

**Changes in Serum Growth Hormone Levels from Fetal to Adult Age in the Rat<sup>1</sup> (38437)**ENRIQUE BLÁZQUEZ,<sup>2</sup> FREDERIC A. SIMON,<sup>2</sup> MERCEDES BLÁZQUEZ, AND PIERO P. FOÀ*Department of Research, Sinai Hospital of Detroit, Detroit, Michigan 48235*

Several hormones play an important role in metabolic regulation during fetal and postnatal development (1, 2). Among them, are insulin that favors the storage of nutrients during fetal life and glucagon that favors their mobilization at birth, when the fetus loses its placental support (3, 4). Evidence that fetal growth hormone is biologically active (5) and that maternal growth hormone does not cross the placental barrier (6), suggests that the fetal pituitary may also play a role in the regulation of fetal and perinatal growth and metabolism, even if not as important as the role that it plays in the 6- to 28-day-old animal (7, 8). We have investigated this problem by measuring the level of serum growth hormone (GH) and the rate of weight gain of rats during that period of development when, according to previous work (10), a correlation exists between growth and the level of serum insulin.

**Materials and Methods.** Female Wistar rats, weighing 200–250 g, with free access to commercial rat chow and water were caged with males until copulation had occurred, as determined by the presence of spermatozoa in the vaginal smear (for details, see 11). On the anticipated 22nd day after fertilization, the animals were kept under observation and the actual time of delivery was noted. Blood of the pups, obtained by decapitation, was collected in pre-chilled test tubes, allowed to clot and centrifuged at 4°. The serum samples were preserved at –20° for subsequent analysis. In other experiments, fetuses were removed by cesarean section, immediately after the mother had been sacrificed by cervical dislocation and their blood was col-

lected as described above. Blood samples of fetuses and of nursing pups of the same litter were pooled, while those of 20-, 30-, and 45-day-old rats were analyzed individually. Growth hormone was measured using a modification of a double antibody radioimmunoassay (12), using standards prepared with rat GH (3.5 U/mg)<sup>3</sup> and an anti-rat-GH serum (ARGHS) prepared in chickens<sup>3</sup>. The second or precipitating antibody (PA) was prepared in rabbits. For this purpose, 10 mg of chicken gamma globulin<sup>4</sup> and 1 ml of 0.9% sodium chloride were mixed with an equal volume of Freund's incomplete adjuvant<sup>5</sup>. This suspension was injected into the foot pad of New Zealand rabbits (2.5 mg/pad) at weekly intervals. After four injections of this mixture, the rabbits received two weekly injections of antigen without adjuvant and were bled ten days after the last injection. Rat GH<sup>6</sup> was labeled with Iodine-131 using the method of Hunter and Greenwood (13) and was analyzed by chromato-electrophoresis and purified on a column of Sephadex G75. The assay consisted of mixing 0.1 ml of diluted sample (see below) and 0.7 ml of buffer or 0.8 ml of standard rat GH solution (0.125–8.0 ng/0.8 ml) with 0.1 ml of ARGHS and 0.1 ml of labeled rat GH solution. After incubation at 4° for 3 days, 0.1 ml of PA, diluted 1:500 with chicken plasma<sup>4</sup>, was added to all tubes. The tubes were mixed, incubated for 18 hr at 4° and spun at 3000 rpm for 20 min in a refrigerated centrifuge. The supernatant was decanted and the radioactivity of the precipitate was measured in an automatic gamma counter.

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<sup>3</sup> Gift of Dr. J. M. Martin, Hospital for Sick Children, Toronto, Ontario, Canada.

<sup>4</sup> Nutritional Biochemical Company, Cleveland, OH 44128.

<sup>5</sup> Difco Laboratory, Detroit, MI 48232.

<sup>6</sup> Rat GH-1-2, National Institute of Arthritis and Metabolic Diseases, Bethesda, MD 20014.

The serum samples were diluted to contain 0.5–4 ng of GH. In this range of concentrations, corresponding to the steepest portion of the standard curve (Fig. 1), the method detects differences of 0.125 ng/ml ( $P < 0.001$ ).

**Results.** (Table I). The concentration of GH in rat serum increased rapidly from the 19th to the 21st day of intrauterine life. During the last day of pregnancy, the hormone level began a period of decline, reaching a plateau toward the end of the nursing period. The rate of weight gain was greatest between the 19th and 20th day of fetal life and declined progressively thereafter except for a brief period between the 5th and the 10th day after birth. Thus, this parameter of growth followed a course that was not entirely parallel to the concentration of serum GH.

**Discussion.** Significant amounts of growth hormone were detected in the serum of 19-day-old rat fetuses. Since this is the age when the hormone first appears in the pituitary gland (14) and since maternal growth hormone does not

cross the placental barrier (6), we can assume that the hormone circulating in the fetus was of fetal origin. Indeed, significant levels of GH biologically, chemically and immunologically similar to the adult hormone (14), have been found in fetal blood and pituitary glands (14–18) of several animals. Nevertheless, the role of GH in fetal growth and its possible relationship to the development of pancreatic function are uncertain and may vary from species to species. Thus, birth length and body weight of most anencephalic human fetuses without pituitary and of most children with hypopituitarism are within the normal range (19–21), while rabbits and mice decapitated in utero continue to grow at a normal rate until birth (22, 23). On the other hand, GH appears to be necessary for normal fetal growth in sheep and cattle (24, 25). The role of the pituitary in the fetal rat growth is controversial. According to Jost (22), it has only a minimal effect, while according to Heggestad and Wells (7, 8), fetal growth, somewhat stunted

#### RADIOIMMUNOASSAY OF RAT GROWTH HORMONE STANDARD CURVE

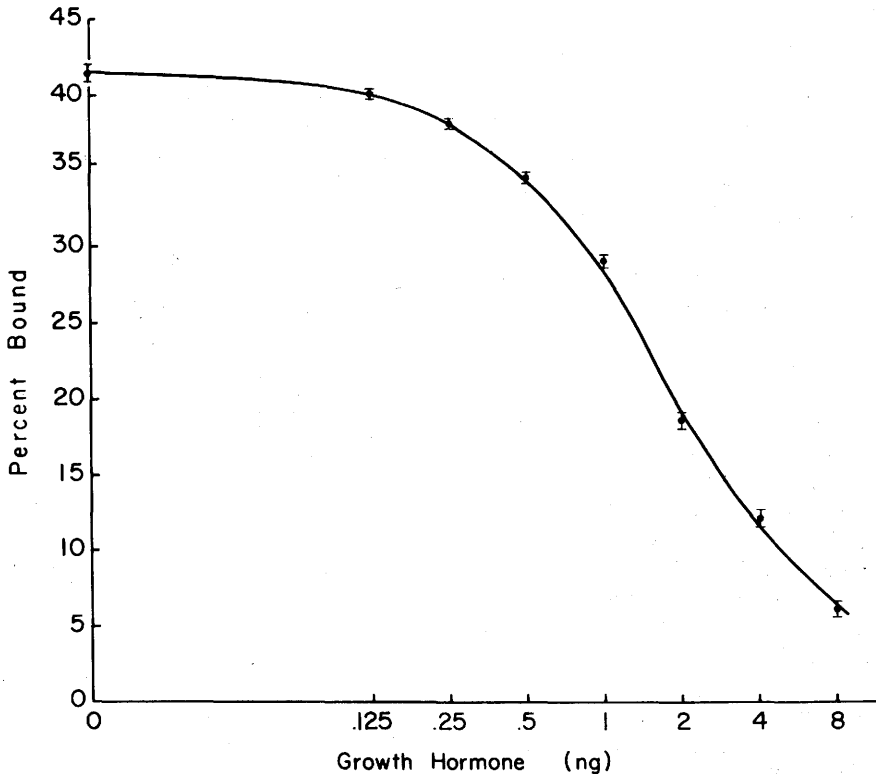


FIG. 1. Radioimmunoassay of rat growth hormone. Each point represents the average of five determinations  $\pm$  SE.

TABLE I. Body Weight, Rate of Weight Gain and Serum Growth Hormone Level in the Developing Rat.

Age	Body wt (g)	Rate of weight gain		Growth hormone (ng/ml)
		g/day	percent/day	
<b>Intrauterine</b>				
19 days	1.9 ± 0.06 (15)	—	—	14.3 ± 0.4 (6)
20 days	3.5 ± 0.11 (15) <sup>a</sup>	1.6	84	58.0 ± 5.5 (6) <sup>a</sup>
21 days	5.4 ± 0.05 (15) <sup>b</sup>	1.9	54	102.5 ± 9.7 (6) <sup>b</sup>
22 days	6.4 ± 0.07 (15) <sup>c</sup>	1.0	16	83.6 ± 9.1 (5) <sup>a</sup>
<b>Extrauterine</b>				
2 hr	—	—	—	78.2 ± 9.5 (4)
6 hr	6.9 ± 0.19 (8)	—	—	81.6 ± 15.7 (5)
1 day	7.3 ± 0.2 (15)	0.9	14	75.7 ± 8.4 (7)
5 days	13.4 ± 0.5 (15) <sup>f</sup>	1.2	12	51.8 ± 4.4 (7)
10 days	25.2 ± 0.7 (15) <sup>g</sup>	2.3	17	32.9 ± 1.9 (7) <sup>d</sup>
20 days	39.6 ± 1.6 (15)	1.4	6	17.2 ± 1.7 (7) <sup>d,e</sup>
30 days	75.9 ± 3.2 (15)	3.6	9	20.2 ± 0.6 (8) <sup>d</sup>
45 days	144.5 ± 5.3 (15) <sup>h</sup>	4.6	6	24.6 ± 2.1 (7) <sup>d</sup>

<sup>a</sup> *P* < 0.01 vs day 19.<sup>b</sup> *P* < 0.01 vs day 19 and 20.<sup>c</sup> *P* < 0.01 vs day 21.<sup>d</sup> *P* < 0.01 vs day 1.<sup>e</sup> *P* < 0.01 vs day 5.<sup>f</sup> *P* < 0.001 vs day 1.<sup>g</sup> *P* < 0.001 vs day 20 and 30.<sup>h</sup> *P* < 0.001 vs day 45.

following hypophysectomy, can be restored to normal by treatment with GH. The results of our experiments suggest that the contribution of GH to growth is relatively small during the last few days of gestation, since during that period, the rate of weight gain decreased, even as the circulating level of GH was rising. After birth, both parameters started to decline, although at this age the role of GH may become more significant, as indicated by the marked growth retardation that follows hypophysectomy (9). This problem has not been resolved because, in the mammalian newborn, the mechanisms for the secretion of GH does not appear to have reached full maturity. Thus, while our results indicate that the serum levels of GH during the perinatal age are not related to the marked changes of glucose, insulin and glucagon which occur at that time (3, 11), other evidence shows that GH release is not modified by the administration of glucose or by hypoglycemia in human, simian and ovine neonates (26–28). On the other hand, there is evidence that insulin may be an important growth factor, since it is actively synthesized and secreted by the fetal pancreas (29, 30) and its serum concentrations, which reach maximum values just before birth (3, 10), correlate well with the weight and length of the newborn (31–33). Other observations demonstrate that injections of insulin into the rat fetus during

the last days of pregnancy increase body weight, nitrogen retention and lipid storage (34, 35) and that insulin stimulates cellular growth (36) and the incorporation of arginine into the protein of fetal rat liver cultures (37). It is also possible that insulin and growth hormone may act synergistically (35, 38). Indeed, GH failed to initiate DNA synthesis by itself, but enhanced the stimulatory effect of insulin and restored B cell function (39) that had been reduced by hypophysectomy (40). These observations and the fact that the rate of weight gain is more closely parallel to the concentration of serum insulin than to that of serum GH support the concept of a synergistic action between the two hormones and suggest that, in relation to growth, GH rather than insulin may play the role of permissive factor.

**Summary.** The concentration of immunoreactive growth hormone (GH) in the serum of rats increased significantly from the 19th to the 21st day of fetal life, began to decrease on the last day of gestation, reached minimum values on the 20th day of extrauterine life and increased again after the pups were weaned. The relative rate of weight gain was greatest between the 19th and the 20th day of fetal life. After this time, it declined progressively, except for a brief period between the 5th and 10th day after birth. Thus, this parameter of growth followed a course that was more closely parallel to previously pub-

lished changes in the level of serum insulin than in the changes in serum GH observed in these experiments. It is possible that, in fetal and young rats, growth may depend upon a combined action of insulin and GH in which the latter, rather than the former, plays the role of permissive factor.

*Addendum.* Since this manuscript was submitted for publication, we found that the oral administration of glucose (2 g/kg) or of a 10-amino acids mixture (41) (1 g/kg) had no effect on the serum growth hormone levels of 19-day-old rat fetuses and of 1-hr, 5-, 10-, 20-, 30-, and 45-day-old pups. These results support the notion that, in the rat, the release of growth hormone does not respond to stimuli that are effective in other species (42, 43). We wish to call the reader's attention to a similar study published very recently (44).

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