collagen fraction of guinea pig skin(11). Since it has been shown that collagen hydroxyproline arises only from the hydroxylation of bound proline(11), it may be concluded that the steroid depression of collagen synthesis is not related to an interference with the subsequent hydroxylation of proline.

Since the reduction in collagen synthesis appears to coincide with a depressed formation of other constituents (no change in collagen concentration), the primary steroid effect may be to depress a more basic cellular metabolic process. Thus, the steroid effect probably is not directed specifically at collagen synthesis, and certainly not at the conversion of bound proline to hydroxyproline.

Summary. Cortisone acetate and dexamethasone administered directly into 5-day-old cotton gauze-induced granulomas significantly reduced the wet and dry weights and the total hydroxyproline (collagen) in the granulomas. The total hydroxyproline remained a constant fraction of the dry weight. The injection of hydrocortisone acetate directly into developing (2 days) Carrageenin-induced granulomas significantly reduced the granuloma wet weight and the C^{14}-proline uptake and conversion to hydroxyproline. The hydroxyproline:proline specific activities ratio was not altered from the control value of 1.0 by steroid treatment.

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Estimation of Thyroid-Stimulating Hormone (TSH) Secretion Rates of New Hampshire Fowls.* (29540)

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A method of estimating the thyroxine secretion rate (TSR) of fowls has been described(1). It has been shown that the mean TSR of mature fowls is significantly lower during summer months than during winter months(2,3). TSR's measured during the period of rapid growth in the chick(4) were significantly higher than were TSR's of mature fowls, determined at comparable seasons of the year(2).

Therefore it seemed of interest to develop a method of directly estimating the thyroid-stimulating hormone secretion rate (TSH-SR) of fowls and to compare it with their

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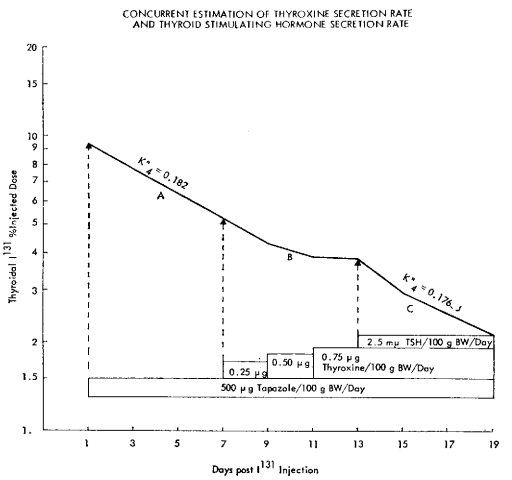

FIG. 1. Release rate A is normal release of thyroidal-I^{131} due to endogenous TSH and with recycling of I^{131} inhibited with the goitrogen tapazole to obtain the true release rate (K''_4). Brownell(11) suggested K'_4 as the thyroidal-I^{131} release rate with re-utilization of I^{131}. Premachandra *et al.*(12) suggested the term K''_4 to represent the "true" release rate when recycling was prevented with a goitrogen. Section B of the graph is period of TSR determination with increasing levels of thyroxine until thyroidal-I^{131} release is inhibited. Release rate C is renewed release of thyroidal-I^{131} with a known level of exogenous TSH (2.5 mμ), in addition to tapazole and TSR level of thyroxine, to determine TSH-SR. If K''_4 of C is decreased or increased from K''_4 of A then C is determined again with an increased or decreased level of TSH, respectively.

estimated TSR. A preliminary report of this research has been published(5). The object of the present paper is to describe the method of estimation of TSH-SR and to relate these observations to the TSR of the same birds.

Materials and methods. The method of estimating TSH-SR involved first the subcutaneous injection of 100 μc of I^{131} to be taken up by the thyroid gland. Then 500 μg of 1-methyl 1-2-mercaptoimidazole (tapazole)/100 g body weight/day was injected subcutaneously to prevent the recycling of I^{131} from metabolized thyroidal-I^{131}. This level of tapazole was given daily throughout the experiment. The true thyroidal-I^{131} release rate was then measured at 48-hour intervals for 6 days. The TSR of each bird was then determined by daily subcutaneous injection of L-thyroxine, the level of thyroxine being increased by 0.25 μg/100 g body weight each 48 hours until thyroidal-I^{131} release was blocked. This level of thyroxine was considered the TSR. Blockage of thyroidal-I^{131} release was then maintained throughout the remainder of the experiment by continued daily injection of this level of thyroxine. In this way endogenous TSH secretion and release were prevented (Fig. 1).

The estimation of TSH-SR is dependent upon the concept that an amount of exogenous TSH which would stimulate a thyroidal-I^{131} release rate equal to that of the endogenous thyroidal-I^{131} release observed, would indicate an equivalent rate of secretion of hormone.

Exogenous TSH at a given level was then injected subcutaneously daily for 6 days and the thyroidal-I^{131} release rate determined at 48-hour intervals. To compare the 2 thyroidal-I^{131} release rates, the data were plotted on semi-log paper since the release rate is exponential. If the endogenous and exogenous TSH-stimulated release rates were essentially equal, the estimated TSH-SR was considered determined. If the exogenous release rate was less or greater, then the amount of TSH injected was increased or decreased and the new 6-day release rate was determined. This procedure was continued until equality in the 2 release rates was obtained (Fig. 1). The TSH‡ preparations used had been labelled with their specific potencies. However, as a precaution, they were again assayed in our fowls and compared to a USP Reference Standard. The method of assay was to inject I^{131} into groups containing 8 hens each. The release of thyroidal-I^{131} was blocked with a TSR level of thyroxine and release of thyroidal-I^{131} was renewed, with each hen receiving 2.5 mμ TSH/100 g BW/day for 6 days. This dosage level of TSH was determined for each preparation according to the potency expressed by the respective manufacturer. The 6-day release of thyroidal-I^{131} was plotted on semi-log paper as a mean of the 8 hens of each group and the slopes of the release rates were determined. In no case did the

‡ Thyroid-stimulating hormone kindly supplied by Nat. Inst. Health, Jensen-Salsbery Labs., Armour Lab., and Parke Davis Co.

TABLE I. Relation of Thyroxine Secretion Rates to Thyrotrophic Hormone Secretion Rates of Fowls.

Group	No. birds/group	TSR, μg/100 g BW/day (mean)	TSH-SR, mμ/100 g BW/day (mean)
I (May-June) 2-year-olds	13	1.63 ± .23*	3.3 ± .43†
II (Sept.-Oct.) 2-year-olds	13	.85 ± .13*	1.7 ± .20†
III (Sept.-Oct.) 1-year-olds	22	.68 ± .10	2.4 ± .31

Group I significantly different from Group II. * $P<.01$ † $P<.005$

potency of a preparation deviate significantly from that expressed by the manufacturer as compared to the USP Reference Standard in these fowls. The results are presented in terms of USP units. Since the amount of hormone secreted is minute, it is expressed as milliunits (1 USP unit = 1000 mμ).

TSH-SR's of 13 2-year-old hens were determined during late spring to early summer and TSH-SR's of 13 2-year-old and 22 1-year-old hens were determined concurrently during late summer to early fall. The birds were maintained under normal environmental conditions. Their diet consisted of 10 lb of a vitamin and mineral premix per 1000 lb of mash mixed by a commercial feed company. No additional iodized salt was added as the feed constituents contained sufficient iodine to prevent iodine deficiency as can be seen by the relative low uptakes of I^{131}.

The thyroid counts were made by placing the birds in a funnel shaped holder, wrapped with lead sheets to block out body radiation and with an aperture in the area of the thyroid. This holder was made stationary 15 cm from a Nuclear Chicago, model DS-5, scintillation counter which included a 2″ × 2″ NaI crystal which was attached to a Picker X-Ray rate meter, model 5866A. The head of the scintillation counter was encased in a lead column which extended upward to the aperture of the holder or thyroid area of the bird. This greatly reduced background radiation.

Results. The first group of 13 2-year-old hens tested during late spring had a mean TSR of 1.63 μg with a range of 0.50 to 2.75 μg thyroxine/100 g BW/day and a mean TSH-SR of 3.3 with a range of 2.0 to 7.5 mμ TSH/100 g BW/day. The second group of 13 2-year-old hens tested during late summer had a mean TSR of 0.85 μg with a range of 0.25 to 1.50 μg thyroxine/100 g BW/day and a mean TSH-SR of 1.7 with a range of 0.5 to 2.5 mμ TSH/100 g BW/day. This was a significantly lower TSR and TSH-SR for these hens which had been subject to the high environmental temperature of the summer months (Table I). The third group of 22 1-year-old hens tested during late summer had a mean TSR of 0.67 μg and a range of 0.25 to 2.00 μg thyroxine/100 g BW and a mean TSH-SR of 2.4 with a range of 0.5 to 8.0 mμ TSH/100 g BW/day. These values do not differ significantly from those of 2-year-old birds tested concurrently.

The correlation coefficients within groups do not show the expected relationship of TSR to TSH-SR as they were +.29 for Group I; −.04 for Group II; and +.10 for Group III. However, only a limited number of birds were available for these experimental groups. A correlation coefficient of +.30 was found for the combined groups of 48 birds which had a mean TSR of 0.98 μg thyroxine/100 g BW and a mean TSH-SR of 2.5 mμ TSH/100 g BW/day (Table II).

The mean uptake of I^{131} by the thyroid was 12.49% with a range of 9.24 to 17.05% for Group I; 9.37% with a range of 5.68 to 18.08% for Group II; and 7.78% with a range of 3.71 to 14.87% for Group III. By group comparison mean uptakes of I^{131} appear to be correlated to mean TSR and TSH-SR. However, on an individual basis for all 48 birds the correlation coefficient of I^{131} uptake to TSR was +.04 and for I^{131} uptake to TSH-SR it was +.08.

Another parameter of thyroid function, the normal release rate of thyroidal-I^{131} was compared to TSR and TSH-SR. This was

TABLE II. Frequency Distribution of TSH and Thyroxine Secretion Rates of Fowls.

TSR, μg/100 g BW/day	Frequency distribution	TSH secretion rate, mμ/100 g BW/day (mean)
.25	7	2.1
.50	14	2.2
.75	9	2.5
1.00	1	2.0
1.25	5	2.6
1.50	4	2.0
1.75	0	—
2.00	4	2.6
2.25	0	—
2.50	3	4.3
2.75	1	4.0

Mean TSH-SR = mμ/100 g BW/day.
Mean TSR = .98 μg thyroxine/100 g BW/day.

done by a comparison of the numerical values of the slope of the line for the 6 days of normal release. Greater numerical value of the slopes indicate faster release rates. In this case the correlation coefficient of release rate of thyroidal-I^{131} to TSR was +.26 and for release rate of thyroidal-I^{131} to TSH-SR it was +.44.

Discussion. The estimation of the TSH-SR proposed is based upon the comparison of the normal thyroidal-I^{131} release rates, where recycling of I^{131} has been prevented, with that induced by subcutaneous administration of exogenous TSH after the TSR level of thyroxine has been administered to block endogenous TSH secretion. Since it is believed that endogenous TSH secretion is at a relatively uniform rate, whereas exogenous TSH by subcutaneous injection would be absorbed at varying rates daily, the estimate would be expected to be somewhat higher than that normally secreted by the anterior pituitary.

The present data indicate that there is little relationship between TSR and TSH-SR of these birds. In other words, some birds of low TSR secrete approximately as much TSH as some birds of high TSR. This would indicate that the thyroid glands of some birds with high TSR are more sensitive or responsive to a given level of circulating TSH than are other birds in their capacity to secrete thyroxine.

Insignificant correlations were observed between uptake of I^{131} by the thyroid and TSR or TSH-SR. This again indicates a possible difference of sensitivity to given levels of circulating TSH. In addition to this it is felt that uptake of I^{131} by the thyroid is a poor indication of thyroid activity in the fowl due to the variability of anatomical location of the thyroid in the fowl. Therefore, to make a meaningful study of thyroidal-I^{131} uptake in the fowl it would be necessary to remove the thyroids to determine accurately thyroidal-I^{131} content.

The normal release rate of thyroidal-I^{131} is suggested to be a much better parameter of thyroid gland function and although a highly significant correlation was not obtained between release rate and TSR or TSH-SR a relationship equal to that found between TSR and TSH-SR was observed.

In studies of the biological half-life ($t_{1/2}$) of TSH in human(6) and in the rat(7) it was shown that exogenous TSH disappears from the blood at faster rates in hyperthyroid states and at slower than normal rates in hypothyroid states. While we have not determined the half-life of TSH in these fowls, it has been shown(8) that in a hyperthyroid state (thyroxine at levels of 12 and 16 μg/100 g body weight) reduced the release rate of thyroidal-I^{131} when 0.1 USP units of TSH were administered.

However, some birds of low TSR have low TSH-SR and some birds of high TSR show high TSH-SR also. Therefore, one probably could not fully account for the differences in TSH-SR's on this basis.

Except in our preliminary report, no one has presented data on TSH-SR of animals by a method similar to the one proposed. The TSH-SR of a single dwarf beef calf was estimated as 40 USP units/100 lb body weight by the amount of TSH required to restore I^{131} uptake to normal in an animal given thyroxine at the TSR level(9). The TSH-SR of normal 350-400 g rats on the basis of 0.02 mμ/ml in the serum and a $t_{1/2}$ of 13.7 minutes was estimated as 16.8 mμ/day(7). On basis of 400 g rats their secretion rate would be 4.2 mμ/100 g BW/day. This represents secretion of about 0.1 of a pituitary content per day based upon a mean

potency of 135 mu/gland. This compares with a mean TSH-SR of 48 birds of 2.5 mμ/100 g body weight. By a similar method (6) the TSH in human plasma was reported as 0.003 mμ/ml, a t½ of 30 minutes, and an estimated TSH-SR of 260 mμ/day. No data on body weight were reported.

The short t½ of 13.7 minutes reported for the normal rat(7) would suggest that the t½ of TSH in the fowl would also be relatively short. Although it is believed that determinations of the blood t½ of hormones can result in valuable data, the t½ does not necessarily lead to an accurate measurement of the time duration in which an injected dose of TSH would exert an effect on the thyroid gland resulting in the release of thyroidal-I^{131}.

In fact, in a previous study(10) of fowls whose thyroidal-I^{131} release had been blocked with thyroxine, intravenous injections of TSH resulted in a renewed release of thyroidal-I^{131} and this release continued to occur 10 to 24 hours beyond the time of injection. This indicates that although TSH is cleared from the blood rapidly its effect on the thyroid is prolonged.

Summary. A method of estimating the daily TSH-SR of fowls is described, depending on a comparison of the normal thyroidal-I^{131} release rate where recycling of I^{131} is blocked by a goitrogen, endogenous TSH release is blocked with thyroxine at the TSR level and by producing a comparable thyroidal-I^{131} release rate with the daily subcutaneous injections of TSH assayed against a USP Reference Standard TSH. TSR and TSH-SR of 2-year-old New Hampshire hens were shown to be significantly higher during late spring than during late summer to early fall. No significant difference was observed between TSR and TSH-SR of 1-year-old and 2-year-old hens tested concurrently during late summer. However, there was no significant correlation between TSR and TSH-SR of these hens.

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Liver Tryptophan Pyrrolase Activity in Fed and Starved Female Rabbits.* (29541)

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Liver tryptophan pyrrolase (LTP) may be induced directly by its substrate l-tryptophan(1,2), or closely related analogues such as d-tryptophan(3,4), α-methyl tryptophan (3,4) and allylisopropylacetamide(5). Glucocorticoids(1,6) will also stimulate LTP activity. These substances are among the effective inducers of LTP in either normal or adrenalectomized rats. Many substances such as sodium chloride(7) and allylisopropyl-

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