Early Effects of Thyrotropin on Biosynthesis of Thyroglobulin in the Rat.* (32476)

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Studies of the incorporation of labeled amino acids into thyroid protein *in vitro*(1,2) and *in vivo*(3,4) indicate that thyroglobulin biosynthesis proceeds by way of sub-unit precursors. It is believed that a 12 S protein, with one-half the molecular weight of 19 S thyroglobulin, forms aggregates with a sedimentation coefficient, in the rat, of approximately 16 S(2,4). Maturation of 16 S into 19 S thyroglobulin probably involves a change in the molecular conformation resulting from iodination of the protein(2).

Stimulation by thyrotropin (TSH) of the incorporation of labeled amino acid into thyroid protein has been observed in thyroid slices(5) from animals pre-treated with the hormone. Such effects of TSH have not been demonstrated, however, when the hormone was added to the preparations *in vitro*(5,6). Klitgaard and coworkers(7) found an increase in the labeling of thyroid protein from ^{14}C L-tyrosine and L-arginine *in vivo* in chicks pretreated with TSH for 2 days. In none of the previous studies were early effects of TSH on protein synthesis described nor were individual thyroid proteins analyzed.

The present work concerns the early effects of TSH on the time-course of labeling of thyroglobulin and its presumed precursors following *in vivo* pulse-labeling of the thyroid glands of rats. The results indicate a prompt stimulation by TSH of several phases of thyroglobulin biosynthesis. Portions of this study have been reported in abstract form (8).

Materials and methods. Male Sprague-Dawley rats, weighing 150 to 200 g, were fed either a low-iodine diet (Remington) for 10 to 14 days or Purina Laboratory Chow. L-thyroxine, 15 μg, was injected subcutaneously at 16 hours and at 2 hours before administration of labeled amino acids, in order to increase the sensitivity of the rats to exogenous TSH. A mixture of uniformly labeled (^{14}C) amino acids (New England Nuclear Corp.), specific activity 40 mc per milliatom carbon, was injected intraperitoneally in a dose of 35 μc per rat. TSH (Thytropar, Armour, or a partially purified preparation of ovine TSH supplied as a gift from the Endocrinology Study Section of NIH) was injected intraperitoneally in a dose of 3 units at various intervals before or after administration of labeled amino acids. From 1 to 6 hours after pulse-labeling, rats were killed by exsanguination from the heart under ether anesthesia. At each time interval, the thyroid glands from 2 or 3 animals were pooled and homogenized in 1.0 ml cold 0.15 M sodium chloride, buffered to pH 6.8 with 0.01 M potassium phosphate. The homogenate was centrifuged at 75,000 $\times$ *g* for 60 minutes. The supernate was dialyzed for 16 hours at 4° against 30 ml of the same buffered saline in order to remove non-protein radioactivity. In some experiments an aliquot of the 75,000 $\times$ *g* supernate was dialyzed against 0.01 M NH_4OH, pH 10. Following dialysis an aliquot was taken for ^{14}C measurement and the remainder of the protein solution was analyzed by sucrose density gradient centrifugation as described previously(4). The optical density of each gradient fraction was read at 280 mμ and the total ^{14}C was determined by liquid scintillation counting.

The extent of contamination of thyroid extracts by labeled plasma protein was estimated from the total ^{14}C content and the sedimentation pattern of plasma proteins obtained at various intervals following injection of labeled amino acids. It was determined that such contamination would account for no more than 10% of the radioactivity in 3-8 S proteins and less than 2% of that in 12-19 S proteins of the thyroid extracts.

Results. The effects of TSH are summarized in Table I. In those experiments in which TSH was injected 2 hours or longer before

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TABLE I. Effect of TSH on Incorporation *in vivo* of ^{14}C Amino Acid into the Soluble Proteins of the Rat Thyroid. (In each experiment the glands from 2 or 3 rats were pooled.) Total incorporation is expressed as counts/min ^{14}C in one gland (2 lobes). Last column lists the sedimentation constant of the major radioactive peak determined by sucrose density-gradient ultracentrifugation.

Diet	Interval between administration and thyroidectomy		^{14}C thyroid protein	
	^{14}C Amino acid	TSH (3 units)	Counts/min per gland	Sed. constant of peak
a) Low-iodine	1 hr	—	788	12 S
	1 hr	4 hr†	1165	12 S
b) " "	2 hr	—	720	12 S
	2 hr	4 hr†	1122	15 S
c) " "	3 hr	—	508	15 S
	3 hr	4 hr*	464	18 S
d) " "	6 hr	—	643	15 S
	6 hr	4 hr*	684	18 S
e) Purina	2 hr	—	578	12 S
	2 hr	4 hr†	1020	18 S
f) "	6 hr	—	438	18 S
	6 hr	4 hr†	382	18 S

* Armour TSH.
† NIH S-4 TSH.

administration of ^{14}C amino acid (Experiments a, b and e), the total amount of ^{14}C incorporated was significantly increased. When TSH was given within 1 hour of pulse-labeling or two hours afterward (Experiments c, d and f), total ^{14}C incorporation was not affected. Since the duration of the pulse following intraperitoneal injection of ^{14}C amino acids to rats is of the order of 10 minutes(4), one would not expect TSH, given after the labeled amino acid, to affect the incorporation of ^{14}C by the thyroid. In 2 experiments (c and d) in which the total ^{14}C content of soluble protein was not affected, TSH did nevertheless cause a shift in the position of label from 15 S to 18 S protein.

This phenomenon is illustrated in Fig. 1 which shows the sedimentation patterns obtained in Experiment c of Table I. In thyroid extracts which had been dialyzed at pH 6.8 prior to density-gradient centrifugation (Fig. 1 a), the major ^{14}C peaks are in 15 S and in 3-8 S fractions. In the TSH-treated group (Fig. 1 b) the label was shifted from 15 S to 18 S.

Portions of the thyroid extracts from the same experiment were dialyzed at pH 10 prior to density-gradient centrifugation. As shown in Fig. 1 c and d, label appears in 12 S protein, presumably as a result of disaggregation of more rapidly sedimenting species (see discussion). In the control group (Fig. 1 c) less than 5 per cent of the total ^{14}C incorporated is found in alkali-stable 18-19 S protein. In the TSH-treated group (Fig. 1 d) 15% of total ^{14}C is in alkali-stable 18-19 S protein. In this experiment, therefore, TSH caused an increase in the amount of label in 18-19 S thyroglobulin and decreased the label in more slowly sedimenting proteins, the total ^{14}C incorporated into all soluble proteins remaining constant.

As shown in Fig. 2, which represents the results obtained in Experiment e of Table I, TSH had a marked effect on both total ^{14}C incorporation and the sedimentation pattern of labeled thyroid protein. The extracts were dialyzed at pH 6.8 prior to density-gradient centrifugation. In the control group, radioactivity was present in 12 S and in 3-8 S proteins. In the rats given TSH (3 units) 2 hours before pulse-labeling, 18-19 S thyroglobulin contains most of the label, and 12 S protein is relatively unlabeled. Although the amount of ^{14}C incorporated into 3-8 S proteins was increased by TSH in this experiment, the ratio of label in 3-8 S over that in the total soluble proteins was diminished by TSH (Fig. 2).

Discussion. The results of the present study show that TSH produces an increased incorporation of labeled amino acid into thyroid protein when the hormone is given 2-4 hours

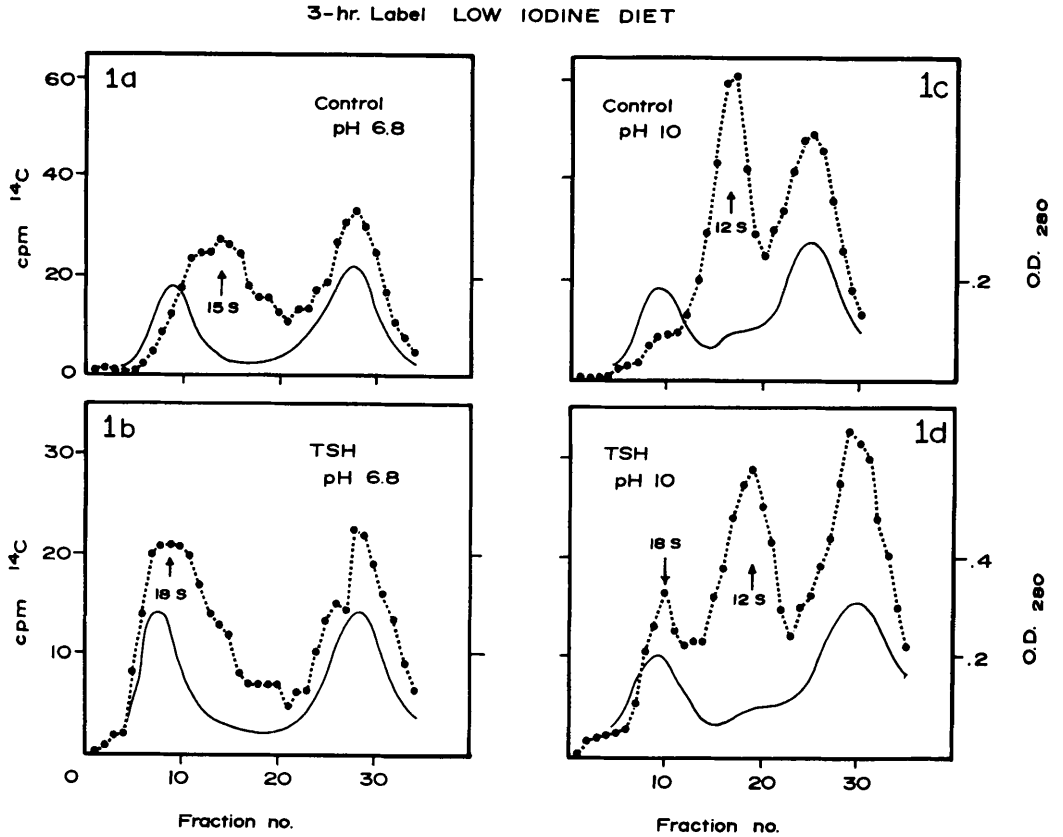

FIG. 1. Sucrose density-gradient centrifugation analysis of soluble thyroid proteins from rats pulse-labeled *in vivo* with ^{14}C amino acids 3 hrs before sacrifice. All animals were given L-thyroxine. Groups (b) and (d) were given TSH 1 hr before labeling (4 hrs before sacrifice). In (a) and (b) thyroid extracts were dialyzed against pH 6.8 buffer prior to sedimentation on the density-gradient. In (c) and (d), portions of the same extracts were dialyzed at pH 10. In each panel, the bottom of the gradient tube is on the left. Optical density is shown by a solid line; radioactivity, by an interrupted line. Sedimentation coefficients were estimated by reference to the optical density peak at 19 S.

before pulse-labeling. The possibility that the enhanced incorporation of label may merely reflect a decreased pool size of amino acid precursors is unlikely since there is evidence that TSH causes an increase in the free amino acid content of the thyroid(9). Therefore, we can assume that the TSH-induced effect on the incorporation of the tracer represents a true stimulation of protein synthesis.

There are several possible mechanisms by which TSH might stimulate the formation of protein in the thyroid. An increase in the uptake of amino acids from the blood is one possibility. There is some evidence from studies on thyroid slices(10) and isolated thyroid cells(11) that TSH, added *in vitro,* stimulates the uptake of alpha-aminoisobutyric acid, a non-metabolized amino acid. However, others(12) have failed to find such an *in vitro* effect of TSH. Another mechanism may involve an increase in ribosomal activity. Hall and Tubman(13) have shown that TSH, added *in vitro,* promptly stimulates the formation of ribonucleic acid from radioactive purine precursors in thyroid slices. These and other related effects of TSH remain to be investigated in the intact animal.

The results of the present work also demonstrate an early effect of TSH on the later stages of thyroglobulin biosynthesis, *i.e.,* aggregation of 12 S sub-units and maturation. The acceleration by TSH of thyroglobulin

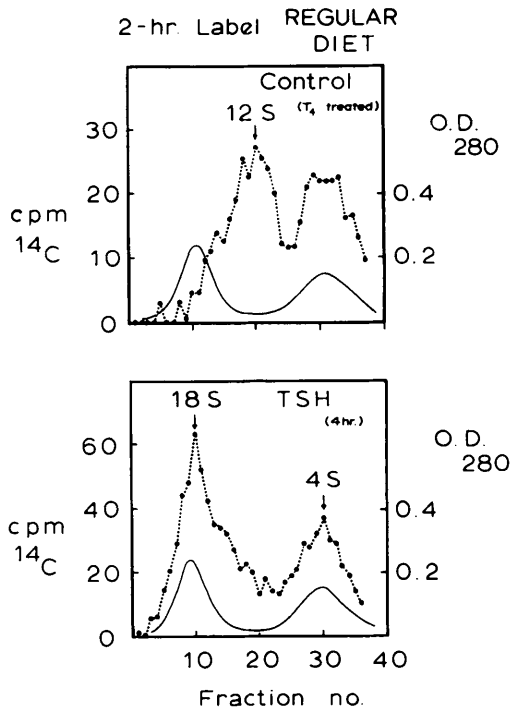

FIG. 2. Sedimentation patterns of thyroid proteins from rats pulse-labeled with ^{14}C amino acids 2 hr before sacrifice. The lower panel shows results in rats given TSH 4 hrs before sacrifice. The thyroid extracts were dialyzed against pH 6.8 buffer prior to analysis.

maturation was evident both from the shift in the sedimentation coefficient of labeled protein from 15-16 S to 18-19 S and from the observed increase in the proportion of label in alkali-resistant 18-19 S protein. Lissitzky *et al*(14) have shown that maturation of thyroglobulin is associated with a decreasing tendency of the molecule to disaggregate into 12 S sub-units at alkaline pH. We have confirmed this finding(4). The apparent stimulation by TSH of aggregation and maturation may be merely secondary to the increase in the synthesis of polypeptide sub-units.

The TSH-induced acceleration of maturation may, on the other hand, be related to the known effects of this hormone on iodine metabolism. An increase in organic binding of iodine(15) and an increase in iodothyronine synthesis(16) have been observed within one hour after injection of TSH in rats. If maturation of thyroglobulin is dependent upon iodination reactions, as has been suggested (2), then the effect of TSH on maturation noted in this study may be secondary to a stimulation of iodine metabolism. Experiments to test this possibility are now in progress.

Summary. The effect of a single dose of TSH on the incorporation *in vivo* of ^{14}C labeled amino acids into thyroid proteins of the rat have been studied. Soluble thyroid proteins were analyzed by sucrose density-gradient ultracentrifugation. TSH, injected 2 to 4 hours before pulse-labeling, increased the total amount of ^{14}C in protein and accelerated the appearance of label in 19 S thyroglobulin. When TSH was given within one hour of labeling or two hours afterwards, total ^{14}C incorporation was unaffected, but the label appeared in mature (18-19 S) thyroglobulin at the expense of label in an immature (15-16 S) precursor protein. The results suggest that TSH produces an early stimulation of several phases of thyroglobulin biosynthesis.

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