PITUITARY AND PLASMA GROWTH HORMONE LEVELS IN BULLS FROM BIRTH TO ONE YEAR OF AGE

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R Elationships between endogenous growth hormone (GH) and growth characteristics have been studied in several mammals including hogs (Machlin et al., 1968; Gerrits, 1968), cattle (Curl et al., 1968; Dev and Lasley, 1969), rats (Peake, Mariz and Daughaday, 1968) and man (Kaplan et al., 1968). Comparisons of these results are frequently difficult, however, because variations in growth have been induced by different methods and because of differences in methods used to assess GH status. For example, variations in growth rates may be due to genetic, environmental, age or size differences while GH status of an animal may be based upon anterior pituitary GH, plasma GH, GH turnover rate, hypothalamic GH releasing hormone, tissue responsiveness to GH or combinations of these.

The primary objectives of this study were to determine levels of pituitary and plasma GH in Holstein bulls between birth and 1 year of age, and to determine whether these levels were related to changes in growth rate. Another objective was to compare estimates of pituitary GH levels measured by bioassay with those measured by radioimmunoassay. Some other endocrine data and descriptions of reproductive development of the same bulls have been published recently (Macmillan and Hafs, 1968a, b; 1969). To our knowledge, GH changes with age have not been investigated previously in bulls.

Materials and Methods

Sixty-five Holstein bulls were killed in groups of five at monthly intervals from birth through 12 months of age. Their nutritional management was described by Macmillan and Hafs (1968a).

Anterior pituitaries were frozen (−79 C) within 20 min. of exsanguination. They were later thawed, homogenized in 10 ml of 0.85% saline and adjusted to 50 mg/ml. Supernatant fluids (pituitary extracts) obtained after centrifugation were pooled within age groups and assayed for GH potency by a modification of the rat tibia test (Evans et al., 1943).

Female rats, hypophysectomized at 28 days of age, were injected subcutaneously with standard GH or with unknowns beginning on the twelfth day after hypophysectomy. The standard NIH-GH-B9 was injected at levels of 25 or 100 micrograms. NIH-TSH-B3 was added 1:1 to the standard GH to compensate for the rat tibial response due to thyroid stimulating hormone (TSH) augmentation of GH in the pituitary homogenates (Greenspan et al., 1950). Pituitary extracts were injected in doses equivalent to 4 or 16 mg of pituitary tissue. Four rats were used at each dose level for each standard or unknown and the total dose was equally divided among twice-daily injections for 4 days. Twenty-four hours after the final injection, the proximal end of a tibia was sagitally sectioned, fixed (Evans et al., 1943) and the width of each epiphyseal plate was measured at eight randomly selected locations. The average width was used in calculating GH potencies by the method for parallel line assays (Bliss, 1952).

Growth hormone in pituitary extracts and in blood plasma from each bull were measured using a double antibody radioimmunoassay patterned after that reported by Niswender et al. (1969) for luteinizing hormone. Bovine GH (NIH-GH-B12) was labelled with 125I (Greenwood, Hunter and Glover, 1963) and the 125I-GH was separated from free 125I on a Biogel P-60 column. When not used within 10 days, the 125I-GH was repurified on a Sephadex G-100 column. The second column chromatograph yielded 3 radioactive peaks. The material from the second peak was used in the assay. A 1:3,200...
dilution of guinea pig antiserum to GH (NIH–GH–B12) was used as the binding antibody. Separation of antibody bound GH from free GH was accomplished with a 1:2 dilution of sheep anti-guinea pig gamma globulin. The precipitate was solubilized with 0.1 ml NCS reagent and $^{125}$I was quantified in Bray’s solution (Bray, 1960) in a Nuclear Chicago Mark I liquid scintillation spectrometer.

Standard tubes containing 0.0, 0.1, 0.3, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 or 5.0 ng standard NIH–GH–B12 were assayed with each lot of 38 unknowns. Radioimmunoassay standard curves were obtained from a multiple regression equation with linear, quadratic and cubic components. Plasma aliquants of 100 or 250 µl, and 100-µl aliquants of extracts containing 4 g pituitary/ml were assayed in duplicate. Dilutions of bovine plasma and of bovine pituitary extracts closely paralleled curves for standard GH. Addition of standard GH to plasma samples resulted in recoveries which were consistently greater than 100%, suggesting that these plasmas contained factors which altered one of the antigen-antibody reactions (Burr et al., 1969). But recoveries exceeded 110%, only at GH levels greater than 3.0 ng per tube, and levels this high were seldom encountered. Bovine TSH, LH, FSH and prolactin caused negligible reductions in binding of labeled GH.

Results and Discussion

The bulls had a constant growth rate (Macmillan and Hafs, 1968b) and their body weight increases (figure 1) were similar to growth standards (Morrison, 1956). Because of this linear growth, an estimate of the percentage of body weight at slaughter (slaughter weight) acquired during the preceding month (specific growth rate) could be obtained from the following equation.

Specific growth rate =

$$\frac{(\text{Slaughter wt} - 22.5 \text{ kg})}{(\text{Age (months)})(\frac{100}{\text{Slaughter wt}})}$$

The value 22.5 is the weight at birth according to the regression of weight (Y) on age (figure 1).

To estimate plasma GH content, plasma volume was calculated as 3.5% of body

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Figure 1. Body weight, anterior pituitary weight and blood plasma growth hormone content of Holstein bulls from birth to 12 months.
weight at slaughter in kilograms. Changes in plasma GH content and anterior pituitary weight with age (figure 1) indicate that a large portion of the variation in these two parameters can be accounted for by variation in body weight at slaughter. Plasma GH levels were higher at birth (figure 2) than at any other age (P<.01), in agreement with observations on humans (Cornblath et al., 1965) which revealed elevated plasma GH for the first 48 hr. after birth. The values for plasma GH concentration (figure 2) agree with others derived from radioimmunoassays in cattle (Trenkle and Burroughs, 1967; Eaton, Klosterman and Johnson, 1968).

Pituitary GH concentrations as measured by radioimmunoassay paralleled those measured by bioassays (figure 2) with a simple correlation coefficient of 0.60. The lack of a closer correspondence may be partially attributed to nine missing observations for the radioimmunoassays. The lower values for GH estimated by radioimmunoassay (figure 2) agree with similar data from rats (Birge et al., 1967; Schalch and Reichlin, 1966). Garcia and Geschwind (1968) discussed situations where results from immunoasays and bioasays of pituitary GH were not compatible and suggested that this may have resulted from the two assays measuring different properties of the GH molecule. Subsequent discussion will concern radioimmunoassay values only.

Pituitary GH (figure 2), measured by radioimmunoassay, was greater at 2 to 4 months than at any other age (P<.01) and comparable patterns have not been reported previously. The end of this peak does, however, correspond to the time when milk was removed from the ration. Blood glucose was not estimated in this study, but Ratcliff, Jacobson and Allen (1958) showed that blood glucose in Holstein calves decreased up to 8 or 9 weeks of age. Hypoglycemia is known to increase plasma GH levels in short term responses (Baylis et al., 1968), but whether similar mechanisms could give rise to long term effects on pituitary GH concentration is not known. Armstrong and Hansel (1956) showed no such peak in the pituitary GH concentrations of Holstein heifers between the ages of 1 and 80 weeks, but their animals were slaughtered at intervals of 16 weeks.

Changes with age in pituitary GH content
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(figure 3) and in pituitary GH content per unit body weight (figure 3) paralleled closely the changes in pituitary GH concentration (figure 2). This result is at variance with others where the changes in pituitary GH concentrations were small and differences between specific growth rates of the anterior pituitary and the body apparently were responsible for positive relationships between specific growth rates and pituitary GH per unit body weight (Baird, Nalbandov and Norton, 1952; Baker et al., 1956; Armstrong and Hansel, 1956). We also found a significant positive correlation between these traits (table 1), but this appeared to be due to the drop in pituitary GH concentrations after 4 months. Pituitary growth, which was essentially linear, paralleled body growth, while increases in pituitary and plasma GH concentrations did not. Thus, the quantity of pituitary GH in these animals probably was not an important determinant of their growth rates, at least during the first 4 months. The changes in pituitary GH concentration (figure 2) or pituitary GH content (figure 3) show no similarity to those reported for rats (Birge et al., 1967; Garcia and Geschwind, 1968). Pituitary GH content in rats increased at a decreasing rate with age for males and females.

Table 1. Among Bull Correlation Coefficients Between Some Measures of GH Status and Some Measures of Growth

<table>
<thead>
<tr>
<th>GH status</th>
<th>Specific growth rate</th>
<th>Body weight at slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma GH concentration</td>
<td>0.33*</td>
<td>-0.19</td>
</tr>
<tr>
<td>Plasma GH content</td>
<td>-0.51**</td>
<td>0.70**</td>
</tr>
<tr>
<td>Pituitary GH concentration</td>
<td>0.34*</td>
<td>-0.53**</td>
</tr>
<tr>
<td>Pituitary GH content</td>
<td>0.21</td>
<td>-0.41**</td>
</tr>
<tr>
<td>Pituitary GH/body weight</td>
<td>0.42**</td>
<td>-0.58**</td>
</tr>
</tbody>
</table>

* P<.05.  ** P<.01.

If the GH status of an animal is considered as the quantity of GH effectively utilized by the tissues, then it probably is a function of the plasma concentration of GH and/or the turnover rate of plasma GH. If GH utilization is determined primarily by plasma GH concentration, then the results of the present study suggest that there is a small relationship between GH status and growth, as plasma GH concentration and specific growth rate were correlated (table 1). Conversely, if utilization is determined primarily by factors other than plasma GH concentration, then GH status may be closely correlated to GH turnover rate in the plasma rather than concentration. Thus, the very

![Figure 3](image-url). Pituitary growth hormone content per unit body weight of Holstein bulls from birth to 12 months.
low relationship between plasma and pituitary concentrations demonstrated in this study \((r=0.006)\) does not necessarily mean that pituitary GH concentration is not a good indicator of GH status. More appropriate criteria of GH status are necessary.

None of the measures of GH status in this study were closely related to measures of growth. In the case of plasma GH concentration, the influence of factors which are known to cause short term fluctuations in blood may have prevented collection of representative samples, but this was probably not the case with pituitary GH concentrations as the mean GH content of the pituitaries \((4.01 \text{ mg})\) was more than 50 times as great as the mean plasma GH content \((77.29 \mu g)\).

The GH bioassay procedure used in this study differed from the normal procedure in that TSH was added to the GH standard. The average index of precision \((\lambda=0.11)\) is less than that \((0.31)\) regarded as acceptable (Greenspan et al., 1950). TSH levels were not measured in these bulls and they may have varied among ages and fallen below the level of TSH needed for augmentation of GH. If the TSH concentrations in pituitary extracts were below this level any attempt to cause short term fluctuations in blood may have prevented collection of representative samples, but this was probably not the case with pituitary GH concentrations as the mean GH content of the pituitaries \((4.01 \text{ mg})\) was more than 50 times as great as the mean plasma GH content \((77.29 \mu g)\).

The correlation coefficient between pituitary GH concentrations measured by radioimmunoassay and by rat tibia test bioassay was 0.60. Body weight increased linearly with age, but pituitary GH concentration and content showed a peak at 3 to 4 months and plasma GH concentration was relatively constant after falling from high levels at birth. None of the measures of GH status was closely related to age or to measures of growth.

Summary

Sixty-five Holstein bulls were killed in groups of five at monthly intervals from birth to 12 months of age to investigate relationships between body growth and pituitary or plasma growth hormone \((GH)\). The correlation coefficient between pituitary GH concentrations measured by radioimmunoassay and by rat tibia test bioassay was 0.60. Body weight increased linearly with age, but pituitary GH concentration and content showed a peak at 3 to 4 months and plasma GH concentration was relatively constant after falling from high levels at birth. None of the measures of GH status was closely related to age or to measures of growth.

Literature Cited


