The Ontogenesis of Human Fetal Hormones: *I. GROWTH HORMONE AND INSULIN*

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I. GROWTH HORMONE AND INSULIN

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ABSTRACT The content and concentration of immunoreactive growth hormone (GH) were measured in 117 human fetal pituitary glands from 68 days of gestation to term and in the pituitary glands of 20 children 1 month to 9 yr of age. Physicochemical and immunochemical properties of GH of fetal pituitary glands and GH from adult pituitary glands were indistinguishable by disc gel electrophoresis, immunoelectrophoresis, starch gel electrophoresis, and radioimmunoassay techniques. In the fetal pituitary gland, the GH content rose from mean levels of 0.44±0.2 μg at 10–14 wk of gestation, to 9.21±2.31 μg at 15–19 wk, to 59.38±11.08 μg at 20–24 wk, to 225.93±40.49 μg at 25–29 wk, to 577.67± 90 μg at 30–34 wk, and to 675.17±112.33 μg at 35–40 wk. There was a significant positive correlation between growth hormone content of the pituitary and gestational age, crown-rump length, and the weight of the pituitary gland.

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Immunoreactive GH was detected in fetal serum at a concentration of 14.5 ng/ml as early as 70 days gestation, the youngest fetus assayed. At 10–14 wk, the mean concentration of serum growth hormone was 65.2±7.6 ng/ml; at 15–19 wk 114.9±12.5 ng/ml; at 20–24 wk 119.3±19.8 ng/ml; at 25–29 wk 72.0±11.5 ng/ml; and 33.5±4.2 ng/ml at term. A significant negative correlation of serum growth hormone with advancing gestational age after 20–24 wk was observed ($P < 0.001$). In 17 fetuses paired serum and pituitary samples were assayed; no significant correlation between the concentration of serum GH and the pituitary content or concentration of GH was demonstrable.

The serum concentration of chorionic somatomammotropin (HCS) in the fetus was unrelated to gestational age. Insulin (1–30 μU/ml) was detected in 42 of 46 fetal sera assayed.

These data suggest that the appearance and development of the secretory capacity for GH by the human fetal pituitary gland coincides with developmental changes in the portal system and hypothalamus. Maturation of inhibitory central nervous system control mechanisms for secretion of GH may not occur until infancy.

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INTRODUCTION

The human fetal pituitary gland secretes polypeptide hormones early in gestation; acidophile cells have been observed in the anterior hypophysis by the 9th wk of gestation by histochemical (1–7), electron microscopic (8), and immunofluorescent techniques (9, 10). Fetal pituitary tissue grown in vitro secretes and synthesizes trophic hormones, including growth hormone (GH),[1] prolactin, gonadotropins, and thyrotropin (11–14). Immunologic techniques have been utilized to detect the presence of pituitary hormones during the first trimester (15–22). Preliminary studies from our laboratory showed incremental changes in the content of HGH in fetal pituitaries (15, 16) and changes in serum HGH throughout gestation (17, 19). The present communication describes in a more complete report, the synthesis and release of pituitary growth hormone prenatally and the relationship to development of hypothalamic regulation in the fetus.

[1] *Abbreviations used in this paper:* FSH, follicle-stimulating hormone; GH, growth hormone; GRF, growth hormone releasing factor; HCS, chorionic somatomammotropin; HGH, human growth hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

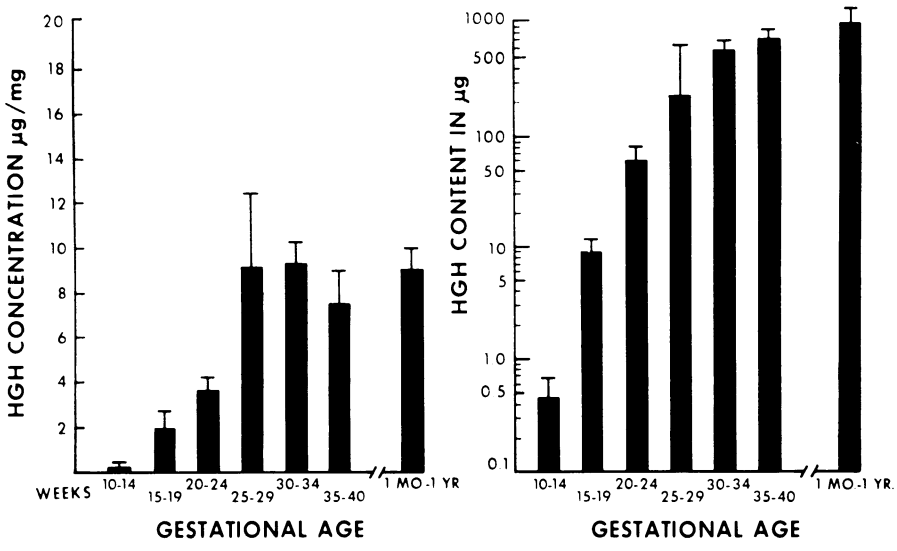

FIGURE 1 The mean (±SEM) concentration and content of HGH in pituitary glands of fetuses 68 days to term (plotted at 4-wk intervals) and in children aged 1 month to 1 yr is shown. The panel on left indicates HGH in micrograms/milligram pituitary gland on a linear scale and in the panel on right as growth hormone content in micrograms per pituitary gland on a semi-logarithmic scale.

METHODS

Pituitary glands were obtained from 117 fetuses (96 from spontaneous abortions and 21 from fetuses removed by hysterotomy) and from 20 children and 4 adults at postmortem examination. Gestational age of the fetuses was estimated from crown-rump measurements and/or from calculations obtained by subtraction of 14 days from the onset of the last menstrual period (23).

The pituitary glands of the aborted fetuses were quick frozen, stored at −20°C, and processed on the day of assay. Pituitaries from some older fetuses, the children, and adults, were dissected free of capsular connective tissue, weighed on an analytical balance, and divided into approximately equal sagittal sections to provide tissue for routine pathologic evaluation. The weight of the intact gland and the portion retained for immunologic assay were both obtained within 10 min after removal from the fossa.

The pituitary specimens were homogenized in glass tissue grinders in small volumes (0.5–1.0 ml) of 0.1 M barbital buffer, pH 8.2, at 4°C on the day the assay was performed. A clear supernatant was obtained after centrifugation of the homogenates at 2500 rpm for 10 min. The homogenization procedure was altered for the adult pituitary glands by the use of 5 ml buffer with a more prolonged period of centrifugation (30 min at 4°C). Portions of the supernatants were used for immunoassay and for analysis by immunoelectrophoresis, disc gel electrophoresis, and starch gel electrophoresis.

Specimens of blood were obtained from 62 of the aborted fetuses, 41 spontaneous and 21 induced; 17 of which had matched pituitary glands.

Immunoassay of HGH (24), HCS (25), and insulin (26) were performed by double antibody methods. Iodination of HGH, HCS, and insulin was carried out by a modification (25) of the method of Hunter and Greenwood (27) followed by separation of the iodinated hormones on Sephadex G75 (HCS and HGH) or Sephadex G50 (insulin) (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) with 0.15 M phospho-saline buffer at pH 7.8. The HGH content of the initial group of 38 fetal pituitary glands was measured by a hemagglutination-inhibition assay using tanned formalinized sheep red cells coated with HGH (28, 29). The specimens were assayed at multiple dilutions in duplicate. The validity of this assay for nonserum-containing fluids has been demonstrated previously (15, 16). Evaluation of data from fetuses of the same age assayed by the two methods was not statistically different ($P > 0.5$); accordingly the data obtained by both methods were combined.

All values are expressed in terms of purified standards: Wilhelmi 840 (HGH), Florini preparation (HCS) (Lederle Laboratories, Pearl River, N. Y.), Lilly preparation of human insulin (Eli Lilly & Co., Indianapolis, Ind.).

Immunoelectrophoretic analysis of the concentrated pituitary homogenates was performed according to micromodification of Scheidegger (30) and of Osserman (31). Starch gel electrophoresis was carried out by the discontinuous buffer system of Ferguson and Wallace (32). Disc gel electrophoresis was performed according to the method of Ornstein (33) using Tris buffer, pH 8.6. Statistical analysis of the data was carried out by a logarithmic transformation and modified Student's t test.

RESULTS

Pituitary growth hormone. Immunoreactive GH was detected in the fetal pituitary gland as early as 68 days of gestation, the youngest pituitary gland assayed. At 10–14 wk of gestation, the mean content of HGH was 0.44±0.20 µg (SEM) per pituitary, and the mean concentration was 0.14±0.9 µg mg (Fig. 1).

Incremental changes throughout gestation were observed in both the content and concentration of pituitary

TABLE I

Statistical Comparison of Pituitary Content and Concentration of HGH, Weight of Pituitary Gland, and Serum HGH at Different Gestational Ages

Gestational age	Pituitary content of HGH in micrograms	Pituitary concentration of HGH (μg/mg)	Weight of pituitary gland	Serum HGH
wk				
10–14 vs. 15–19	<0.02*	<0.05*	<0.05*	<0.001*
10–14 vs. 20–24	<0.001	<0.001	<0.005	<0.005
10–14 vs. 25–29	<0.001	<0.001	<0.005	<0.005
15–19 vs. 20–24	<0.001	<0.005	<0.005	>0.5
15–19 vs. 25–29	<0.001	<0.005	<0.005	>0.5
20–24 vs. 25–29	<0.001	>0.1	<0.005	>0.5
20–24 vs. 30–34	<0.001	<0.001	<0.005	<0.025
25–29 vs. 30–34	<0.001	>0.5	<0.005	<0.025
30–34 vs. 35–40	>0.5	>0.5	<0.02	>0.5

* *P* value.

growth hormone (Figs. 2 and 3). At 15–19 wk the mean GH content was 9.21±2.31 μg/pituitary and mean concentration of 2.02±0.55 μg/mg; at 20–24 wk the mean content was 59.38±11.08 μg and mean concentration of 3.83±0.60 μg/mg; at 25–29 wk mean content was 225.93 ±40.49 and mean concentration 9.24±3.25 μg/mg; 30–34 wk the mean content was 577.67±90.0 with a mean concentration of 9.34±1.22 μg/ml; and at 35–40 wk the mean content was 675.17±112.33 and the mean concentration was 7.50±1.47 μg/mg (Fig. 1). Significant differences were observed both in content and concentration of HGH at different gestational ages with the exception of those during the last 2 months of fetal life (Table I).

The increment in content and concentration of pituitary GH bore a significant relationship to gestational age, crown-rump length, and to the weight of the pituitary gland (Table II and Figs. 2 and 3).

The concentration and content of GH in the pituitaries of children less than 1 yr of age was comparable to the levels detected in fetal pituitaries at 32–40 wk of gestation with a mean content of 1202±252 μg and a mean concentration of 10.1±1.4 μg/mg (Fig. 1). A wide variation in the GH content was noted in the small sampling of pituitaries from children 1–9 yr of age with a mean of 5577.0±1235 μg. The pituitary concentration of growth hormone in this group of children ranged from 3.9 to

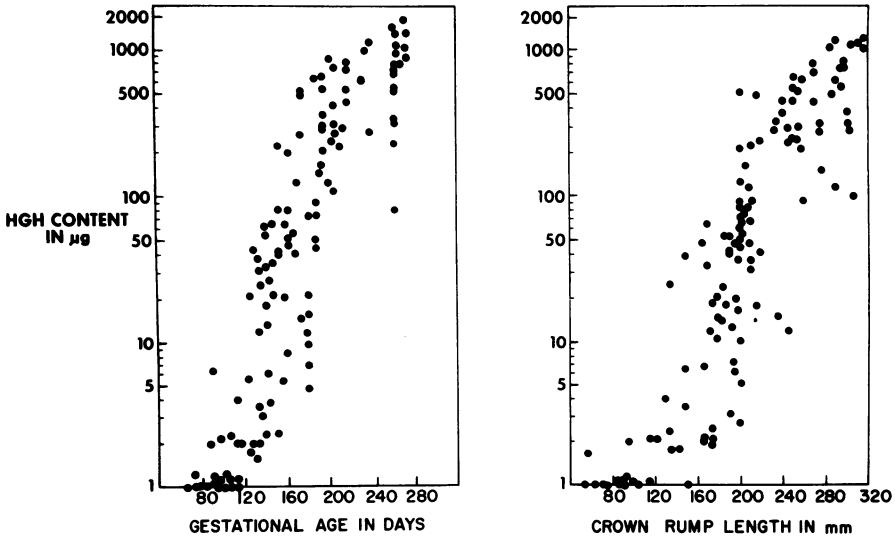

FIGURE 2 The content of HGH in fetal pituitary gland is plotted on a semilogarithmic scale against gestational ages in days on the left panel and crown-rump length in millimeters on the right panel.

TABLE II

Correlation of Gestational Age, Weight of Pituitary Gland, Content and Concentration of Pituitary HGH, and Serum HGH

	Gestational age vs. content of HGH in pituitary	Gestational age vs. concentration (μg/mg) HGH in pituitary	Gestational age vs. weight of pituitary gland	Weight of pituitary gland vs. content of HGH in pituitary gland	Gestational age vs. serum HGH	Paired serum GH vs. content of HGH in pituitary gland
N	115	115	115	115	78	17
r	0.77	0.32	0.80	0.73	−0.48	−0.11
t	12.6	3.6	14.8	11.4	−4.8	−0.43
P	<0.001	<0.001	<0.001	<0.001	<0.001	>0.5

65.8 μg/mg. The content of GH in the pituitary glands of the four adults ranged from 6.5 to 12.0 mg and the concentration of GH was 12.3–28.6 μg/mg pituitary gland.

Immunologic and physicochemical data. The immunologic reactivity of HGH in the serum and homogenates of fetal pituitary glands was compared to that of adult pituitaries. Serial dilutions of pituitary homogenates and sera of fetuses bore a parallel relationship to the standard curve for purified human growth hormone (Fig. 4).

Further evidence for the similarity of the GH in the pituitary gland of the fetus and adult was the comparable physicochemical properties observed after disc electrophoresis (Fig. 5). The position of the major band for the pituitary homogenates is similar, but the major band of the purified HGH was slightly more anodal. Similar observations were made after immunoelectrophoresis. The crest of the precipitin arc for the fetal pituitary homogenate when reacted with antiserum to HGH was located in slightly cathodal position and was similar to that observed for homogenates of pituitary glands of the children and adults (Fig. 6). The precipitin band of the purified pituitary HGH was slightly more anodal. Starch gel electrophoresis of pituitary homogenates of fetuses, children, and adults demonstrated comparable mobility of the major bands for GH.[2]

Serum growth hormone. GH was present at a concentration of 14.5 ng/ml in the youngest of 62 fetuses studied from 70 to 245 days of gestation. A rise to peak levels was observed by 20–24 wk with a gradual decrease of up to 60% in the concentration of serum GH by term (Fig. 7). The mean concentration at 10–14 wk was 65.2±9.6 ng/ml; at 15–19 wk mean concentration was 114.9±12.5 ng/ml; at 20–24 wk mean concentration was 119.3±19.8 ng/ml; at 25–29 wk the mean concentration was 72.0±6.5 ng/ml; and at 30–40 wk the mean concentration was 26.5±11.5 ng/ml. The mean concentration in umbilical venous serum specimens obtained at delivery was 33.5±4.2 ng/ml.

The concentration of serum HGH showed a significant negative correlation with gestational age ($P < 0.001$) (Table II).

A significant difference was demonstrated in the mean concentration of serum HGH at 10–14 wk compared with all other gestational periods ($P > 0.05$) (Table I). No significant difference was demonstrated between the mean concentration at 15–19 wk, 20–24 wk, or 25–29 wk. The mean concentration of serum GH at 25–29 wk was significantly greater than at 30–40 wk ($P < 0.02$).

In 17 fetuses from which matched serum and pituitary specimens were obtained, there was no significant correlation of the concentration of serum HGH to the pituitary content of HGH ($P > 0.5$) (Table II, Fig. 8).

Serum HCS. HCS was detected in all 48 fetal sera tested in concentrations of 2–240 ng/ml. The levels were higher in some fetuses at mid-gestation than at term. There was no correlation of the concentrations of serum HCS with gestational age or the concentration of serum HGH (Fig. 9). The levels of fetal HCS were in sharp contrast to the expected levels in the maternal circula-

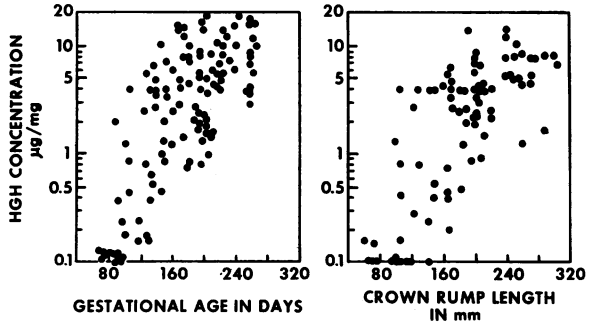

FIGURE 3 The concentration of HGH in fetal pituitary is plotted on a semilogarithmic scale against gestational age in days on the left panel and crown-rump length in millimeters on the right panel.

[2] Deamidation and other structural alterations of pituitary HGH during purification procedures affect its electrophoretic mobility (34).

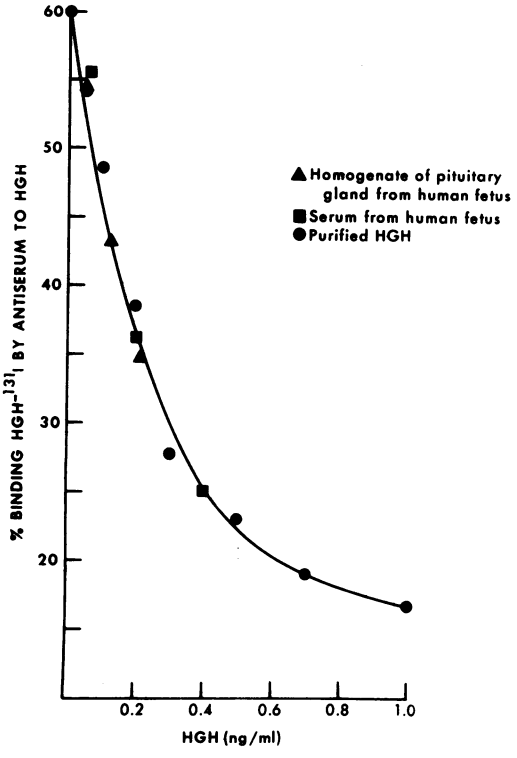

FIGURE 4 Standard curve for HGH in double antibody radioimmunoassay method. The per cent binding of HGH-^{131}I to anti-HGH serum is indicated on the ordinate and HGH concentration in nanograms per milliliter on the abcissa.

tion at that gestational period. Although elevated levels of HCS can affect the measurement of HGH, cross-reactivity with HCS was unlikely since the HCS concentration in the diluted serum samples utilized in the assay was less than 0.5 ng/ml. This concentration of HCS would have an insignificant effect on the measured concentration of GH and an equivalent concentration of HGH would not affect the determination of HCS.

Insulin.[8] The mean concentration of serum insulin measured in 42 fetuses with a gestational age of 84–245 days was 8.0±1.5 μU/ml with a range of 1–30 μU/ml. Immunoreactive insulin was not detected in four fetuses. There was no relationship to gestational age.

DISCUSSION

The ontogenesis of GH in the human fetal pituitary is described in these studies. Early in gestation, the fetal pituitary synthesizes and secretes GH which is similar immunologically and physicochemically to that of the child and adult. These observed changes correlate with morphogenetic development of the pituitary gland and hypothalamus during gestation.

The anlage of the anterior hypophysis appears during the 7th to 8th wk of fetal life (1, 5) and by the 9th to 10th wk acidophiles (2–5) and immunoreactive GH are present in the pituitary (10, 17–19). Cytologic differentiation proceeds concomitantly with increases in pituitary weight (1–3, 5) and incremental changes in pituitary content of GH. A 4-fold increase in the weight of the pituitary gland occurs between the 10th and the 24th wk of gestation which is associated with a 15-fold increase in the content of GH. By term, the weight of the pituitary gland increases 7- to 10-fold over the mean weight observed at 24th wk of gestation, with a 5-fold further increment in GH content.

The findings are consistent with those reported previously from our laboratory (15, 17, 19) and by Matsuzaki, Irie, and Shizume (18). Gitlin and Biasucci (13), Pasteels, Brauman, and Brauman (11), Solomon, Grant, Burr, Kaplan, and Grumbach (12), and Gailani, Nussbaum, McDougall, and McLimans (14) have demonstrated the secretion of immunoreactive and biologically active GH by human fetal pituitary glands (14–24th wk of age) grown in tissue culture. Growth hormone activity has been detected by other workers in fetal pituitaries of the human (35, 36) and pig (37) in the first trimester of gestation.

The sequential rise in the pituitary content of growth hormone and its concentration in serum from 70 to 150 days is associated with the development of the hypo-

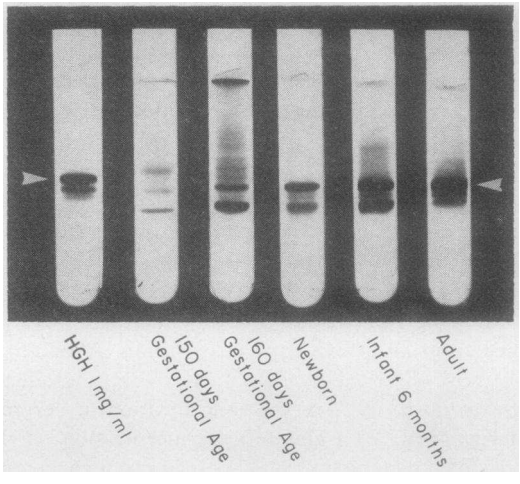

FIGURE 5 Disc gel electrophoresis of homogenates from pituitary glands or fetuses at 150 and 160 days of gestation, a newborn infant, a 6 month old infant, and from an adult are compared to the pattern observed for a purified Wilhelmi preparation of HGH. Note that the major band for HGH (indicated by arrow) in pituitary homogenates is similar in electrophoretic mobility at all ages listed but less anodal than the major band of the purified HGH.

[8] The radioimmunoassay does not discriminate between insulin and proinsulin.

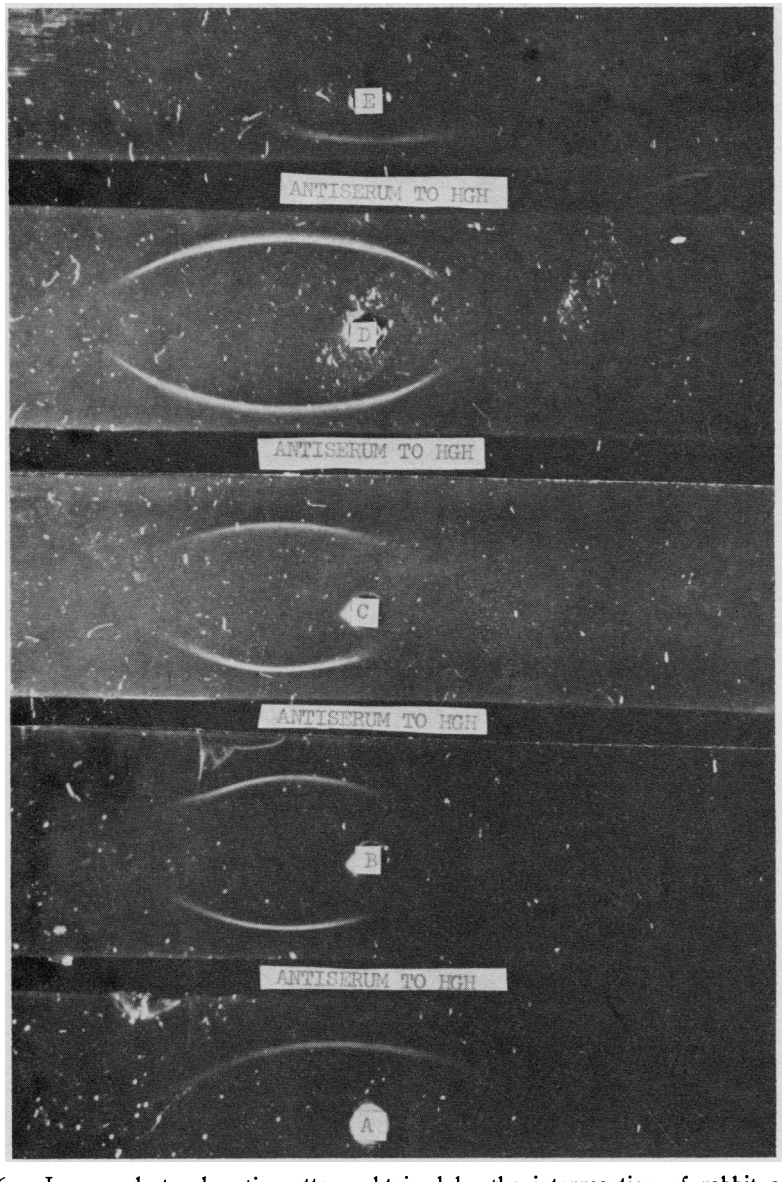

FIGURE 6 *a* Immunoelectrophoretic pattern obtained by the interreaction of rabbit anti-HGH serum (absorbed with human serum proteins) added to troughs with the following antigens added to the wells: (1) HGH (Raben) 200 μg/ml in well A; (2) homogenate of pituitary gland from a 22 wk old human fetus in well B; (3) homogenate of a pituitary gland from a 29 wk old human fetus in well C; (4) homogenate of a pituitary gland from a 2½ yr old child in well D; and (5) HGH (Raben) 50 μg/ml in well E.

thalamus and portal system. The first hypothalamic nuclei and fibers of the supraoptic tract are seen by 55 days of gestation (38), soon after the appearance of the anlage of the pituitary gland. By the 16th wk of gestation, at a time when differentiation of the pars tuberalis, median eminence, and remainder of the hypothalamic nuclei occurs, there is a 20-fold increase in pituitary HGH content. Neurosecretory material is present in the supraoptic and paraventricular nuclei at this time (39, 40). Monoamine fluorescence appears in the hypothalamus of the human fetus by the 10th wk and in the median eminence by the 13th wk (41). The hypothalamic nuclei continue to increase in size until the early postnatal period when structural development is completed. The primary plexus of the portal vascular system is initiated by 100 days, at which time capillaries are in abundance in the pituitary gland (42, 43). Peak GH content of the fetal pituitary seems to coincide with the establishment of the continuity

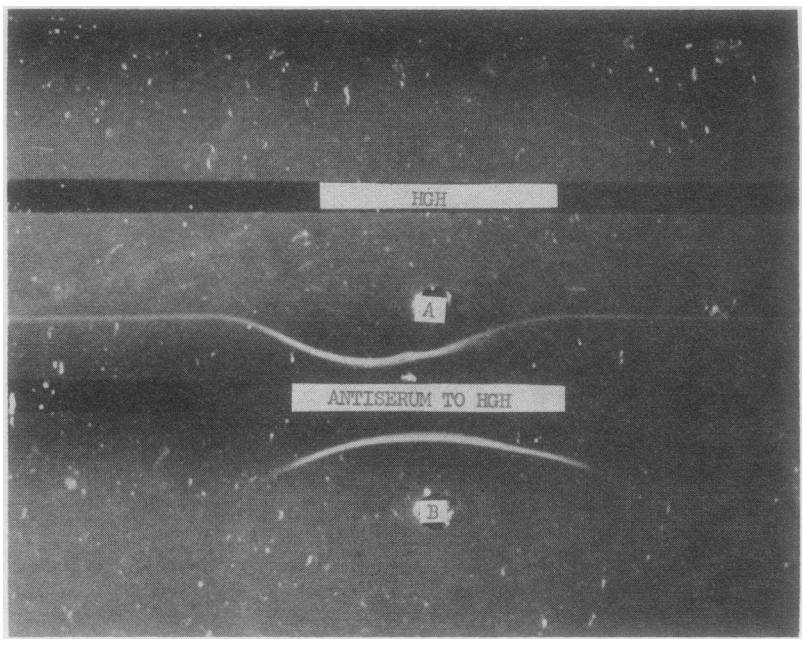

FIGURE 6 *b* Immunoelectrophoretic analysis of a homogenate of a pituitary gland from a 31 wk old fetus (well A) by the Osserman technique. Rabbit antiserum to HGH was added to the lower trough and HGH (Raben) 100 μg/ml to upper trough. The purified HGH diffused from the trough and formed a straight line of precipitation with the antiserum. This line of precipitation fused with the cathodal and anodal tips of the precipitin arc formed by the reaction of the electrophoresed pituitary homogenate with the rabbit antiserum to HGH, thus indicating immunologic identity of the two antigens. HGH (Raben) 200 μg/ml added to well B and subjected to electrophoresis has reacted with rabbit antiserum to HGH to form a precipitin arc.

of the primary and secondary plexus of the portal system and maturation of the hypothalamus by 130 to 150 days of gestation.

These data and recent evidence on the diminished response to provocative tests for GH release in the fetus and newborn suggest that CNS regulation of the secretion of growth hormone releasing factor (GRF) may not be fully operative until the postnatal period. Mintz, Chez, and Horger (44) and Chez, Mintz, Horger, and Hutchinson (45) were unable to elicit an increased growth hormone response to arginine or to hypoglycemia nor suppression of the elevated fetal GH by administration of glucose in the simian fetus. Similar findings were observed in sheep by Bassett, Thorburn, and Wallace (46). The poor suppressibility of GH after administration of glucose has been reported in the human neonate (47, 50) and is comparable to studies in the simian neonate (44) and in the lamb (51). By 1 month of age, glucose suppression of GH secretion is demonstrable in the human and simian neonate (44, 47). In contrast, an increase in serum GH after insulin-induced hypoglycemia or arginine infusion can be observed in the newborn human and monkey (45, 47).

This pattern of GH secretion in the human fetus may reflect the immaturity of the neurophysiologic function of the brain. Bergstrom has suggested that the electrical activity of the brain progresses from a simplistic primitive stage during the first 70–120 days of gestation to a phasic asynchronous stage, and finally in late gestation and the early postnatal period synchronous activity with inhibitory restraint is apparent (52).

The electroencephalographic changes and motor activity in the human fetus support this interpretation. No EEG or motor activity is apparent until the second month of gestation (53). By 5 months of fetal life, increased brain wave activity is noted with onset of electrical activity in the diencephalic area (54, 55). Increased motor activity is present but is not fully coordinated. Serum growth hormone is at peak concentration during this period.

By the 8th month of fetal life, a distinction between sleep and wakefulness stages can be demonstrated on EEG, but asynchrony between hemispheres persists throughout the immediate neonatal period (52, 55, 56). Motor activity is restrained, coincident with the appearance of inhibitory circuits in the higher brain centers. The secretion of GH is comparatively reduced postnatally. Synchronous hemispheric activity with EEG changes in response to sleep becomes apparent by 2 months of age (57). The absence of sleep-induced GH

release has been demonstrated in the human neonate during the first 2 months of life (48–60).

The chronology of events in the development of hypothalamic-hypophyseal function may be interpreted as follows: Onset of GH secretion is coincident with appearance and increase in acidophiles in the fetal pituitary and at this stage autonomous secretion by the fetal pituitary may occur. The absence of a contiguous portal system does not eliminate the possibility that GRF stimulation of growth hormone secretion can occur at this time by simple diffusion. By mid-gestation, with appearance of hypothalamic nuclei and electrical activity of the diencephalon, secretion of GRF may occur with resultant unrestrained release of GH by the fetal pituitary. In late gestation, the neural inhibitory influences become operative and can lead to decreased GRF and GH secretion. Regulatory mechanisms for control of GH secretion may not become fully functional until infancy, at which time myelination, cortical development, and synchronous EEG activity are at a mature stage (Fig. 10). A similar developmental cycle may be operative in the control of secretion of other fetal pituitary hormones, such as fetal follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (20) and fetal thyroid-stimulating hormone (TSH) (21, 22).

Absent or deficient GRF secretion throughout the gestational period with autonomous release of growth hormone by the fetal pituitary is an alternate explanation for the observed data. This is not consistent with

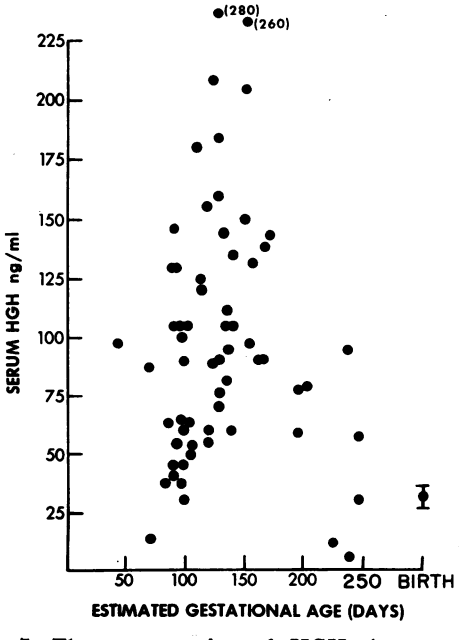

FIGURE 7 The concentration of HGH (nanograms/milliliter) in fetal serum is plotted on the ordinate against gestational age in days on the abscissa.

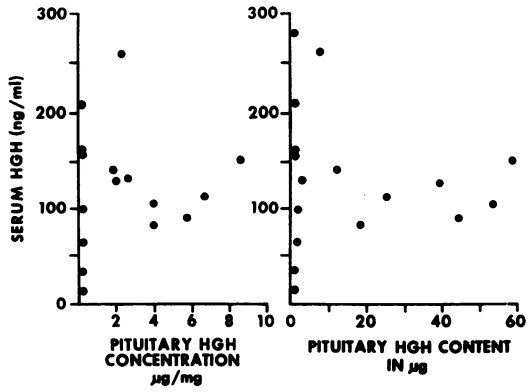

FIGURE 8 The concentration of serum growth hormone (nanograms/milliliter) on the ordinate is compared with the concentration of pituitary growth hormone (micrograms/milligram) (left panel) and with content of pituitary growth hormone (right panel) in paired serum and pituitary homogenate samples obtained from 17 human fetuses.

the demonstration of low levels of serum growth hormone (17, 61) in anencephalic fetuses, most of whom have acidophile cells in the pituitary and anomalous development of the hypothalamus and adjoining structures.

One must consider the possibility that the stress of parturition and fetal distress from other factors may be responsible in part for the marked elevations in the concentration of serum growth hormone observed in the fetus at mid-gestation (62–64). In the present study, however, the concentration of growth hormone in the serum or pituitaries from fetuses removed by hysterotomy was similar to that of spontaneously aborted fetuses. Turner, Schneeloch, and Paterson (62) have noted that in fetuses delivered by hysterotomy at 20–24 wk of gestation, the concentration of serum growth hormone is threefold higher in peripheral blood (mean 90±51 ng/ml) obtained postdelivery than that noted at delivery in

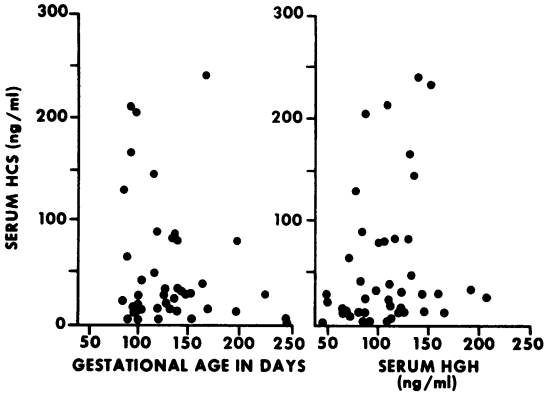

FIGURE 9 The concentration (nanogram/milliliter) of chorionic somatomammotropin (HCS) is plotted on ordinate against gestational age in days (left panel) and against serum HGH in nanograms/milliliter (right panel).

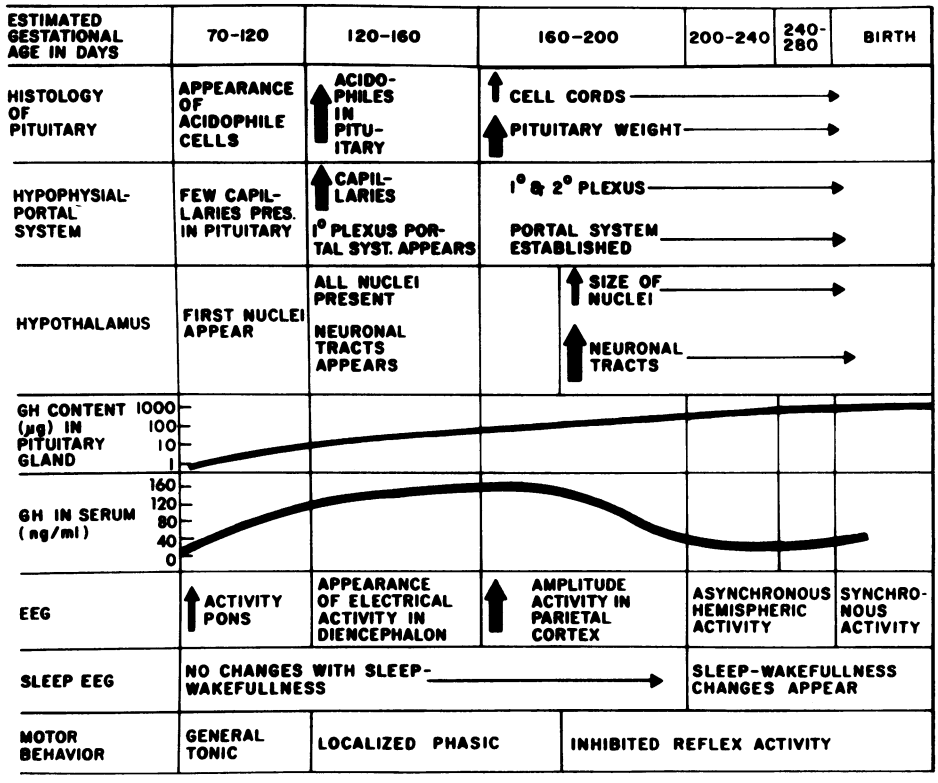

FIGURE 10 The ontogeny of GH secretion by the human fetus as correlated with histologic changes in the pituitary and the development of the portal system and central nervous system.

umbilical cord blood (mean 59±22 ng/ml). At term, the concentration of growth hormone is lower in umbilical vein samples (65–67) than in fetuses at 20–24 wk of gestation. Further, Aubert, Sistek, and Bossart (64) showed that the magnitude of the serum GH rise 1 hr postdelivery is less in fetuses delivered at term than at mid-gestation (62). This is in accord with the observations of Cornblath, Parker, Reisner, Forbes, and Daughaday (47). The serum GH rise in response to acidosis or anoxia of the fetus is significantly less in full-term fetuses than in those delivered at mid-gestation (62, 64). This difference in the growth hormone responsiveness to stress with maturation of the fetus would be consistent with our hypothesis of a persistent immature state or incomplete development of control mechanisms for the release of growth hormone until early infancy.

Delayed removal of GH from the fetal circulation could contribute to the elevated levels of serum GH. The disappearance rate of exogenously administered GH was comparable in the premature infant (47) to that observed in the child and adult. In the sheep fetus, Bassett and associates (46) reported a slight prolongation in the disappearance rate of GH (34 min) when compared to that observed in neonatal lambs (17 min). Hence, it is unlikely that delayed disposal of growth hormone is a major factor in the elevated serum GH values in the fetus. Transplacental passage of HGH from the maternal circulation is negligible (68) and does not contribute to the elevated fetal GH levels.

The content of GH in the pituitary glands of children was significantly higher than that observed throughout fetal life. This is in contrast to the study of Gershberg (69) in which age-related differences in bioassayable HGH was not demonstrated.

The characteristics of a human placental hormone, chorionic somatomammotropin (HCS), which bears many similarities to HGH have been well documented in the past and detailed in a recent review (70). Transplacental passage of HCS is limited as evidenced by the low concentration of HCS in umbilical venous sera (71), in fetal sera in contrast to maternal sera, and by the limited transfer to the fetus of HCS-^{131}I administered to the mother (72). The high levels (> 100 ng/ml) present in a few of the fetal serum samples may reflect increased placental permeability or absorption from amniotic fluid. The latter seems more likely since the concentration of HCS is high in amniotic fluid and meconium (67, 72). The concentration of HCS in the fetal sera, however, is only 1/20th to 1/300th of that present in the maternal circulation at that gestational period.

There is no apparent relationship between the levels of serum HCS and serum HGH in the fetus throughout gestation.

Insulin secretion by the human fetal pancreatic islets was detected by 84 days in the present study in agreement with histologic data (73, 74) and immunoassay measurements of fetal insulin extracted from pancreatic islets (75–79). Immunoreactive insulin can be localized in the islets utilizing the fluorescent antibody technique by 80 days gestation, a week after the first histologic evidence of islet formation (76). Van Assche demonstrated a positive correlation between the number of beta cells per islet and the extractable immunoreactive insulin content of the human fetal pancreas (77). A progressive rise in pancreatic insulin content in the human fetus from 10 to 12 wk of gestation until 24 wk of gestation was noted by Rastogi, Letarte, and Fraser (78). A further increase in pancreatic insulin content occurs between 34 and 40 wk of gestation (75). Not only is the relative percentage of islet tissue per total pancreas higher (mean 5.1±1.6%) in the fetus and the newborn of normal mothers than in the normal adult (1.5%) (77), but the insulin concentration is higher in the fetus than in the adult (78, 80).

The concentration of serum insulin does not appear to rise with advances in fetal development based on the present study and the data of Adam, Teramo, Räiha, Gitlin, and Schwartz (81), Paterson, Page, Taft, Phillips, and Wood (82), and Thorell (83), nor is insulin secretion affected significantly by acute administration of glucose either in vivo (44, 81, 84–86) or in vitro (87, 88). At term, insulin responsiveness to glucose has been variable (48, 89–91). Intravenous arginine does not induce insulin release in the simian (44), ovine (46), or human fetus (50, 92), although Grasso, Messina, Saporito, and Reitano (93) demonstrated a rise in insulin secretion in premature infants after infusion of a mixture of essential amino acids. This latter discrepancy remains unresolved. Insulin secretion can be increased in the fetus by glucagon administration (85) or by combined theophylline and glucose infusions (94). Chez, Mintz, and Hutchinson (94) suggest that in the fetus a defect in insulin release rather than synthesis exists which may be a consequence of decreased availability of cyclic AMP.

Is fetal GH physiologically active in the fetus? The biological activity of human and animal fetal GH has been demonstrated in animals by classic bioassay methods. However, available evidence suggests that neither maternal nor fetal human growth hormone is essential for normal fetal growth. The birth length of the apituitary fetus, anencephalic fetus, or children with idiopathic hypopituitarism is usually within the normal range (95–100).[4] Similar findings have been observed in mice with congenital pituitary dwarfism (101) and after hypophysectomy of fetal rats (102), rabbits (102), sheep (103, 104), and monkeys (105). Children born to women with isolated growth hormone deficiency (106, 107) or to mothers hypophysectomized during gestation do not show evidence of growth retardation (105, 108, 109).

The possible role of insulin as a "growth hormone" in the fetus is not inconsistent with its known biological actions. Administration of insulin to the rat fetus induces changes in length and weight as well as an increase in body content of proteins and lipids (110, 111). Body weight and the width of the tibial epiphysis is increased in hypophysectomized rats treated with insulin (112). Elevated circulating insulin levels in the fetus as in infants of diabetic mothers and in those with transposition of the great vessels is associated with increased birth weight and length (113, 114). Conversely, infants with congenital diabetes tend to be of low birth weight and length (115).

The importance of other factors, including somatomedin on skeletal growth in the fetus remains speculative. Somatomedin, the plasma mediator of the anabolic effects of growth hormone (116–118) is present in the fetus.[5] The hormones or factors which promote synthesis and secretion of somatomedin in the fetus are unknown. Indirect evidence suggests that somatomedin may affect fetal growth. The birth length of children with an hereditary form of dwarfism associated with decreased plasma somatomedin activity is significantly retarded (119, 120) in contrast to the normal or near normal birth length of apituitary fetuses and children with hypothalamic hypopituitarism. Hence, there may be other effectors of somatomedin synthesis in the fetus. Since the absence of growth hormone is not rate-limiting in terms of fetal growth, other humoral growth factors as yet unidentified may be present in the fetus. Nerve growth factor (121), recently shown to have similarities in structure and function to that of proinsulin (122) may be representative of other inducer substances present during fetal life which may influence fetal development.

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[4] Kaplan, S. L., and M. M. Grumbach. Unpublished data.

[5] Van Wyk, J. J. Personal communication.

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