rats was 1.3 ug/100 g body weight, with a range of 0.5 to 2.5 ug. The mean TSR of a group of 16 lactating rats of similar age and weight was 2.2 ug/100 g body weight, with a range of 0.5 to 3.0 ug. This is an increase of about 70 percent in TSR. The estimation of TSR was begun on day 4 of lactation and was complete on days 8 to 14 (Grosvenor and Turner, 1958).

A group of 10 lactating rats weighing 280 g had a mean TSR of 1.76 ug/100 g body weight when estimated during the first 14 days of lactation in comparison with a control group having a TSR of 0.83 ug. On day 14 their mean milk yield was 18.1 g. A foster litter was then placed with the dams and lactation continued for a second 14 day period. During this peri-

Table 12. Normal Thyroid Hormone Secretion Rate of Female Rats
(No Treatment)

No. of Animals	Body Weight (g)	Mean TSR (ug/100 g b.w.)	Range	Reference
20	260-300	1.30	0.5-2.5	Grosvenor & Turner (1958)
14	244	1.24		Grosvenor & Turner (1959)
18	217	1.08±0.06		Moon & Turner (1960)
41	240	1.09±0.08		Moon & Turner (1960)
10	265	1.10±0.16		Moon & Turner (1960)
12	245	1.20±0.16		Moon & Turner (1960)
81		1.11±0.05		Moon & Turner (1960)
25		0.97		Anderson et al. (1961)
12		0.916		Grossie & Turner (1962)
24		0.937		Grossie & Turner (1962)
12		1.08		Grossie & Turner (1962)
6	222	0.99±0.05		Djojoseobagio & Turner (1964)
15	210	1.26±0.06		Ishibashi et al. (1966)
23	226	1.02±0.04		Kumaresan & Turner (1966)
18	250	1.03±0.04		Kumaresan & Turner (1966)
54	200-230	1.16±0.32		Bauman & Turner (1967)
14	166	1.21±0.08		Narang et al. (1967)
14	203	1.12±0.10		Narang et al. (1967)
14	228	0.88±0.10		Narang et al. (1967)
24	217	1.30±0.08		Singh et al. (1969)
24	248	1.36±0.08		Singh et al. (1969)
24	236	1.63±0.09		Singh et al. (1969)
24	233	1.40±0.11		Singh et al. (1969)
22	261	1.63±0.05		Singh et al. (1969)
25	226	1.24		Singh & Turner (1969)
16	117	1.03±0.18		Singh et al. (unpubl.)
15	193	1.23±0.08		Singh et al. (unpubl.)
28	232	1.04±0.04		Singh et al. (unpubl.)
26	241	1.04±0.01		Singh et al. (unpubl.)

od a second TSR was determined, which showed a reduction to 0.98 ug/100 g body weight. The milk yield on day 28 was reduced to 13.8 g.

A second group of 10 lactating rats weighing 270 g showed a mean TSR of 2.1 ug/100 g body weight when estimated during the first 14 days of lactation. The mean milk yield was 20.8 g at the test milking. The young were removed and the TSR was again determined. The mean TSR had declined to 0.75 ug/100 g body weight. The individual milk yields on day 14 of the two groups (20) were related to the individual TSR. A significant positive correlation coefficient (r = + .70) was observed (Grosvenor, 1961).

L-T₄ Excretion in Lactating Rats

Grosvenor (1962) studied the percentage of the dose of L-T$_4$-I[131] excreted by rats on day 14 of lactation during a period of 16 hours compared to

Table 13. Thyroid Hormone Secretion Rate of Rats (Various carriers added)

No. of Animals	Carrier Solution	Body Wts. (g)	Mean TSR ug/100 g b.w.		Range	Reference
			Before	After		
			Females			
6	Sesame oil	187	0.786	0.846		Djojosoebagio & Turner (1964)
6	1.6 g glycerol + 2 g phenol 10 100 ml H_2O	207	0.886	0.893		Djojosoebagio & Turner (1964)
14	0.1 ml olive oil	244	1.24±0.10			Grosvenor & Turner (1959)
			Males			
20	Sesame oil	351	1.19±0.04		0.75-1.5	Kumaresan & Turner (1967b)

non-lactating rats. The non-lactating rats excreted 17.8 percent in the urine and 18.5 percent in the feces, or a total of 36.3 percent. The lactating rats excreted 9.9 percent in the urine, 10.5 percent in the milk, and 29.2 percent in the feces, or a total of 49.6 percent. The increased rate of excretion of I^{131} in the feces of the lactating rat is probably due to the great increase in feed consumption during lactation. It was shown in both types of rats that there was a high correlation between fecal weight and fecal I^{131}, so that rats eating more feed would excrete the larger amounts of iodine.

Table 14. Normal Thyroid Hormone Secretion Rate of Male Rats

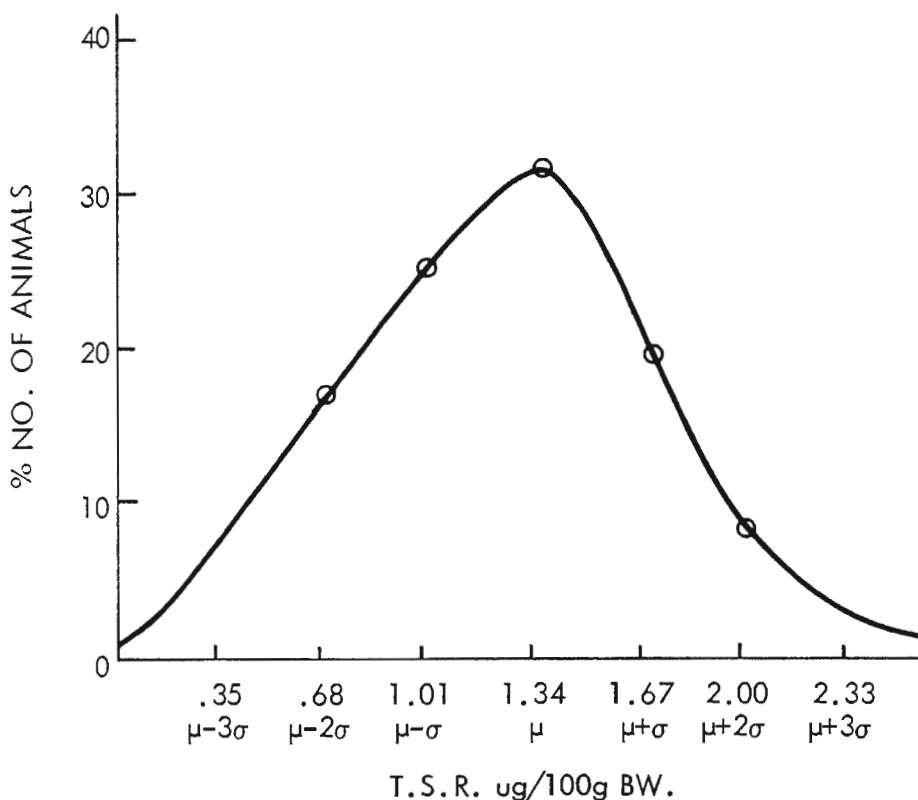| No. of Animals	Body Weight (g)	Mean TSR (ug/100 g b.w.)	Range	Reference
34		0.93±0.05		Anderson et al. (1961)
76	257	1.17±0.02	0.75-1.50	Kumaresan & Turner (1967a)

T.S.R. ug/100g BW.

FIGURE 3. Frequency distribution of normal thyroid hormone secretion rate (TSR) of Sprague-Dawley-Rolfsmeyer male rats about one month of age. Mean daily TSR value was 1.34 ug/100 g b.w., with ± one standard deviation equal to 33 ug. Number of rats 118, with a mean of 145 g b.w. (Kumaresan and Turner, 1967).

The 10.5 percent of I^{131} secreted into the milk is of interest. It has been shown that L-T_4 is not secreted into milk; therefore, the presence of I^{131} in the milk represents the deiodination of L-T_4-I^{131}.

It has been suggested that there may not be sufficient iodine to make possible the increased TSR and increased excretion of I^{131} by the lactating rats; however, the PBI of the lactating rats of 3.4 ug percent did not differ significantly from that of the non-lactating group (3.6 ug percent, indicating that adequate iodine was present for normal L-T_4 secretion.

THE ACCUMULATION OF I^{131} BY THE
LACTATING MAMMARY GLAND

The amount of I^{131} secreted in 8 hours into milk of lactating rats was determined on day 14 by Grosvenor (1960). The mammary glands and kidneys each trapped 35-40 percent of the injected I^{131} when it was injected when the glands were empty. The amount of I^{131} eliminated via milk or urine was not affected by the dietary level of iodide. When the glands were partially full of milk when I^{131} was administered, the I^{131} content of the milk was only ¼ to 1/5 as much per gram of milk. A significant reduction in the thyroidal-I^{131} uptake was observed when the glands were free of milk. It was suggested that lactating rats require greater dietary intake of iodide to compensate for that lost in the milk in order to insure adequate amounts for L-T_4 synthesis.

Grosvenor (1963) reported that the mammary glands of lactating rats fed a low-iodine diet accumulated 50 percent of an injected dose of I^{131} or 5 times as much as the thyroid gland and twice as much as excreted by the kidneys in 17 to 24 hours. Rats which accumulated greater amounts of I^{131} in their mammary glands had significantly lesser thyroidal I^{131} uptake. Methimazole significantly reduced accumulation of I^{131} in milk. It acts primarily in reducing the binding of I^{131} to milk protein. The concentration of I^{131} in milk was significantly reduced by the addition of iodide to the diet. The amount of I^{131} in the milk was not correlated with the amount of milk secreted.

Injection of L-T_4 inhibited thyroidal-I^{131} uptake but not mammary gland uptake. Injection of TSH restored thyroidal-I^{131} uptake but not by the mammary gland.

STIMULUS OF INCREASED TSR IN LACTATION

It has been shown that there is a marked increase in TSR during lactation in rats. The cause of this increase has been studied by Grosvenor (1964), who observed a significant increase in plasma PBI^{131} following 6 hours of normal nursing of the mammary glands. However, no increase in plasma PBI^{131} occurred following 6 hours of nursing when milk removal was prevented by the ligation of the galactophores.

When the pelvic mammary glands were deprived of somatic sensory innervation by spinal cord transection and the contained milk was removed by nursing young with the aid of oxytocin injection to the mothers, the PBI^{131} level after 6 hours increased significantly and was comparable with that following normal nursing.

Neither nursing of similar glands without oxytocin, and hence without milk removal, nor injecting oxytocin into rats non-nursed during the same period, altered the plasma PBI[131] levels. No detectable increase in PBI[131] occurred when only part of the milk was removed from normal mammary glands.

The nursing-induced increase in PBI[131] was blocked with exogenous L-T_4 and the PBI[131] was elevated in non-nursed lactating rats following injection of TSH.

These data suggest that the nursing stimulus elevates TSR in the lactating rat as the result of the effects of milk removal and/or milk reaccumulation by the mammary gland rather than as the result of excitation of neural receptors in the nipple or skin overlying the mammary glands. The data suggest that the effect on the thyroid gland is mediated by the release of TSH.

ENVIRONMENTAL FACTORS INFLUENCING THYROID HORMONE SECRETION RATE (TSR)

Effect of Food Restriction on TSR

In many experiments where endocrine glands are removed or animals are treated with various hormones, the voluntary feed intake may be reduced. In order to evaluate such effects upon the TSR, it was considered important to determine the effect of feed restriction upon the TSR.

Grossie and Turner (1962) determined the effect of feed restriction of 3 to 14 days. An 8 percent reduction in food had no effect on TSR; a 17 percent reduction resulted in an 8 percent reduction in TSR; a 22 percent feed reduction depressed TSR by 13 percent; and a 48 percent reduction in feed caused a 28 percent depression in TSR.

When the restriction in feed extended for 17 to 27 days, the 8 percent feed reduction reduced TSR 23 percent; on a 17 percent reduction in feed, the TSR was reduced 15 percent; on a 22 percent feed reduction, 42 percent; and on a 48 percent feed reduction the TSR was reduced 54 percent.

Effect of Graded Levels of Protein on TSR

The effect of a protein-free diet and levels of 5, 10, 15, and 20 percent casein added was compared to the feeding of Purina Lab Chow containing 23.4 percent protein on the TSR of mature female rats by Singh et al (1967).

The TSR was significantly decreased 16.9 percent ($P\langle 0.01$) and 27.7 percent ($P\langle 0.005$) after 10 and 25 days, respectively, on the protein-free diet (Table 15). In other groups there were non-significant decreases in TSR. A significant decrease in feed consumption was observed in groups on the protein-free ($P\langle 0.001$ and $P\langle 0.005$), the 5 percent protein ($P\langle 0.01$ and $P\langle 0.001$), the 10 percent protein ($P\langle 0.001$ and $P\langle 0.001$), and the 15 percent protein ($P\langle 0.001$ and $P\langle 0.001$), but not on 20 percent protein. No decrease in body weight was observed in the groups fed 10 percent or more protein in the diet. Decreases in body weight of 46 g in the protein free and 36 g in the 5 percent protein group after 25 days were significant.

It was observed that a 10 percent protein diet did not result in a loss

Table 15. Effect of Decreased Dietary Protein on TSR and Feed Consumption in Rats

Group	No. of Animals	Type of Diet	Length of Treatment (days)	Body Weight (g) Mean±S.E.	TSR L-T₄ ug/100 g b.w. Mean±S.E.	Change (%)	Feed Consumption Mean (g)	Per 100 g b.w. (g)	Change (%)
A	24	Control (Normal)		217±0.08	1.30±0.080		13.20±0.40	6.10±0.140	
B	24	Protein-free	10	202±1.5	1.08±0.005 P⟨0.01	-16.9	9.50±0.30	4.70±0.190 P⟨0.001	-23.0
C	24	Protein-free	25	171±1.5	0.94±0.080 P⟨0.005	-27.7	9.00⟩0.30	5.26±0.240 P⟨0.005	-13.8
D	24	Control (Normal)		248±1.3	1.36±0.080		13.50±0.20	5.70±0.200	
E	24	Casein (5%)	10	230±2.7	1.29±0.080	-5.1	11.80±0.25	5.11±0.112 P⟨0.01	-10.4
F	24	Casein (5%)	25	212±3.5	1.24±0.120	-9.8	10.80±0.40	5.00±0.050 P⟨0.001	-12.3
G	24	Control (Normal)	.	236±0.8	1.63±0.090		14.80±0.31	6.31±0.120	
H	24	Casein (10%)	10	246±1.4	1.50±0.090	-8.0	12.10±0.28	4.93±0.130 P⟨0.001	-21.9
I	24	Casein (10%)	25	246±1.7	1.46±0.80	-10.4	10.70±1.04	4.38±0.080 P⟨0.001	-30.6
J	24	Control (Normal)		233±2.5	1.40±0.110		13.40±0.48	5.76±0.160	
K	24	Casein (15%)	10	244±0.9	1.49±0.080	+6.4	12.48±0.15	5.12±0.140 P⟨0.001	-11.1
L	24	Casein (15%)	25	246±4.0	1.22±0.070	-12.9	11.44±0.23	4.65±0.090 P⟨0.001	-19.3
M	22	Control (Normal)		261±5.7	1.63±0.050		15.30±0.22	5.80±0.030	
N	22	Casein (20%)	10	260±2.3	1.59±0.070	-2.5	15.70±0.46	6.00±0.010	3.4
O	22	Casein (20%)	25	262±2.4	1.58±0.070	-3.1	15.40±0.39	5.81±0.020	0.2

S.E. = Standard error
P = Student's "t" test, level of significance.

of body weight or in a reduction in TSR, although a significant 30 percent reduction in feed consumption was observed. It would appear from these results that the protein content of the diet does not become a limiting factor on TSR until it is lower than 5 percent. It was suggested that the intake of calories plays a more important role in regard to TSR than low protein intake.

Effect of a Low Iodine Diet on TSR

Low iodine diets in domestic animals have induced enlargement of the thyroid gland (goiter) and other indications of reduced thyroid hormone secretion such as hairless pigs.

The effect of two low-iodine diets on TSR in female rats has been studied by Sinha et al (1969a). The first diet was obtained from the Nutritional Biochemical Corporation and contained 0.06 ppm. The second diet was prepared by the Agricultural Chemistry Department, University of Missouri-Columbia, and contained less than 0.05 ppm.

These two diets fed for 2 and 3 months did not significantly decrease the TSR in comparison with the controls. The PBI and thyroid size were slightly increased. On the low-iodine diet, the percentage uptake of thyroidal-I^{131} was greatly increased and the biological half-life ($t\frac{1}{2}$) was significantly reduced, indicating a faster turnover of the hormones. It was concluded that on the low-iodine diet the rat thyroid gland has the capacity to increase its activity to compensate for the iodine deficiency for at least 2 to 3 months and maintain a normal TSR.

Effect of Vitamin A on Thyroid Hormone Secretion Rate

The effect of two levels of vitamin A on the TSR of mature female rats was determined by Kumaresan and Turner (unpublished). A group of 42 rats having a body weight of 234 g had a mean TSR of 1.56 ug L-T_4/100 g body weight. They were then divided into three equal groups of control, 5000 I.U. vitamin daily for 20 days, and 10,000 I.U. of vitamin A. The group injected with 5000 I.U/day had a mean TSR before administration of 1.6 ug L-T_4 and 1.64 ug during its injection, a non-significant difference. The group on 10,000 I.U. of vitamin A had a mean TSR of 1.52 ug prior to and a mean of 1.46 ug during its administration. Thus neither level of vitamin A had any direct effect upon TSR.

However, these levels of vitamin A had a significantly depressing effect upon daily feed consumption, a reduction of 16 and 30 percent, respectively, being observed. Since it has been shown that reductions of this magnitude in feed consumption depress TSR, it was suggested that vitamin A might have a stimulating effect upon TSR which was masked by the decline in feed consumption.

Effect of Dietary Nitrate on Thyroid Function

The effect of 0.5, 1.0, and 2.5 percent of the nitrate KNO_3 in the feed of 200 to 300 g rats upon the I^{131} uptake by the thyroid gland has been studied by Bloomfield et al (1962). It was shown that as the level of nitrate increased, the uptake of I^{131} was depressed.

Welsch et al (1961) fed a corn-soybean oil meal diet (iodine 72 ppm)

to 10 female rats weighing 300 g. To it was added 2.5 percent KNO_3 for 30 days. A second similar group was fed 1.8 percent KCl. The rats fed KNO_3 had thyroid glands weighing 8.58 mg/100 g body weight, whereas the KCl group weighed 5.36. The difference was significant (P⟨0.005). In 14 rats weighing 100 g, 2.5 percent KNO_3 was fed for 75 days. The thyroid glands weighed 12.3 mg/100 g body weight compared to 8.35 mg, a significant difference (P⟨0.001).

In a further trial, Welsch et al (1962) fed 120 g female rats on the same rations but the rats were maintained for 27 days at 2°C. On the KNO_3 ration, the rats showed significant enlargement of the thyroid gland with reduced rate of gain as compared to those fed KCl. When similar rats were fed for 10 days on an iodine supplemented diet (1081 ppm), no difference was observed. They suggest that increased iodine will overcome the adverse effects of the nitrate in the cold.

In a further study using 150 g male Wistar strain rats on the same diet, Bloomfield et al (1962) reported that rats maintained in a normal environment showed a decreased thyroidal-I^{131} uptake after 7 hours on a 2.5 percent KNO_3 diet, but were able to overcome this effect after two weeks. Initially the nitrate flushed I^{131} from the thyroid gland; however, this effect was overcome in one week. After 5 weeks, in the absence of tapazole, the nitrate-fed rats fixed more I^{131} as PBI^{131} than did those fed 1.8 percent KCl.

In a further study, Bloomfield et al (1964) reported that the inhibitory effect of nitrate feeding on thyroid function which was enhanced in a cold environment could be overcome by the feeding of 0.2 percent iodized salt in the diet.

Welsch et al (1964) fed 24 male rats for 4 months on a low-iodine corn-soybean ration. The TSR of this group was 1.44 ug/100 g body weight. Then 10 rats were continued on the same ration and 14 were given 2.5 percent KNO_3 for 3 months. The TSR of the normal group was 1.38 ug/100 g body weight and those fed the nitrate was 1.34 ug. It is thus evident that when iodine is present in adequate amounts in the ration, the nitrate effect can be overcome.

Effect of Light and Darkness on TSR

The mean TSR of a group of 62 female rats at 30 days of age was observed to be 1.24 ug/100 g body weight by Singh and Turner (1969a). The rats were then divided into three groups with equal TSR. Group I was kept under control conditions of $78 \pm 1°F$ and in a room artificially illuminated during normal daylight hours. Group II was placed in nearly constant total darkness. Group III was placed in continuous light. After 56 days of exposure, the mean TSR of the controls was 0.81 ug/100 g body weight; those in continuous darkness had a mean TSR of 0.87 ug, a nonsignificant difference; whereas those in continuous light had a mean TSR of 0.98 ug, a 20.98 percent increase. It was suggested that continuous light depressed melatonin secretion to approximately the pinealectomy level, whereas continuous darkness had little stimulating effect upon melatonin secretion.

Effect of Varying Temperatures on TSR

Various indices of thyroid activity have indicated that low temperature increases thyroid hormone secretion rate. Bauman and Turner (1967) determined the TSR of 54 mature female rats weighing 200-230 g maintained at a temperature of 78°F (25.5°C). The mean TSR was 1.16 ug L-T$_4$/100 g body weight. The rats were then placed in a chamber at 40°F (4.5°C) and the TSR again determined. In the 4 to 10 days required to complete the assay, the TSR had increased to 1.76 ug, an increase of 52 percent; after 20 days of exposure the TSR was 2.15 ug, an increase of 85 percent; after 30 days to 2.3 ug, an increase of 98 percent; and after 50 days to 2.59 ug, or 123 percent. After 90 days the TSR increased to 2.8 ug or 141 percent. In each case the increase was based on the individual rats' prior TSR.

A group of 12 male Holtzman rats weighing 400-600 g, which were raised at 83°F (28.5°C) showed a mean TSR of 1.0 ug. After 10 days of being held at 40°F (4.5°C), their TSR increased to 1.76 ug, an increase of 76 percent; after 30 days to 2.58, an increase of 158 percent; and after 80 days to 2.88 ug, an increase of 188 percent.

Thus in both groups, the low temperature caused a marked increase in TSR.

In a further study, 24 male Holtzman rats which had been raised at a temperature of 48°F (8.8°C) were placed in a room at 78°F (25.5°C). The mean TSR at 48°F (8.8°C) was 1.31 ug: after 8 days at 78°F (25.5°C), the TSR was reduced to 1.26 ug, a decrease of 4 percent; after 40 days to 1.06 ug, a decrease of 19 percent; and after 80 days no further change was observed. In this group of rats, the increase in environmental temperature had only a moderately depressing effect upon the TSR.

Effect of L-T$_4$ on Survival of Rats at 4.5°C

When rats were transferred from a temperature of 78°F (25.5°C) to 40°F (4.5°C), it was observed that many began to die. The mean survival time was 37 days, with only 12 of 54 surviving to 90 days. It is interesting that no correlation between the TSR at 78°F (25.5°C) and survival at 40°F (4.5°C) was observed (Bauman and Turner, 1967).

In order to determine the effect of L-T$_4$ on survival time, a group of 50 rats was injected with 3.0 ug L-T$_4$/100 g body weight/day for 7 days, then placed in a room at 40°F (4.5°C). Their mean survival time was increased to 79.5 days and 42 survived to 90 days.

A second group of 50 rats received 1.25 mg/day of corticosterone. Their survival time was increased to 63 days and 28 survived for 90 days.

A third group of 50 rats received both L-T$_4$ and corticosterone. Their survival time was 88 days and 45 survived for 90 days. It was seen that the two hormones gave the best protection against cold, but were not greatly superior to L-T$_4$ alone.

Effect of High Altitude on Thyroid Function

Nelson and Anthony (1966) exposed rats to a reduced pressure (380

mm Hg) simulating an altitude of 18,000 feet. This resulted in a markedly elevated I^{131} uptake in the thyroid, a relative increase in the amount of intrathyroidal I^{131} MIT, and a lowered I^{131} DIT. The hypoxia induced alteration of thyroid hormone synthesis was found to be a transient response since the I^{131} MIT/DIT ratio returned to control levels after 60 hours of exposure.

Table 16 gives a summary of the physiological and environmental factors influencing TSR.

Table 16. Summary of Factors Influencing TSR —
Physiological and Environmental

Factor	Effect
Physiological	
Advancing age	Decline of about 40%
Sex	No significant difference
Lactation	70% increase
Involution after lactation	Decrease to normal
Environmental	
Food Restriction:	
8% (17 to 27 days	23% reduction
17% (17 to 27 days	15% reduction
22% (17 to 27 days)	42% reduction
48% (17 to 27 days)	54% reduction
Protein-free diet (25 days):	28% reduction
5% protein	10% reduction
10% protein	10% reduction
15% protein	13% reduction
20% protein	No reduction
Low Iodine diet	No reduction
Vitamin A: 5,000 I.U.	No reduction
10,000 I.U.	No reduction
Nitrate - 2.5% KNO_3	No reduction when iodine is adequate
Temperature reduction 78°F to 40°F (25.5°C to 4.5°C)	
20 days	85% increase
30 days	98% increase
50 days	123% increase
90 days	141% increase

ENDOCRINE FACTORS INFLUENCING THYROID HORMONE SECRETION RATE (TSR)

Effect of Various Hormones on the TSR of Normal Rats

A series of experiments has been conducted to determine the effect of various hormones upon TSR. In these experiments, the normal TSR was first determined, then single or graded levels of the hormone were administered and the TSR redetermined.

Effect of Growth Hormone on Thyroxine Secretion

The TSR of 20 nonlactating rats with a mean body weight of 262 g was 1.3 ug/100 g body weight. A group of 15 rats weighing a mean of 245 g were injected with 1 mg of growth hormone and the TSR then determined. The GH did not alter TSR or affect the rate of release of thyroidal-I^{131}, although body weight was increased 2 g/day (Grosvenor and Turner, 1959).

Effect of Growth Hormone on Thyroxine Secretion of Lactating Rats

It was shown that a group of 16 lactating rats had TSR of 2.2 ug/100 g body weight. When a group of 24 lactating rats was given 1 mg GH/day from days 8 to 16 of lactation, the TSR increased to 2.9 ug/100 g body weight, a 32 percent increase. The rate of release of thyroidal-I^{131} was also increased significantly. These data suggest that GH in some way influences the release of TSH (Grosvenor and Turner, 1959).

Effect of Estrogen upon Thyroxine Secretion Rate

The effect of estradiol benzoate (EB) upon the TSR of female rats was determined. Injections of 1.0, 3.6, 15.0 or 50.0 ug EB/day were given for 5 days, and then TSR was estimated. The 14 control animals had TSR's of 1.24 ug, whereas those receiving 3.6 ug EB/day weighed 244 g and had a mean TSR of 1.68 ug/100 g body weight, an increase of 35.5 percent. The other levels were without significant effect (Grosvenor and Turner, 1959).

Influence of Testosterone on TSH Secretion in the Rat

Lampe and Noach (1962) observed that treatment of rats with testosterone propionate (TP) resulted in a diminished thyroidal uptake of I^{131}. They also demonstrated that TP caused an increased clearance of I^{131} from the blood, possibly by an enhanced urinary excretion.

Van Rees *et al* (1965) reported that castration of male rats decreased both pituitary and serum TSH levels. TP increased serum TSH levels in castrate males. TP in physiological doses prevented the decrease induced by castration, whereas high doses resulted in low pituitary TSH content. TP administered to L-T_4 maintained thyroidectomized rats had the same effects as mentioned above, but if L-T_4 was omitted, no effect of TP on pituitary and serum TSH levels was observed.

Kumaresan and Turner (1967a) injected normal mature male rats with 2 mg of TP for 10 days prior to and during the estimation of TSR. The TSR was increased 25 percent above that observed prior to the TP injection. Following castration, the TSR of similar rats was reduced 12 percent. When castrate rats were injected with 2 mg TP daily, the TSR was increased 21 percent in comparison with the normal TSR and a significant 35 percent in comparison with the mean TSR after castration.

Effect of Neonatal Testosterone Propionate on TSR of Female Rats

Kumaresan and Turner (1966b) injected a group of female rats when 36 to 48 hours old with 2 mg of testosterone propionate (TP). The TP produced a condition of constant estrus. At 120 days of age, these 24 animals

showed a mean TSR of 1.06±0.05 ug/100 g body weight in comparison with a mean TSR of 25 normal animals of 1.26±0.06 ug/100 g body weight. The TP injected group thus showed a significant decline of 16 percent in TSR as compared to the control group.

Effect of Glucocorticoids on TSR

Anderson *et al* (1961) reported a decline in TSR in male and female rats after adrenalectomy. When 150 ug/day of hydrocortisone was administered, the TSR of the males was increased to 1.05 ug (normal 0.93 ug) and the females to 1.13 ug (normal 0.97 ug). These increases were not significant but indicated that glucocorticoids injected into normal animals might stimulate increased TSR.

That thyroid activity in rats exposed to cold may be slightly depressed by the injection of 1.25 mg corticosterone/day was indicated by Bauman and Turner (1967). After 60 days at 40°F (4.5°C), a corticosterone-treated group had a mean TSR of 2.13 ug L-T$_4$/100 g while the control group had a mean TSR of 2.43 ug/100 g.

Effect of Insulin upon TSR

A group of 23 female rats with a mean body weight of 226 g showed a mean TSR of 1.02±0.04 ug/100 g body weight. The rats were then injected with 3 u of protamine-zinc insulin for 10 days prior to and during the estimation of TSR. The TSR was increased to 1.22±0.06 ug, a significant increase of 20 percent (Kumaresan and Turner, 1966c).

A second group with a mean TSR of 0.97±0.03 ug was similarly injected with 3 u of insulin. The insulin was then withdrawn and the TSR determined. The mean TSR of this group was 1.23±0.04 ug, an increase of 27 percent, indicating that insulin has a favorable effect upon TSR for a period after its withdrawal.

Effect of Parathyroid Extract upon TSR

Djojosoebagio and Turner (1964) determined the normal TSR of 12 female rats weighing 209 g as 0.835±0.57 ug/100 g body weight. The rats were then injected with 30 U.S.P. units of parathyroid extract/100 g body weight/day for 13 days, then the TSR was again estimated as 1.01±0.049 ug, an increase of 21 percent.

Singh and Turner (1969b) gave graded doses of parathyroid extract (20, 30, and 40 U.S.P. units) to groups of female rats. The TSR during treatment increased 25.4, 36.5, and 54.1 percent, respectively. These data indicate that the parathyroid hormone stimulates the thyroid hormone very markedly.

Effect of Calciferol, Hytakerol and Dihyrotachysterol upon TSR

Djojosoebagio and Turner (1964) administered calciferol at a level of 0.2 mg/100 g body weight, crystalline dihydrotachysterol at a level of 100 ug/100 g body weight, and hytakerol, which contains dihydrovitamine D$_2$II, to groups of normal female rats. Their TSR, after treatment, increased 48, 55 and 103 percent, respectively.

Singh and Turner (1969b) injected calciferol at levels of 0.1, 0.15, and 0.2 mg/100 g body weight to female rats. Levels of 0.1 and 0.15 had no effect on TSR, but 0.2 mg stimulated a significant 26.7 percent increase.

Effect of Melatonin on TSR

Ishibashi *et al* (1966) determined the mean TSR of 16 immature female rats weighing 128 g as 1.53 ug/100 g body weight. A comparable group of 17 rats weighing 129 g had a mean TSR of 1.28 ug, after the daily injection of 20 ug/day of melatonin for 10 days prior to the estimation of TSR, a decrease of 16 percent.

Narang and Turner (1966) reported that the TSR of female rats at 55 days of age was reduced 23 percent by the daily injection of 50 ug melatonin/day for a week before and during TSR estimation. When 85 days of age, the injection of 75 ug/day of melatonin caused a 15 percent reduction in TSR. At 115 days of age, 100 ug/day of melatonin reduced TSR 9 percent.

In a further study, Singh, Narang and Turner (1969) determined the mean TSR of 62 female rats at 33 days of age. At 55 days of age they were divided into four groups of equal TSR and administered melatonin at levels of 50, 75 and 100 ug/rat/day. Compared to the control group, the TSR of these groups were reduced by 13.6 percent, 19.4 percent, and 29.1 percent, respectively. The melatonin was then withdrawn for at least 20 days and the TSR again determined. The TSR at 89 days of age increased markedly to a level from 10 to 14 percent above the control TSR and to 51.7 percent, 66.3 percent, and 91.8 percent, respectively, above the graded levels of melatonin observed at 55 days of age.

At 140 days of age, groups 1 and 2 were combined as a control group and groups 3 and 4 were again administered melatonin at a level of 250 ug/rat/day (about 1 ug/g body weight) for a period of 7 days before and during the period of TSR estimation. It was without effect in depressing TSR of these same rats. At 180 days of age after withdrawal of melatonin for 30 days, the TSR was unaltered, indicating that in mature rats neither the injection nor withdrawal of melatonin had an effect.

Table 17 shows a summary of endocrine and hormone factors influencing TSR.

EFFECT OF VARIOUS HORMONES ON THE TSR OF NORMAL RATS

The effect of several hormones on the TSR of normal rats has been studied. The growth hormone at a level of 1 mg/day had no effect upon the TSR of normal rats. However, in lactating rats, growth hormone increased TSR by 32 percent above that of lactating rats.

Estrogen at a level of 3.6 ug of estradiol benzoate/day increased TSR 35.5 percent above the controls. In male rats, testosterone propionate at a level of 2 mg/day increased TSR 25 percent; insulin at a level of 3 units/100 g body weight/day increased TSR 20 percent; and parathyroid hormone at a level of 30 U.S.P. units/100 g body weight increased TSR 21 percent. In a second study, 20, 30, and 40 U.S.P. units/100 g body weight

Table 17. Summary of Factors Influencing TSR —
Endocrine - Hormones

Factor	Effect
Growth Hormone:	
Normal rats	No effect
Lactating rats	32% increase
Estrogen-Female:	
3.6 ug EB/day	35.5% increase
Testosterone-male:	
2 mg/day	25% increase
Females at 2 days of age	
At 120 days of age	16% decrease
Glucocorticoids	Slight increase
Insulin - 3 units/100 g b.w.	20% increase
Parathyroid Hormone:	
30 U.S.P. units/100 g	21% increase
20 U.S.P. units	25% increase
30 U.S.P. units	36% increase
40 U.S.P. units	54% increase
Melatonin:	
20 ug/day	16% decrease
50 ug/day (55 days of age)	23% decrease
75 ug/day (85 days of age)	15% decrease
100 ug/day (115 days of age)	9% decrease
50 ug/day (55 days of age)	14% decrease
75 ug/day (55 days of age)	19% decrease
100 ug/day (55 days of age)	29% decrease
250 ug/day (140 days of age)	3% decrease

increased TSR 25, 36, and 54 percent, respectively. The effect of a combination of these hormones which increase TSR has not been studied.

Normal rats vary considerably in TSR. These data suggest the possibility that part of the difference in TSR might be due to differences in the rates of secretion of these hormones in normal animals. If this is true, the rats with higher normal TSR might also be higher in the secretion of other hormones.

Effect of the Removal of Several Endocrine Glands on TSR

The effect of the removal of certain endocrine glands upon the TSR has been studied. Part of the effect observed may be due to the absence of the hormone and part may be due to alteration in voluntary feed intake, which may influence the TSR.

Effect of Ovariectomy and Replacement Therapy on TSR

Moon and Turner (1960) determined the effect of ovariectomy of rats upon the thyroxine secretion rate. The first group of 18 control animals showed a mean TSR of 1.08 ug/100 g body weight. When TSR was estimated starting 2 to 6 days following the operation, the mean TSR of 16

rats was 0.72 ug/100 g body weight, a reduction of 33 percent. Increasing the duration of ovariectomy prior to TSR estimation had little further effect in reducing the TSR (Table 18).

Table 18. Summary of Factors Influencing TSR —
Endocrine, Gland Removal-Replacement Therapy.

Factor	Effect
Ovariectomy	33% reduction
1 ug EB/day	Return to normal
1 ug EB+3 mg Progesterone (P)	Return to normal
3 mg P/day	No effect
Castration - male	12% reduction
2 mg/day testosterone propionate	21% increase above normal
2 mg/day testosterone propionate	35% above castrate
Adrenalectomy	
Males	17% reduction
Females	32% reduction
150 ug hydrocortisone	
Males	Above normal
Females	Above normal
Pinealectomy	11.8% increase
Pancreas (destruction of B-cells by alloxan)	41% decrease
3 u insulin/day	98% increase in alloxan-treated
	17% increase above normals

A group of 12 rats showed a mean TSR of 1.2 ug/100 g body weight. After ovariectomy they were given 1 ug estradiol benzoate/day and TSR estimated 2 to 8 days later. Their mean TSR was 1.13 ug/100 g body weight, indicating that the estrogen maintained normal secretion. When 1 ug estrogen plus 3 mg progesterone was administered, the mean TSR was 1.17 ug/100 g body weight. When progesterone, 3 mg/day, was given alone to ovariectomized rats, the TSR was 0.73 ug/100 g body weight, indicating that it was without benefit.

Grosvenor (1962) reported that EB injected at a level of 1.5 ug/100 g body weight increased the TSR of ovariectomized female rats from 0.70 ug/100 g body weight to 1.05 ug. This effect was not mediated by a direct action on the thyroid gland since EB failed to evoke discharge of thyroidal I^{131} when TSH was totally suppressed by endogenous L-T_4, whereas marked acceleration of thyroidal-I^{131} release occurred during partial TSH suppression.

Daily injection of 1.5 ug/100 g body weight of EB for 4 weeks to ovariectomized rats significantly increased 24- and 48-hour urinary and fecal excretion of I^{131} following a single injection of L-T_4-I^{131}, without significantly altering serum PBI or thyroid weight. These data suggest that EB in the proper dosage may stimulate TSR while also increasing its rate of degradation.

Effect of Castration of Male Rats on TSR

A group of 18 male rats weighing 248 g showed a mean TSR of 1.22 ± 0.03. After gonadectomy, the TSR was repeated. The mean value was reduced to 1.07 ± 0.04 ug, a significant reduction of 12 percent (Kumaresan and Turner, 1967a). When similar rats were injected with 2 mg TP daily, the TSR significantly increased to 1.44 ug, or 21 percent, in comparison to normal TSR and to 35 percent in comparison to castrated animals.

Effect of Adrenalectomy on Thyroxine Secretion Rate

Anderson *et al* (1961) determined the mean TSR of 34 male rats to be 0.93 ug/100 g body weight/day and of 25 females to be 0.97 ug. The animals were then adrenalectomized and maintained on 1 percent NaCl in the drinking water. The TSR was then redetermined in the survivors after 2 weeks. The 18 males showed a mean TSR of 0.75 ug, a decrease of 17 percent, while the 21 females showed a mean TSR of 0.67 ug, a decrease of 32 percent.

Replacement therapy was then started at a level of 150 ug/day of hydrocortisone for 2 weeks and TSR redetermined. The 10 males showed a mean TSR of 1.05 ug and 15 females showed a mean TSR of 1.13 ug, both slightly above normal. Body weight was not affected by the treatments.

Effect of Pinealectomy on TSR

A group of 15 female rats weighing 210 g were sham-operated. Their mean TSR was 1.26 ± 0.06 ug/100 g body weight. Following pinealectomy, a second group of 15 rats weighing 219 g showed a TSR of 1.43 ± 0.07 ug/100 g body weight, an increase of 11.8 percent (Ishibashi *et al*, 1966).

Effect of Alloxan on TSR

The administration of alloxan to rats in suitable doses caused the destruction of the B-cells of the pancreas and a loss of insulin secretion. A permanent condition of diabetes is induced.

Kumaresan and Turner (1966) determined the TSR of a group of 18 mature rats as 1.03 ± 0.04 ug L-T_4/100 g body weight. After alloxan diabetes was induced, the rats showed a mean TSR of 0.61 ± 0.07 ug, a highly significant decrease of 41 percent compared with the normal TSR values of the same rats. After replacement therapy with 3 units of insulin/day, the rats showed a mean TSR of 1.21 ± 0.12 ug, a highly significant increase of 98 percent. In comparison with the normal TSR, replacement therapy induced a non-significant increase of 17 percent.

The Effect of the Removal of Endocrine Glands on TSR

The removal of certain endocrine glands has been shown to decrease TSR and the administration of the hormones has been shown to restore TSR to a normal level or above. Thus ovariectomy has been shown to reduce TSR 33 percent and the administration of estrogen but not progesterone has been shown to restore TSR to normal. In castrate males there was a reduction of 12 percent in TSR and 2 mg of testosterone propionate increased TSR 21 percent above normal.

Adrenalectomy caused a 17 percent reduction in TSR in males and a 32 percent reduction in females and hydrocortisone increased the TSR of both sexes above normal

The destruction of the B-cells of the pancreas by alloxan in females decreased TSR by 41 percent and the TSR was increased above normal by insulin.

The observations that the lack of certain hormones depresses TSR and the injection of these hormones returns the TSR to normal or above is further evidence that the level of secretion of these hormones in some way influences TSR.

Use of TSR Method in Estimating Potency of Analogues of L-T₄

In the estimation of TSR in rats, the minimal amount of $L-T_4$ required to block the release of TSH and in turn the release of thyroidal-I^{131} in animals given methimazole to prevent the recycling of metabolized I^{131} is considered the TSR.

By the substitution of thyroxine analogues for $L-T_4$, the biological activity of the analogue in the suppression of TSH release may be determined.

In female rats, Pipes and Dale (1963) reported that $L-T_4$ was 10 times as active biologically on an equimolar basis as $D-T_4$. It was shown also that $L-T_3$ was 2.2 times as active as $L-T_4$.

In a comparable study with male rats, $D-T_4$ was found to be 1/10 as active as $L-T_4$. whereas $L-T_3$ was 2.6 times as active as $L-T_4$ (Bauman *et al*, 1965).

The compound 3,5-diiodo-3'-isopropyl-1-thyronine, although containing only 2 atoms of iodine, was shown to be slightly more active than $L-T_3$ and 2.9 times as active as $L-T_4$.

Use of TSR Method of Determining Oral Biological Activity of Thyroidal Substances

The TSR method can be used, also, to determine the oral activity of various thyroidal substances. While thyroidal substances are orally active since they are iodinated amino acids, it has been recognized that they are less effective than when injected.

Bauman and Turner (1966a) used male rats in a study of oral activity. The subcutaneous administration of $L-T_4$ was used as a standard. $L-T_4$ was shown to be 37.9 percent effective orally. $L-T_3$ was 95 percent as effective as $L-T_4$ on an equimolar basis when administered orally. Since $L-T_3$ is 2.6 times as active as $L-T_4$ by injection, its oral effectiveness would be 36.9 percent.

Thyroprotein, which contains 1 percent of $L-T_4$ was found to be 36.2 percent effective orally. Possible causes of the reduced oral effectiveness of $L-T_4$ in the rat were studied by Cheng and Van Middlesworth (1964), who reported that during 1 hour 20 to 50 percent of a tracer dose of $L-T_4-I^{131}$ was absorbed *in vivo* from washed intestinal loops of rats. The absorption was much slower during the succeeding 5 hours. Their studies suggested that protein, possibly plasma albumin, secreted into the intestinal lumen reduced the absorption of $L-T_4$ from the washed intestinal loop.

In order to study this problem further, Bauman *et al* (1967) added 45 ml of rat plasma to the orally administered $L-T_4$. It was shown that rat plasma given orally alone had no effect on TSR. In this experiment, when $L-T_4$ was administered orally in saline, it was found to be 48 percent as effective as when injected subcutaneously. However, when rat plasma was

given, the effectiveness was reduced to 34 percent, a significant reduction of 30 percent.

These data suggest that the reduced biological effectiveness of oral L-T_4 may be due in part to a plasma-binding protein (TBP) in the intestines to which the L-T_4 becomes bound and fails to be absorbed.

The higher oral effectiveness of L-T_4 in this group (48 percent) as compared to the previous study (38 percent) indicates that groups of rats may show differences as well as individual differences in oral effectiveness of L-T_4.

That there are marked differences in the oral absorption of thyroidal substances by species is shown by the approximately 10 percent absorption by ruminant animals (cattle) indicated by Bauman and Turner (1965) and the much higher oral absorption by female fowls of about 55 percent (Srivastava and Turner, 1967).

EFFECT OF RESERPINE ON THYROXINE SECRETION RATE

Groups of female rats weighing between 230 and 280 g were injected subcutaneously with reserpine at levels of 5, 10, or 50 ug/100 g body weight/day. Reserpine at levels of 5 and 10 ug reduced TSR from 1.4 ug/100 g body weight to 0.23 ug/100 g body weight, whereas at the 50 ug level the TSR was reduced to 0.135 ug. This is a decrease of 84 to 90 percent. The first two levels produced a slight gain in body weight, but the higher level resulted in a 22 percent loss in body weight (Moon and Turner, 1959a). Thyroidal-I^{131} release rate was significantly reduced.

In a study to determine the mode of action of reserpine upon TSR by Moon and Turner (1959b), it was shown that reserpine had no significant effect upon thyroidal-I^{131} release rate when TSH was injected into young female rats whose endogenous secretion of TSH was blocked with L-T_4. It was shown further that reserpine prevented thyroid enlargement when tapazole was administered. It was suggested that reserpine inhibited TSR in the rat through TSH suppression.

BIOLOGICAL HALF-LIFE (t½) OF L-T_4 AND L-T_3 IN THE RAT

Feldman (1960) compared the t½ of L-T_4 and L-T_3 in controls and rats on an iodine-deficient diet. Iodine deficiency had no effect on the t½ of L-T_4 but L-T_3 had a significantly shorter t½ (Table 19).

Pittman et al (1964) studied the effect of thyroxine analogues on the t½ of L-T_4 in thyroidectomized rats maintained in a euthyroid state with L-T_4, D-T_4, DL-T_3, 3,3',5-triiodothyropropionic acid, and 3,5-diiodothyroacetic acid lengthened the t½ of L-T_4-^{131}I, increased the recovery in the feces, and decreased the amount in the urine.

Grossie et al (1965) observed a mean t½ of control rats of 19.5 hours, whereas 7 days after thyroidectomy the t½ increased to 23.7 hours.

Anderson et al (1968) compared the t½ of L-T_4 by blood sampling and by whole body count of females. Normal rats had a t½ of 21.8 hours by blood count and 24.8 hours by whole body count. When methimazole was given, the t½ was 20.3 and 21.2 hours, respectively. In hypophysectomized rats the t½ was 20.7 and 47.1 hours, respectively.

Table 19. Half-life (t½) in the Rat

	No. of Animals	L-T$_4$ (hours)	L-T$_3$ (hours)	Remarks	Reference
Male	7	18.2	11.0	Normal	Feldman (1960)
Female	7	18.6	10.3	Iodine deficient	Feldman (1960)
Thyroidectomized + 1.5 ug L-T$_4$/100 g b.w.	38	16.6	-	-	Pittman et al. (1964)
Controls	10	19.5	-	-	Grossie et al. (1965)
Thyroidectomized	19	23.7	-	-	Grossie et al. (1965)
Thyroidectomized +1.0 ug L-T$_4$/100 g b.w.	23	17.8	-	-	Grossie et al. (1965)
Methimazole, 30 days (400 ug/100 g b.w./day)	10	18.5	-	-	Grossie et al. (1965)
Female, normal	14	21.2 (blood)	-	-	Anderson et al. (1968)
		24.8 (whole body)	-	-	Anderson et al. (1968)
Female, methimazole	14	20.3 (blood)	-	-	Anderson et al. (1968)
		21.2 (whole body)	-	-	Anderson et al. (1968)
Female, hypophysectomized	22	20.7 (blood)	-	-	Anderson et al. (1968)
		47.1 (whole body)	-	-	Anderson et al. (1968)
Male	36	18.2		(28°C), 60 days	Yousef & Johnson (1968)
Male	36	18.2		(28°C), 110 days	Yousef & Johnson (1968)
Male	36	18.2		(28°C), 220 days	Yousef & Johnson (1968)
Male	36	21.0		(34°C), 60 days	Yousef & Johnson (1968)
Male	36	21.7		(34°C), 110 days	Yousef & Johnson (1968)
Male	36	20.4		(34°C), 220 days	Yousef & Johnson
Male	36	23.1		(28°C), 60 days*	Yousef & Johnson (1968)
Male	36	20.4		(28°C), 110 days*	Yousef & Johnson (1968)
Male	36	21.7		(28°C), 220 days*	Yousef & Johnson (1968)
Male	36	27.7		(34°C), 60 days*	Yousef & Johnson
Male	36	21.7		(34°C), 110 days*	Yousef & Johnson (1968)
Male	36	23.1		(34°C), 220 days*	Yousef & Johnson (1968)

*Feed restricted to intake at 34°C.

Animal	Sex	Number	Range of TSR	Mean (ug L-T$_4$/ 100 g b.w.)	Environmental Conditions	Reference
Albino Mouse						
(Swiss Webster)	F	16		1.38	78°F	Pipes *et al.* (1960)
P. maniculatus graculis		12		1.45	July	Eleftheriou & Zarrow (1962)
P. maniculatus bairdii		12		0.75	July	Eleftheriou & Zarrow (1962)
		8		1.12	Dec.	Eleftheriou & Zarrow (1962)
Hamster			0.5-0.75	0.62	Room Temp.	Premachandra (1962)
Hamster	F	88	0.45-0.70	0.58	Room Temp.	Bauman *et al.* (1968)
Guinea pig			0.1-0.75	0.48	Room Temp.	Premachandra (1962)
Mole	M + F	20	1.75-2.50	1.96	78°F	Leach *et al.* (1962)
Opossum	M + F	51	0.75-2.50	1.72	Feb.-May Adults	Bauman & Turner (1966)
Opossum	M + F	50	1.00-5.75	2.66	Feb.-May Juveniles	Bauman & Turner (1966)
Raccoon	M + F	29	0.03-0.40	0.12	Room Temp. Adult & juvenile	Bauman & Turner (1965)
	M + F	22	0.03-0.40	0.12	Adult	Bauman & Turner (1965)
	M + F	7	0.08-0.15	0.10	juvenile	Bauman & Turner (1965)
Tree Shrews	M + F	6	1.00-1.50	1.33	15-20°C	
	M+ F	4	0.75-1.50	1.25	25°C	
	M + F	4	0.75-1.75	1.31	37.5°C	Sorenson & Bauman (unpub.)
Chinchillas (normal)	M			2.30		Vanjonack & Johnson (1969)
Chinchillas (fur chewers)	M			3.10		Vanjonack & Johnson (1969)

Yousef and Johnson (1968) determined the $t\frac{1}{2}$ of L-T_4 in growing rats held at 28°C. No difference was noted in rats from 60 to 220 days (Table 19). Groups of rats held at 34°C showed a significantly higher $t\frac{1}{2}$. When the rats were restricted to the feed consumption of the rats held at 34°C, they were also increased.

RELATION OF TSR TO HORMONE CONTENT OF THE THYROID GLAND

The hormone secreted by the thyroid gland is stored as thyroglobulin in the lumen of the thyroid follicles. Sinha *et al* (1969b) studied the relation of the daily TSR to the magnitude of the hormone stored in the gland. They reported that the thyroid glands contained a mean of 3.87 times the daily TSR with a range from 2.09 to 6.22 days. These observations indicated a relatively rapid turnover rate of the hormone.

COMPARISON OF THE THYROID HORMONE SECRETION RATE OF THE RAT WITH OTHER SPECIES

While the factors influencing the TSR of the rat have been studied very extensively in this laboratory, it is interesting to relate the normal TSR of the rat with comparable determinations of TSR in other laboratories and wild species of small mammals (Table 20).

Of the various species studied, the TSR of the raccoon appears to be the lowest. The next lowest TSR was observed in the guinea pig, with the hamster only slightly higher. The TSR of tree shrews and mice appear to be slightly higher than the rat, while the mole and opossum have the highest TSR per 100 g body weight of the species so far examined.

It appears evident that these differences are not directly related to body weight. Tissue sensitivity to thyroid hormones and metabolic response to low levels of hormone in the maintenance of normal body temperature appear to play roles in the differences observed.

SUMMARY

With the aid of radioactive iodine (I^{131}), a method has been developed in this laboratory for the estimation of the thyroid hormone secretion rate (TSR). Using this method, an extensive study was initiated to determine the factors influencing the TSR of the rat.

Starting in the 1950's, the results of these studies have been published in various endocrine journals. The object of the present publication is to summarize these observations and point the way for future research.

Of the physiological factors influencing TSR, it was noted that sex had little or no effect. However, there was observed a significant decline in TSR with age from birth to maturity. While there is believed to be a further decline in old age, further research is required. There is no clear evidence for a change in TSR during pregnancy. There was observed, however, a marked increase during lactation, which returns to normal during involution.

It has been suggested for a long time that the functional activity of the thyroid gland is altered by changes in environmental temperature. In this study it was shown that low temperature stimulates a marked increase in TSR, which is maintained or increased further by continued cold. On the other hand, the change from cold to a warm or hot environment quickly reduces the TSR.

A reduction in food intake has been shown to cause a reduction in TSR which is increased as the restriction in food intake is increased to 50 percent. That this is caused primarily by a reduction in calories rather than in protein was shown by the feeding of a protein-free diet and graded levels of protein in the diet. While a protein-free diet reduced TSR about 28 percent, 5, 10, and 15 percent protein reduced the TSR only about 10 percent.

To our surprise, it was observed in the rat that a ration very low in iodine was without effect upon the TSR. The study indicated that the rat has mechanisms by which the iodine is conserved. There is great efficiency in the uptake of iodine by the thyroid gland and the same system recovers and conserves the iodine produced in the metabolism of the hormone. There is evidence that under these conditions, more $L-T_3$ is secreted, which has over twice the biological activity of $L-T_4$.

Increased vitamin A in the diet had no effect on TSR. On the other hand, it was observed that calciferol (vitamin D_2) at a level of 0.2 mg/100 g body weight increased TSR 26.7 percent.

Nitrate at a level of 2.5 percent KNO_3 caused a reduction in TSR but this could be corrected when the iodine content of the diet was increased.

The influence of other hormones on TSR has been studied by the administration of these hormones to normal animals, by the removal of the endocrine gland and by replacement therapy of the various hormones. These studies indicate that the sex hormones (estrogen and testosterone), insulin, and parathyroid hormones increase the TSR of normal animals. Melatonin, from the pineal gland, however, depressed TSR.

When the ovary, testis, adrenal glands, and pancreas (destruction of the B-cells) were removed, the TSR was depressed. The pineal gland removal, however stimulated an increase. By replacement therapy, the TSR was returned to normal or above.

These observations clearly indicate that the TSR is influenced by the

level of secretion of other hormones. If the secretion rate of a hormone is low, the TSR of the animal would be low. If optimal levels of several hormones are being secreted, this should tend to increase the TSR. If this is true, then rats with higher normal TSR might also have optimal secretions of the other hormones. This could be demonstrated by the comparison of the secretion rates of various hormones in groups of rats which differ markedly in TSR.

The question of the effect of a high TSR or by maintaining a high level of $L-T_4$ in the blood by replacement therapy upon the secretion rate of other endocrine glands should be explored.

The method of estimating the TSR of animals is also useful in determining the biological potency of analogues of $L-T_4$. By this method, $D-T_4$ has been observed to be only $1/10$ as active as $L-T_4$. Using $L-T_3$, it has been observed to be more than two times as active as $L-T_4$.

Comparisons of the subcutaneous and oral administration of thyroidal materials may also be made. In the rat, $L-T_4$, $L-T_3$ and thyroprotein (containing 1 percent $L-T_4$) were found to be about 37 percent as effective orally as by injection. In female fowls, the oral effectiveness has been shown to be about 55 percent, whereas in cattle the oral effectiveness is only about 10 percent.

The marked variation in the oral effectiveness of thyroidal substances in mammals and birds requires further study.

The use of a method for estimating the TSR of the rat has contributed greatly to our understanding of the functional activity of the thyroid gland. However, much further research must be conducted to determine the mode of inheritance of TSR and the development of strains of animals with optimal secretion of $L-T_3$ and $L-T_4$.

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