The relationship of plasma levels of growth hormone to meatiness and growth rate in swine

David Gard Siers
Iowa State University

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THE RELATIONSHIP OF PLASMA LEVELS OF GROWTH HORMONE TO MEATINESS AND GROWTH RATE IN SWINE

by

David Gard Siers

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Animal Breeding

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Ames, Iowa

1968
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INTRODUCTION

Swine breeding goals are directed toward producing the maximum amount of lean red meat per unit of feed, time and labor. In seeking this goal, animals have been selected for growth rate, muscling and a lack of fat. Selection has been reasonably successful as evidenced by the observed changes in growth rate and carcass composition. These readily observable changes must correspond to physiologic changes within the animal. These physiologic changes are not readily observable and have not been extensively investigated.

The physiologic changes are of interest to population geneticists because they may be highly correlated with economically important traits. If so, they could be used to estimate genetic potentials for traits which cannot be evaluated unless animals are sacrificed, and to decrease the error in estimates of genetic potential for lowly heritable and for sex-limited traits.

Many types of physiologic traits could be correlated with readily observable phenotypes. Circulating growth hormone level is a physiologic trait that is probably related to rate of gain, protein deposition and fat catabolism. An immunoassay sensitive enough to measure circulating levels of growth hormone is being used in many species.
The purpose of this research was to develop an immunoassay to measure circulating levels of porcine growth hormone and to investigate the relationships of circulating hormone level to growth rate and carcass composition in genetically diverse groups of swine. The experiment was designed so that it afforded an opportunity to investigate the accuracy of the assay as well as the relationship of hormone level to the age of the pigs.
REVIEW OF LITERATURE

The literature review is divided into four main sections. Section I is concerned with the relationship between growth hormone and carcass composition. The research in this area is of two main classes. The first class involves treatment of intact and hypophysectomized animals with exogenous growth hormone. The second class involves attempting to measure the amount of growth hormone available and correlating this measure with growth and carcass composition.

Section II includes investigations into growth hormone action which brings about the observed variations in growth rate and carcass composition.

Section III contains literature related to growth hormone action at the cellular level. This section deals mainly with the effect of growth hormone upon amino acid transport and protein synthesis.

Section IV is concerned with the theory of immunoassay, the technique used to measure circulating levels of growth hormone.

Section I: Relationship of Growth Hormone to Growth Rate and Carcass Composition

Bierring and Nielson (1932) found that rats treated with an alkaline pituitary extract gained weight faster than control animals not receiving pituitary extract. The
carcasses of the treated rats contained higher percentages of protein and water and less fat. Lee and Schaffer (1934) reported that rats treated with bovine anterior pituitary extract gained faster and increased body length faster than controls. The treated rat carcasses contained more total nitrogen, water and ash with less total fat. The treated and control rats were pair-fed so that the differences were not due to differential feed consumption.

Young (1945) reported that pituitary extract treatment of rats, dogs and cats led to faster weight gains and increased nitrogen retention. The carcasses of treated rats contained less fat and more protein and water than their pair-fed controls. Evans, Simpson and Li (1948) treated 210-day old female rats with growth hormone for 437 days. The treated rats increased body length and live weight faster than the controls. The average weight gain for the 437 day period was 289 grams for the treated rats while that for the controls was 59 grams. Li, Simpson and Evans (1948) found that growth hormone treated rats fed ad libitum had carcasses containing less fat and more water and protein than untreated control rats.

Samuels, Reinecke and Baumann (1943) demonstrated that force-fed hypophysectomized rats deposited less protein nitrogen and more fat than did intact controls. Turman and Andrews (1955) treated seven barrows with growth hormone and
maintained seven as controls. The treated barrows did not gain significantly faster than the controls. The control animals required significantly more feed per 100 pounds of gain (384 pounds vs. 310 pounds). The carcasses of the growth hormone treated barrows contained more water and protein and less fat. The treated barrows had significantly longer carcasses and the average live animal backfat probe of the treated barrows was 0.37 inches less than that for the controls.

Li and Evans (1948) demonstrated that the reduced growth rate of hypophysectomized rats can be partially corrected by treatment with growth hormone.

Baird, Nalbandov and Norton (1952), utilizing 86 pigs, investigated the pituitary growth hormone content of two lines of pigs selected for fast and slow gain. The growth hormone content of the pituitaries was determined by the tibia test in young hypophysectomized rats. The pituitary growth hormone content of the fast growing line was significantly higher than that of the slow growing line at all weights and at all ages above 56 days. The difference between lines in anterior pituitary potency was almost constant at all ages. The total anterior pituitary growth hormone content of the fast growing line increased from 56 days to 115 days and dropped significantly at 154 days of age. In the slow growing line the total pituitary growth hormone content
increased from 56 days to 75 days and dropped at 154 days of age.

Baker, Jr. et al. (1956) investigated the relationship of total pituitary growth hormone to age and mature size in pigs. They found that the total pituitary potency per unit body weight declines from birth to 225 days of age and maintains the 225 day level thereafter. Baird et al. (1952) also reported that the amount of pituitary growth hormone per unit body weight decreases with age. Armstrong and Hansel (1956) investigated the relationship of pituitary growth hormone content to plane of nutrition and age in Holstein heifers. They observed six animals in each age by nutrition level subclass. The pituitary growth hormone content per unit live weight decreased with age. They reported a correlation of 0.37 between growth hormone per unit pituitary mass and the percent increase in body weight during the 16 weeks prior to slaughter. The correlation between pituitary growth hormone per 100 pounds live weight and the percent increase in body weight during the 16 weeks prior to slaughter was 0.66. The correlation between the percent increase in wither height in the last 16 weeks prior to slaughter and growth hormone per unit pituitary weight was 0.42 while the correlation of pituitary growth hormone per 100 pounds live weight with the percent increase in wither height was 0.54. The level of nutrition had no significant
effect upon growth hormone per unit pituitary weight or
growth hormone per 100 pounds live weight.

The literature cited in this section suggests that
growth hormone influences the following:

a) protein deposition and water retention;

b) fat deposit or the mobilization and catabolism of
fat;

c) weight gain and skeletal growth.

Section II: Growth Hormone and Protein
(amino acid) Conservation

Greenbaum (1953) studied the effect of level of feed
consumption and growth hormone treatment upon rats. He had
three treatment groups; 1) controls, 2) fed ad libitum and
received growth hormone, and 3) limited food intake and
received growth hormone. After 70 days the group 1 rats had
increased their protein content by 25%, the group 2 rats had
increased their protein content by 86%, and the group 3 rats
had increased their protein content by 57%. The control
(group 1) rats maintained a constant percent fat over the
70 days. The group 2 rats at first lost fat and then in-
creased it slowly until at the end of the 70-day experiment
they contained 70 percent as much as did the controls. The
group 3 rats lost fat and contained only one-half as much as
the controls at the end of the 70-day experiment. The period
of fat loss in the treated rats corresponds to a period during which the animals exhibited a lowered respiratory quotient, indicating that a larger than normal portion of their energy was being derived from fat catabolism.

Knobil (1959) demonstrated that adipose tissue from growth hormone treated hypophysectomized rats released significantly more non-esterified fatty acids in vitro than does adipose tissue from saline injected hypophysectomized rats. Knobil and Greep (1959) reported that glucose and food abolished the increased plasma non-esterified fatty acid response to growth hormone treatment in the monkey. They also demonstrated that the fat mobilizing effect of the growth hormone was not due to contamination by thyroid stimulating hormone, leuteothrophin or adrenocorticotrophic hormone. Neither TSH or LTH caused fat mobilization. ACTH caused fat mobilization but did not reduce insulin sensitivity or glucose tolerance as did the growth hormone treatment. Goodman and Knobil (1959) found that hypophysectomized monkeys exhibit elevated plasma levels of non-esterified fatty acids upon fasting. The elevation was much less in hypophysectomized than in intact monkeys. In the simian growth hormone treated hypophysectomized monkey the non-esterified fatty acid rise in response to fasting was of the same magnitude as the fatty acid rise seen in normal intact monkeys after fasting. Barrett et al. (1938) reported that growth hormone treatment
induced a rapid increase in the rate of transfer of peripheral fat depots to the liver and depressed the respiratory quotient. Greenbaum and McLean (1953) found that growth hormone treatment resulted in an increased rate of lipid mobilization to the liver. Goodman and Knobil (1961) demonstrated that removal of the pituitary gland, the adrenal gland, or the thyroid gland reduced the degree of non-esterified fatty acid rise in response to fasting. These authors concluded that the pituitary, adrenal, and thyroid glands are necessary for the fatty acid response to fasting but they do not initiate the response.

Dole (1956) found that fasted individuals exhibit elevated plasma levels of non-esterified fatty acids. He also reported that oral glucose, a meal, or injected insulin depressed the non-esterified fatty acid rise during fasting. Winkler et al. (1964) demonstrated that growth hormone treated dogs exhibit elevated plasma non-esterified fatty acids. In addition they noted that the rate of fatty acid oxidation is dependent upon the rate of uptake which in turn is dependent upon the plasma levels. Through this chain of events growth hormone does indirectly effect the rate of fatty acid oxidation.

Raben and Hollenberg (1959) found that the non-esterified fatty acid rise during fasting could be abolished by food consumption, oral glucose plus insulin, and insulin alone. They also reported elevated plasma non-esterified fatty acids
in response to growth hormone treatment. This occurred in both normal and diabetic dogs. Normal human subjects exhibited elevated plasma non-esterified fatty acids when treated with human or simian growth hormone.

Roth et al. (1963) reported that hypoglycemia induced by either exercise or insulin would result in raised plasma levels of growth hormone. No growth hormone rise was observed after exercise or hypoglycemia if the animals were pre-treated with glucose. Administration of 2-deoxy-D-glucose, which inhibits intracellular glucose utilization, results in hyperglycemia with hypoglycemic symptoms within the cell. Treatment with 2-deoxy-D-glucose resulted in elevated plasma levels of growth hormone. This suggests that glucose utilization at the intracellular level is important in eliciting the elevated growth hormone levels observed in times of energy shortage.

Hunter et al. (1965) reports that exercise elicits a rise in plasma levels of growth hormone and non-esterified fatty acids while lowering plasma levels of glucose. The respiratory quotient is also reduced during exercise. Ingestion of glucose during exercise tended to nullify the rise in plasma levels of growth hormone and non-esterified fatty acids while the respiratory quotient remained normal. These results agree with the findings of Roth et al. (1963) and suggest that, if energy is readily available, the plasma
growth hormone level is not elevated during periods of great energy utilization.

From the literature reviewed in Section II one can safely conclude that growth hormone has an effect upon the rate of fatty acid mobilization and fat catabolism. During periods when glucose is not available in adequate quantities to supply the energy needs of the animal, plasma levels of growth hormone and fatty acids are raised. As indicated by the lowered respiratory quotient, fatty acids are used as an energy source. If the fatty acid levels were not raised in response to reduced available glucose, energy needs would be met through protein and amino acid utilization. The fat mobilizing effect of growth hormone can be referred to as a protein and amino acid conserving effect.

Section III: Growth Hormone and Amino Acid Transport and Protein Synthesis

The increased rate of weight gain observed in growth hormone treated animals is accompanied by increased nitrogen retention. (Li and Evans 1948, Smith et al. 1955) Increased nitrogen retention could result from increased protein synthesis and/or decreased amino acid and protein catabolism. Li and Evans (1948) demonstrated that growth hormone administered to hypophysectomized rats caused an increased rate of $^32P$ uptake. This indicates that the rate of
nucleic acid synthesis was increased, an expected happening if the increased nitrogen retention were the result of increased protein synthesis.

Korner (1959, 1961) demonstrated, using a cell-free system, that hypophysectomy depressed the ability of ribosomes to assemble amino acids into polypeptide chains. The same author in 1965 demonstrated that growth hormone administered after actinomycin treatment resulted in increased protein synthesis. Actinomycin blocks ribonucleic acid synthesis. Therefore the increased protein synthesis resulting from growth hormone treatment is not completely due to increased ribonucleic acid synthesis. Korner (1965) suggests that growth hormone may cause polyribosome formation from single ribosomes, thus providing more ribonucleic acid "readers" and increased protein synthesis.

Kostyo and Schmidt (1962a, 1962b) demonstrated that growth hormone treatment will induce amino acid accumulation within the cells of a muscle in vitro. The same authors (1962a) found that prolactin was the only other pituitary hormone tested which caused increased intracellular accumulation of an amino acid. The effect of growth hormone on intracellular amino acid accumulation in vitro was shown to be independent of insulin. Kostyo and Engel (1960) found that adrenocorticotropic hormone did not stimulate intracellular amino acid accumulation in vitro. Riggs and Walker
found that amino acid accumulation in rat muscle cells was reduced by hypophysectomy. They also demonstrated that treatment with growth hormone returned the level of intracellular amino acid accumulation to normal in hypophysectomized rats.

Noall et al. (1957) demonstrated that growth hormone treatment of adult female rats elicited an increased accumulation of intracellular labelled amino acids. Knobil and Roth (1963) found that growth hormone did not cause intracellular accumulation of valine when protein synthesis was blocked with puromycin. Puromycin treatment did not stop intracellular accumulation of the nonmetabolizable amino acid, alpha-amino isobutyric acid.

It has been demonstrated that growth hormone treatment results in increased intracellular accumulation of amino acids. This effect could result from direct stimulation of an amino acid transport mechanism or by stimulation of protein synthesis which would result in more amino acid transport into the cell. Regardless of whether growth hormone affects amino acid transport directly or indirectly it does cause an increase in protein synthesis.
Section IV: Immunoassay Theory

The principle of immunoassay can be illustrated by the following schematic taken from a paper by Bearson et al. (1964).

\[
\text{labelled hormone} + \text{antibody against the hormone} \rightarrow \text{labelled hormone antibody complex} \\
+ \\
\text{unlabelled hormone} \\
\downarrow \\
\text{unlabelled hormone antibody complex}
\]

This system contains a constant amount of labelled hormone and antibody. The amount of unlabelled hormone in the system varies. The quantity of antibody present will bind 55 to 65% of the labelled hormone when no unlabelled hormone is present. The unlabelled hormone added will compete with the labelled hormone for the limited quantity of antibody present. Due to this competition, there is an inverse relationship between the amount of unlabelled hormone added and the amount of labelled hormone that binds to the antibody. The points on a standard curve are gotten by adding various amounts of unlabelled hormone (e.g., 0.5, 1.0, 2.0, 3.0 μg) to a constant amount of labelled hormone and antibody.

Many types of standard curves are used to assay micro quantities of hormone in serum. The most frequently used
curve is constructed by plotting the B/F ratio against the quantity of unlabelled hormone added. Here B is equal to the radioactivity (counts per minute) of labelled hormone bound to antibody and F is equal to the radioactivity (counts per minute) of the labelled hormone that is free in solution. Figure 1a shows a curve of this type. A second type of standard curve is obtained when the B/F ratio is plotted against the logarithm of the amount of unlabelled hormone added (Figure 1b). Herbert et al. (1965) used a standard curve constructed so that it is linear with a positive slope. They plotted B/B' against the quantity of unlabelled hormone added to the standard tubes. B equals the radioactivity (counts per minute) of the labelled hormone bound to antibody when no unlabelled hormone is added and B' is the counts per minute of labelled hormone bound to antibody in the standard tubes containing various quantities of unlabelled hormone (Figure 1c). The type of standard curve used in this study is illustrated in Figure 1d. This curve is constructed by plotting the percentage of the total radioactivity added that is bound to antibody against the quantity of unlabelled hormone present in the tubes.

The amount of hormone in a serum sample is estimated by combining a small quantity of serum (0.1 ml.) with labelled hormone (same amount used in standard tubes) and antiserum (the same quantity and dilution as in the standard
Figure 1. Standard curves used to assay micro quantities of hormone in serum
tubes). The hormone contained in the serum will compete with the labelled hormone for the limited quantity of antibody available. The percent of the total radioactivity added that is bound to the antiserum is determined. The percent bound is located on the ordinate of the standard curve and a horizontal straight line is drawn from this point through the standard curve. This line is the dashed line on Figure 1d, the point of standard curve intersection is marked P. A second straight line, parallel to the ordinate, is drawn from point P through the abscissa. This is the dotted line on Figure 1d. The point where this dotted line intersects the abscissa (incremented in μg hormone) provides the estimate of the quantity of hormone in the unknown serum sample.

The following chart illustrates the various types of tubes that must be made up to run as assay.
<table>
<thead>
<tr>
<th>Tube No.</th>
<th>0.1 to 0.2μg labelled PGHa</th>
<th>Antibodyb</th>
<th>Standard diluentc</th>
<th>10μg/ml unlabelled PGHd</th>
<th>Serum</th>
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<tr>
<td>0</td>
<td>100μl</td>
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<td>100μl</td>
</tr>
<tr>
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<td>100μl</td>
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<td>500μl</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>100μl</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>100μl</td>
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<td>11</td>
<td>100μl</td>
<td>100μl</td>
<td>250μl</td>
<td>50μl</td>
<td>100μl</td>
</tr>
</tbody>
</table>

Chart 1. Protocol for an assay

aSolution of labelled porcine growth hormone diluted in standard diluent contains one to two μg PGH per ml.

bDiluted so that 100 lambda binds 55 to 65 percent of labelled hormone added.

cStandard diluent -.25% BSA Veronal buffer pH 8.6 (I=1).

dSolution of unlabelled porcine growth hormone diluted in standard diluent contains 10 μg PGH per ml.

The 0 tubes are called serum controls and the 1 tubes are called no antibody controls. Tubes 2 through 9 are points on the standard curve while tubes 10 and 11 are examples of unknown serum tubes.
IMMUNOASSAY PROCEDURE

The immunoassay procedure used in this study consisted of a mixture of many procedures described in the literature. Each of the following objectives must be accomplished before unknown serum samples can be assayed to determine their growth hormone content:

1) obtain a pure standard hormone sample;
2) label a quantity of the standard hormone with a radioactive isotope;
3) obtain an antiserum that will bind the labelled and unlabelled standard hormone;
4) find a method of separating the labelled hormone bound to antibody from the labelled hormone not bound to antibody;
5) determine what dilution of the antiserum will bind 55 to 65 percent of the 0.1 μg to 0.2 μg of labelled hormone added to all tubes;
6) combine the labelled hormone, properly diluted antiserum and unlabelled hormone to get a standard curve;
7) run trials to determine the extent of serum damage to the labelled hormone.

Objective number one was expediently achieved due to the generosity of Dr. A. E. Wilhelmi who kindly provided a
5 mg sample of porcine growth hormone.

Greenwood et al. (1963), Utiger et al. (1962), Glick et al. (1963), Yalow and Bearson (1960), and Hunter and Greenwood (1962) describe techniques for labelling proteins with radioactive iodine. The protein labelling procedures described in the publications cited above are similar to the procedure used in this study.

The procedure used to label porcine growth hormone in this study is described below. Five micrograms of porcine growth hormone in five lambda of solution was combined with 1-1/4 millicuries of iodine-125 in 25 lambda of 0.5M phosphate buffer, pH 7.6. Twenty-five lambda of chloramine-T (35 mg/10ml) was added to the above mixture. After gently shaking for thirty seconds, 100 lambda of sodium-meta-bisulfite (24mg/10ml) was added and the mixture was allowed to stand four to five minutes. Then 200 lambda of potassium iodide (10mg/10ml) was added and the entire mixture was transferred to a 25 ml sephadex G-100 column. The mixture was eluted with 0.015 M veronal buffer (pH 8.6).

The 25 ml. sephadex column was presaturated with one milliliter of bovine serum albumen in veronal buffer (30 mg BSA/ml). This presaturation minimizes the quantity of labelled hormone that adsorbs to the glass column and sephadex beads. One milliliter elutes were collected in tubes containing 200 lambda of a one percent solution of
bovine serum albumen in veronal buffer (0.015 M, pH 8.6). A typical elution pattern is depicted in Figure 2a. The first peak consists of labelled growth hormone whose secondary structure has been destroyed. The second peak is the intact labelled growth hormone and the third peak consists of free iodine.

Before the labelled hormone is used in an assay it is further purified by running a 100 to 200 lambda sample of the labelled hormone solution on a 50 ml sephadex G-100 column. This column is presaturated with bovine serum albumen and the elution is carried out with 0.015 M veronal buffer, pH 8.6. One milliliter elutes are collected in tubes containing 200 lambda of the one percent bovine serum albumen buffer. A typical elution pattern appears in Figure 2b.

The middle peak consists of the intact labelled growth hormone molecule which binds very readily with the antibody against growth hormone. The first peak consists of molecules whose effective molecular size (rotating radius) is greater than that of the intact growth hormone molecule. This first peak contains hormone molecules whose secondary structure has been destroyed. The last peak contains molecules whose effective size is smaller than that of the immunologically active growth hormone molecule. This peak consists of fragments of growth hormone molecules. The
Figure 2. Figures illustrating elution patterns and an antibody dilution trial
Figure 2a

8x10^6

Activity cpm

Tube Number

Figure 2b

2x10^6

Activity cpm

Tube Number

Figure 2c

% Bound to Antibody

1:10

Antibody Dilution

1:10000
middle peak is used in the assay after it has been diluted so that the concentration of labelled hormone is one to two millimicrograms per milliliter. It is diluted in one-quarter percent bovine serum albumen veronal buffer pH 8.6. This BSA buffer is referred to as standard diluent in the remainder of this dissertation.

The antiserum used in this study was produced in guinea pigs by repeated subcutaneous injections of porcine growth hormone, obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Five milligrams were injected each time over a period of five months. Each animal received six injections, the first three with complete Freund's adjuvant and the last three without adjuvant. Antisera to human growth hormone has been produced in the rabbit but our attempts to produce an antiporcine growth hormone antibody in rabbits failed. Techniques used to produce antiserum against growth hormone (human) are given by Read and Bryan (1960) and by Utiger et al.

To determine the quantity of labelled hormone bound to antibody, the labelled hormone not bound to antibody must be separated from that bound to antibody. This separation can be accomplished by paper electrophoresis (Bearson et al. 1964, Yalow and Bearson 1960, and Hunter and Greenwood 1964). The pattern of radioactivity on the electrophoresis strip will vary depending on what hormone is being assayed. For porcine growth hormone, the free labelled hormone would be electrophoretically immobile and would therefore be adsorbed.
at the origin. The porcine growth hormone bound to antibody would migrate toward the cathode. These electrophoresis strips are counted in an automatic strip counter or are cut into smaller pieces and each piece is counted separately in a conventional sodium iodide crystal scintillation detector.

The separation of antibody bound from free labelled hormone can be accomplished by using a second antibody. (Schalch and Parker, 1964, Machlin et al. 1968, Schalch and Reichlin 1966, and Utiger et al. 1962). This second antibody is produced against gamma-globulin from the species used to produce the antibody against the hormone. This second antibody will precipitate the labelled hormone-antibody complex leaving the labelled hormone not bound to antibody in solution. Herbert et al. (1965) used dextran coated charcoal to adsorb the free labelled hormone from solution, leaving the labelled hormone-antibody complex in the supernatant. Norit A neutral pharmaceutical grade charcoal is coated with dextran 80, average molecular weight 80,000. The dextran coat on the charcoal acts as a molecular sieve. The free labelled hormone is adsorbed to the charcoal while the larger labelled hormone-antibody complex is not adsorbed since it cannot penetrate the dextran coat.

In the present study dextran coated charcoal was used to separate the labelled hormone-antibody complex from the free labelled hormone. This technique was first applied un-
successfully in the porcine growth hormone system because the dextran coated charcoal adsorbed both the labelled hormone-antibody complex and the free labelled hormone from solution. When this happens there appears to be no labelled hormone bound to antibody, since essentially all the added radioactivity is adsorbed to the charcoal. Adding serum (porcine, bovine, rabbit, guinea pig) to the tubes immediately before adding the dextran coated charcoal prevents the adsorption of the labelled hormone-antibody complex. Since the free labelled hormone is adsorbed, the technique is quite successful.

Since quantity of serum in the tube when the dextran coated charcoal is added influences the adsorption of the free labelled hormone to charcoal, all tubes should contain equal amounts of serum when the dextran coated charcoal is added.

Antisera dilution trials were run to determine at what dilution 100 lambda of the antiserum would bind 55 to 65% of the quantity of labelled hormone being used. Antiserum dilution tubes contain 400 lambda of standard diluent, 100 lambda of labelled hormone (0.1 to 0.2 mg), and 100 lambda of antiserum at various dilutions. Duplicate no antibody control tubes are included. These tubes contain 0.5 ml of standard diluent and 100 lambda of labelled hormone. All tubes were incubated two days at 4°C. The tubes are shaken daily during
the incubation period.

At the end of the incubation period, 100 lambda of serum is added to each tube. Immediately after this addition, 2 ml of the dextran coated charcoal is added to each tube. The tubes are then placed in the refrigerator at 4°C. After 15 minutes the tubes are removed from the refrigerator and inverted to thoroughly mix the contents. The tubes are then centrifuged (1000g) for thirty minutes at 4°C.

During centrifugation the dextran coated charcoal, with the adsorbed free labelled hormone, is packed in the bottom of the tube. After centrifugation the supernatant which contains the labelled hormone-antibody complex is removed by aspiration. The tubes containing the charcoal (unbound labelled hormone) are counted. The counter used was a well-type sodium-iodide crystal scintillation detector.

The radioactivity (counts per minute) of the no antibody control tubes is the total radioactivity available to the dextran coated charcoal. This total available radioactivity will represent 90 to 97 percent of the total radioactivity added to these tubes. The counts per minute of the tubes containing the various antibody dilutions are expressed as percentages of the total available radioactivity. These percentages are the percentage unbound (not bound to antibody) radioactivity. The percent antibody bound radioactivity is obtained by subtracting the percent unbound from 100 percent.
The antibody was tried at dilutions of 1:10, 1:100, 1:1000, 1:2000, 1:3000, 1:4000, 1:5000 and 1:10,000. The percent antibody-bound radioactivity is plotted against the antibody dilution. This graph is shown in Figure 2c. The dilution of antibody used is that one at which 100 lambda of the antibody solution will bind 55 to 65 percent of the total available radioactivity.

The points on a standard curve are obtained by combining a constant amount (0.1ml) of antibody at the proper dilution with different amounts of unlabelled hormone and standard diluent. Each tube contains a final volume of 0.5 ml. These tubes are incubated at 4°C for two days. During incubation the tubes are shaken daily. At the end of the two-day incubation period, each tube receives 0.1 ml of the labelled growth hormone solution containing 0.1 to 0.2 mg of the labelled hormone. After this addition the tubes are incubated for two more days at 4°C. They are again shaken daily. At the end of the fourth day of incubation all tubes receive 0.1 ml serum and 2 ml dextran coated charcoal. The tubes are held at 4°C for 15 minutes and are inverted to mix the contents. They are centrifuged (1000 g for 30 minutes) at 4°C and the supernatant is aspirated.

The tubes containing the charcoal are counted and the count is expressed as a percentage of the total radioactivity available. The total radioactivity available is determined
by running no antibody control tubes, just as in the antibody
dilution trial. The percent antibody-bound radioactivity is
calculated. The percent antibody-bound radioactivity is
plotted against the amount of unlabelled hormone added to
the various standard curve tubes. See Figure 1d for an
example standard curve.

The amount of growth hormone in a particular serum sample
is estimated by replacing the unlabelled hormone of the
standard tube with the serum being assayed. These tubes con-
tain labelled hormone, antibody, serum, and enough standard
diluent to make a final volume of 0.6 ml. The antibody,
serum, and standard diluent are incubated for two days.
The labelled hormone is added and the tubes are incubated
for two more days. Separation of antibody-bound radio-
activity from free radioactivity is accomplished by using
dextran coated charcoal.

The only real difference between a standard curve tube
and a tube containing a serum sample is the presence of the
serum. The samples are treated exactly alike until the end
of incubation. At this time 0.1 ml serum is added to all
standard curve tubes, bringing their total volume to 0.7
ml. The serum unknown tubes receive 0.1 ml of standard
diluent, bringing their final volume to 0.7 ml. Therefore,
when 2 ml of dextran coated charcoal is added, all tubes
contain equal quantities of serum and the same final volume.
The percent antibody-bound radioactivity is determined for the tubes containing the serum samples being assayed.

Tubes which do not contain antibody but do contain labelled hormone and serum during incubation are called serum control tubes. They are expected to have the same percent unbound radioactivity as the no antibody control tubes. This expectation is not realized, because the amount of labelled hormone (radioactivity) available to the dextran coated charcoal is reduced by the presence of serum during incubation. This reduction in available radioactivity is called "serum damage" and must be corrected for when estimating the percent unbound radioactivity for the serum sample under assay.

The presence of 100 lambda of serum during incubation will reduce the amount of radioactivity adsorbed to the charcoal by 4 to 12 percent and 200 lambda will cause a reduction of 10 to 20 percent. This problem seems to be particularly acute when dealing with porcine growth hormone. The various publications dealing with immunoassay of hormones in other species give very little attention to the problem of serum damage. Other authors treat serum damage as a constant effect common to all serum samples assayed and they correct their observations accordingly or not at all.

The usual serum correction procedure is to run serum control tubes. These tubes contain serum and standard diluent
for the first two days of incubation and they also contain 0.1 ml of labelled hormone during the last two days. The charcoal adsorbed radioactivity of the serum control tubes is lower than the charcoal adsorbed radioactivity in the no antibody control tubes. This difference is expressed in counts per minute and designated "serum damage." The correction for serum damage can be made by simply adding the serum damage counts per minute to the observed charcoal adsorbed counts per minute for each serum sample assayed. A proportional correction can be made. In this case the average charcoal adsorbed radioactivity of the serum control tubes is divided by the charcoal adsorbed radioactivity in the no antibody control tubes. This correction factor (K<1) is used to correct the charcoal adsorbed radioactivity of the tubes containing the serum samples being assayed.

The proportional correction method assumes that the following relationship holds.

\[
\text{(cpm observed with serum present)} = K \times \text{(cpm observed without serum)}
\]

This implies that the serum damages a constant proportion of the labelled hormone not bound to antibody, resulting in its not adsorbing to dextran coated charcoal. The correction will be less for tubes which exhibit low counts per minute on the charcoal (low level of unlabelled hormone) and more for those having higher counts per minute on the charcoal (higher
level of unlabelled hormone). Therefore, the charcoal adsorbed radioactivity of serum samples containing large amounts of growth hormone receive large corrections while serum samples with low levels of hormone receive low corrections. The additive method of correction assumes that all tubes, regardless of unlabelled growth hormone content, receive the same amount of serum damage. An attempt was made to determine which serum damage correction was best. This was done by running three "standard curves". Set #1 was a typical standard curve, set #2 was a series of standard curve tubes each of which received 50 lambda of hypophysectomized pig serum and set #3 was a series of standard curve tubes each of which received 100 lambda of normal pig serum. The percent antibody bound radioactivity was calculated and the conventional standard curve was plotted using the set #1 tubes. The quantity of unlabelled growth hormone in each of the set #2 and set #3 tubes was estimated using this conventional standard curve. These estimates were then corrected for serum damage using the two proposed correction methods. The resulting estimates of growth hormone level appear in the body of Table 1. The assay indicated that the hypophysectomized pig serum contained no growth hormone. Serum damage (lowered charcoal adsorbed activity) was evident in normal serum and hypophysectomized serum. This is apparent since at every hormone level the growth hormone content of
Table 1. Indicated growth hormone levels using two methods of serum damage correction

<table>
<thead>
<tr>
<th>Amount of PGH added to the tube</th>
<th>1/2 μg</th>
<th>1 μg</th>
<th>1-1/2</th>
<th>2 μg</th>
<th>3 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No serum correction Set #2</td>
<td>0 μg</td>
<td>.9 μg</td>
<td>1.4 μg</td>
<td>1.85 μg</td>
<td>2.25 μg</td>
</tr>
<tr>
<td>No serum correction Set #3</td>
<td>0</td>
<td>.9 *</td>
<td></td>
<td>1.83</td>
<td>2.52</td>
</tr>
<tr>
<td>Proportional serum correction Set #2</td>
<td>.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.47</td>
</tr>
<tr>
<td>Proportional serum correction Set #3</td>
<td>.68</td>
<td>1.13</td>
<td>*</td>
<td>2.25</td>
<td>3.08</td>
</tr>
<tr>
<td>Additive serum correction Set #2</td>
<td>.75</td>
<td>1.08</td>
<td>1.53</td>
<td>2.05</td>
<td>2.7</td>
</tr>
<tr>
<td>Additive serum correction Set #3</td>
<td>.97</td>
<td>1.44</td>
<td>*</td>
<td>2.55</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Indicates no value at this level of unlabelled hormone.
the tubes of set #2 and #3 was underestimated if no correction was made for serum damage. The amount of underestimation was less in the set #3 tubes. This is understandable since the normal serum probably contained growth hormone.

With one exception the proportional correction procedure raised the estimated growth hormone content of the set #2 tubes to the level expected if there had been no growth hormone in the hypophysectomized serum. The exception occurred in the tube containing 3 μg of hormone. Here the proportional correction was not large enough, resulting in an underestimate of the hormone content. The proportional serum damage correction of the tubes in set #3 gave estimates of hormone content greater than the amount of hormone added. This is reasonable since the normal serum probably contained growth hormone in addition to that added.

In the set #2 tubes the additive correction procedure overestimated the amount of growth hormone present at every level except the highest (3 μg added). This overestimation was greatest in the tube containing 1/2 μg of hormone. The amount of hormone estimated in the tubes of set #3 was greater than the amount added at every level of addition.

These results suggest that the proportional correction would be best at the lower levels of hormone while the additive correction is superior at higher levels of hormone. These results also suggest that the degree of serum damage depends
upon the level of unlabelled hormone present. If this is true and different serum samples contain different levels of hormone, it follows that different serums will cause different amounts of serum damage.

The solution adopted for this study was to have at least one serum control tube for each serum sample assayed and to use the additive correction for serum damage.

Preliminary observation of many standard curves revealed that variation between the standard curves is to be expected. The most frequently observed inconsistency was the large variation in the slope of the curve between zero and one-half μg of unlabelled hormone. Preliminary study also led the author to suspect that a high proportion of the 0.1 ml serum samples assayed would contain less than 1/2 μg of hormone. This level of hormone would correspond to the most inconsistent portion of the standard curve. Therefore, one-half μg of unlabelled hormone was added to each tube containing 0.1 ml of serum being assayed. One-half μg of hormone was also added to each serum damage control tube so that it would, with the exception of antibody, have the same contents as the unknown serum tubes. The amount of hormone present in a serum sample was found by subtracting one-half μg from the level indicated by the assay.
SOURCE OF DATA AND EXPERIMENTAL DESIGN

The eighty gilts used in this study were chosen from parents which differed widely in rate of gain. This was done in order to have a wide range of growth rates within the experimental group. Only gilts were used in order to avoid differences that might exist between sexes in the traits measured.

The pigs were fed ad libitum and provided about 200 square feet of pen space per pig. The pigs were provided shelter and shade in the dirt lots. Twenty to thirty pigs were housed in each of the three lots. The pigs were born and housed for the whole experiment on the swine breeding farm at Napier.

Each pig was weighed at birth and again at weaning, at about 56 days of age. Each litter, selected to participate in this experiment was placed in one of the lots when weaned. The pigs were weighed each week so that their weight the following week could be predicted reasonably well. Blood samples were taken at weaning when the pigs weighed 25 to 40 pounds, when they weighed 100 pounds and when they weighed 200 pounds. All pigs were slaughtered at 200 pounds. Carcass information consisting of body length, backfat, loin eye area and ham and loin percentage was obtained.

Research indicates that in other species the plasma
level of growth hormone is greatly elevated in response to stress. Therefore it was thought necessary to minimize the amount of stress experienced by the pigs just prior to bleeding. To minimize stress, the pigs were not weighed until after they had been bled and they were bled as quickly as possible with a minimum of handling. The blood samples were obtained by slashing a vein on the back of the ear. Preliminary trials indicated that this technique afforded the best opportunity to quickly obtain a small (1/2 to 1 ml) blood sample.

When the blood samples were being collected it was thought that 0.3 ml of serum would be enough to permit making duplicate determinations of growth hormone level. However, preliminary results indicated that serum damage would differ from one serum sample to another. In an effort to test this preliminary observation and to control serum damage, at least one serum damage control tube was run for each serum sample assayed. Where possible, duplicate serum damage control tubes and duplicate hormone level determinations were made on each serum sample. When serum samples were not large enough to permit this, duplicate hormone level determinations were run with one serum damage control. A few serum samples were so small that only one hormone level determination and one serum damage control could be run. The assay procedure was such that 30 serum samples could be analyzed in one
operation (assay). Therefore eight assays were conducted. Ten pigs were randomly assigned to each of the eight assays.

The following chart illustrates the experimental design.

<table>
<thead>
<tr>
<th>Pig</th>
<th>Weaning</th>
<th>100 lbs</th>
<th>200 lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1</td>
<td>(\text{XX}^a)</td>
<td>00(^b)</td>
<td>XX</td>
</tr>
<tr>
<td>Assay 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,10</td>
<td>XX</td>
<td>00</td>
<td>XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8,1</td>
<td>XX</td>
<td>00</td>
<td>XX</td>
</tr>
<tr>
<td>Assay 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8,10</td>
<td>XX</td>
<td>00</td>
<td>XX</td>
</tr>
</tbody>
</table>

Chart 2. Experimental design

\(^a\text{XX represents two serum damage determinations.}\)
\(^b\text{00 represents two hormone level determinations.}\)

The hormone level determinations were expressed in \(\mu g\) of hormone per milliliter of serum. The serum damage values were expressed as the percent reduction in charcoal adsorbed radioactivity in the serum control tubes.

The following model illustrates the statistically controllable sources of variation affecting an observed growth hormone level or serum damage value.

\[Y_{ijkl} = \mu + A_i + P_{ij} + T_{ijk} + d_{ijkl}\]
Where:

\[ Y_{ijkl} = \text{an observed hormone level (mg/ml) or an observed serum damage value (percent serum damage)} \]

\[ \mu = \text{mean hormone level or serum damage value common to all } Y\text{'s} \]

\[ A_i (i=1,\ldots,8) = \text{effect common to all observations in the } i\text{th assay} \]

\[ P_{ij} (j=1,\ldots,10) = \text{effect common to all observations on the } j\text{th pig within the } i\text{th assay} \]

\[ T_{ijk} (k=1,2,3) = \text{effect common to all observations on the } k\text{th age or time when the sample on the } j\text{th pig within } i\text{th assay} \]

\[ d_{ijkl} (l=1,2) = \text{a random error associated with the } l\text{th observed value for hormone level or serum damage in the } k\text{th blood sample from the } j\text{th pig within the } i\text{th assay} \]
Table 2a. Assay means for serum damage and growth hormone

<table>
<thead>
<tr>
<th>Assay</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean hormone level</td>
<td>6.36</td>
<td>1.65</td>
<td>4.79</td>
<td>2.83</td>
<td>3.74</td>
<td>2.29</td>
<td>2.69</td>
<td>5.23</td>
</tr>
<tr>
<td>Mean serum damage</td>
<td>5.97</td>
<td>4.98</td>
<td>5.67</td>
<td>4.53</td>
<td>4.32</td>
<td>6.18</td>
<td>3.08</td>
<td>4.13</td>
</tr>
</tbody>
</table>

Table 2b. The AOV of serum damage values

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean sq.</th>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assays</td>
<td>7</td>
<td>50.34*</td>
<td>.86</td>
<td>12.57</td>
</tr>
<tr>
<td>Pigs/A</td>
<td>72</td>
<td>10.01*</td>
<td>.89</td>
<td>12.97</td>
</tr>
<tr>
<td>Ages/P/A</td>
<td>148</td>
<td>5.72*</td>
<td>1.06</td>
<td>15.47</td>
</tr>
<tr>
<td>Determinations</td>
<td>144</td>
<td>4.05</td>
<td>4.05</td>
<td>58.99</td>
</tr>
</tbody>
</table>

*Significant at the .001 level.

Table 2c. The AOV of growth hormone levels

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean sq.</th>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assays</td>
<td>7</td>
<td>135.36*</td>
<td>2.33</td>
<td>22.81</td>
</tr>
<tr>
<td>Pigs/A</td>
<td>72</td>
<td>13.69</td>
<td>.18</td>
<td>1.81</td>
</tr>
<tr>
<td>Ages/P/A</td>
<td>148</td>
<td>12.28*</td>
<td>5.70</td>
<td>55.94</td>
</tr>
<tr>
<td>Determinations</td>
<td>190</td>
<td>1.98</td>
<td>1.98</td>
<td>19.45</td>
</tr>
</tbody>
</table>

*Significant at the .001 level.
RESULTS AND DISCUSSION

Serum Damage

The total variation of the serum damage values was partitioned into four sources. The analysis of variance appears in Table 2b on page 39. The sources of variation are (1) differences between assays, (2) differences between pigs within assays, (3) differences between serum samples collected from the same pig at three different ages, and (4) differences between determinations made on the same serum sample. All mean squares are highly significant. In this analysis the experimental unit is the serum sample.

Determinations mean square

The determinations mean square represents the difference between duplicate determinations made on the same serum sample. This mean square estimates the true random experimental error and is used to test the mean square for ages. The two major sources of error that contribute to differences between duplicate serum damage values are counting errors and errors in laboratory technique.

Errors in laboratory technique cause the contents of duplicate serum damage control tubes to differ in total volume and composition. The total volume that a tube contains when the dextran coated charcoal is added influences the adsorption of labelled hormone to the charcoal. There-
fore variation in total volume causes differences between duplicate serum damage determinations.

The composition of the contents of a tube influences the adsorption of labelled hormone to the dextran coated charcoal. Serum damage control tubes contain standard diluent, serum, and labelled hormone. Variation in labelled hormone content influences the amount of labelled hormone that adsorbs to the dextran coated charcoal. The amount of labelled hormone that adsorbs to the dextran coated charcoal is influenced by the quantity of serum present. This effect of serum upon the charcoal adsorption of labelled hormone was described earlier.

Errors in counting the charcoal adsorbed radioactivity contribute to the variation between duplicate serum damage determinations. The counting error associated with a particular count is a function of the length of the count and the total number of counts detected. If a total count, $C$, is observed over a period of time, $t$ minutes, the mean and variance of the total count is $C$. Therefore the standard deviation of a counting rate, $R=C/t$, is given by

$$\sigma_R = \frac{\sqrt{C}}{t}$$

The counting error can be reduced by increasing the time of the count.

In this study, all tubes were counted five minutes.
The counts ran from 6,000 to 13,000. This means that the standard deviations of the counting rates range from 23 to 15 counts per minute.

The mean square for ages within pigs

This mean square contains a component attributable to random error and a component which reflects differences between serum samples taken from the same pig at three different ages. The mean square for differences between ages is significant. This indicates that serum samples taken from the same pig at different times (ages) cause significantly different amounts of serum damage.

Many serum characteristics probably change as the pig becomes older. Any of these changes could cause significant differences between serum damage values for samples taken from the same pig at different ages. One readily observable serum characteristic that may contribute to the age component in the analysis of serum damage values is the degree of hemolysis. This possibility should be investigated in subsequent studies.

The mean square for pigs within assays

This mean square contains a random error component, a component for ages within pigs, and a component for pigs within assays. The mean square for pigs within assays is significant when tested against the ages mean square. This test measures the significance of the component attributable.
to pigs, since the expected mean squares for ages and pigs differ only by the pig component. The significant F value indicates that serum samples from different pigs cause significantly different amounts of serum damage. Serum characteristics that differ from pig to pig with consistency over the three ages are responsible for the significant mean square for pigs. The nature of these characteristics is not known.

The mean square for assays

The assay mean square, tested against the mean square for pigs, proved to be highly significant. This indicates that the degree of serum damage differs significantly from one assay to the next. The mean serum damage values for the eight assays are presented in Table 2a on page 39.

The assay component will be influenced by assay characteristics which vary from assay to assay but are common to all observations within an assay. The amount and age of the labelled growth hormone used will vary from assay to assay and will influence the observed serum damage values.

Growth Hormone Level

The total variation of the growth hormone determinations was partitioned into the same four sources as was the serum damage determinations. The analysis of variance is given in Table 2c on page 39. The determinations mean square is the
measure of the true experimental error and is estimated by the difference between duplicate determinations made on the same serum sample. The square root of this mean square estimates the standard deviation (σ = 1.407 mg) of a growth hormone determinations.

**The determinations mean square**

This mean square is influenced by two sources of error. The errors associated with counting radioactivity contribute to the differences between duplicate determinations. The nature and extent of the counting error was discussed earlier. Errors resulting from laboratory technique contribute to the observed determinations mean square. Errors in laboratory technique cause duplicates to differ in total content and composition of the contents.

Variations in total volume of the contents influence the reaction between antibody and antigen. The total volume when dextran coated charcoal is added influences the adsorption of labelled hormone to the charcoal.

Variation between duplicates in serum content contributes to the determinations mean square in two ways. First, the serum content during incubation affects the antigen-antibody reaction because the serum contains growth hormone which is the antigen. Secondly, the serum content influences the adsorption of labelled hormone to the dextran coated charcoal.

Duplicate tubes may receive different amounts of labelled
and unlabelled growth hormone. The quantity of hormone (labelled or unlabelled) present during incubation influences the antigen-antibody reaction and therefore contributes to the observed difference between duplicate tubes. Variation in the quantity of antibody added affects the antigen-antibody reaction and thus cause differences between duplicates.

Errors in serum damage determinations contribute to the error involved in estimating the growth hormone content of a serum sample. The errors between serum damage determinations on the same serum sample do not contribute to the variation between growth hormone determinations made on the same serum sample. This is true because the average of the two serum damage determinations is used as a correction factor for both of the growth hormone determinations made on the same serum sample.

The mean square for ages

Changes with time in the hormone content of a pig's serum are reflected in the age component. The age component is influenced by any factor which changes from one bleeding to the next. Factors which do not change with time affect the age component if the pig's reaction to these factors changes with time. The degree of stress to which a pig is subjected at bleeding varies with time and the pig's reaction to a constant amount of stress may change with time. Both of these factors contribute to the age component.
The amount of stress is probably one of the largest sources of extraneous variation influencing the observed growth hormone level. An attempt was made to minimize the effect of stress by collecting the blood samples quickly and with a minimum of handling. If blood samples are collected in the same way at every age, this should help make the effect of stress constant for all ages.

The mean square for age is significant, indicating that the change in growth hormone level with age is too great to have occurred by chance. The mean hormone level at weaning was 5.41 μg/ml. The standard deviation of this estimate was 0.486 μg. The mean weaning weight was 37.8 pounds. The mean hormone level at 100 pounds was 3.33 μg/ml with a standard of 0.273 μg/ml. The average age of pigs at the bleeding (100 pounds) was 110.3 days and the average pig weighed 113.5 pounds at this age. The mean hormone level at the third bleeding (200 pounds) was 2.82 μg/ml with a standard deviation of 0.204 μg. The average age and weight of pigs at the third bleeding were 174 days and 203.7 pounds, respectively.

The circulating growth hormone level decreases with age. This agrees with the results of Machlin et al. (1968). Baker Jr. et al. (1956) and Baird et al. (1952) reported that pituitary growth hormone content per unit body weight decreased with age. Relating the results of Baird and Baker
to those of Machlin and myself, we see that both the circulating hormone level and the pituitary hormone content per unit body weight decreases with age. This does not suggest a positive relationship between circulating hormone level and pituitary hormone content per unit body weight at a given age.

The mean square for pigs within assays

This mean square is not significant, indicating that the differences between pigs in growth hormone level averaged across ages could easily be due to chance variation. The pig component could be inflated by any factor which did not change with time but did differ from one pig to the next. For example, different pigs react differently to stress and this reaction may not change with time. If this is the case, the reaction to stress could contribute to the pig component without influencing the age component.

The mean square for assays

The mean square for assays is significantly larger than the mean square for pigs within assays. The significant difference between assays could scarcely be due to sampling variation in the assignment of pigs to assays, because the pigs were randomly assigned to assays. The mean hormone levels for the eight assays are given in Table 2a on page 39.

The significant assay mean square results from the large assay component. This component is influenced by
assay characteristics which vary from assay to assay but are constant within an assay. The amount and age of the labelled growth hormone used varies from one assay to the next and influences the observed growth hormone level. There are errors in antibody dilution and errors in making up the standard growth hormone solution. These errors contribute to the assay component but do not contribute to the other components because only one dilution of antibody and one dilution of standard hormone was made per assay.

Correlations of Growth Hormone Levels with Growth Rate and Carcass Traits

Growth hormone is a complex chemical produced by the anterior pituitary. Research into the nature of its action indicates that its functions are many and quite complicated. Growth hormone has a positive effect upon growth rate and protein deposition in many species. It has also been implicated as a regulator of fat deposition and catabolism. Research dealing with hypophysectomy and growth hormone replacement therapy emphasizes the vital role played by growth hormone in the physiological well-being of an animal.

In spite of the voluminous writings on growth hormone, very little is actually known about it. The correlations presented and discussed on the next few pages are based upon a small number of observations and the errors associated with
these observations are admittedly quite large. Therefore, the following discussion should be regarded as a collection of ideas which may or may not contain truths.

The correlations presented in Table 3 on page 50 were calculated by pooling the within-assay product moment correlations. Therefore, these correlations are not influenced by differences between assays. The correlations among the growth hormone levels at the three ages are about what one would expect. The positive (0.20) correlation of hormone level at weaning with hormone level at 100 pounds is not significant but does exceed the correlation between weaning hormone level and the hormone level at 200 pounds. The (0.36) correlation between the hormone level at 100 pounds and that at 200 pounds is significant. The hormone level at each age is positively and significantly correlated with hormone level averaged over ages. This is to be expected, since the average level is calculated as the mean of the three determinations.

The work of Baird et al. (1952) shows a positive relationship between growth rate and growth hormone content of the anterior pituitary. The relationship between plasma hormone level and pituitary hormone content is not known. By some mechanism, possibly feedback, the release of hormone from the pituitary is regulated so that the circulating level is kept relatively constant for pigs of a particular age. The
Table 3. Correlations of growth hormone levels with rate of gain and carcass traits\textsuperscript{a,b}

<table>
<thead>
<tr>
<th></th>
<th>Weaning GH level</th>
<th>100# GH level</th>
<th>200# GH level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth hormone at 100 pounds</td>
<td>+.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth hormone at 200 pounds</td>
<td>-.07</td>
<td>+.36</td>
<td></td>
</tr>
<tr>
<td>Average of 3 GH levels</td>
<td>+.80</td>
<td>+.74</td>
<td>+.41</td>
</tr>
<tr>
<td>21-Day weight</td>
<td>+.26</td>
<td>-.02</td>
<td>-.10</td>
</tr>
<tr>
<td>Weaning weight</td>
<td>+0</td>
<td>-.03</td>
<td>-.16</td>
</tr>
<tr>
<td>Carcass length</td>
<td>-.04</td>
<td>-.03</td>
<td>-.18</td>
</tr>
<tr>
<td>Loin Eye Area</td>
<td>-.20</td>
<td>-.12</td>
<td>+.08</td>
</tr>
<tr>
<td>Ham and Loin %</td>
<td>-.03</td>
<td>-.12</td>
<td>-.21</td>
</tr>
<tr>
<td>Carcass Backfat</td>
<td>-.13</td>
<td>0</td>
<td>+.23</td>
</tr>
<tr>
<td>ADG (Birth-weaning)</td>
<td>-0</td>
<td>-.05</td>
<td>-.18</td>
</tr>
<tr>
<td>ADG (Weaning-100#)</td>
<td>-.36</td>
<td>0</td>
<td>+.05</td>
</tr>
<tr>
<td>ADG (100#-200#)</td>
<td>-.26</td>
<td>-.14</td>
<td>-.20</td>
</tr>
<tr>
<td>ADG (Weaning-200#)</td>
<td>-.35</td>
<td>-.09</td>
<td>-.07</td>
</tr>
</tbody>
</table>

\textsuperscript{a}To be significant at the 5% level $r \geq .27$.

\textsuperscript{b}To be significant at the 1% level $r \geq .36$.

analysis of variance of hormone levels indicated that there was no significant difference between pigs in average plasma growth hormone level. If this is the case, the rate of hormone turnover or the rate of hormone release from the pituitary would be positively correlated with growth rate.
In this system the circulating hormone level would probably be negatively correlated with growth rate. This would occur because faster-growing animals would remove the hormone from the blood faster and their circulating hormone level would be near the lower boundary of the range in hormone level normal for pigs of that age. The slower growing animals would exhibit hormone levels near the upper boundary of the range in hormone level.

The observed correlations between circulating hormone levels and measures of growth rate are negative with one notable exception. The exception is the 0.26 correlation between hormone level at weaning and the 21-day weight. This correlation is not significant but suggests that the plasma hormone level is positively related to rate of growth when both are observed in young animals.

The correlations between hormone levels and rate of growth in later life are negative. This suggests that the faster-growing animals exhibit lower plasma hormone levels. This observation supports the idea that faster-growing animals remove the hormone from circulation faster and therefore exhibit lower circulating hormone levels.

The observed correlations between circulating hormone levels and the carcass traits are all nonsignificant. The weight gained by a pig is due to an increase in total mass. This mass consists mostly of muscle, fat and bone. The
proportion of the total increase in mass that is attributable to each of these three components differs with the age of the animal. In the early growth stages (from birth to about 150 pounds) the increase in weight is due largely to an increase in muscle. This period is one of rapid protein deposition and during this time the ratio of protein deposition to fat deposition is high. As the animal becomes older this ratio decreases. In mature animals most of the weight change is attributable to changes in the fat content of the body.

The circulating growth hormone levels at weaning and 100 pounds are negatively correlated with loin eye area. The hormone levels at all three ages are negatively correlated with the percentage ham and loin. Both loin eye area and percentage ham and loin are positively associated with carcass protein content and negatively correlated with carcass fat content. Carcass length is negatively correlated with circulating growth hormone level at all three ages. Many authors have reported that growth hormone has a positive influence upon protein deposition and bone growth. If those animals which are utilizing growth hormone at the faster rates are the animals which exhibit lower plasma hormone levels, the above negative correlations agree with the accepted positive relationship of growth hormone to protein deposition and bone growth.

The correlations between carcass backfat and the
circulating hormone level at weaning, 100 pounds and 200 pounds are -.13, 0, and 0.23 respectively. During the later (after 150 pounds) growth stages the amount of fat deposition is increasing. It is the plasma hormone level observed during this time which shows a positive correlation with carcass backfat. This suggests that those animals having a high plasma hormone level at 200 pounds also yield carcasses with high backfat thickness. If the plasma hormone level is negatively related to the rate of hormone utilization, these observed correlations support the idea that growth hormone has a positive effect on fat catabolism and therefore a negative effect upon fat content.
CONCLUSIONS

The first objective of this work was to develop an immunoassay to measure circulating levels of growth hormone in the pig. This objective has been achieved.

Finding that different serum samples cause significantly different amounts of damage to the labelled growth hormone is an important result. This result suggests that careful control and correction for serum damage is of great importance and should possibly be given more attention in subsequent work. One might also wonder about this in other species.

This study supports the conclusions of Machlin et al. (1968) regarding the decline in circulating growth hormone level with age. The results of this work strongly suggest that a negative relationship exists between the circulating growth hormone level and the rate of growth hormone utilization. This point could be investigated further by doing a rate of utilization or rate of turnover study using labelled growth hormone. The relationship of the rate of utilization with growth rate, carcass traits and circulating hormone levels could provide conclusive evidence about the postulated negative relationship between rate of hormone utilization and circulating hormone level.
LITERATURE CITED


ACKNOWLEDGMENTS

I thank you Dr. Hazel for having enough faith in me to allow me to attempt this project and for the help you provided. I am indebted to Dr. Trenkle for his suggestions and for his generosity in loaning me equipment without which this work could not have been completed.

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Last but surely not least, I thank you Lady for your indulgence during my seemingly endless education. If I could relive my life, one item definitely would not be changed and that item is you Lady.

I would like to express my gratitude to Dr. H. E. Wilhelmi of Emory University, Atlanta, Georgia for the porcine growth hormone sample used as a standard in this research. The hormone sample (lot no. P522-A) had 2.0 units of activity. The activity was determined by the weight gain test using a bovine growth hormone standard.