Ontogeny of Pituitary Growth Hormone and Growth Hormone mRNA in the Chicken (43519)

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Abstract. **The changes in pituitary growth hormone (GH) mRNA levels have been determined by Northern blot analysis and laser densitometry during embryonic development and posthatch growth of white Leghorn cockerels. Pituitary GH mRNA levels were observed to progressively increase between 18 days of embryonic development to a maximum at 4 weeks of age (posthatch). Subsequently, pituitary GH mRNA levels declined between 4 and 8 weeks of age, and between 12 weeks of age and adulthood. Pituitary GH contents showed increases during embryonic development and posthatch growth that paralleled the rise in GH mRNA. The decline in pituitary GH mRNA levels between 4 weeks of age and adulthood occurs when GH secretion has been observed previously to decline.** [P.S.E.B.M. 1993, Vol 202]

In chickens, plasma concentrations of growth hormone (GH) rise in late embryonic development and early posthatch growth to a maximum between 2 and 6 weeks, and then decline to relatively low levels before sexual maturation (reviewed in [1]). The decline in plasma concentrations of GH reflects decreased GH secretion and elevated metabolic clearance (2), with the decrease in secretion being due, at least in part, to reduced GH secretory responses to secretagogues (3, 4) and increased sensitivity to inhibition (5, 6). Also, intracerebroventricular injection of GH has inhibitive effects on circulating GH levels (7).

Presently, there is no information on *in vivo* changes in GH mRNA in the chicken, and relatively little on other species. In sheep, hypothalamo-pituitary disconnection does not affect GH mRNA levels, despite the lack of releasing factors or the hypothyroidism resulting from the surgical manipulation (8). Nutritionally deprived sheep have elevated levels of pituitary GH mRNA (9), which is reversed by refeeding (10). Ontogenic or age-related changes in GH mRNA have received little attention (except for Refs. 11–13) and, moreover, there have been no complete longitudinal studies.

The purpose of the present study was to determine the ontogenic profile of pituitary GH mRNA in the chicken. Additionally, pituitary GH content was determined to characterize GH ontogeny in the pituitary. Changes in pituitary GH content for most of the posthatch growth period have been reported previously (14). In the present study, patterns similar to those reported (14) were observed, and observations were extended to older birds and to the perihatch period. These studies will be helpful in further elucidating the developmental control of GH.

Materials and Methods

Animals. White Leghorn male chickens were obtained at 1 day after hatching from Avian Services (Frenchtown, NJ). Birds were housed in floor pens and they received *ad libitum* a commercial diet (Broilermaker; Agway, Allentown, NJ) and water. White Leghorn embryos were incubated at Rutgers University research facilities or were received from Avian Services.

In Study 1, ontogenic changes in the levels of pituitary GH mRNA were determined. Embryos were

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Received February 7, 1992. [P.S.E.B.M. 1993, Vol 202]
Accepted July 9, 1992.

0037-9727/93/2021-0109$3.00/0

sacrificed by decapitation at 18 and 20 days of incubation. At each embryonic age, approximately 20 anterior pituitary glands were combined for each determination of GH mRNA levels (with three replicates per age). For the estimation of GH mRNA levels after hatching, two replicate samples for 8 weeks were collected, four replicates for 4 weeks and adulthood, and three replicates for all other time points studied. These samples consisted of pools of five to 15 anterior pituitary glands, depending on age and pituitary size. Pooled adenohypophysial tissue was obtained after hatching from 1 day, and 2-, 4-, 8-, 12-, and 54 (adult)-week-old chickens immediately after decapitation. In Study 2, to monitor ontogenic changes in GH content in individual pituitary glands, embryos were sacrificed at 12, 15, and 18 days of incubation. Birds were sacrificed 1, 3, and 7 days after hatching, and 2, 5, 9, 12, 14, 19, and 24 weeks after hatching. In addition, in Study 3, anterior pituitary gland weights were determined at 1 day and at 5, 12, 24, and 49 weeks.

RNA Isolation. Total cellular RNA was isolated by the acid guanidinium thiocyanate/phenol chloroform extraction method of Chomczynski and Sacchi (15). The RNA was re-extracted twice with equal volumes of phenol and chloroform/isoamyl alcohol and precipitated by the addition of one tenth a sample volume of 3 *M* sodium acetate and at least two sample volumes of ethanol. Samples containing precipitated RNA were stored at −70°C before electrophoresis.

Northern Blot Analysis of Pituitary GH mRNA. Concentrations of RNA were determined by UV absorbance spectrophotometry at 260 nm and quality of the RNA was assessed using the 260 nm:280 nm ratio. Subsequently, RNA samples were electrophoresed in 1.2% agarose and 6% formaldehyde gels at 90–100 V. Equal quantities of total cellular RNA (1 μg) were loaded for each sample. Loading errors were controlled for by checking similarity in 28 S ribosomal bands. Three replicate gels were run, each including separate RNA samples from the different ages. RNA was Southern-transferred (16) to Genescreen nylon membrane (Dupont NEN, Boston, MA) overnight. Membranes containing the UV cross-linked RNA were washed for 1 hr in 1% sodium dodecyl sulfate at 42°C to remove any residual agarose. Membranes were prehybridized at 55°C for 4 to 6 hr and hybridized at 42°C for 18 to 24 hr in hybridization cassettes (BIOS, New Haven, CT). Prehybridization and hybridization solutions contained: 0.2% bovine serum albumin (Armour, Blue Bell, PA), 0.2% polyvinylpyrrolidone (mol wt 40,000; Sigma, St. Louis, MO), 0.2% Ficoll (mol wt 400,000; Sigma), 50 m*M* Tris-HCl (pH 7.5), 0.1% sodium pyrophosphate, 1.0% sodium dodecyl sulfate, 50% deionized formamide, 10% dextran sulfate, 1 *M* NaCl, and 0.1 mg/ml of salmon sperm DNA (Sigma).

The chicken GH cDNA probe obtained from the pUC 119 vector (17) was labeled with [^{32}P]dCTP by nick translation (BRL, Gaithersberg, MD). The probe was separated from free nucleotides by use of a NucTrap push column (Stratagene, La Jolla, CA). After hybridization, the membranes were washed to remove any nonspecific radioactivity. The washes consisted of 0.3 *M* sodium chloride, 0.03 *M* sodium citrate (2× saline-sodium citrate), and 1% sodium dodecyl sulfate for 20 min at room temperature, followed by 20 min at 42°C with fresh solution. Then, membranes were washed a final time at room temperature with a solution containing 75.0 m*M* sodium chloride and 7.5 m*M* sodium citrate (0.5× saline-sodium citrate). Membranes were exposed to Kodak X-AR film and subsequent autoradiographs were analyzed by laser densitometry (Electro Scan X-Excel; Pharmacia, Piscataway, NJ) and the bands identified as GH mRNA were expressed as a percentage of the GH mRNA level found in the 4-week-old chicken pituitary samples of each blot.

Pituitary GH Content. The GH content of individual pituitary glands was measured by a homologous radioimmunoassay for GH (18). Frozen glands were homogenized in 0.5–1 ml of ice-cold water and an aliquot used in the assay. Interassay and intraassay coefficients of variation were less than 10%.

Statistical Analysis. Pituitary GH mRNA data were analyzed by one-way analysis of variance and least significant difference multiple range test. All differences were considered to be significant if $P < 0.05$. Pituitary GH content and weight data were analyzed by one-way analysis of variance ($P < 0.0001$).

Results

Figure 1 shows a representative Northern blot of pituitary RNA concentrations from all ages of chickens investigated. Only one RNA transcript was detected (approximately 0.8 kb) at any age investigated. It appears that GH mRNA levels in the anterior pituitary gland increased during development/growth, peaked around 4 weeks of age, and subsequently decreased. To quantify these changes, laser densitometry was performed. The highest level of pituitary GH mRNA (rel-

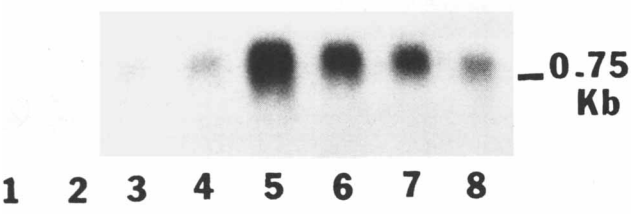

Figure 1. Northern blot analysis of anterior pituitary RNA from different aged chickens hybridized with a ^{32}P-labeled cDNA probe for chicken GH. Lane 1: 18ed (ed = days of incubation of embryos); Lane 2: 20ed; Lane 3: 1 day; Lane 4: 2 weeks; Lane 5: 4 weeks; Lane 6: 8 weeks; Lane 7: 12 weeks; Lane 8: adult (54 weeks).

ative to total cellular RNA) was detected at 4 weeks of age and all data on RNA levels from all age groups were expressed as a percentage of this group (Fig. 2). A very large (at least 38.8-fold) increase ($P < 0.05$) in the levels of pituitary GH mRNA was observed between the 18th day of incubation and 4 weeks after hatching. These changes can be described by a linear increase ($R^2 = 0.997$). The pituitary GH mRNA levels increased between the 18th day of incubation and 2 weeks after hatching ($P < 0.05$), and between 2 weeks and 4 weeks after hatching ($P < 0.05$). There was a tendency for GH mRNA levels to increase between the 18th day of embryonic incubation and Day 1 after hatching. The accumulated GH mRNA transcripts decreased by 75.3% between 4 weeks of age and 54 weeks (adult) of age. The profile of GH mRNA in the pituitary gland is similar to previously reported ontogenic changes in plasma GH concentrations (Fig. 2).

The changes in pituitary GH content and pituitary weight during growth and development are summarized in Figure 3. The increase in pituitary GH content is linear between Day 12 of embryonic development and 24 weeks after hatching ($R^2 = 0.78$; $P < 0.001$) (Fig. 3A). Alternatively, the GH content appeared to increase logarithmically between 12 days of embryonic development and 9–12 weeks of age (Fig. 3C). After 9–12 weeks of age, the pituitary GH content achieved a plateau level. Similarly, the weight of the anterior pituitary gland increased during posthatch growth (Fig. 3B). The increase in GH content (62.9-fold between 1 day old and 5 weeks of age [Study 2]) was disproportionally greater than the 5.54-fold increase in the anterior pituitary gland weight over the approximate similar

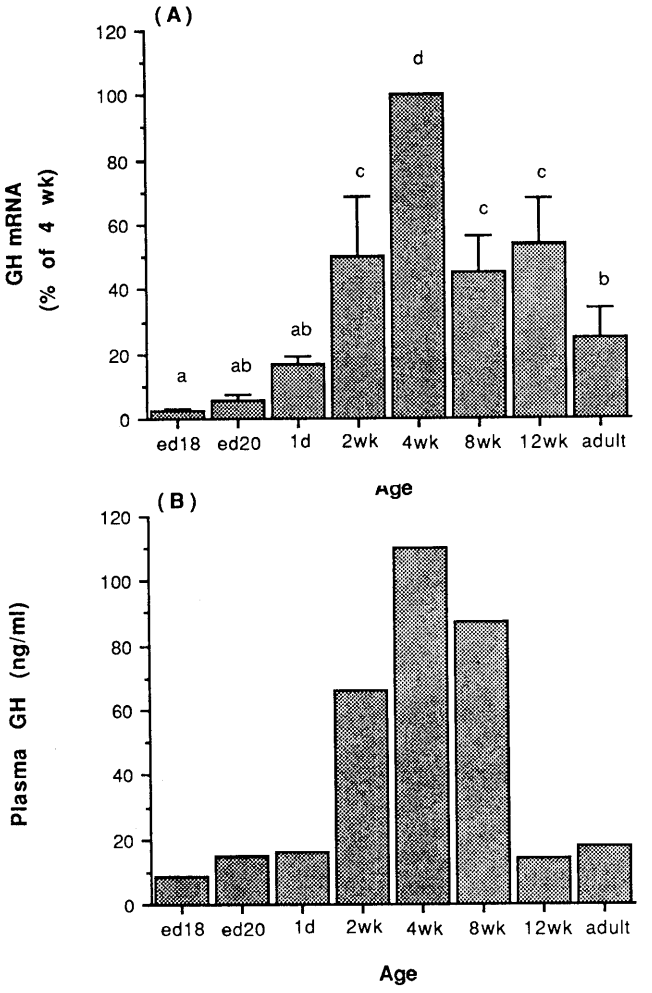

Figure 2. (A) Variation in the accumulation of GH mRNA in the anterior pituitary gland during development of the chick embryo and during posthatch growth. RNA is expressed as a percentage of the 4-week level; ed = days of incubation of embryos; vertical bars indicate SE ($n = 3$, except at 8 weeks of age where $n = 2$, and at 4 weeks of age and adulthood where $n = 4$). Means with different superscript letters are different ($P < 0.05$) by analysis of variance and least significant difference; if percent data are arcsin transformed, substantially similar differences are observed. (B) Profile of plasma GH during growth in white Leghorn males (redrawn from Refs. 19 and 20).

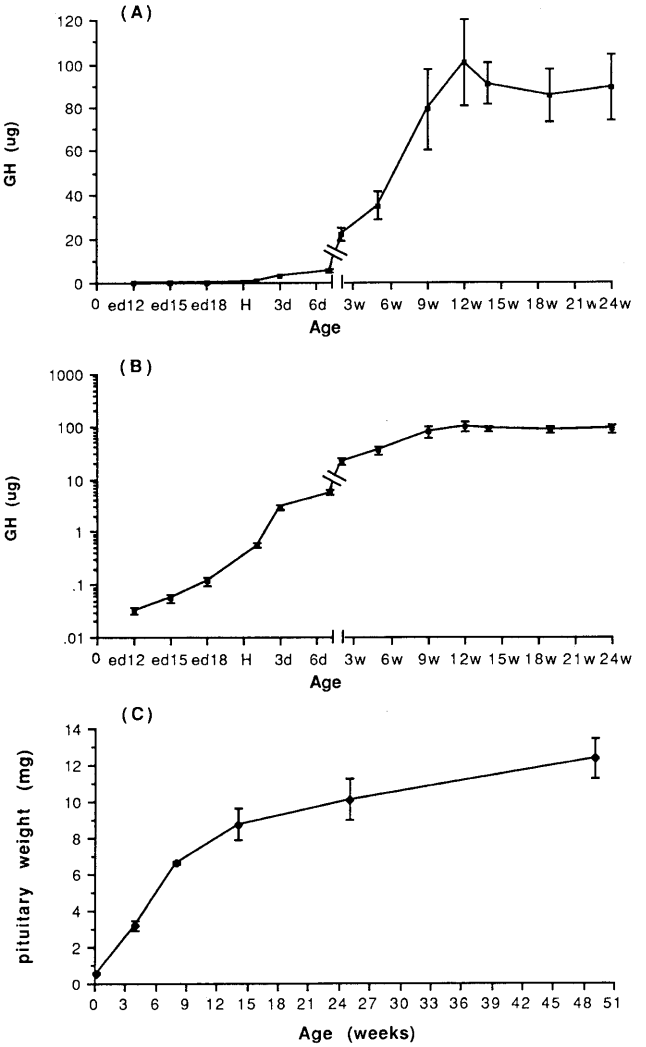

Figure 3. Changes in pituitary GH content ([A] μg per gland; [B] log μg) during embryonic development and posthatch growth (ed = days of incubation of embryos; H = hatching; vertical bars indicate SE [$n = 4$–11]). (C) Changes in pituitary weight during posthatch growth ($n = 4$).

time period (Study 3). In contrast, the changes in GH content between 5 weeks of age and 12 weeks, and between 12 weeks of age and adulthood (156.3% increase and 11.0% decrease, respectively) were relatively similar to those for pituitary weight between 4 weeks of age and 14 weeks, and between 14 weeks of age and adulthood (175.0% increase and 15.6% increase, respectively). The increase in pituitary weight over the time period studied was also linear ($R^2 = 0.78$; $P < 0.02$).

Discussion

The patterns of increase in levels of pituitary GH mRNA and GH content in late embryonic/early posthatch development are very similar, with progressive increases in late embryonic development and early posthatch life (Figs. 2 and 3). The changes in pituitary GH and GH mRNA levels during this early growth/development are comparable to the increases in plasma GH reported previously (Fig. 2). It would appear, therefore, that the perihatching elevation in plasma concentrations of GH corresponds to increased GH content in pituitary gland and, hence, GH available for release. Taken together, the parallel rise in pituitary GH and GH mRNA would suggest that the ontogenic rise in GH is controlled at the transcriptional rather than the translational level. Similar ontogenic changes in GH mRNA levels have been observed by *in situ* hybridization in the mouse (12) and by Northern blot analysis in the human fetus (13).

It is not clear whether the increase in GH mRNA accumulation is due to genesis and proliferation of somatotrophs, or to a higher GH mRNA transcription rate per somatotroph. The former is supported by immunocytochemical studies in changes in the percentage of somatotrophs during embryonic development/early posthatch growth in chickens (S. Malamed, J. Gibney, F. Perez, and C. G. Scanes, unpublished observations); the percentage of somatotrophs was observed to increase from 3.4% of the adenohypophysial cells at 18 days of embryonic development to over 20% of pituitary cells 4 days later (1-day-old chicks).

The increase in pituitary GH content observed between 1 and 12 weeks of age was very similar to that reported previously in a broiler strain of chickens (14). In both studies, pituitary GH content reached a plateau at between 5 and 9 weeks of age and remained high for the rest of the growth period examined. In the current study, it was possible to verify that the GH levels in the pituitary remained high throughout the second half of the growth period (after 9 weeks of age) and into adulthood. Preliminary results in our laboratory indicate that pituitary GH concentrations, as well as content, remain stable during this period.

After 4 weeks of age, GH mRNA levels were observed to decrease (Figs. 1 and 2). An analogous decline in GH mRNA content has also been reported during aging in mice (11). It is of note that both GH mRNA levels and plasma concentrations of GH declined after 4 weeks of age (Fig. 2). The reduction in plasma concentrations of GH reflects decreased GH secretion (2). It is, therefore, evident that during much of the middle and later phases of growth, both GH secretion and synthesis are declining. Moreover, the present data suggest that GH synthesis and release are tightly coupled during later phases of growth, as is the case during perihatch development (see above). The change in GH mRNA and GH release may represent a desensitization to secretagogues for GH during development. Also, increased circulating insulin-like growth factor-I levels during growth/development (21), which result from the increased plasma GH, may then inhibit secretion. In turn, transcription may be affected by some intracellular mechanism due to the consistently higher stored levels of GH in the somatotroph and/or the lower GH release from the pituitary. The discrepancy between the decrease in GH mRNA (and presumably GH synthesis) and the plateau in pituitary GH content may also be explained by an ontogenic change in the rate of intrapituitary GH degradation.

In summary, it seems that during ontogeny, GH synthesis is regulated primarily at the transcriptional level. Moreover, GH secretion and synthesis appear to be tightly coupled during growth/development. The factors that control the transcriptional regulation of GH mRNA during growth/development in chickens are presently being investigated.

This is a paper of the Journal Series, New Jersey Agricultural Experiment Station (Project 06140), supported by State and Hatch Act funds.

1. Scanes CG. The physiology of growth, growth hormone and other growth factors in poultry. CRC Poult Biol **1**:51–106, 1987.
2. Lauterio TJ, Scanes CG. Effect of age and protein restriction on the clearance and secretion of growth hormone in the domestic fowl. Poult Sci **67**:120–125, 1988.
3. Scanes CG, Harvey S. Stimulation of growth hormone secretion by human pancreatic growth-hormone-releasing factor and thyrotropin-releasing hormone in anaesthetized chicken. Gen Comp Endocrinol **56**:198–203, 1984.
4. Harvey S, Scanes CG. Comparative stimulation of growth hormone secretion in anaesthetized chickens by human pancreatic growth hormone-releasing factor (hpGRF) and thyrotropin-releasing hormone (TRH). Neuroendocrinology **39**:314–320, 1984.
5. Scanes CG, Harvey S. Triiodothyronine inhibition of thyrotropin-releasing-hormone- and growth hormone releasing factor-induced growth hormone secretion in anaesthetized chickens. Gen Comp Endocrinol **72**:477–484, 1989.
6. Scanes CG, Harvey S. Somatostatin inhibition of thyrotropin-releasing-hormone- and growth hormone-releasing factor-induced growth hormone secretion in young and adult anaesthetized chickens. Gen Comp Endocrinol **75**:256–264, 1989.
7. Lea RW, Harvey S. Feedback effects of growth hormone on anterior pituitary function in chickens. J Endocrinol **125**:409–

415, 1990.
8. Hamernik DL, Crowder ME, Nilson JH, Nette TM. Measurement of messenger ribonucleic acid for luteinizing hormone β-subunit, α subunit, growth hormone, and prolactin after hypothalamic pituitary disconnection in ovariectomized ewes. Endocrinology **119**:2704–2710, 1986.
9. Landefeld TD, Ebling FJP, Suttie JM, Vannerson LA, Padmanabhan V, Beitins IZ, Foster DL. Metabolic interfaces between growth and reproduction. II. Characterization of changes in messenger ribonucleic acid concentrations of gonadotropin subunits, growth hormone, and prolactin in nutritionally growth limited lambs and differential effects of increased nutrition. Endocrinology **125**:351–365, 1989.
10. Thomas GB, Mercer JE, Karaliss T, Rao A, Cummins JT, Clarke IJ. Effect of restricted feeding on the concentrations of growth hormone (GH), gonadotropins, and prolactin (PRL) in plasma, and on the amounts of messenger ribonucleic acid for GH, gonadotropin subunits, and PRL in the pituitary glands of adult ovariectomized ewes. Endocrinology **126**:1361–1367, 1990.
11. Crew MD, Spindler SR, Walford RL, Kiozumi A. Age-related decrease of growth hormone and prolactin gene expression in the mouse pituitary. Endocrinology **121**:1251–1255, 1987.
12. Nogami H, Suzuki K, Enomoto H, Isikawa H. Studies on the development of growth hormone and prolactin cells in the rat pituitary gland by *in situ* hybridization. Cell Tissue Res **255**:23–28, 1989.
13. Suganuma N, Seo H, Yamamoto N, Kitkawa F, Oguri H, Narita O, Tomodo Y, Matsui N. The ontogeny of growth hormone in the human fetal pituitary. Am J Obstet Gynecol **160**:729–733, 1989.
14. Vasilatos-Younken R. Preparation and culture of dispersed avian pituitary cells and age-related changes in donor pituitary weight and growth hormone content. Gen Comp Endocrinol **64**:99–106, 1986.
15. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem **162**:156–159, 1987.
16. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Press, 1989.
17. Lamb IC, Galehouse DM, Foster DN. Chicken growth hormone cDNA sequence. Nucleic Acids Res **16**:9339, 1988.
18. Harvey S, Scanes CG. Purification and radioimmunoassay of chicken growth hormone. J Endocrinol **73**:321–329, 1977.
19. Scanes CG, Harvey S. Growth hormone and prolactin in avian species. Life Sci **28**:2895–2902, 1981.
20. Harvey S, Davison TF, Chadwick A. Ontogeny of growth hormone and prolactin secretion in the domestic fowl (Gallus domesticus). Gen Comp Endocrinol **39**:270–273, 1979.
21. Huybrechts LM, King DB, Lauterio TJ, Marsh J, Scanes CG. Plasma concentrations of somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as determined by heterologous radioimmunoassay. J Endocrinol **104**:233–239, 1985.