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Goitrogenic Effect of Prolonged Treatment with LATS on Mice Immunologically Tolerant to Human Immunoglobulin G[1] (37802)

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(Introduced by L. Van Middlesworth)

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Thyroid-stimulating hormone (TSH) promotes the synthesis and release of thyroid hormone, but also stimulates many of the metabolic processes of the thyroid gland, such as oxygen consumption (1), glucose oxidation (2), phosphorus metabolism (1–5), and nucleic acid synthesis (6–9). Long-acting thyroid stimulator (LATS) of Graves' disease shares some of these metabolic effects of TSH (10–13). It has been suggested, therefore, by several investigators that TSH and LATS do not differ in their mode of action. Some of the metabolic effects of LATS on the thyroid gland could simply be due to secondary processes involving energy production which follow the primary stimulus of initiation of secretion of preformed hormone. Alternatively, LATS may have a tropic action on the thyroid gland similar to that of TSH, so that, if it were administered over a prolonged period of time, it would cause cell growth resulting in the formation of a goiter (14,15).

Reports are available in the literature in which the effect of prolonged administration of LATS has been studied. The earliest report of this kind was that of McKenzie (11, 16) who treated mice for 2–5 days with unfractionated serum obtained from hyperthyroid patients. In these studies, he observed that following such treatment the mice showed thyroid hyperactivity with respect to histological, chemical, and radiobiological parameters. He therefore concluded that there was some factor in the serum of patients with hyperthyroidism which activated the thyroid gland of the treated mice. Ochi and DeGroot (17) observed the increased formation of RNA and phospholipid in the mouse thyroid following injections of purified LATS. In their study, animals were sacrificed only 8 hr after the injection of LATS. They also observed some effects of the administration of LATS in mice for longer periods of time (15). Following treatment of the animals for 5–8 days, there was stimulation of protein synthesis and increased gland weight, and thyroidal uptake of ^{131}I at 24 hr was markedly increased.

The experiments reported in this paper were intended to answer the question of whether or not LATS has a true tropic effect on the thyroid gland. The problem of immunogenicity of LATS which would arise with chronic treatment was overcome by rendering the recipient mice tolerant to human gamma globulin. Thus, for this study, mice were first made tolerant to human IgG and were then treated with LATS for varying periods of time.

Materials and Methods. Male black mice (c57 BL/6 J Strain obtained from Jackson Memorial Laboratories, Bar Harbor, Maine, were made tolerant to human IgG following a modified method of Golub and Weigle (18).

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For induction of tolerance, each mouse was injected intraperitoneally with 1 mg soluble human IgG daily for 2 consecutive days. The IgG was obtained as human Fraction II from Nutritional Biochemicals Corporation. It had been freed of aggregates by centrifugation at 100,000*g* for 45 min. The success of the establishment of the tolerant state was determined as follows: 1 wk following induction, the mice were challenged with a subcutaneous injection of 0.1 mg aggregated IgG together with incomplete Freund's adjuvant. The IgG had been aggregated by heating at 63° for 20 min. A noninduced group of mice which received the same challenge served as immunized controls. One week after the challenge, i.e., at the beginning of the third week after the first injection, both of these groups, and also a third unimmunized group, received intraperitoneal injections of 10 μg of ^{131}I-labelled IgG. The specific activity of the ^{131}I-labeled IgG varied among the experiments depending upon the completeness of iodination. The protein was labeled as described by Williams and Chase using chloramine T and sodium metabisulphate (19). The third unimmunized group of injected mice served as the control for the measurement of ^{131}I decay. Each whole mouse was counted for radioactivity in a well counter. Evidence for the existence of the tolerant state in the treated mice was determined by comparing the rates of clearance of ^{131}I IgG by the treated, immunized, and control groups of mice.

In order to suppress endogenous TSH secretion, commencing one week before the course of chronic LATS-IgG injection, the mice were kept on water containing thyroxine (5 μg/ml), and throughout the experiment, they were maintained on the thyroxine water and normal food. The thyroxine water was freshly made each day.

LATS-IgG was prepared from the serum of a patient with active Graves' disease. The IgG was separated by a standard method of $(NH_4)_2$-SO_4 precipitation followed by DEAE sephadex column chromatography (20). The column was eluted with 0.01 *M* Na_2HPO_4 buffer, pH 7.8. The IgG used for the chronic study was always checked for purity by immunoelectrophoresis. One milligram of this purified LATS elicited a response of about 1000% at 8 hr in the McKenzie bioassay (21).

The tolerant mice received LATS-IgG, either 2 mg/day for 8 days or 4 mg/day for 15 days. In each experiment, corresponding control groups, also tolerant to IgG, received equivalent amounts of pure commercial IgG for the same length of time. After the last injection of LATS-IgG or normal IgG, each mouse received an intraperitoneal injection of 5 μCi ^{131}I. Twenty-four hours later, uptake of the radioactivity by the thyroid was determined either by holding the neck region of the mouse close to the well of a gamma counter, or by counting the excised tracheas with attached thyroid glands in the gamma counter.

Another group of mice received 100 milliunits of TSH/day for 8 days. TSH was obtained from Armour Laboratories. These mice were also on thyroxine (5 μg/ml) for 1 wk before and during the entire treatment. Controls for the TSH group received injections of equal volumes of saline. For histology, glands were fixed in Bouin's fluid. Six micron paraffin sections were stained by the P A Schiff technique and counterstained by hematoxylin. Some sections were stained simply with hematoxylin-eosin. Because of the difficulty of weighing individual excised glands, all samples of each group were pooled to yield a single measurement, and the mean values for thyroid gland weights were calculated accordingly.

Protein was estimated by the Folin–phenol method (22) following homogenization of the glands in 1 ml cold distilled water. Total RNA was extracted by the method of Scott *et al.* (23) in which the RNA was recovered quantitatively in hydrolyzed form from the acid insoluble precipitates of a tissue homogenate.

Results. Figure 1 shows the ^{131}I elimination curves of the control, tolerant, and immunized groups. It can be seen clearly that the immunized group rapidly lost ^{131}I-IgG, so that there was very little radioactivity left in their bodies at the end of the seventh day. The control group also eliminated the ^{131}I rapidly, although not as rapidly as the immunized group. The tolerant group on the contrary,

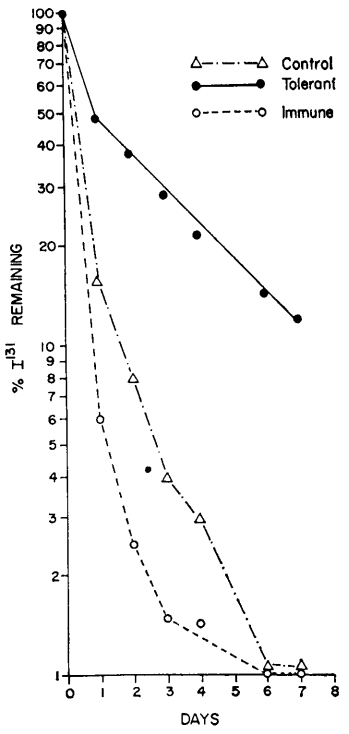

FIG. 1. Elimination of ^{131}I-Human Gamma Globulin from Immunized, Tolerant, and Control Mice.

retained a substantial amount of radioactivity, thereby demonstrating the successful development of tolerance or hyporesponsiveness as defined by Weigle (18). Our experiments on chronic LATS administration were performed on these tolerant animals.

Table I shows the mean body weight of mice before and after chronic treatment with LATS and TSH. In Expt I, 2 mg of LATS was injected per day for 8 days, and in Expts II and III, 4 mg of LATS was injected per day for 14 days. TSH injection continued only for 8 days at a dose of 100 milliunits per day. In all experiments except No. III, a definite increase in body weight was observed both in control and experimental groups. However, the gain in body weight was more in the control than in the LATS-treated groups which sustained a small but definite inhibition of growth.

The effect of LATS and TSH treatment on body weight and thyroid gland of the animals is shown in Table I. In both control and experimental groups, the ratio of gland weight to body weight was determined. The ratios of these ratios are tabulated in the last column of the table. It is obvious that both LATS and TSH markedly stimulated growth of the thy-

TABLE I. Effect of Chronic Treatment of Mice with LATS and TSH on Body Weight and Thyroid Gland Weight.

Experiment	Body weight (g) Before	After	% Change	Thyroid wt post-RX (mg)	$\frac{\text{Gland}}{\text{body}} \times 10^5$(R)	$\frac{\text{R (Treated)}}{\text{R (Control)}}$
I						
Control (5)	16.3	17.7	+8.5	0.52	2.9	3.6
Treated (8)	21.1	22.1	+4.7	2.28	10.3	
II						
Control (3)	16.3	20.0	+22.6	0:80	4.0	3.2
Treated (6)	16.6	18.6	+12.3	2.40	12.8	
III						
Control (10)	20.5	20.1	−1.95	0.56	2.8	2.4
Treated (13)	18.7	18.0	−3.80	1.20	6.8	
IV						
Control (8)	20.7	22.0	+5.60	1.80	8.2	1.8
Treated (9)	21.3	22.3	+4.60	3.30	14.8	

Animals were treated with 2 mg LATS/day for 7 days in Expt I, and with 4 mg LATS/day for 14 days in Expts II and III. In Expt IV, animals were treated with 100 mU TSH/day for 8 days. All animals, control and experimental, were tolerant to human IgG. Control groups for LATS-treated animals received equivalent doses of normal human IgG. Control groups for TSH-treated animals received equal volumes of saline. Numbers in parentheses represent numbers of animals.

TABLE II. Effect of LATS and TSH on ^{131}I Uptake and on Protein and RNA Content of Mouse Thyroid Gland.

Experiment		^{131}I		Protein		RNA	
		CPM	% Uptake	μg / mg gland	μg / gland	μg / mg gland	μg / gland
I	Control	3185	0.35	57.1	29.7	1.5	0.8
	Treated	7336	4.8	56.4	128.6	3.2	7.3
II	Control	3722	2.7	60.0	48.0	3.5	2.8
	Treated	20,358	14.0	64.0	153.6	4.4	10.6
III	Control	6682	0.5	64.0	35.8	4.0	2.2
	Treated	241,755	18.0	71.0	85.2	5.2	6.2
IV	Control	4772	0.35	69.0	124.2	3.9	7.0
	Treated	114,288	8.5	78.0	257.4	4.1	13.5

Control and experimental animals treated as in Table I. In Expts I and II, radioactivity was measured by holding the neck region of the mouse over the well of a crystal scintillation counter, while in III and IV, radioactivity was counted directly by placing the excised thyroid gland directly in the counting tubes.

roid gland itself. It is not clear why the thyroid glands of the saline-treated TSH control mice were so much larger than the normal IgG-treated controls.

Table II shows the data on ^{131}I uptake by the thyroid glands of control and treated groups. In every instance, there was an increase of ^{131}I uptake by the thyroid gland following TSH and LATS administration. A greater increase in ^{131}I uptake was noticed

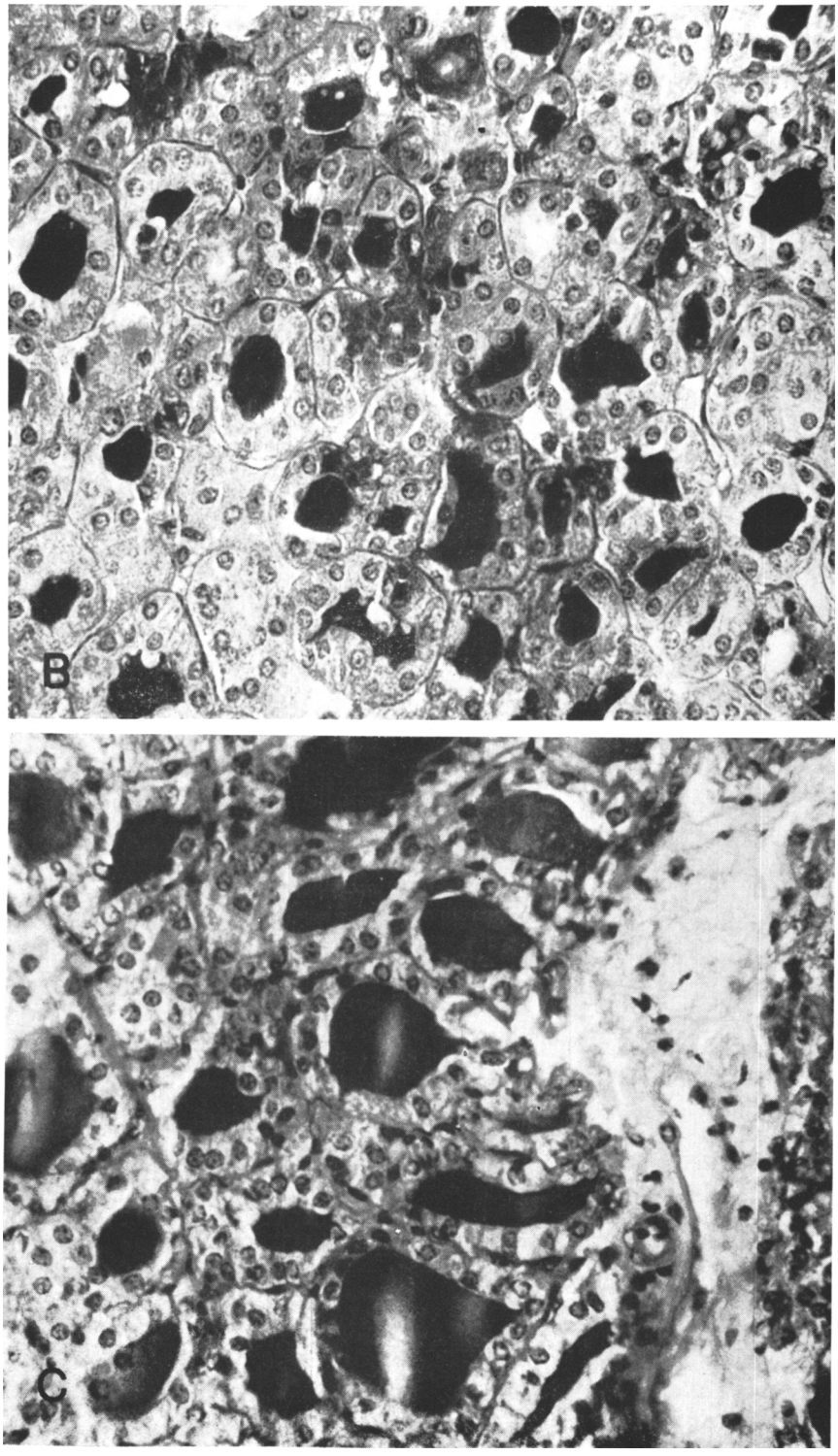

FIG. 2A. Thyroid from Control, Untreated Mouse. Figure 2B: Thyroid from TSH-Treated Mouse. Figure 2C: Thyroid from LATS-Treated Mouse. All mice were immunologically tolerant to human gamma globulin. Magnification ×400.

when higher doses of LATS were used. The difference in absolute counts between Expts I and II and Expts III and IV were due to different doses of ^{131}I and different methods of counting.

Table II shows the changes in protein and RNA content of thyroid glands following LATS and TSH administration. It is obvious that there is a significant increase of both protein and RNA in the hypertrophied glands following LATS as well as TSH administration, indicating a stimulation of both RNA and protein synthesis by both tropic materials.

Following chronic TSH and LATS treatment, the thyroid gland was not only increased in size, but showed significant histological changes. Figure 2-A is from a control mouse which showed the normal features of the thyroid gland. Typical histological changes were observed following injection with TSH (Figure 2-B). Cuboidal cells with enlarged nuclei, less colloid and also signs of colloid resorption were obvious. Sections obtained after chronic LATS treatment (Figure 2-C) were similar to those after TSH, i.e., the cells as well as the nuclei were very much enlarged. The colloid follicles, however, in contrast with those of the TSH-treated glands, contained considerable quantities of colloid and there was very little sign of colloid resorption.

Discussion. The present problem was investigated in order to determine whether or not the long-acting thyroid stimulator (LATS) of Graves' disease has a true tropic effect on the thyroid gland. Evidence for such tropic activity was not to be based simply on the promotion of secretion from the thyroid gland or the acceleration of various intermediary metabolic pathways. The data sought was whether LATS had a goitrogenic capacity similar to that of TSH. This tropic activity of TSH is noted, for instance, by the formation of a goiter induced by increased secretion of TSH during the blocking of formation of iodinated thyroglobulin by propylthiouracil and the resultant absence of feedback inhibition of TSH secretion by circulating thyroid hormones. Hyperfunctioning goiters have been noted in newborn infants born of mothers with Graves' disease during the late stages of pregnancy. Since the manifestations of the neonatal hyperthyroidism diminished in parallel with the decay of LATS from the newborn's circulation, it has been postulated that this is evidence of thyrotropic activity of LATS. This is obviously, however, only circumstantial, and is not a direct demonstration of the goitrogenic activity of LATS.

The data reported here was obtained following the prolonged administration of relatively small amounts of LATS to mice which had been made tolerant to human IgG. This approach is unique in that, although LATS has been used by previous investigators for prolonged periods of time, they have used 30–60 mg per day or 7–15 times more than that used in these experiments. The requirement for higher doses may have been due to the inactivation of LATS by the development of antibodies by the mice to the foreign human protein. This was not a problem in the immunologically hyporesponsive mice used here.

Our results demonstrated that in all of the parameters measured, there was an indication of thyroid hyperactivity in treated mice following prolonged LATS administration. It is unlikely that this increased thyroid activity could have been due to increased TSH secretion, since the latter was inhibited by continual administration of exogenous thyroxin to the animals. During the period of administration of LATS, the thyroid gland of the mice increased in absolute weight when compared to that of the control mice. At the same time, there was a slight loss of body weight of the experimental animals, particularly when compared with control animals which continued to gain weight during the experimental period. There was an increase in macromolecular synthesis by the LATS-treated glands. While the glands increased in size, the amount of RNA and protein per mg of glandular tissue remained relatively constant, indicating that there was a true hypertrophy of tissue rather than an increase in weight due to other mechanisms such as edema. Finally, the hyperactivity stimulated by both LATS and TSH in the thyroid gland was confirmed by the marked increase in ^{131}I uptake.

In addition to the biochemical and meta-

bolic parameters, LATS-induced thyroid hyperactivity was also determined from the histological data. Both TSH and LATS administration caused enlargement of the cells as well as the nuclei, although interestingly, colloid resorption was different. The difference could be due simply to the particular arbitrary doses chosen for both TSH and LATS, and it is possible that higher doses of LATS might well have shown colloid resorption similar to TSH. Shishiba *et al.* (24) have shown in their study on the early histological changes produced by LATS and TSH, that there is a definite time sequence for the changes produced and the rate of change is very much dose-dependent. They reported that when they injected doses of TSH and LATS with similar activity (based on the criterion of their being approximately equal in the McKenzie bioassay effect at 2 hr) colloid droplet formation by TSH was much earlier than that of LATS. This phenomenon, therefore, could account for the difference in the resorption of colloid noted here.

The results presented in this report indicate that the chronic administration of LATS can lead to goiter formation by causing accelerated cell growth. Thus, LATS has a tropic activity similar to TSH. This raises the question of whether LATS can activate the genetic apparatus of the thyroid gland. If LATS has true tropic activity, it should be able to cause an increase in protein and RNA synthesis, not only in the whole gland, where follicles are present containing preformed hormone, but it should also be able to stimulate isolated thyroid cells where there will not be any interference with preformed hormone due to disruption of follicles. This question has been investigated and is the subject of a second paper (25).

Summary. In order to determine whether LATS has a true tropic or goitrogenic effect on mouse thyroid glands, mice were made hyporesponsive to human IgG so that they could receive prolonged treatment with low doses of human LATS-IgG. Groups of mice were treated with TSH, two different doses of LATS, normal human IgG, and saline. The LATS- and TSH-treated mice were found to have lost weight when compared to controls, to have enlarged thyroid glands which had increased rates of RNA and protein synthesis, and to have elevated rates of ^{131}I uptake. The LATS- and TSH-treated glands showed histologic evidence of hyperactivity, although the LATS glands, in contrast to those treated with TSH, did not show marked colloid resorption. These results derived from prolonged therapy with LATS-IgG in tolerant mice, confirm the thyrotropic activity of LATS.

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