The Effect of LATS and TSH on Protein and RNA Synthesis in Isolated Thyroid Cells[1] (37432)

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(Introduced by S. Rogers)

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LATS (Long-Acting Thyroid Stimulator) shares with TSH several metabolic effects on the thyroid gland (1–6). It is believed that the two hormones exert their effects by binding to the thyroid cell membrane (7) which results in an increase in the conversion of ATP to cAMP (8). The two hormones differ in their molecular weight (9, 10), their origin, biological half lives (11), and possibly other properties.

The present report lends additional evidence for a possible difference in the action of TSH and LATS on isolated thyroid cells. Under the conditions of these experiments the incorporation of ^{3}H-leucine and ^{3}H-uridine into protein and nucleic acid, respectively, by thyroid cells was stimulated slightly or not at all by TSH. LATS, on the other hand, stimulated the incorporation of the protein and nucleic acid precursors by about 2-fold. The data also show that the stimulation of protein synthesis is partially dependent upon new RNA synthesis.

Materials and Methods. Chemicals. TSH was purchased under the trade name Thyrotropar from Armour and Co., Kankakee, Illinois. Leucine-4, 5-^{3}H (54 Ci/mmole) and uridine 6-^{3}H (10 Ci/mmole) were purchased from New England Nuclear Co., Boston, Mass. Actinomycin D was purchased from Schwarz/Mann, Orangeburg, New York. Puromycin and human gamma-globulin were purchased from Nutritional Biochemical Co., Cleveland, Ohio. Minimum Essential Media (MEM), and calf serum were obtained from North American Biologicals, Rockville, Md. The calf serum was dialyzed for 3 days against NKM solution (130 m*M* NaCl, 5 m*M* KCL and 8 m*M* $MgCl_2$) before being used.

Preparation of LATS-IgG. LATS-IgG was isolated according to the method of Sober and Paterson (12). An equal volume of saturated ammonium sulfate was added to the serum of blood from patients with Graves' disease and the mixture was stirred for 30 min in the cold. The precipitate was separated by centrifugation at 500 rpm in a Sorval RC-2 refrigerated centrifuge. The precipitate was suspended in 0.01 *M* phosphate buffer, pH 7.8, containing normal (0.15 *M*) saline (PBS) and then the ammonium sulfate precipitation and centrifugation were repeated. The precipitate was again suspended in PBS and dialyzed overnight against 0.01 *M* phosphate buffer at pH 7.8 at 4°. The dialysate was applied onto a 2.5 × 45 cm preconditioned DEAE–cellectacel column. The LATS-IgG fraction was eluted with 0.01 *M* phosphate buffer, pH 7.8. The eluate was concentrated by Amicon filtration. The purity of the IgG fraction was established by immunoelectrophoresis of the protein. The biological activity of the LATS-IgG was determined by the McKenzie method (13).

Isolation of thyroid cells. Bovine or porcine thyroid glands obtained from Armour and Co., Memphis, Tennessee, were placed on ice shortly after the animals were slaughtered. Within one hour, the glands were freed of fat and connective tissue, placed in Puck's saline solution, and minced. The minced tissue was washed in cold Puck's saline solution several

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times. Aliquots were placed into sterile flasks which contained, per gram of tissue: Puck's saline, 50 ml; collagenase, 15 mg; penicillin-G, 500 units; streptomycin 500 μg; and trypsin, 10 mg/ml. The mixture was incubated at 37° with constant stirring for two hours. The supernatant containing separated cells was passed through a Renwal filter and was then centrifuged at 500*g* for 10 min. The pellets were washed several times with Puck's saline to remove fat and red blood cells. The clean cells were suspended in 1 ml of MEM without glutamine or serum.

Incubation conditions for the uptake of ^{3}H-leucine and ^{3}H-uridine by isolated thyroid cells. In all experiments, Eagle's Minimum Essential Medium without glutamine was used with the following additions: glutamine (2 m*M* final concentration); calf serum (10%); and antibiotics (streptomycin and penicillin) 0.1%. In all experiments, an accurate measurement of the packed-cell volume was used in the incubation flasks and this volume was the criterion for equivalent numbers of cells. In the protein synthesis studies, 5 μCi of ^{3}H-leucine/ml of incubation mixture was added. For the RNA synthesis experiment, 10 μCi of ^{3}H-uridine were added per milliliter of incubation mixture. The cells were allowed to equilibrate at 35° in moisturized air containing 5% CO_2 for 5 min before the labeled precursors were added. Actinomycin D and puromycin were added at the concentrations and times indicated in the figures. For the LATS-IgG studies, normal IgG was utilized as the control. The TSH controls contained all the ingredients of the reaction mixture, except for the stimulators and normal IgG.

The incorporation of ^{3}H-leucine was determined by collecting the cells by centrifugation at 500*g* and treating them with 0.5 ml of 10% trichloroacetic acid (TCA). The samples were heated at 90° for 15 min. Five milliliters of 5% TCA was added and the precipitate was collected. The precipitate was washed once with 5% TCA and once with 70% ethanol. It was then dissolved in 0.6 ml NCS reagent and 0.5 ml of the solution was diluted with 15 ml of scintillation fluid containing toluene and 4% Omnifluor (14). The counts were determined in a Nuclear Chicago Mark I Liquid Scintillation Spectrometer, and the data reported as cpm/0.5 ml of the NCS solution.

The uptake of ^{3}H-uridine was determined by isolating the total cellular RNA (15). Cells were sedimented after incubation for the desired time and were washed with acetate buffer at pH 5.1. The washed cells were then suspended in the acetate buffer containing 0.3% dodecyl sulfate (SDS) and were homogenized in a tightly fitted homogenizer. When the cells were completely disrupted, an equal volume of saturated phenol containing 8-OH quinoline and cresol was added to the homogenate. The mixture was incubated at 65° for 15 min and then centrifuged. The aqueous layer was removed and an equal volume of the saturated phenol was added. The mixture was centrifuged and the aqueous layer was removed. The RNA was precipitated from the combined saturated phenol washings by adding 2.5 volumes of ethanol containing 2% sodium acetate. The precipitated RNA was collected and the optical density and the radioactivity were determined. The results were calculated as cpm/OD.

For the nuclear RNA studies, the nuclei were isolated by homogenizing the cells in 5% citric acid (16) until nuclei were free of any cytoplasmic material when observed under the microscope. The homogenate was centrifuged and the precipitate was suspended in 0.25 *M* sucrose containing 1.5% citric acid. The mixture was underlayered with 0.88 *M* sucrose that contained 1.5% citric acid, and the nuclei were pelleted by centrifugation. The nuclear pellet was suspended in a 0.3% SDS solution and then homogenized. An equal volume of aqueous saturated phenol was added to the homogenate and the RNA was isolated as above.

The ribosomal RNA isolation was achieved by washing the cells with 50 m*M* Tris buffer at pH 7.4 containing 50 m*M* KCl and 5 m*M* $MgCl_2$. The cells were homogenized in the same buffer and the homogenate was centrifuged at 10,000*g* for 10 min. The supernatant was made 0.5% with respect to deoxycholate, rehomogenized, and then centrifuged at 30,000*g* for 10 min (17). The supernatant was placed in polycarbonate tubes and under-

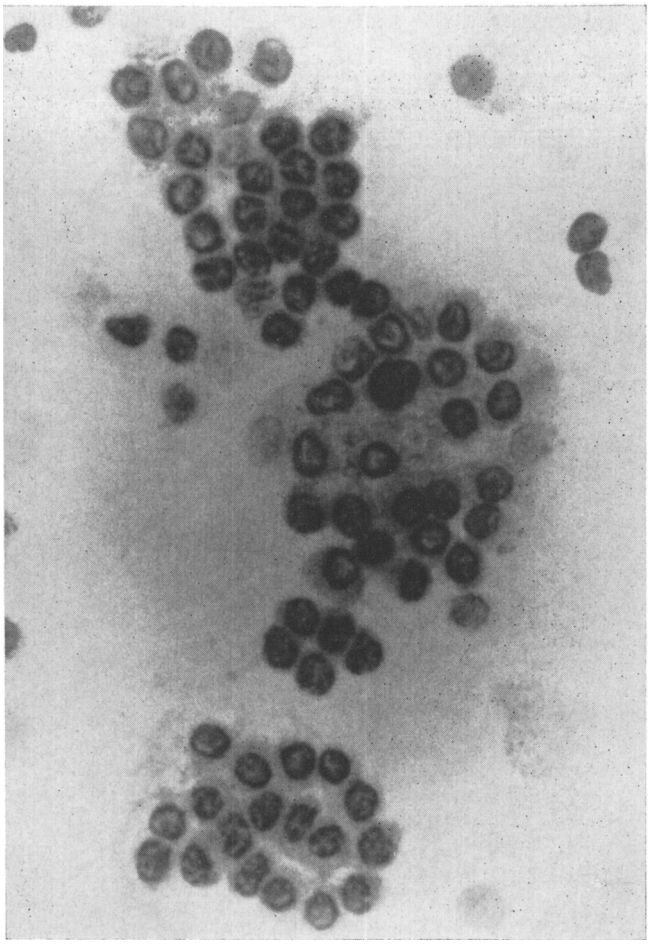

FIG. 1. Thyroid cells isolated from bovine thyroid glands by a combination of trypsin and collagenase digestion as outlined in "Methods." Magnification is 400×.

layered with ⅓ volume of 1 *M* sucrose solution and centrifuged at 105,000*g* for 16 hr. The RNA was isolated from the pelleted polysomes by the SDS and phenol method.

Results. Isolation of thyroid cells. Figure 1 demonstrates a group of cells representative of those obtained by the collagenase and trypsin continuous digestion. The cells were free of any connective tissue, and although they were frequently clumped or in sheets, no structural organization remained. The viability of the cells ranged 70–90% as determined by the exclusion of the dye trypan blue. Under the conditions of isolation, the cell yield varied from 0.4 to 1 ml of packed cells/50 g tissue. The main problem encountered in isolating the cells was the difficulty of separating fat and red blood cells. The yield in general was greater when pork thyroids were utilized, rather than bovine.

Effect of TSH and LATS-IgG on the uptake of ^{3}H-uridine by isolated thyroid cells. The isolated thyroid cells incorporated ^{3}H-uridine slowly but progressively during the 3-hr incubation in the presence of no added stimulator. The effect of adding IgG was variable, but in general was negligible. In the presence of LATS-IgG, which displayed biological activity as shown in Table I, there was a 2-fold stimulation of the labeled nucleotide incorporation into total cellular RNA over the incorporation by cells incubated with normal IgG (Fig. 2 represents the data of a sample experiment). Table II

TABLE I. Comparison of Activity in the McKenzie Assay of LATS-Containing Serum and LATS-IgG Derived from the Serum.

Stimulant	% Increase in ^{131}I content of blood[a]		
	Interval after injection		
	3 hr	7 hr	24 hr
Patient: M. P.			
Serum, 0.5 ml	215	530	222
LATS-IgG, 3 mg (Prep. 1)	103	226	181
Patient: M. P.			
Serum, 0.5 ml	68	315	163
LATS-IgG, 3 mg (Prep. 2)	30	139	95
Patient: M. P.			
Serum, 0.5 ml	210	683	345
LATS-IgG, 3 mg (Prep. 3)	139	318	232
Patient: C. H.			
Serum, 0.5 ml	107	407	285
LATS-IgG, 3 mg	50	290	154

[a] Five mice were injected with each stimulant. The % calculation was made from the average cpm of the 5 mice, and was compared to the count at zero time.

reports percent increase in the incorporation of labeled neucleotide into the cellular RNA in the presence of LATS-IgG over that occurring in the presence of normal IgG. The results are taken from duplicates in each run. The data also indicate that TSH exerted no stimulatory effect on the uptake of the labeled nucleotide by the isolated thyroid cells.

Effect of LATS-IgG and TSH on ^{3}H-leucine incorporation into protein of isolated thyroid cells. Figure 3 illustrates the influence of both TSH and LATS-IgG on the incorporation of labeled leucine into hot trichloroacetic acid (TCA) insoluble material from thyroid cells. The net effect of TSH, under these experimental conditions, was negligible, although in individual experiments, there

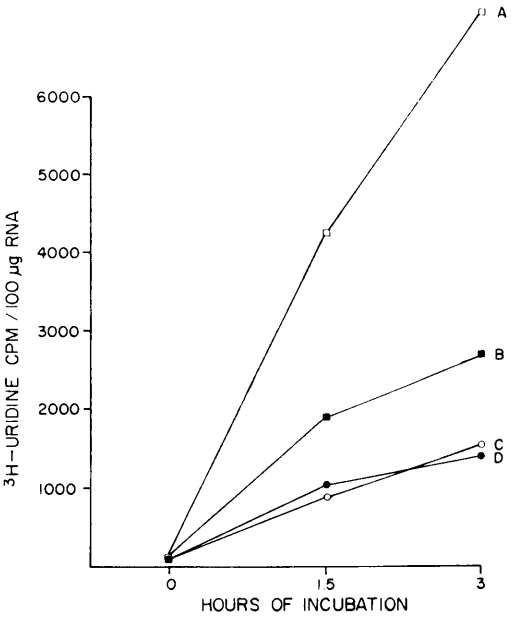

FIG. 2. Effect of LATS-IgG, TSH and normal IgG on ^{3}H-uridine incorporation into RNA of isolated thyroid cells. The cells were incubated in Eagle's MEM containing antibiotics (1%); calf serum (10%); L-glutamine (2 m*M*) and ^{3}H-uridine at a concentration of 1 nmole/ml) (10 µCi/ml). The mixture was incubated at 35° for time indicated. (A) LATS-IgG (3 mg/ml); (B) normal IgG (3 mg/ml); (C) TSH (200 mU/ml); (D) TSH—control, to which nothing was added.

TABLE II. Increase of ^{3}H-Uridine Incorporation into RNA of Isolated Thyroid Cells in the Presence of LATS-IgG or TSH.

Experiment	% Increase over control			
	Incubation time in hours			
	LATS-IgG (3 mg/ml of media)		TSH (200 mU/ml media)	
	1.5	3	1.5	3
1	114.2 ± 2.66	133.1 ± 0.17	−2.8 ± 2.36	−1.6 ± 1.84
2	106.6 ± 1.73	137.4 ± 2.66	3.5 ± 1.28	3.9 ± 1.32
3	107 ± 0.98	127.9 ± 2.83	3.3 ± 1.15	2.5 ± 0.52

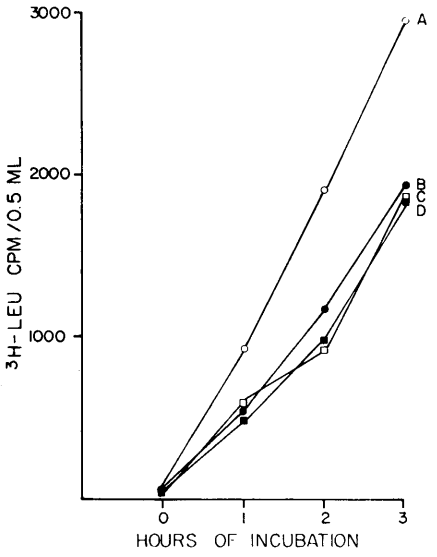

FIG. 3. Effect of LATS-IgG, TSH and normal IgG on the incorporation of ^{3}H-leucine by isolated thyroid cells. Incubation was carried out at 35° in modified Eagle's MEM containing antibiotics (1%); calf serum (10%); L-glutamine (2 m*M*) and 0.1 nmole of ^{3}H-leucine (5 μCi/ml). (A) LATS-IgG (3 mg/ml); (B) normal IgG (3 mg/ml); (C) TSH (200 mU/ml); (D) control for TSH, to which nothing was added.

occasionally was slight stimulation or slight inhibition (Table III). LATS-IgG, alone, as in the case of uridine incorporation, consistently exerted up to one- to two-fold stimulation of leucine incorporation when compared to normal IgG.

In these experiments, LATS-IgG was added on the basis of protein concentration per assay tube rather than LATS activity. The maximal stimulation by LATS-IgG was obtained at a protein concentration of 3 mg/ml of incubation mixture. An increase in the protein concentration caused an inhibitory effect which can be seen in Fig. 4, while at lower protein concentration LATS was less effective. Thus, 3 mg of protein/ml was used in all experiments. Also, at the protein concentration of 3 mg/ml, the LATS-IgG reached nearly its maximal effect after three hours of incubation, and this was taken as the time to terminate the incubation in all experiments unless stated otherwise.

Effect of puromycin and actinomycin D (AMD) on LATS-IgG activity. Puromycin at a concentration of 20 μg/ml, when added at mid-incubation, inhibited any further incorporation of ^{3}H-leucine by cells in the presence of either LATS-IgG or normal IgG (Fig. 5). A statistical analysis of the data is presented in Table IV. Puromycin inhibited all leucine incorporation, as expected, when added at the beginning of the incubation. At lower concentrations (5 μg/ml), the inhibition was minimal.

At a concentration of 5 μg/ml, AMD displayed no significant inhibition of the incorporation of labeled uridine into cellular RNA. An inhibition of about 40%, however, was observed with a concentration of 10 μg/ml. At a higher concentration, 20 μg/ml, the antibiotic inhibited 70% of the labeled nucleotide incorporation. The inhibitory effect was displayed whether the antibiotic was added at the outset of the incubation or midway in the incubation, *i.e.*, 1½ hr after LATS-IgG has been added to the incubation mixture. IgG from the serum of patient with high titers of LATS in McKenzie

TABLE III. ^{3}H-Leucine Uptake by Isolated Thyroid Cells in the Presence of LATS-IgG, Normal IgG or TSH.

	% Increase over control			
	Time of incubation in hours			
	LATS-IgG (3 mg/ml of media)		TSH (200 mU/ml media)	
Experiment	1.5	3	1.5	3
1	41.7 ± 0.63	62.6 ± 0.15	—4.9 ± 3.18	—6.2 ± 3.89
2	42.9 ± 0.4	63.1 ± 0.40	6.2 ± 2.36	8.1 ± 3.62
3	42.0 ± 0.18	62.2 ± 0.63	6.1 ± 2.30	6.2 ± 2.36
4	41.8 ± 0.15	61.5 ± 0.40	—1.3 ± 1.40	—1.6 ± 1.84

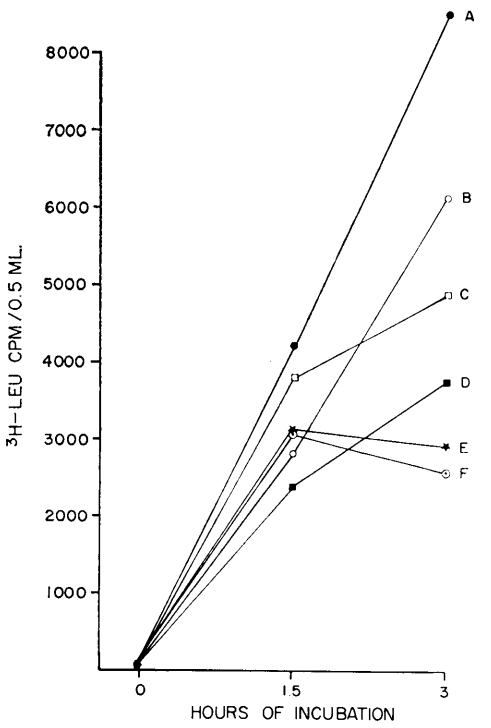

FIG. 4. Study of the relationship of LATS-IgG and normal IgG concentration to their effect on ^{3}H-leucine incorporation by thyroid cells. The incubation conditions as outlined for Fig. 3. (A) LATS-IgG (3 mg/ml); (B) normal IgG (3 mg/ml); (C) LATS-IgG (1.5 mg/ml); (D) normal IgG (1.5 mg/ml); (E) LATS-IgG (4.5 mg/ml); (F) normal IgG (4.5 mg/ml).

units consistently gave a reproducible, proportional stimulation, although the absolute number of counts varied from experiment to experiment, whether the source of LATS-IgG was the same or different. The variation in the counts, within experimental error, was attributed to the number of viable cells in the assay mixture. In contrast to LATS, TSH displayed no significant stimulatory effect on the incorporation of the nucleotide into total cellular RNA.

Since TSH did not stimulate the incorporation of the labeled nucleotide into cellular RNA, the effect of exogenous cyclic AMP on the incorporation in both the presence and the absence of thyrotropin was studied. The isolated thyroid cells were incubated in the tissue culture media with or without TSH, labeled nucleotide and 10 μg/ml of cAMP. The cyclic nucleotide was added either at the outset of incubation of 1½ hr after incubation started. The results of the experiment presented in Table V indicate that cAMP in the presence of TSH exerted a definite stimulatory effect (80%) on the incorporation of labeled uridine into total cellular RNA. The stimulation appears to be a combined effect exerted by both the cAMP and the thyrotropin, since cAMP alone exerted only a small stimulation (20%). A similar stimulatory effect by TSH on the uptake of the labeled leucine (36%) was observed in the presence of cAMP (10 μg/ml incubation media). cAMP alone exerted a minimal effect on the leucine incorporation, but functioned in a synergistic manner with TSH as shown in Table VI.

To determine whether or not the stimulation of protein synthesis by LATS was correlated with and dependent upon the RNA synthesis, the cells were incubated under the following conditions: (a) Tissue culture

TABLE IV. Effect of Puromycin[a] on the Uptake of ^{3}H-Leucine in the Presence of LATS-IgG and Normal IgG.

	% Increase between LATS-IgG/normal-IgG			
	Incubation time in hours			
	Without puromycin		With puromycin	
Experiment	1.5	3.0	1.5	3.0
1	39.3 ± 0.74	81.8 ± 0.34	40.1 ± 0.34	40.8 ± 0.28
2	40.4 ± 0.11	80.7 ± 0.91	41.3 ± 0.35	42.1 ± 0.45
3	42.0 ± 0.81	82.5 ± 0.75	40.8 ± 0.06	41.2 ± 0.05

[a] Puromycin (20 mg/ml) was added after 1½ hr of incubation.

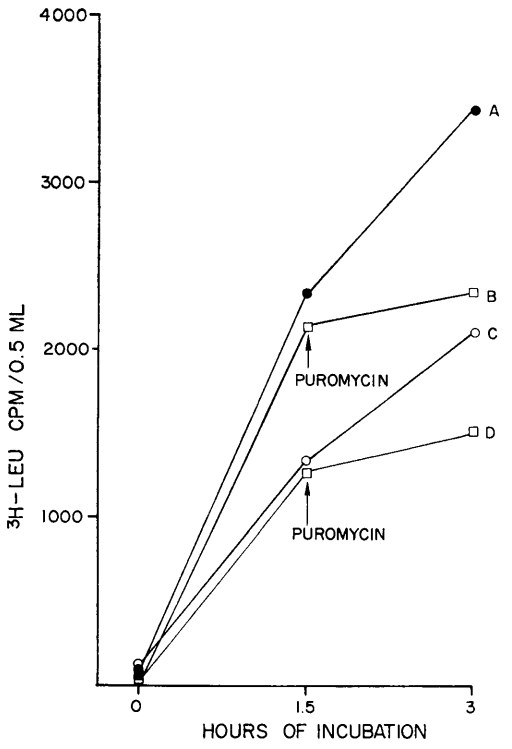

FIG. 5. Effect of puromycin on the incorporation of ^{3}H-leucine in the presence of LATS-IgG and normal IgG. Cells were incubated at 37° with LATS-IgG (3 mg/ml) or normal IgG (3 mg/ml) and ^{3}H-leucine. At the end of 1½ hr incubation, puromycin (20 μg/ml) was added. (A) LATS-IgG without puromycin; (B) LATS-IgG with puromycin added after 1½ hr; (C) normal IgG without puromycin; (D) normal IgG with puromycin added after 1½ hr.

media containing labeled leucine and LATS-IgG or normal IgG, represented by the lines A and B in Fig. 6, respectively; (b) Media containing either LATS-IgG (line C) or normal IgG (line D), but with labeled leucine added after 1½ hr of incubation; (c) Media with AMD and labeled leucine added at zero time in the presence of either LATS-IgG (line E) or normal IgG (line F); and (d) Media containing AMD added at zero hour of incubation in the presence of either LATS-IgG (line G) or normal IgG (line H), with ^{3}H-leucine added after 1½ hr of incubation. The result presented in Fig. 6 is a compilation of two experiments, and indicates that the stimulatory effect of LATS-IgG on protein synthesis was inhibited by the AMD in concentrations (20 μg/ml) sufficient to inhibit about 70% of new RNA synthesis. Since AMD did not completely inhibit the RNA synthesis, LATS continued to exert its stimulatory effect as can be seen in line E compared to line F for normal IgG, in Fig. 6. The inhibition of leucine incorporation was paralleled with the inhibition of the incorporation of labeled uridine into cellular RNA. This finding suggests that the stimulation of protein synthesis by LATS is dependent, at least in part, on the stimulation in RNA synthesis.

Effect of LATS-IgG on the nuclear RNA (nRNA) and ribosomal RNA (rRNA.) In order to determine whether LATS stimulated the synthesis of specific species of RNA, the total cellular RNA was fractionated into nuclear and ribosomal RNA. The data in Table VII demonstrates that LATS promotes the synthesis of both RNA's. The variation in values from one experiment to another was probably due to the titre of LATS in the IgG fraction as well as the number of viable cells in the incubation mixtures. This data does not rule out the promotion of synthesis of individual species of nuclear RNA which are

TABLE V. The Effect of cAMP and TSH on the Incorporation of ^{3}H-Uridine by Isolated Thyroid Cells.[a]

Addition		^{3}H-Uridine incorporation (cpm/OD)		
		Incubation time		
Stimulator	cAMP	0 hr	2 hr	% Increase
None	None	125	2861	
None	@ 0 hr	111	3515	23.0%
None	@ 1 hr	140	3240	13.0%
TSH	None	98	3300	
TSH	@ 0 hr	105	6000	80.0%
TSH	@ 1 hr	123	4650	41.0%

[a] The incubation mixture (2 ml) contained isolated thyroid cells (0.5 ml); Eagle's Minimum Essential Media supplemented with 2 m*M* glutamine; antibiotics (1%); calf serum (10%); cyclic AMP [cAMP (10 μg/ml)]; thyrotropar (200 mU/ml); and ^{3}H-uridine (10 μCi/ml). Incubation was at 37°. Thyrotropar was omitted from the control flasks.

TABLE VI. The Effect of cAMP and TSH on the Incorporation of ^{3}H-Leucine by Isolated Thyroid Cells.[a]

Addition		^{3}H-Leucine incorporation (cpm/0.5 ml)		
		Incubation time		
Stimulator	cAMP	0 hr	2 hr	% Increase over control
None	None	177	2230	
None	@ 0 hr	125	2735	17.7%
None	@ 1 hr	167	2545	14.0%
TSH	None	170	2377	
TSH	@ 0 hr	155	3222	36.0%
TSH	@ 1 hr	163	2870	20.8%

[a] Isolated thyroid cells (0.5 ml) were added to flasks containing 1.5 ml of Eagle's Minimum Essential Medium supplemented with calf serum (10%); antibiotics (1%); and glutamine (2 m*M*); ^{3}H-leucine (5 μCi/ml); cAMP (10 μg/ml); and TSH (200 mU/ml). The mixture was incubated at 37°. For control the TSH was omitted from the reaction mixture.

not detected by this assay.

Discussion. Adiga *et al.* (18) reported that LATS-IgG enhanced the incorporation by thyroid slices of labeled uridine and orotic acid into RNA and labeled leucine into protein. On the other hand, if TSH was added along with the precursors at the beginning of the incubation period, there was a marked inhibition of the incorporation of the labeled uridine and leucine. This inhibition was overcome by incubating the thyroid slices with TSH for 2–3 hr followed by washing the tissue prior to the addition of labeled RNA and protein precursors. The inhibition effect by TSH was attributed to the inhibition of uridine kinase which would affect both the RNA and protein synthesis.

Our data show that the incorporation of ^{3}H-uridine and ^{3}H-leucine into RNA and protein, respectively, was neither consistently inhibited nor stimulated by TSH added to the isolated cells at the beginning of incubation prior to the addition of the labeled nucleotide and amino acid. However, stimulation in the incorporation of the labeled precursors was achieved when cyclic AMP was added along with TSH. This stimulation could not be entirely due to cAMP alone, since without TSH the cyclic nucleotide caused only half the stimulation of that achieved in the presence of both. In contrast, LATS-IgG was added at the beginning of the incubation consistently produced a stimulation of labeled uridine into RNA and labeled leucine into protein by the isolated cells. This was consistent with the findings of Adiga *et al.* (18). These findings suggest

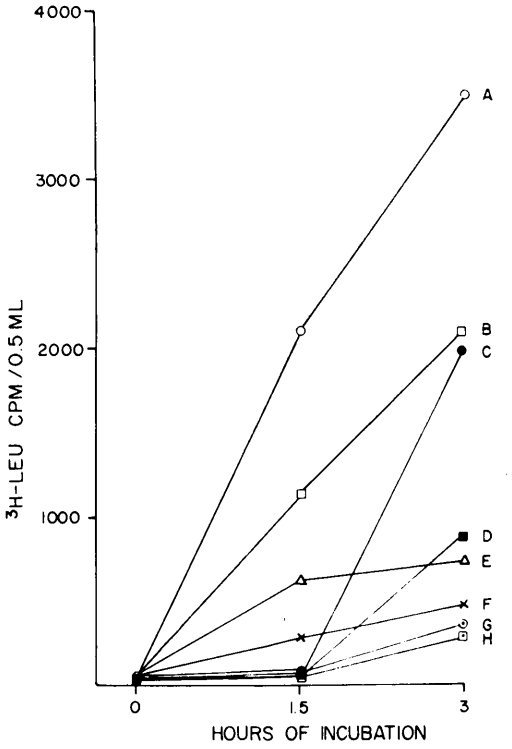

FIG. 6. Actinomycin D inhibition of ^{3}H-leucine uptake by thyroid cells in presence of LATS-IgG or normal IgG. The incubation conditions and concentration of LATS-IgG and normal IgG are as that for Fig. 2. Actinomycin D (20 μg/ml) was added as indicated below. (A) LATS-IgG effect without actinomycin D and ^{3}H-leucine added at zero time; (B) normal IgG without actinomycin D and ^{3}H-leucine added at zero time; (C) LATS-IgG effect without actinomycin D while ^{3}H-leucine added at 1½ hr; (D) normal IgG without actinomycin D while ^{3}H-leucine added at 1½ hr; (E) LATS-IgG effect with AMD and ^{3}H-leucine added at zero time; (F) normal IgG effect with AMD and ^{3}H-leucine added at zero time; (G) LATS-IgG effect with AMD added at zero time while ^{3}H-leucine was added after 1½ hr; (H) normal IgG—at condition similar to G.

TABLE VII. ^{3}H-Uridine Incorporation into nRNA and rRNA in the Presence of LATS-IgG or Normal IgG.[a]

		Incubation time (cpm/OD)			
		IgG		LATS-IgG	
RNA	Expt.	0 hr	3 hr	0 hr	3 hr
nRNA	1	26[b]	1250	28	2603
	2	11	1330	12	3680
	3	18	1700	16	3460
rRNA	1	32	1711	43	3197
	2	31	1265	23	4190
	3	16	1600	18	5100

[a] Cells were incubated in Eagle's Minimum Essential Medium containing calf serum (10%); antibiotics (1%); L-glutamine (2 m*M*); uridine-6-^{3}H (10 μCi/ml); LATS-IgG (3 mg/ml) or normal IgG (3 mg/ml) were added to the incubation mixture in a total volume of 2 ml, and incubated at 37°. The RNAs were isolated as described in "Materials and Methods."

[b] The numbers represent the average of duplicates from different experiments.

that the initial binding of TSH and of LATS is not at the same site on the thyroid cell membrane. This is not illogical, since the two molecules are very different chemically and structurally. It would appear that the procedure for producing isolated cells, which includes a limited digestion with two proteolytic enzymes, may have altered or damaged the site for binding the TSH, but not that for binding of LATS. The binding site may not be totally destroyed, however, since the effect of TSH can be at least partially restored in the presence of cAMP. This finding is further suggestive that the effect is at the cell membrane, since cAMP does not penetrate the cell to a significant extent (19). The binding site for LATS may be less complex than that for TSH and possibly more resistant to damage, in that it may consist of a simple repeated antigenic sequence which is recognized by the antigen-binding sites on the LATS immunoglobulin molecule. The data do not indicate whether TSH and LATS have different mechanisms of action beyond the binding step, and, in fact, the subsequent reactions are probably more similar than dissimilar (20, 21).

In our experiments, it was found that a high concentration of AMD (20 μg/ml) was needed to achieve a significant inhibition of RNA synthesis both in the presence and the absence of LATS-IgG. This could be due to the inability of the antibiotic to penetrate the thyroid cellular membrane. It was observed, furthermore, that at a level of the antibiotic sufficient to inhibit 70% of RNA synthesis, the protein synthesis was inhibited to approximately the same extent. This suggests that the stimulation of protein synthesis by LATS in the thyroid cells is at least partially dependent upon new RNA synthesis. Our finding that the LATS stimulation to protein synthesis was inhibited by AMD is not in agreement with Tong's (22) report in which he found that TSH stimulation of protein synthesis is independent of new RNA synthesis. Also, this is not in agreement with Wilson's (23) finding which showed that TSH exerted stimulatory effects on the RNA synthesis. However, the difference could be due to the method of cell isolation.

In conclusion, the following can be deduced from the present data: (a) LATS and TSH, under similar conditions, exerted different effects on RNA and protein synthesis in isolated thyroid cells; (b) the differences could be due to some alteration in the binding mechanism of TSH but not in that of LATS; (c) the stimulation of protein synthesis by LATS is dependent on the stimulation in RNA synthesis.

Summary. The effects of Long-Acting Thyroid Stimulator (LATS) and of pituitary thyrotropin on macromolecular biosynthesis by isolated thyroid cells were measured. LATS consistently produced up to 3-fold stimulation of the incorporation of protein and RNA precursors by such thyroid cells. The stimulation was linear with time. TSH under similar conditions, on the other hand, did not exert any significant stimulation of the uptake of labeled leucine or uridine by the thyroid cells. In the presence of cyclic AMP, however, TSH stimulated the uptake of leucine up to 36% and that of uridine up to 80%. Actinomycin D at a concentration of 20 μg/ml inhibited, by 70%, the stimulatory effect of LATS on both RNA and pro-

tein synthesis. The antibiotic effect indicates that the stimulation of LATS of protein synthesis is dependent upon new RNA synthesis. The data suggest that the kinetics of the binding of LATS and of TSH to thyroid cells, as demonstrated by their subsequent effects on subcellular macromolecular synthesis, are not identical, but does not indicate whether their mechanism of action are different.

1. Shishiba, Y., Solomon, D. H., and Beall, G. N., Endocrinology **80**, 957 (1967).
2. Scott, T. W., Good, B. F., and Ferguson, K. A., Endocrinology **79**, 949 (1966).
3. Field, J. B., Remer, A., Bloom, G., and Kriss, J. B., J. Clin. Invest. **47**, 1553 (1968).
4. Burke, G., Endocrinology **82**, 1170 (1968).
5. Hall, R., and Tubman, J., J. Biol. Chem. **240**, 3132 (1965).
6. McKenzie, J. M., Trans. Ass. Amer. Physicians **78**, 174 (1965).
7. Burke, G., Endocrinology **83**, 1210 (1968).
8. Burke, G., Clin. Res. (Abstract) **17**, 521 (1969).
9. Meek, J. C., Jones, A. E., Lewis, U. J., and Vanderlaan, W. P., Proc. Nat. Acad. Sci. **52**, 342 (1964).
10. McKenzie, J. M., J. Clin. Invest. **42**, 955 (1963).
11. Adams, D. D., Endocrinology **66**, 658 (1960).
12. Sober, H. A., and Peterson, E. A., Fed. Proc. **17**, 1116 (1958).
13. McKenzie, J. M., Endocrinology **63**, 372 (1958).
14. Selawry, H. S., and Starr, J. L., J. Immunol. **106**, 349 (1971).
15. Nakamura, T., Prestayko, A. W., and Busch, H., J. Biol. Chem. **243**, 1368 (1968).
16. El-Khatib, S. M., Ro-Choi, T. S., Choi, Y., and Busch, H., J. Biol. Chem. **245**, 3416 (1970).
17. Littlefield, J. W., and Keller, E. B., J. Biol. Chem. **224**, 13 (1957).
18. Adiga, P. R., Murthy, P. V. N., and McKenzie, J. M., J. Biochem. **10**, 702 (1971).
19. Butler, R. W., and Sutherland, E. W., J. Biol. Chem. **238**, 3016 (1963).
20. Field, J. B., Zor, U., and Kaneko, T., Program 51st Meeting, Endocrine Society (Abstract), 1969, p. 98.
21. Levey, G. S., and Pastan, I., Life Sci. **9**, 67 (1970).
22. Tong, W., Endocrinology **80**, 1101 (1967).
23. Wilson, B. D., and Wright, R. L., Biochem. Biophys. Res. Comm. **41**, 217 (1970).

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Erratum

Volume **142**, No. 3 (1973), in the article, "Effect of Inhibitors on Sodium-Independent Lysine Transport in Intact Intestine," by Paul K. Frederick, Sheldon Reiser, and Philip A. Christiansen, pp. 988–992:

Page 990, Table I, column heads under "Without sodium," should read: Inhibitor absent; Inhibitor present.

Erratum

Volume **142**, No. 1 (1973), in the article, "Platelet Factor 4 in Platelet Disorders —Storage Location and the Requirement of Endogenous ADP for Its Release," by H. J. Weiss and J. Rogers, pp. 30–35:

Page 31, column 1, line 25. Insert after the second plus sign: 10 units of bovine thrombin (Parke, Davis & Co.) per ml of normal saline. The latter was prepared from a stock solution containing . . . .