Triiodothyronine Reduces Growth Hormone Secretion and Pituitary Growth Hormone mRNA in the Chicken, *in Vivo* and *in Vitro* (43716)

STEVEN V. RADECKI,* DANIEL R. DEAVER,† AND COLIN G. SCANES*,[1]

Department of Animal Science, Rutgers—The State University of New Jersey, New Brunswick, New Jersey 08903 and Department of Dairy and Animal Science,† College of Agriculture, Pennsylvania State University, University Park, Pennsylvania 16802*

Abstract. **The influence of triiodothyronine (T_3) on growth hormone (GH) mRNA and GH secretion has been examined in the chicken. Initially T_3 treatment in the diet for three days did not alter plasma concentrations of GH. Plasma concentrations of GH were depressed with seven and 14 days of T_3 treatment (1 or 5 ppm in the diet). There was a concomitant decline in pituitary GH mRNA with T_3 treatment. Pituitary GH content was reduced with 14 but not seven days of T_3 treatment. No effect of T_3 was observed on the percentage by somatotroph cells in the anterior pituitary gland by fluorescence flow cytometry analysis. While exposure of pituitary cells *in vitro* from young chickens to GHRF for 2 hr increased GH mRNA, no effect was observed with T_3. The presence of T_3, for 48 hr *in vitro*, tended to reduce GH mRNA in adenohypophyseal cells from young chickens and decreased GH mRNA with anterior pituitary cells from adult chicks. It is concluded that T_3 chronic administration of T_3 depresses circulating concentrations of GH, at least in part, by decreasing GH mRNA and hence GH synthesis.** [P.S.E.B.M. 1994, Vol 205]

Thyroid hormones are thought to exert a predominantly stimulatory role on growth hormone (GH) secretion and synthesis. In rodent models, thyroid hormones consistently have been found to elevate circulating concentrations of GH (e.g., 1–5) and GH release *in vitro* (6, 7). Moreover, triiodothyronine (T_3) is reported to increase GH mRNA in rodent pituitary tissues (7–12). While T_3 seems to be required for GH synthesis in some species, T_3 can also reduce GH mRNA levels in others. For instance, T_3 depressed GH mRNA levels in bovine pituitary cells *in vitro* (13). Similarly, when rat pituitary cells transfected with the human GH gene were incubated with T_3, transcription of this gene was reduced (14).

In chickens, GH release is inhibited by thyroid hormones. Circulating concentrations of GH are reduced by the administration of either T_3 or T_4 (15–19) but elevated in hypothyroidism (19–23). In addition, T_3 blocks either GHRF- or TRH-induced GH release *in vivo* (16, 24) and *in vitro* from chicken pituitary cells (25, 26). Moreover, T_3 attenuates GH release *in vitro* in the presence of 8-bromo cAMP or phorbol esters (26). There is relatively little information on effects of T_3 on GH synthesis in the fowl with the exception of the report that T_3 depresses the accumulation of newly synthesized GH in the pituitary (22). The present studies examine the effects of T_3 on GH mRNA *in vitro* and *in vivo* in the chicken.

Materials and Methods

Animals. All studies utilized white Leghorn male chickens. These were obtained at one day old from

[1] To whom requests for reprints should be addressed at Department of Animal Science, Bartlett Hall, Cook College, Rutgers University, New Brunswick, NJ 08903.

Received September 1, 1993. [P.S.E.B.M. 1994, Vol 205]
Accepted December 21, 1993.

0037-9727/94/2054–0340$10.50/0

Avian Service (Frenchtown, NJ). Prior to experimentation chicks had feed (commercial chicken diet; Agway, Flemington, NJ) and water available *ad libitum*.

To Examine the Effect of T_3 on GH Release and GH mRNA *in Vitro*. Anterior pituitary glands were obtained from young (4- to 8-week-old) or adult chickens immediately following sacrifice. Adenohypophyseal cells were dispersed by the method of Vale and colleagues (28), as modified in our laboratory (29). After dispersion, cells (1×10^6 cells per well in 6-well Falcon plates) were incubated in M199 supplemented with 20% fetal bovine serum, 2.0 m*M* glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml fungizone (Grand Island, NY), in a humidified incubator (5% CO_2, 95% air) at 37°C. Two incubation times were employed: chronic exposure to test agents for 48 hr or exposure to treatments for 2 hr (acute) after a 48-hr preincubation period. For either acute or chronic studies, T_3 (100 ng/ml media, Sigma Chemical) and/or GHRF (GHRF, 5 ng/ml; Peninsula Laboratories, Inc., Belmont, CA), were added to the cell cultures in 2×2 factorial arrangement of treatments (for either 48 or 2 hr).

To Examine the Effect of T_3 on GH Secretion and Pituitary GH mRNA *in Vivo*. Three studies were performed each with two treatments: control and T_3 (Study 1, 1 ppm in the diet; Study 2 and 3, 5 ppm). For each treatment there were three or four cages each containing 10 chicks. Birds (four to five weeks old) were killed on Day 7, 8, and 9 (data are combined from the three days) (Study 1), Day 7 (Study 2), and Day 3, 7, and 14 (Study 3). Blood was collected following decapitation from six to eight birds per treatment; the separated plasma being stored at −20°C prior to hormone assay. Anterior pituitary glands were dissected out immediately following death. Pools of 10 pituitary glands were used for GH mRNA analysis. In addition, pituitary GH content was determined with four chicks per treatment (Study 3). Somatotroph number was estimated in pools of six pituitary glands per treatment with adenohypophyseal cells dispersed as in the *in vitro* studies.

Radioimmunoassay. Plasma and anterior pituitary glands were assayed for GH by a homologous chicken GH radioimmunoassay (RIA) (30). In each experiment, samples were assayed in a single RIA, and the intra-assay coefficient of variation was less than 5%. Plasma concentrations of T_3 were determined using an RIA kit (ICN Biomedicals, Irvine, CA).

RNA Isolation and mRNA Analysis. Immediately following removal, anterior pituitary tissue was homogenized in an ice-cold solution of 4 *M* guanidine thiocyanate (Amresco, Solon, OH), 25 m*M* sodium citrate (pH 7.0) and 0.1 *M* β-mercaptoethanol. RNA was then isolated by the method of Chomczynski and Sacchi (31). RNA was quantitated by uv absorbance, and the quality of RNA assessed by calculating the 260/280 nm ratio. GH mRNA was detected by either slot blot (31) or northern analysis. For the slot blots, 1 and 10 μg total RNA was loaded per slot. RNA was uv cross-linked to a nylon membrane (Genescreen; New England Nuclear, Boston, MA). Slot blots were hybridized as described below.

For northern analyses, 2–5 μg total RNA was subjected to electrophoresized on a formaldehyde, 1.2% agarose gel. The RNA was then transferred to a nylon membrane (Genescreen; New England Nuclear, Boston, MA) by capillary transfer, followed by uv cross-linking. The nylon membranes containing the RNA were prehybridized at 55°C for 4–6 hr in a prehybridization buffer (50% deionized formamide, 10% dextran sulfate, 1 *M* NaCl, and 20% 5× hybridization buffer [1% BSA, 1% polyvinyl pyrrolidone, 10% ficoll {MW 400,000}, 250 m*M* Tris HCl {pH 7.5}, 0.5% $Na_4P_20_710H_20$, and 5% sodium dodecylsulfate SDS]) and containing 10 μg salmon sperm DNA (Sigma Chemicals, St. Louis, MO) per ml of buffer.

Following the prehybridization, membranes were incubated at 42°C for 18–24 hr with a ^{32}P-labeled chicken GH cDNA probe (33; kindly donated by Dr. Foster, University of Minnesota, St. Paul, MN). The probe was labeled by nick translation (Gibco/BRL, Grand Island, NY). Membranes were exposed to X-ray film (Eastman, Kodak), at −70°C, until developing. Autoradiographs were subjected to laser-densitometry (Pharmacia, Piscataway, NJ) to assess the relative intensity of the band produced by the probe. To assess equal loading of RNA, ethidium bromide staining intensity was evaluated.

Fixation, Staining, and Flow Cytometry of Cells. The flow cytometric immunofluorescence staining of anterior pituitary gland cells was performed as described by Hatfield and Hymer (35) with minor modifications (34). An aliquot of each pool of dispersed anterior pituitary cells was fixed with PBS-buffered 4% w/v paraformaldehyde for 30 min. Cells were then incubated for four wash periods with PBD-azide (0.01% w/v), 0.1% BSA, 0.025% w/v Triton X–100 for 20 min each wash step. After washing, the suspension of cells in the PBS-azide/BSA/Triton X–100 buffer was stored at 4°C until used for flow cytometric analysis. Cells were stained for GH by incubating 4×10^5 cells with 500 μl of a 1:4000 dilution of the primary antisera (rabbit anti-chicken GH 30) and incubating for 12–16 hr at 22°C. Nonspecific binding tubes were incubated with 500 μl of a 1:4000 dilution of normal rabbit sera. After incubation with the primary antisera, cells were rinsed and subsequently incubated with 500 μl of a 1:4000 dilution of fluorescein isothiocyanate (FITC)–conjugated goat anti-rabbit IgG (Organon Teknika, West Chester, PA) for 1 hr at 22°C. Cells were then rinsed and incubated with 25 μl

RNase (40 μg/ml; Serva Fine Biochemicals, Inc., Westbury NY). Immediately prior to analysis of the cells on the flow cytometer, propidium iodide nuclear staining solution (PI, Serva) was added to the cells and the suspension was filtered through 46 μm nylon mesh. Cells were analyzed on an EPICS 750 Series Dual Laser Flow Cytometer (Coulter Electronics, Hialeah, FL). A gate was set using the PI peak so that only single, nucleated cells were analyzed. The percentage of GH-positive cell was determined from the plot of Forward Angle Light Scatter (FALS) versus FITC fluorescence.

Statistical Analysis. The data for each experiment were subjected to ANOVA (36). In Study 3, bird weight, pituitary weight and GH content, and plasma GH were analyzed in a repeated measures analysis. However, since only one time point (Day 14) was used for the GH mRNA analysis, differences between the two means were analyzed by one-way ANOVA.

Results

***In Vitro* Studies.** As expected, GH release was increased ($P < 0.01$) in the presence of GHRF for 2 hr with anterior pituitary cells from young chickens (Fig. 1). The presence of T_3 had no discernible effect on GH release irrespective of the presence or absence of GHRF (Fig. 1). While GHRF increase ($P < 0.02$) GH mRNA levels in adenohypophyseal cells from young chickens, T_3 treatment had no effect (Fig. 1). In contrast, with pituitary cells from adult chickens, neither GHRF nor T_3 affected GH mRNA.

There were no effects of either GHRF or T_3 on GH release with prolonged incubation (for 48 hr) of pituitary cells from either young or adult chickens (Fig. 2). Following incubation in the presence of T_3, there was a decrease ($P < 0.05$) in GH mRNA with pituitary cells from adult chickens.

***In Vivo* Studies.** Treatment with T_3 for seven or 14 days had no effect on body weight in any study (Table I). As was expected, T_3 treatment consistently elevated ($P < 0.001$) plasma concentrations of T_3 (e.g., Study 1 control 3.62 ng/ml, T_3; 13.4 ng/ml pooled SEM 2.4 ng/ml [$n = 15$] per treatment; Study 3 see Fig. 3). Treatment with T_3 consistently decreased plasma concentrations of GH (Fig. 3 and 4). In Study 1 and 2, plasma concentrations of GH were reduced by 57% and 81% following treatment with 1 ppm and 5 ppm T_3, respectively, for 7 days. In Study 3, T_3 treatment decreased ($P < 0.01$) plasma concentrations of GH after seven days (by 74%) and 14 days (by 77%), but this effect was not evident after three days treatment (Fig. 3). No effect of T_3 treatment on pituitary GH concentrations was observed after seven days (data not shown). However a marked $P < 0.02$ (60.3%) reduction in pituitary GH was evident when exposure to T_3 was extended to 14 days (Fig. 5).

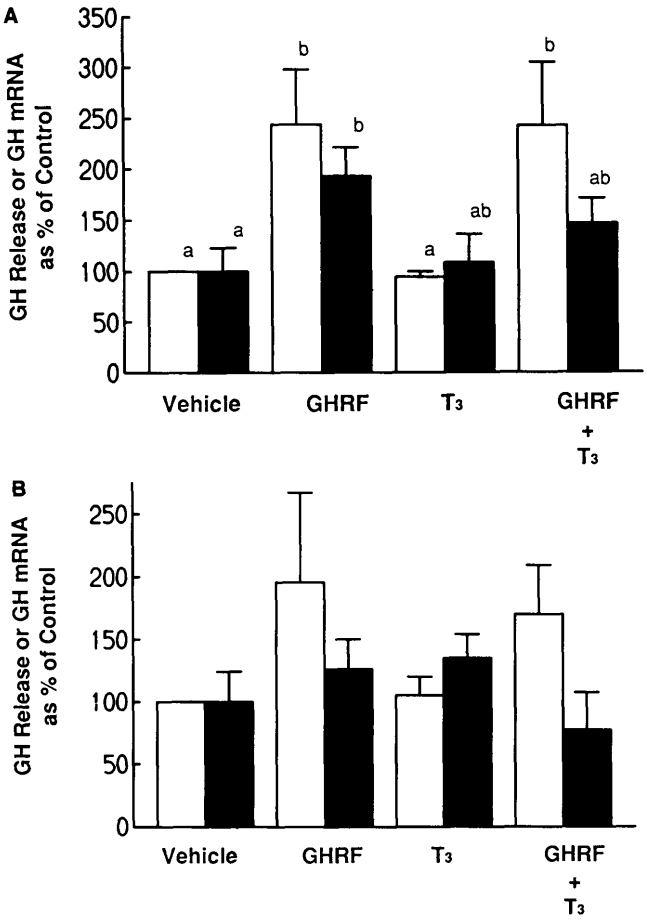

Figure 1. Effect of a 2-hr exposure *in vitro* to T_3 (100 ng/ml) and/or GHRF (5 ng/ml) on GH release (open bars) and GH mRNA (solid bars) in anterior pituitary cells from young (A) and adult (B) chickens. Bars with different superscript letters differ $P < 0.05$. Vertical lines indicate SEM.

The level of GH mRNA in the anterior pituitary glands was decreased ($P < 0.05$) in chicks receiving T_3 treatment. The reduction in pituitary GH mRNA was observed consistently irrespective of whether T_3 treatment was for seven days (Study 1 and 2) (Fig. 4) or for 14 days (Study 3) (Fig. 5).

In view of the possibility that the decrease in pituitary GH content reflects a shift in the number of GH producing cells, the proportion of somatotrophs in the adenohypophysis was examined in control and T_3-treated chicks by FACS analysis. No change in somatotrophs, as a percentage of anterior pituitary cells, was observed (Fig. 5). The GH content per somatotroph tended to be depressed in T_3 chicks as reflected by intensity of the immunofluorescence (LIG FL-volts control 0.0525 ± [$N = 4$]SEM 0.0062; T_3 treated 0.0467 ± [4]0.0094). No difference in somatotroph cell volume/diameter was evident (FALS control 61.3 ± [4]2.16; T_3 treated 60.2 ± [4]0.7).

Discussion

In rodents T_3 exerts a stimulatory role on GH secretion; this being mediated by two mechanisms. Thy-

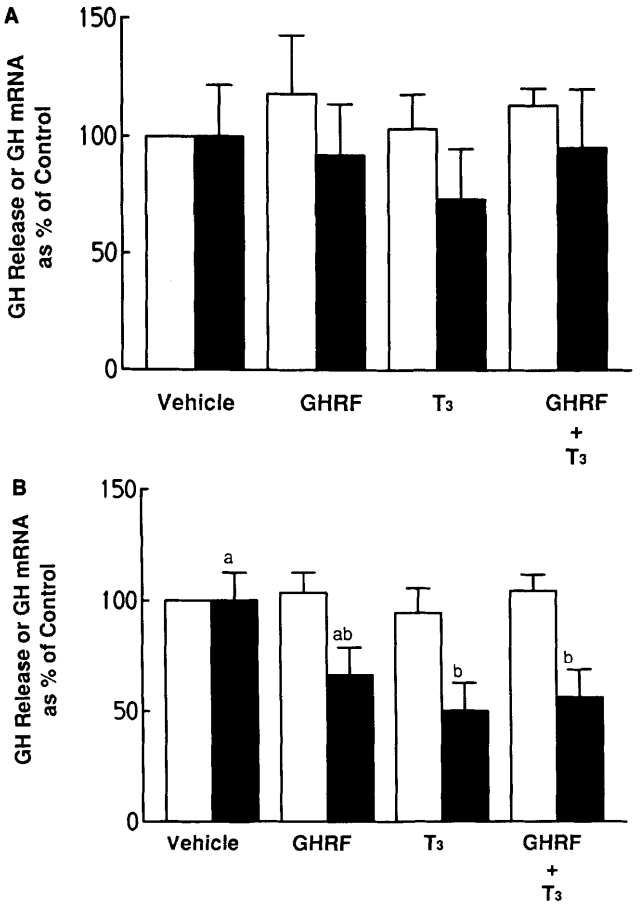

Figure 2. Effect of prolonged *in vitro* exposure to T_3 (100 ng/ml) and/or GHRF (5 ng/ml) on GH release (open bars) and GH mRNA (solid bars) in anterior pituitary cells from young (A) or adult (B) chickens. Vertical bars indicate SEM.

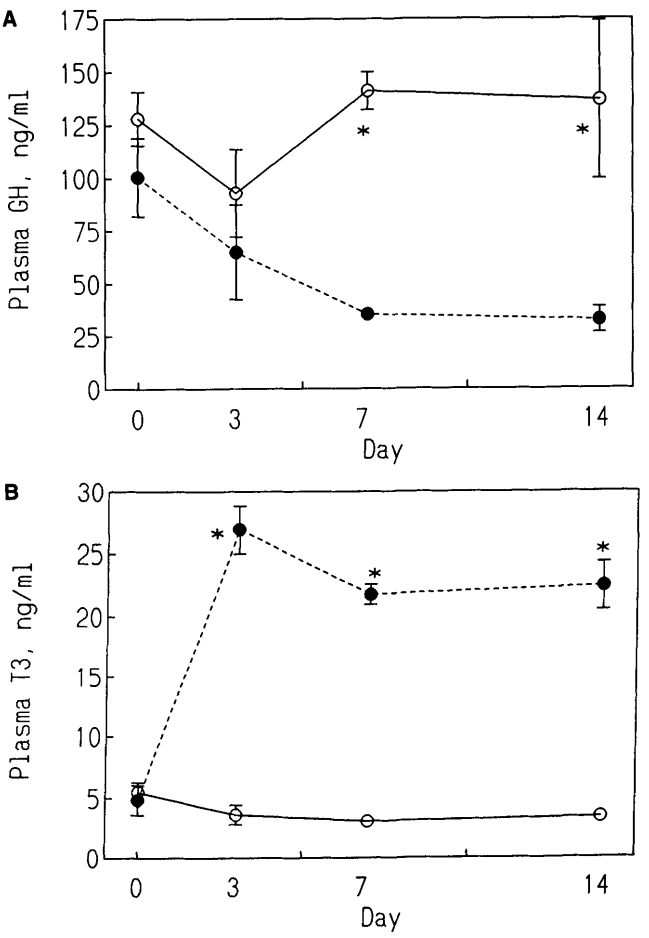

Figure 3. Effect of T_3 (5 ppm in the diet) on the plasma concentrations of GH (A) and T_3 (B) in young chickens. Vertical bars indicated SEM (N = 8); ○—○ controls, ●------● T_3-treated. *difference ($P < 0.05$) between T_3 treated and control chicks in Study 3.

roid hormone increases both GH synthesis and pituitary GH mRNA in rat anterior pituitary tissue (6–11). In addition, T_3 influences pituitary responsiveness to GH secretagogues. For instance hypothyroid rats show decreased GH release in response to TRH and GHRH (1–5). The effects of T_3 on GH synthesis and secretion are different in the chicken. In the present studies, T_3 was observed to reduce GH release in young chickens *in vivo* as indicated by plasma concentrations of GH. This is similar to previous reports (e.g., 19, 26). Administration of T_3 *in vivo* also decreased the GH mRNA in the anterior pituitary gland. This may be explained by terms of T_3 directly reducing transcription of chicken GH gene or indirect effects due say to a reduction of secretagogue-induced GH synthesis/transcription.

Table I. Effect of Triiodothyronine on Growth (Body Weight) in Young Chicks

	Body weight mean ± SEM (N)[+]	
	Control	T_3
Study 1 (1 ppm T_3)		
7-Day treatment	402 ± .11 (3)	386 ± 4.3 (3)
Study 2 (5 ppm T_3)		
7-Day treatment	358 ± 5.2 (4)	350 ± 32.3 (4)
Study 3 (5 ppm T_3)		
7-Day treatment	397 ± 26.4 (4)	395 ± 29.3 (4)
14-Day treatment	553 ± 21.4 (4)	512 ± 32.3 (4)

[+] N = three or four cages (each with eight to nine birds per cage) per treatment.

The present *in vivo* studies provide support for the hypothesis that T_3 can directly reduce somatotroph GH mRNA presumably via decreased transcription. Incubation of adenohypophyseal cells for 48 hr in the presence of T_3 decreased GH mRNA with tissue from adult chickens. The absence of an acute effect of T_3 in a 2-hr incubation is consistent with the time periods normally required for T_3 to exert effects. As T_3 reduces GH mRNA in the chicken pituitary gland under both *in vivo* and *in vitro* conditions, it would seem clear that the inhibitory effects of T_3 on GH secretion from chicken adenohypophyseal cells are mediated at least partially at the level of GH mRNA and presumably GH gene transcription.

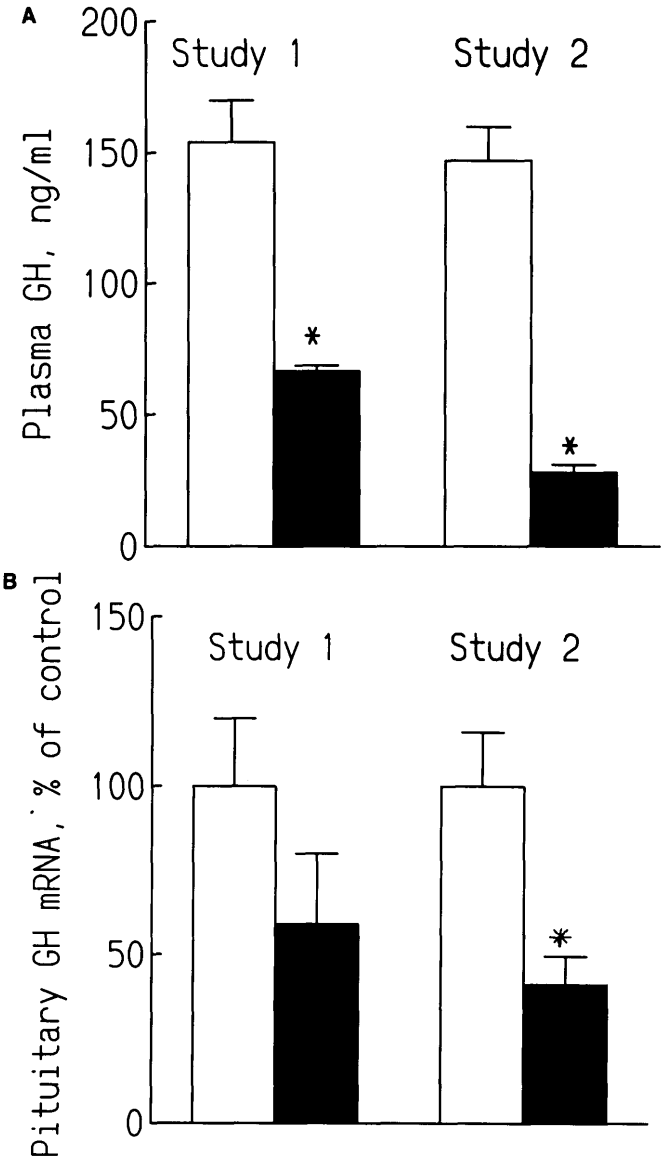

Figure 4. Effect of *in vivo* T_3 treatment on circulating concentrations of GH (A) and pituitary GH mRNA (B) in young chicks. In Study 1, T_3 was administered in the diet at 1 ppm while in Study 2, the dietary concentration of T_3 was 5 ppm. Open bars, control; solid bars, T_3. Vertical lines indicate SEM (N = 3–4 for GH mRNA and 8 for plasma GH). *difference $P < 0.05$.

In vivo administration of T_3 depressed pituitary GH content after 14 days. This is consistent with the *in vivo* observations of decreased adenohypophyseal GH content after 48-hr incubation with T_3 (26). Thus, it might seem that GH release declines following T_3 administration due to there being less GH available for release. Alternatively, it can be argued that reduced release of GH requires less synthesis of GH. The relationship between GH release and synthesis is not fully characterized. The temporal pattern of T_3 reduction of GH release and GH synthesis was similar, suggesting tight coupling between release and synthesis. The decline in pituitary GH content was, however, delayed. This would suggest a later accommodation.

In acute (2 hr) *in vitro* studies with adenohypophyseal cells from young chicks, GHRF increase GH mRNA. The observed stimulatory effects of GHRF on GH mRNA on chicken pituitary cells is similar to that reported for rats (33, 34), cattle (12), and sheep (35). These data are consistent with there being a common promoter mechanism for GHRF stimulating GH transcription and also GH release in mammals, birds, and their common ancestor—the divergence of mammals and birds occurring about 316 million years ago (40). The inhibitory effects of T_3 on both GH release and GH mRNA *in vivo* might be mediated by T_3 affecting GHRF receptors. If T_3 suppressed GHRF receptors on the surface of the chicken somatotroph, as T_3 does with TRH receptors (41), then T_3 may be exerting its inhibitory effect by effectively eliminating the stimulatory influence(s) of GHRF. There is evidence for this from *in vitro* studies with chicken pituitary cells. Chronic exposure of chicken anterior pituitary cells to T_3 for 48 hr prevented any short-term (2-hr) GH secretory response to GHRF, but only attenuated somewhat the response to signal transductors which bypass the GHRF receptor diacylglycerol (26). In the present *in vitro* studies, T_3 did not affect GH release either in acute (2-hr) or chronic (48-hr) studies. The absence of an acute effect is not surprising in view of the time required for T_3 to exert its effect. The absence of an effect of prolonged exposure on basal GH release is similar to previous studies from our laboratory (26).

There was some evidence that GHRF exerted a greater effect *in vitro* on GH mRNA on pituitary cells from young chickens. This is in good agreement with observations on age differences in the ability of GHRF to stimulate GH release in chickens; the maximal GH secretory response to GHRF (increase in plasma concentrations of GH) being considerably greater in young than adult chickens (42). Similarly T_3 appeared to be more effective in reducing *in vitro* GH mRNA with pituitary cells from adult than from young chicks. This appears to be analogous to the greater ability of T_3 to reduce secretagogue induced GH release in adult than young chicks (24).

The administration of T_3 *in vivo* for 14 days reduced both pituitary GH content and pituitary weight in chickens. Possible explanations for this include decreased number and/or size of the GH producing cells. However, flow cytometric analysis indicates no change in somatotroph diameter/volume. Moreover, no change in the percentage of somatotrophs was evident. In view of the decrease in pituitary weight (and hence volume) with T_3 treatment, it is reasonable to conclude that T_3 treatment decreases the number of somatotrophs. A corollary to this is that, if the percentage of somatotrophs does not change, then there should be similar reductions in the number of other adenohypophyseal cell types (e.g., thyrotrophs).

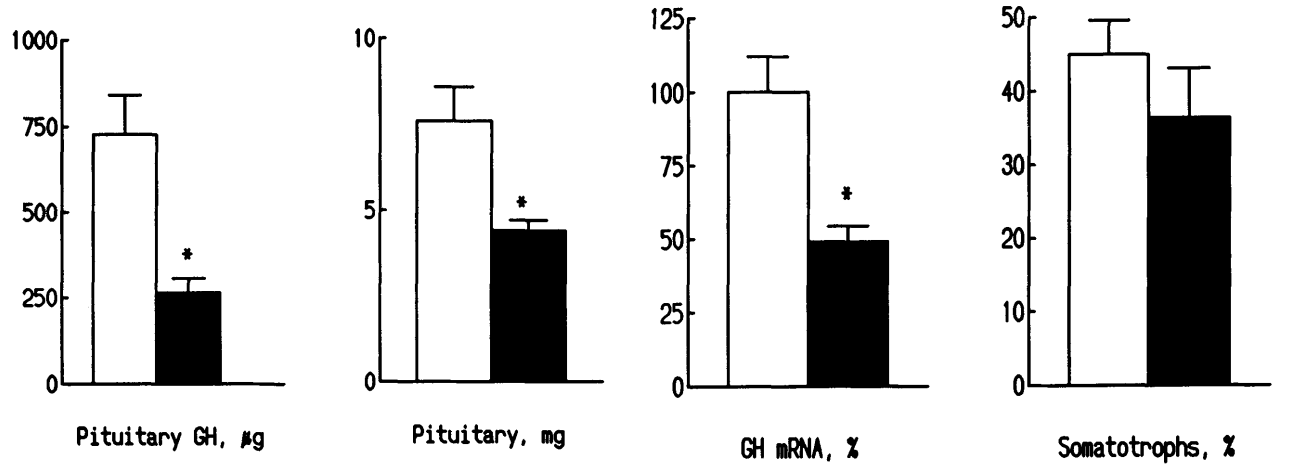

Figure 5. Effect of T_3 treatment (5 ppm in the diet) on pituitary GH content, anterior pituitary weight, pituitary GH mRNA, and percentage of somatotrophs in the anterior pituitary gland. Open bars, control; solid bars, T_3; vertical lines indicate SEM ($N = 4$); *difference between control and T_3 ($P < 0.05$).

This is a paper of the Journal Series, New Jersey Agricultural Experiment Station (Project 06140) supported by State and Hatch Act Funds and the Pennsylvania State University Agricultural Experiment Station Hatch Project 3113. The authors thank Elaine Kunze for her assistance with the Flow Cytometric Analyses.

1. Hervas F, Morreale de Escobar G, Escobar Del Ray F. Rapid effects of a single small dose of L-thyroxine and triiodo-L-thyronine on growth hormone, as studied in the rat by radioimmunoassay. Endocrinology **77**:91–101, 1975.
2. Chihara K, Kato Y, Ohgo S, Iwasaki Y, Maeda K, Mikamoto Y, Imura H. Effects of hyperthyroidism and hypothyroidism on rat growth hormone release induced by thyrotropin-releasing hormone. Endocrinology **98**:1396–1400, 1976.
3. Coiro V, Braverman LE, Christianson D, Fang S-L, Goodman HM. Effect of hypothyroidism and thyroxine replacement on growth hormone in the rat. Endocrinology **105**:641–646, 1979.
4. Szabo M, Ruestow PC, Krawer DE. Growth hormone response to thyrotropin-releasing hormone in the urethane-anesthetized rat: Effect of thyroid status. Endocrinology **117**:330–337, 1985.
5. Root JL, Duckett GE, Sweetland M, Strzelecki JA, Root AW. Hypothyroidism blunts the growth hormone (GH) releasing effect of human pancreatic GH-releasing factor in the adult male rat *in vivo* and *in vitro*. Endocrinology **116**:1703–1706, 1985.
6. Vale W, Vaughan J, Yamamoto G, Spiess J, Rivier J. Effect of synthetic human pancreatic (tumor) GH-releasing factor and somatostatin, triiodothyronine, and dexamethasone on GH secretion *in vitro*. Endocrinology **112**:1553–1555, 1983.
7. Melamed S, Yamashita S. Insulin-like growth factor I action on hypothyroid rat pituitary cells: Suppression of triiodothyronine induced growth hormone secretion and messenger ribonucleic acid levels. Endocrinology **118**:1483–1490, 1986.
8. Samuels HH, Shapiro LE. Thyroid hormone stimulates *de novo* growth hormone synthesis in cultured GH cells: Evidence for the accumulation of a rate limiting RNA specie in the induction process. Proc Nat Acad Sci USA **73**:3369–3373, 1986.
9. Evans RM, Birberg NC, Rosenfield MG. Glucocorticoid and thyroid hormones translationally regulate growth hormone gene expression. Proc Natl Acad Sci USA **79**:7659–7663, 1982.
10. Spindler SR, Mellon SH, Baxter JD. Growth hormone gene transcription is regulated by thyroid and glucocorticoid hormones in cultured rat pituitary tumor cells. J Biol Chem **257**:11627–11632, 1982.
11. Yaffe BM, Samuels HH. Hormonal regulation of the growth hormone gene. J Biol Chem **259**:6284–6291, 1984.
12. Kumara-Siri MH, Surks MI. Regulation of growth hormone mRNA synthesis by 3,5,3′-triodothyronine in cultured growth hormone producing rat pituitary cells (GC cells). J Biol Chem **260**:529–537, 1985.
13. Silverman BL, Kaplan SL, Grumbach MM, Miller WL. Hormonal regulation of growth hormone secretion and messenger ribonucleic acid accumulation in cultured bovine pituitary cells. Endocrinology **122**:1236–1241, 1988.
14. Cattini PA, Anderson TR, Baxter JD, Mellon P, Eberhardt N. The human growth hormone gene is negatively regulated by triiodothyronine when transfected into rat pituitary tumor cells. J Biol Chem **261**:13367, 1986.
15. Harvey S. Thyroid hormones inhibit growth hormone secretion in domestic fowl (*Gallus domesticus*). J Endocrinol **96**:329–334, 1983.
16. Lauterio TJ, Scanes CG. The role of thyroid hormones in the growth hormone response to protein restrictions in the domestic fowl (*Gallus domesticus*). J Endocrinol **117**:223–228, 1988.
17. Scanes CG, Denver RJ, Bowen S. Effect of thyroid hormone secretion in broiler chickens. Poultry Sci **65**:384–390, 1986.
18. Leung FC, Taylor JE, Van Inderstine A. Effect of dietary thyroid hormones on growth, plasma T_3 and T_4, and growth hormone in normal and hypothyroid chickens. Gen Comp Endocrinol **59**:91–99, 1985.
19. Harvey S, Klandorf H, Scanes CG. Participation of triiodothyronine and metabolic clearance rate in the inhibition of growth hormone secretion in thyroxine-treated domestic fowl. J Endocrinol **124**:215–223, 1990.
20. Chiasson RB, Sharp PJ, Klandorf H, Scanes CG, Harvey S. The effect of rapeseed meal and methimazole on levels of plasma hormones in growth broiler cockerels. Poultry Sci **58**:1571–1583, 1979.
21. Harvey S, Sterling RJ, Klandorf H. Concentrations of triiodothyronine, growth hormone, and luteinizing hormone in the plasma of thyroidectomized fowl. Gen Comp Endocrinol **50**:275–281, 1983.
22. Decuypere E, Buyse J, Scanes CG, Huybrechts L, Kuhn ER. Effects of hyper- and hypothyroid status on growth, adiposity and levels of growth hormone, somatomedin C and thyroid metabolism in broiler chickens. Reprod Nutr Dev **27**:555–565, 1987.
23. Scanes CG, Marsh J, Decuypere E, Rudas P. Abnormalities in the plasma concentrations of thyroxine, triiodothyronine and

growth hormone in sex-linked dwarf and autosomal dwarf white Leghorn domestic fowl (*Gallus domesticus*). J Endocrinol **97**:127–135, 1983.
24. Scanes CG, Harvey S. Triiodothyronine (T_3) inhibition of thyrotropin-releasing hormone (TRH) and growth hormone–releasing factor (GRF)—Induced secretion of chicken growth hormone (GH) *in vivo*. Gen Comp Endocrinol **73**:477–484, 1989.
25. Donoghue DJ, Perez FM, Diamante BSA, Malamed S, Scanes CG. Influence of catecholamines prostaglandins and thyroid hormones on growth hormone secretion by chicken pituitary cells *in vitro*. Domest Anim Endocrinol **7**:35–42, 1990.
26. Donoghue DJ, Scanes CG. Triiodothyronine (T_3) inhibition of growth hormone secretion by chicken pituitary cells *in vitro*. Gen Comp Endocrinol **84**:344–354, 1991.
27. Denver RJ, Harvey S. Thuyroidal inhibition of chicken pituitary growth hormone: Alterations in secretion and accumulation of newly synthesized hormones. J Endocrinol **131**:39–48, 1991.
28. Vale W, Grant M, Amos M, Blackwell R, Guillemin R. Culture of enzymatically dispersed anterior pituitary cells: Functional validation of a method. Endocrinology **91**:562–572, 1982.
29. Perez FM, Malamed S, Scanes CG. Growth hormone secretion from chicken adenohypophyseal cells in primary culture: Effects of human pancreatic growth hormone–releasing factor, thyrotropin-releasing hormone, and somatostatin on growth hormone secretion. Gen Comp Endocrinol **65**:408–414, 1986.
30. Harvey S, Scanes CG. Purification and radioimmunoassay of chicken growth hormone. J Endocrinol **73**:321–329, 1977.
31. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem **162**:156–159, 1987.
32. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning—A Laboratory Manual (2nd ed). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
33. Lamb IC, Galehouse DM, Foster DN. Growth hormone cDNA sequence. Nucleic Acid Res **16**:9339, 1988.
34. Hatfield JM, Hymer WC. Flow cytometric analysis and sorting of live male rat anterior pituitary cell types by forward angle and perpendicular light scatter. Endocrinology **119**:2670–2682, 1986.
35. Perez FM, Deaver DR, Hymer WC. Why use a flow cytometer for endocrine cell analysis. In: de Pablo F, Scanes CG, Weintraub B, Eds. Handbook of Endocrine Research Techniques. San Diego: Academic Press, pp157–180, 1993.
36. Gill J. Design and Analysis of Experiments. Ames, IA: Iowa State University Press, 1977.
37. Baringa M, Yamonoto G, Rivier C, Vale W, Evans R, Rosenfeld MG. Transcriptional regulation of growth hormone gene expression by growth hormone–releasing factor. Nature **306**:84, 1988.
38. Gick GG, Zeytin FN, Brazeau P, Long NC, Esch FS, Bancroft C. Growth hormone-releasing factor, regulates growth hormone mRNA in primary cultures of rat pituitary cells. Proc Natl Acad Sci USA **81**:1553, 1984.
39. Silverman BL, Bettendorf M, Kaplan SL, Grumbach MM, Miller WL. Regulation of growth hormone (GH) secretion by GH-releasing factor, somatostatin, and insulin-like growth factor I in ovine fetal and neonatal pituitary cells *in vitro*. Endocrinology **124**:84–89, 1989.
40. Benton MJ. Phylogeny of the major tetrapod groups; Morphological data and divergence. J Mol Evol **30**:409–424, 1990.
41. Harvey S, Baidwan JS. Thyroidal inhibition of growth hormone secretion in fowl: Triiodothyronine induced down regulation of thyrotrophin-releasing hormone binding sites in pituitary membranes. J Mol Endocrinol **4**:127, 1990.
42. Harvey S, Scanes CG. Comparative stimulation of growth hormone secretion by human pancreatic growth hormone–releasing factor (hpGRF) and thyrotrophin-releasing hormone (TRH). Neuroendocrinol **39**:314–320, 1984.