SOME RELATIONSHIPS OF GROWTH HORMONE LEVELS TO BOVINE GROWTH AND CARCASS QUALITY

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Abstract

SOME RELATIONSHIPS OF [illegible] TO BOVINE GROWTH AND [illegible]

By

Robert W. Purdue

Relationships between [illegible] growth hormone (GH) and some other [illegible] were investigated. Forty heifers were [illegible] groups of ten. Four were slaughtered [illegible] and the remaining six were ovariectomized at breeding age. [illegible] groups received [illegible] (0.5 mg/animal/day) from [illegible] level of nutrition [illegible] trol acetate, Upjohn [illegible] were ovariectomized at 0.45 mg/animal/day from [illegible] groups, while two groups received the same [illegible] estrus. The remaining four groups [illegible]

Measurements of carcass composition [illegible] obtained for all animals, included separable [illegible] of the right flank, weight of the right [illegible] muscle area and specific gravity of the right [illegible] animals, the separable fat, lean and bone of the right round and the moisture and petroleum ether extract of the [illegible] right round were also determined. For the [illegible]

ABSTRACT

SOME RELATIONSHIPS OF GROWTH HORMONE LEVELS TO BOVINE GROWTH AND CARCASS QUALITY

By

Roger W. Purchas

Relationships between carcass quality, growth hormone (GH) and some other endocrine parameters of 90 Holstein heifers were investigated. Of the nine groups of heifers, four were slaughtered at first estrus and five were slaughtered at breeding size (120 cm withers height). Three groups received a normal level of nutrition (0.9 kg grain/animal/day) from 2.5 mo of age and six groups were on a high level of nutrition (4.5 kg grain/animal/day). MGA (Melengestrol acetate, Upjohn Co., Kalamazoo, Michigan) was administered at 0.45 mg/animal/day from 2.5 mo of age to three groups, while two groups received the same level from first estrus. The remaining four groups received no MGA.

Measurements of carcass composition, which were obtained for all animals, included separable fat and lean of the right flank, weight of the right forecannon bone, eye muscle area and specific gravity of the right round. For 40 animals, the separable fat, lean and bone of the right round, and the moisture and petroleum ether extract of the boneless right round were also determined. For the same 40 animals,

club steaks were assessed subjectively for color and objectively for tenderness.

A double antibody radioimmunoassay involving guinea pig anti-bovine GH and sheep anti-guinea pig gamma globulin was developed in order to assay for GH in bovine blood plasma. This assay did not cross react with bovine prolactin, LH, FSH, or TSH, and the dose response curves of anterior pituitary extracts and plasma paralleled that for standard bovine GH. Radioimmunoassay values agreed satisfactorily with bioassay values. Growth hormone concentration was measured in blood plasma collected at slaughter and in anterior pituitary extracts of all animals. Blood samples drawn by syringe from the jugular vein of 40 animals were also assayed for GH. These samples were taken at monthly intervals from four to ten months of age.

Thyroid activity was assessed in the same 40 animals by measuring plasma protein bound iodine concentration and thyroid follicular cell heights. Procedures for the extraction, purification and fluorometric quantification of cortisol and corticosterone were adapted for use with bovine plasma and adrenal homogenates. They were used to assess the adrenal cortical activity of 30 animals.

Several statistical approaches were used in attempts to characterize the relationships being investigated. Simple correlation coefficients were calculated and regression analyses involving linear and quadratic components were made in several instances. General least squares models

were employed to assess the effects of endocrine parameters on carcass quality after corrections had been made for the effects of nutrition, age at slaughter, MGA and carcass weight. A similar influence of nutrition, age at slaughter, MGA or carcass weight on both a carcass quality and an endocrine parameter was considered indicative of a relationship between those two parameters. Measures were also made of the degree to which age, nutrition, MGA and carcass weight affected carcass quality indirectly through endocrine effects.

The mean of the seven jugular GH levels was significantly and negatively related to average daily carcass weight gain, both on the basis of a simple correlation coefficient ($r = -0.37$) and a least squares model ($P = 0.01$). Plasma GH level at slaughter was not related to the mean jugular level ($r = -0.07$), and was not consistently related to any carcass quality traits. Growth hormone concentration in anterior pituitary extracts was significantly correlated with carcass weight in the animals slaughtered at breeding size ($r = -0.51$), which implies that the animals with high pituitary GH levels had less carcass weight per unit withers height. This was supported by significant correlations showing that high levels of pituitary GH were associated with decreased fatness, decreased dressing percent and an increased percentage round. For the same animals, pituitary GH concentration was significantly and negatively related to average daily carcass weight gain ($r = -0.49$). The absence

of any significant effects of pituitary GH levels in the least squares models may have been because carcass weight was included as a covariate.

Measures of the concentration and the total content of cortisol and corticosterone in both the plasma and in adrenal homogenates were significantly and negatively ($P < 0.01$) correlated with average daily carcass weight gain. Only adrenal corticosterone concentration had a significant negative effect on average daily gain in the least squares models.

Plasma levels of cortisol and corticosterone accounted for 32.3 and 33.6 percent of the variation in tenderness, respectively, when quadratic components were included in the regression equations. High levels of corticosteroids tended to decrease tenderness.

Plasma protein bound iodine concentration was significantly and positively related to the weight of lean in the round according to a least squares model, but it was not significantly correlated with percent lean in the round ($r = 0.17$). No clear relationships between measures of thyroid activity and growth rates were shown.

Although not all significant, correlations between measures of GH and corticosteroids were consistently positive, suggesting that the stress at slaughter may have affected these measurements similarly.

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INTRODUCTION

Any procedure that facilitates the measurement of productive characteristics of live meat producing animals is of value to the meat industry. Such procedures are particularly valuable if they enable one to predict future or potential productivity as well as the current status. Potential productive characteristics are generally more difficult to predict than the actual, and the ease with which different productive characteristics may be measured varies widely. Rate of growth, for example, is more easily measured than carcass composition, which in turn may be more accurately estimated than meat quality.

Many procedures enabling the prediction of productive characteristics have been proposed. Most of these have involved the evaluation of parameters which are determined by particular productive characteristics. Thus, in the estimation of body composition, body density is essentially determined by fat content while the amount of carcass ^{40}K is determined mainly by muscle content.

An alternative approach, but one which has not been widely used, involves the evaluation of parameters contributing to the determination of a particular productive characteristic. For example, appetite may at least in part

determine growth rate. A factor which plays a role in the determination of a trait is likely to be more closely related to the basic genetic material than one which is determined by that trait, and as a result, may be more highly heritable. However, if several factors contribute to the variation of a productive characteristic, then the correlation between that characteristic and any one of these factors may be lower than the correlation between the characteristic and traits determined by it.

Of the physiological parameters, which may control productive characteristics and can be assessed in the live animal, those associated with the endocrine system would appear to be the most promising. This is a result of the general controlling influence of the endocrine system in such body functions as growth where growth hormone (GH) has frequently been implicated.

Although the hypothesis that GH plays some role in determining growth characteristics is supported by considerable indirect data, direct evidence is more scarce and the results of different experiments are sometimes difficult to compare. This difficulty arises from the different manners in which variations in growth may be induced, and in which GH status may be measured. Thus, differences in growth may be due to genetic, environmental, age or size effects while measurement of GH status may include measures of anterior pituitary GH concentration, plasma GH concentration, plasma

GH turnover rate, hypothalamic concentration of GH releasing factor or tissue responsiveness to GH.

Although many factors besides GH are known to make important contributions to variation in the rate and composition of growth, the only ones which will decrease the correlation between GH and growth are those that affect growth in ways other than through GH.

The study reported herein involved Holstein-Friesian heifers reared on different nutritional regimes and slaughtered at different ages. The objectives of the study were to investigate certain endocrine parameters of these animals in relationship to the rate and composition of growth and to meat quality.

REVIEW OF LITERATURE

Productive Characteristics

For the purposes of discussion, the important productive characteristics of meat type animals are divided into growth characteristics and meat quality characteristics.

Growth Characteristics

Warwick (1968) included rate, efficiency and composition of growth as important characteristics to be considered in selection schemes for beef cattle. He defined efficiency as the gain per unit of food consumed and noted that ". . . the genetic relationship between rate and efficiency of gain is high enough that selection for rate of gain is a reasonably effective means of selecting for efficiency." Phenotypic relationships between the rate and efficiency of gain have also been shown to be close by Botkin (1955), by Broadbent and Bowman (1964) and by Spedding (1968). The desirability of selecting for growth rates will be determined to some extent by the relationships between this parameter and other important characteristics (Warwick, 1968; Taylor, 1968), such as mature size and mature productivity. Dickinson (1960) has suggested that developmental growth rate and mature size are different

genetic characteristics, but the analyses of Taylor and Craig (1965) indicated that, even if they are different, they are not necessarily unrelated. Using 120 pairs of uniformly raised monozygotic and dizygotic twin dairy heifers, they showed that genetic correlations between linear measurements taken at 3 month intervals up to 2 years of age were generally very high. Increased growth rates have also been shown to be negatively related to subsequent productivity in some cases (Schultz, 1969), but Warwick (1968) noted that this relationship is more likely to be phenotypic than genetic.

Patterns of Growth

Compared with the rate and efficiency of growth, the composition of growth is more difficult to measure and assess in terms of desirability, and its relationships with other growth parameters are much less clear (Warwick, 1968). Of the nutrients that are digested by an animal, the proportions utilized by different organs and by different tissues depend on the quantity of nutrients, the age of the animal, the size of the animal, and the animal's genotype, providing the available nutrients constitute a well balanced diet (McCance and Widdowson, 1962). The relative importance of age and of size in determining the growth or development of a particular body part in rats has been studied by Widdowson and McCance (1960). Using rats of the same age but widely differing sizes, obtained by varying litter size, they showed that changes in linear measurements and weights were generally

weight dependent, while some physiological processes such as brain development, teeth erruption and eye opening were primarily age dependent. Palsson (1955) has reviewed the work and theories developed by Hammond and his co-workers at the University of Cambridge regarding the development of meat producing animals. He discussed the theory of partition of nutrients whereby the less a particular process is affected by undernutrition, the higher priority it is considered to have for the available nutrients. This means that at a particular age or size, the way in which the available nutrients are partitioned is determined by the priority of the different tissues, with an increasing proportion going to the low priority tissues as the quantity of nutrients increases. According to Palsson (1955) changes in age or size do not affect tissue priorities appreciably but the partitioning of a particular quantity of nutrients will change due to changes in the growth rates of different tissues and also of the same tissues in different parts of the body. He reviewed considerable evidence which indicated that each tissue has a sigmoid shaped growth curve and that the age or size of the animal at the point of inflexion (maximum growth rate) differs for different tissues. He then indicated that the growth rate of nervous tissue is the first to reach a peak, followed by bone, muscle and finally fat. The time or size at which peak growth rate is reached also varies within a given tissue between different parts of the body. Thus, with increasing time or size, regions of

maximum growth rate pass from the anterior to the posterior along the main axis and centripetally along the limb axes. More recent evidence supporting these growth gradients has been reviewed by Everitt (1968).

The work discussed by Palsson (1955) indicates that nervous tissue with a high priority for available nutrients will be mainly dependent on age and relatively independent of size, because size will be largely determined by tissues that have a lower priority for nutrients. Thus, their growth will be inhibited by less severe restrictions in nutrient availability than will nervous tissue growth. Growth of fatty tissue, on the other hand, will be the first to be affected by quantitative nutrient restrictions so that any effect of plane of nutrition on total body growth will also affect fat growth. It follows that body weight and fat weight are likely to be closely related. Because it has the lowest priority for nutrients, the presence of fatty tissue complicates the relationship between any other tissue and total body weight, which has led several workers (Tulloh, 1963; Elsley et al., 1964; Berg and Butterfield, 1966) to use bone plus muscle weight or fat free body weight as a basis for comparison with other tissues.

The use of the allometric equation of Huxley (1932) to describe relationships between the weights of different body parts has greatly facilitated investigations in this area (Huxley, 1950; Zar, 1968). The allometric equation is,

in one form, a linear regression equation with the logs of the weights of the two components as the two variables.

$$y = bx^a$$

or $$\log y = \log b + a \log x$$

By differentiation $\frac{d\ (\log y)}{d\ (\log x)} = a$,

and since $\frac{d}{dx}\ (\log_a u) = (\log_a e)\ \frac{1}{u}\ \frac{du}{dx}$,

then $d\ (\log_{10} x) \propto \frac{dx}{x}$,

and $$\frac{d\ (\log y)}{d\ (\log x)} = \frac{\frac{dy}{y}}{\frac{dx}{x}} = \frac{\frac{dy}{dt}\frac{1}{y}}{\frac{dx}{dt}\frac{1}{x}} = a$$

Thus, 'a,' the slope of the regression of log y on log x, is equal to the ratio of the specific growth rates of the two components as indicated by Medawar (1950). Huxley (1950) pointed out that if the allometric equation effectively accounts for the relationship between two components, it implies that: (1) growth is essentially multiplicative, (2) the multiplicative rate differs in different body parts giving rise to different specific growth rates, and (3) some factor operates to keep the ratio of specific growth rates constant.

Butterfield and Berg (1966), using the allometric equation with total muscle weight as the independent variable, classified muscles and groups of muscles as high, average or low impetus according to whether the slope of

the regression line was significantly ($P < 0.01$) greater than, not significantly different from or significantly less than one, respectively. Butterfield and Johnson (1968) have discussed the effect of rate of growth on the relative contribution of high and low impetus muscles to total muscle weight. They showed that the fast growing animals had a higher proportion of high impetus muscles and a lower proportion of low impetus muscles than the slow growing groups of the same age. Such results would be expected as high impetus muscles have higher specific growth rates than total muscle, and, consequently must increase as a percentage of total muscle with an increase in total muscle weight. On comparing animals at the same ages, the fast growing group had a greater total muscle weight, and therefore, a greater percentage of high impetus muscles. No differences in the proportions of high and low impetus muscles were shown between the fast and slow growing groups when comparisons were made at the same total muscle weight. Thus, the data indicated that the relationships between individual muscles or muscle groups and total muscle were dependent mainly on muscle weight and were relatively independent of age.

The suggestion by Palsson (1955) that ". . . the animal's form can be controlled at will by changing the plane of nutrition at different stages of growth . . ." is based on the assumption that changes in growth rates that determine the relationships within and between tissues are to some extent age dependent. If such relationships are age

dependent then increasing the quantity of available nutrients at the time of maximum growth rate for a particular tissue and decreasing the quantity as the growth rate decreases should increase the proportion of that tissue in the mature animal. Palsson (1955) further reviewed work which has shown this to be the case, especially when the relationship between a particular tissue weight and total body weight is considered. If, however, the relationship being considered is independent of age and is dependent only on weight, then this relationship, and therefore, this aspect of animal form, will not be influenced by plane of nutrition.

The allometric equation and other methods have been used by several workers (Wallace, 1948; Wilson, 1954; Tulloh, 1963; Elsley et al., 1964; Everitt, 1968; Seebeck, 1968; Burton and Reid, 1969) in analyses similar to that of Butterfield and Johnson (1968) to indicate that the level of nutrition does not affect certain important relationships in meat producing animals. These relationships include those between individual muscles and total muscle, between individual bones and total bone, between muscle weight and muscle plus bone weight, and between bone weight and muscle plus bone weight.

Although use of the allometric equation effectively describes many of the relationships between body components, Medawar (1950) has pointed out that it should be considered as a useful empirical approach rather than as a

basic developmental law. Fowler (1968) discussed situations where expected allometric relationships have not been demonstrated and suggested that in some cases this may be due to the expected changes in form being over-ridden by functional priorities. For example, Palsson and Verges (1952) showed that after adjustment to the same total bone weight, bones of the head in lambs on a low plane of nutrition made up a significantly higher proportion of total bone weight than similar bones of lambs on a high plane of nutrition. Widdowson (1964) also noted that pigs which were severely undernourished from the early suckling period had larger heads than normally fed pigs of the same weight. Fowler (1968) suggested that the effects of nutrition on the bones of the head and on the entire head are a result of the functional relationship of these components with the brain, the development of which is relatively independent of plane of nutrition. He discussed several other comparable examples and suggested that ". . . the animal tends to adjust to environmental (nutritional) changes in such a way that the vital functional relationships between essential body components are preserved, or modified to a form which gives the animal its best chance of survival and successful reproduction."

Fowler (1968) also pointed out that if a particular treatment affects a body component so as to change its allometric relationship with other components, and if this component makes up part or all of the independent variable

in a covariance analysis using the allometric equation, then there will appear to be a treatment affect on the dependent variable. With regard to choosing an independent variable, he further pointed out that one should select the relationships most likely to give answers to the questions being asked, whether they are economic, anatomical, physiological or chemical. The degree of environmental stress necessary to bring about deviations from allometric relationships and to cause permanent stunting, and the changes in susceptability of various species to stress with changes in age have been discussed by Dickinson (1960), Widdowson (1964), and Everitt (1968).

Measures of Composition

Methods that have been used to assess body composition in meat producing animals have been reviewed by Pearson (1965), Barton (1967) and Hedrick (1968). Seebeck (1968) has pointed out that meat scientists are primarily interested in carcass composition and that this may be defined in terms of physical components (lean, fat, bone), chemical components or anatomical components. The distribution of muscle and fat is also an important aspect of composition and an important determinant of value of meat producing animals (Barton, 1967). On studying cattle of widely differing appearance and genetic background, Butterfield (1965) showed very little variation in the distribution of muscle tissue. In the case of fat or adipose tissue, however,

studies such as those of Callow (1962b) suggest that there is appreciable variation in fat distribution throughout the body. He compared the fat content of muscular tissue, adipose tissue and the total carcass in three breeds of steers and showed that at the same overall level of fatness, Shorthorns and Friesians had a higher percentage of fat in their fatty tissues than Herefords, while Shorthorns had a higher percentage of fat in their muscular tissues than Herefords and Friesians. In the same study, considerable variation was shown between breeds in the ratio of mesenteric fat to total carcass fat. However, no attempt was made to investigate breed differences in the relationships between fat sites by using the allometric equation.

Because direct measurement of body composition is expensive and time consuming, many indirect methods of measurement have been investigated (Hedrick, 1968). Seebeck (1968) in discussing this approach pointed out that although the use of indirect methods will probably result in a loss of sensitivity and accuracy on individual animals, the increase in simplicity and economy may improve the overall accuracy by enabling more animals to be assessed. The composition of sample joints has been used to estimate carcass composition, and in cattle, the round constitutes a good sample cut because it makes up a large portion of the carcass (Hedrick, 1968). Also the flank has been shown to be a good sample cut in animals approaching maturity, apparently because it is one of the last regions of the carcass

to mature (Butterfield, 1965; Palsson, 1955; Luitingh, 1962). The late maturation of the flank indicates that the rate at which its composition is changing in animals approaching maturity is likely to be greater than that of the carcass as a whole.

Table 1 summarizes a number of studies showing the usefulness of the round and flank as indicators of bovine carcass composition. Other relevant but unpublished information has been reviewed by Hedrick et al. (1963) and Hedrick (1968). The data in Table 1 suggest that the flank should be at least as effective as the round for estimating carcass fat content but will probably not give as good an indication of muscle content. As expected, flank bone, which consists of only a small portion of the 13th rib, is not highly correlated with total bone. However, the cannon bone, which is of negligible economic value, provides a good indication of total bone in beef cattle (Callow, 1962a; Orme et al., 1959).

Cuts equivalent to the bovine flank do not appear to have been widely used in other species, but the leg in lambs has been shown to be closely correlated to carcass composition (Barton and Kirton, 1958; Field et al., 1963; Timon and Bichard, 1965; Khandekar et al., 1965), as has the ham in pigs (McMeekan, 1941; Joblin, 1966). The relationship between body composition and body weight is very close under many conditions (Reid et al., 1968), and if body weight accounts for variation in body composition that is not accounted for by the composition of a cut, then both these

Table 1. A summary of work relating bovine flank and round composition to carcass composition

Experimental material	Number of animals	Component	Relationship to carcass component (y)		Reference
			Correlation coefficient	Regression equation	
Hereford, Shorthorn and Friesian steers	24	Tibia-Fibula	0.97	y = 2.61 + 12.44 x ±0.74	Callow (1962a)
		Leg muscle	0.80	y = 36.69 + 2.78 x ±7.68	
		Fat	0.59	y = 24.58 + 4.37 x ±7.99	
Steers of British beef breeds	120	Round fat trim	0.85		Dikeman (1968)
		Round bone	0.96		
		Round retail yield	0.97		
		Flank fat trim	0.95		
		Flank bone	0.00		
		Flank retail yield	0.78		
Steers of British beef breeds	80	Round muscle	0.97		Allen (1966)
		Round separable fat	0.90		
		Round bone	0.98		
		Flank muscle	0.86		
		Flank separable fat	0.94		
		Flank bone	0.15		
British, Brahman and dairy breeds. Steers, cows and heifers	50	Round separable lean	0.95		Cole et al. (1960)
Steers	47	Trimmed round	0.88		Miller et al. (1965)
Steers. Increasing level of nutrition from lot 1 to lot 4	156	Round water Lot 1	0.86		Kelly et al. (1968)
		Round water Lot 2	0.82		
		Round water Lot 3	0.95		
		Round water Lot 4	0.92		
		Round fat Lot 1	0.97		
		Round fat Lot 2	0.95		
		Round fat Lot 3	0.97		
		Round fat Lot 4	0.96		
		Flank water Lot 1	0.84		
		Flank water Lot 2	0.91		
		Flank water Lot 3	0.94		
		Flank water Lot 4	0.84		
		Flank fat Lot 1	0.96		
		Flank fat Lot 2	0.93		
		Flank fat Lot 3	0.92		
		Flank fat Lot 4	0.95		

variables should be included in prediction equations. Using the data of Callow (1962a), Harrington and King (1963) showed that the accuracy of predicting side composition from round composition could be improved by including side weight as a second independent variable.

The rationale of using specific gravity measurements to estimate body composition has been discussed by Pearson et al. (1968), while the application and accuracy of this technique in the estimation of the carcass composition of meat animals was reviewed by Garrett (1968) and Garrett and Hinman (1969). Evaluation of the relationships between the specific gravity and the composition of cuts and of the carcass by Kelly et al. (1968) showed that when ether extract of the carcass was less than 20%, specific gravity was not highly correlated to carcass composition.

Meat Quality Characteristics

In discussing identification of quality factors in meat, Pomeroy (1968) pointed out that concepts of quality are not definite but vary between groups of people, and also between producers, distributors and consumers. He stated that the main meat quality factors can be divided into those associated with composition, which have been discussed earlier herein, and those concerned with appearance and palatability. He noted that the most important factors in the latter group are tenderness, juiciness, flavor and color, with the relative significance given to each of these in any

particular situation being generally determined by the degree to which it deviates from optimum. Thus, Batcher et al. (1969) suggested that: "A consumer may accept or reject a piece of lamb meat on the basis of its flavor or odor, whereas acceptability of beef may also be based on tenderness, and pork and turkey acceptability on juiciness." It is generally accepted, however, at least in the case of beef, that tenderness is often the most important palatability characteristic (Brady, 1957; Weir, 1960; Brayshaw and DeLoach, 1963; Pearson, 1966; Pomeroy, 1968). The principle factors which determine beef tenderness were discussed by Pearson (1966).

The measurement of tenderness may be carried out subjectively using taste panels, or objectively by the use of mechanical or chemical methods. Pearson (1963) in a review of methods used to assess tenderness pointed out that objective devices had advantages in their objectivity and simplicity, while Kramer (1969) noted that although objective methods in general may be superior to subjective methods in precision and calibratability, they ". . . cannot possibly be an improvement in accuracy over a subjective method since the accuracy of an objective procedure can be determined only by its degree of correlation with the subjective evaluation sought." Kramer (1969) also discussed a suggestion by Guilford (1942) that it may be appropriate to divide the correlation between objective and subjective measures by the square root of the correlation between

successive subjective measures, since poor repeatability of subjective assessments will obviously decrease the probability of high correlations.

Endocrinological Aspects

Growth Hormone

General reviews concerning the physiology of growth hormone (GH) which have appeared recently include those by Knobil (1961), Knobil and Hotchkiss (1964), Weil (1965), Pecile and Müller (1966a), Evans et al. (1966), Greenwood (1967), Daughaday (1968), Hunter (1968) and Pecile and Müller (1968). The above reviews suggest that the aspects of GH physiology which are most likely to influence the productivity of meat producing animals are those concerned with lipid metabolism, protein metabolism and growth rates. These three aspects together with GH measurement and control of secretion will be considered in the following sections.

Growth Hormone and Lipid Metabolism

Based mainly on results from rats, Knobil (1961) stated: "The body composition of animals treated with growth hormone, when compared with that of their pair-fed controls, characteristically reveals an increase in the proportion of protein and water and a reduction in the proportion of fat. . . ." In discussing the relationship between growth hormone and the mobilization of fatty acids, Rabin and Hollenberg (1959) pointed out that although growth

hormone tended to promote ketosis, lower respiratory quotient, diminish fat stores, cause a rapid transfer of fat from adipose tissue to the liver, and increase plasma free fatty acids *in vivo*, its effect on adipose tissue *in vitro* was inconsistent. They concluded that ". . . the mechanism by which growth hormone augments the release of fatty acids from adipose tissue remains obscure."

Weil (1965) reviewed work suggesting that GH affects fat and carbohydrate metabolism, primarily by increasing catabolism of triglycerides with the resulting production of free fatty acids. He also discussed evidence supporting the hypothesis of Rabinowitz and Zierler (1963) whereby GH and insulin act sequentially between times of food intake so as to maintain a supply of energy to the tissues. They suggested that insulin and GH act together in stimulating protein synthesis, but with increasing time after food intake the emphasis changes from insulin, with glucose as the main energy source, to GH with free fatty acids. The hypoglycemia which results from the action of insulin is an effective stimulant to growth hormone release (Roth *et al.*, 1963), and the inhibition of glucose utilization in muscle by free fatty acids (Randle *et al.*, 1964) would assist in the change from glucose to free fatty acids as an energy supply.

In summarizing his work concerning the relationships between GH and metabolism of carbohydrates and lipids in adipose tissue, Goodman (1968a) pointed out that the effect

of GH on rat adipose tissue consists of an early "insulin-like" phase in which the utilization of carbohydrate and storage of fat is increased, followed by an "anti-insulin-like" phase of prolonged duration involving reduced glucose utilization and the release of increased quantities of free fatty acids. The initial effects, which are due to increased permeability of adipose cell membranes to sugars, are overcome by cellular processes involving synthesis of RNA and protein. Although the injection of GH in vivo results in marked reduction in glucose utilization within a few hours, similar administration in vitro has no such effect even over much longer periods of time. Release of free fatty acids in response to GH is much less consistent in vitro also.

More recently, Goodman (1968b) has suggested that GH has at least three separate effects on adipose tissue. He showed that pre-incubation of epididymal fat segments from normal and hypophysectomized rats with GH increased glycerol and free fatty acid release only in the fat from normal rats. On preincubation of the tissue with GH plus theophylline, however, the fat tissue from both normal and hypophysectomized animals responded. This effect was obtained even in the presence of inhibitors of RNA and protein synthesis indicating that it was independent of the effects of GH acting in combination with certain corticosteroids. This combination significantly increased in vitro lypolysis in adipose tissue from normal but not from hypophysectomized

rats although neither of these hormones gave consistent effects alone (Goodman, 1968b; Fain et al., 1965). The third effect discussed by Goodman (1968b) was the delayed in vivo effect on free fatty acid release as discussed above. In further work on the effect of a combination of GH and glucocorticoid on lipolysis, Goodman (1969) reported variable results on epididymal fat from normal rats with the effect on a particular animal generally being either to increase the basal level of lipolysis or to increase the response to other lipolytic substances such as epinephrine. He noted that such results were ". . . compatible with the suggestion that in increasing lipolysis the combination of growth hormone and glucocorticoid may not act as the actual initiator of lipolysis but rather might merely potentiate other endogenous lipolytic signals present in the tissue at the time of excision."

Growth Hormone and Protein Metabolism

Considerable evidence indicates that administration of GH has an anabolic effect on proteins (Knobil and Hotchkiss, 1964), but the mechanisms involved in this action have not been completely elucidated. Korner (1965, 1967) has reviewed work in this area, especially that carried out in cell free systems of rat liver from either normal, hypophysectomized of hypophysectomized and GH treated rats. Early work with this system (Korner, 1967) indicated that GH caused a decrease in the activity of the ribosomal

fraction of the cell, due at least partly to the larger number of polysomes present in GH treated rats. However, the presence of these polysomes does not appear to be due to a specific effect on messenger RNA as GH has a general stimulating effect on cellular RNA synthesis (Brossard and Nicole, 1969). Further evidence that GH does not stimulate protein synthesis through its effect on RNA synthesis was provided by the finding that actinomycin inhibited the latter effect but not the former. Korner (1967) suggested that GH may influence some factor in the cell fluid that is necessary for ribosomal function and that increased RNA synthesis may be a secondary effect. Using the same rat liver cell free system, Korner (1969) presented evidence suggesting that inhibition of polypeptide chain initiation with dextran sulfate did not suppress the GH effects on protein synthesis. This evidence did not show whether GH affected the chain initiation or not, but it did show that some step other than chain initiation was affected, possibly through an effect on the ratio of active to inactive ribosomes on the polysomes.

Jefferson and Korner (1967) pointed out that all attempts to demonstrate an action of GH on protein synthesis in vitro in a cell free system of liver had failed. However, they showed that GH can stimulate the incorporation of precursor into protein and nucleic acid in perfused rat livers in situ, which indicated to them that the effect of GH on the liver was direct and not through an effect on other body tissues. More recently Clemens and Korner (1969) have

demonstrated the stimulation of precursor incorporation into protein and nucleic acid of rat liver slices by bovine GH. However, the concentration of amino acids required for a GH concentration of 10 ng/ml to cause a stimulation was six times that normally found in plasma. The entry of amino acids into liver cells is also increased by GH, and although the work with cell free systems has shown that this is not the primary action, it may be a necessary action in order to permit other effects to be expressed (Snipes, 1968).

Measurement of Growth Hormone

Methods for the assay of GH have been reviewed by Russel (1955), Papkoff and Li (1962) and Greenwood (1967). After reviewing the principle bioassay methods, Papkoff and Li (1962) concluded that ". . . for the general laboratory assay of purified preparations of mammalian growth hormone, the tibia test stands out as the best procedure in terms of sensitivity, precision and specificity. The tibia test does not lend itself to the assay of body fluids, and here it would appear the use of a sensitive immunological test is indicated." The tibia test of Greenspan et al. (1949) is based on the effect that GH has on the width of the tibial epiphysis in hypophysectomized rats. The specificity of this assay has been questioned as a number of other hormones are known to affect the width of the epiphysis (Ailabouni et al., 1966). Although attempts have been made to assay plasma GH levels by the use of the tibia test, either with

or without prior concentation, this approach has not been widely used (Greenspan, 1950; Gemzell, 1959; Tweed et al., 1962). Recently it has been reported that the precision of the tibia test may be considerably improved by measuring the epiphyseal width by X-ray techniques before and after treatment on the same rats (Leget et al., 1969).

Although all immunological techniques are based on the reaction of an unknown quantity of hormone with a fixed quantity of antibody, the way in which the quantity of hormone is estimated varies considerably. Li et al. (1960) used quantitative precipitation tests followed by Ouchterlony plate or precipitin ring tests to determine the equivalence point of each sample for a fixed quantity of antibody. They were able to detect concentrations of 200 ng GH/ml in acromegalic subjects.

Read et al. (1962) discussed an assay based on the inhibition of hemagglutination by GH. This involved coating red blood cells with the antigen and then reacting a standard quantity of the coated cells with a standard quantity of antibody in order to bring about agglutination of the cells. Pre-incubation of the antibody with free antigen decreased agglutination in proportion to the quantity of free antigen. The main disadvantages of this method are its subjectiveness with regard to assessing the degree of agglutination and the fact that there are interfering factors in plasma, which should be removed by prior purification (Dominguez and Pearson, 1962).

Complement fixation is dependent on the formation of the antibody-antigen complex, and consequently, the degree of fixation in a system containing a standard amount of antibody will be proportional to the antigen concentration. This method has been applied to the assay of GH (Tashjian et al., 1968) and is claimed to have an advantage over the other immunological methods discussed above in that the concentration of complement can be measured optically.

The immunological method, which has been most widely used and accepted for GH as well as for many other antigenic substances, is the radioimmunoassay. This method which has been discussed by Yalow and Berson (1968) is based on the competition between a fixed quantity of radioactively labelled hormone and an unknown quantity of unlabelled hormone for a fixed but limited quantity of antibody. Ekins et al. (1968) also considered the theory of this type assay while other discussions have been written by Wright and Taylor (1967) and Hunter (1967). In a theoretical approach to radioimmunoassays, Yalow and Berson (1968) assumed that the antigen and antibody were in equilibrium with the antigen-antibody complex according to the law of mass action. They then defined sensitivity as the rate of change in response (the ratio of bound to free labelled hormone -- B/F) with change in antigen concentration, and precision as the rate of change in response with fractional changes in concentration of the antigen. Using these concepts, they

showed that "For a combination of satisfactory sensitivity and precision, a trace B/F ratio of about 2 to 3:1 will frequently give best results."

Ekins et al. (1968) disagreed with the definitions of sensitivity and precision used by Yalow and Berson (1968) and claimed that any such definitions should take into account the errors involved in the method. They defined precision as the change in hormone concentration required to change the response (free to bound ratio) by an amount equal to the error associated with the calculation of the response at that particular hormone concentration. Sensitivity was defined as precision in the absence of unlabelled hormone and was, therefore, a measure of the smallest detectable quantity of hormone. Based on these definitions, they derived equations enabling them to predict optimum conditions under certain circumstances. The conclusions reached with regard to optimum conditions differed from those of Yalow and Berson (1968).

Miles and Hales (1968) reported on a type of radioimmunoassay that differed from that discussed above in that the antibody was labelled rather than the antigen. This immunoradiometric assay was based on the reaction of the antigen with excess labelled antibody, and then the removal of remaining antibody with a solid antigen-immunoabsorbent. They claimed the following advantages for this approach: (1) all the unknown antigen is involved in the assay; (2) a zero baseline will be obtained; and (3) there is no

risk of the labelled antigen acting in a different way to the unlabelled antigen as may be the case for other radioimmunoassays. Apart from this major deviation in approach, the main differences in the many radioimmunoassays presently in use are in the methods used to separate the bound and free hormone. Hunter (1967) pointed out that the best method in a particular situation depends on the requirements with regard to simplicity, sensitivity, precision and economy. He discussed the advantages and disadvantages of separation by electrophoresis, chromatography and chromatoelectrophoresis, ion exchange paper chromatography, solvent precipitation systems, methods depending on molecular size and the double antibody system.

Three other systems that have been successfully used in the separation of bound from free GH are: (1) solid phase radioimmunoassay, in which the antibody is coupled to an insoluble compound (Catt et al., 1966; Wide and Porath, 1966); (2) the removal of free GH by adsorption onto substances such as charcoal (Wool and Selenkow, 1968); and (3) the radioimmunoassay by enzyme partition (Mitchell et al., 1969). In the latter study, the proteolytic enzyme ficin was used to destroy the free antigen and then the bound portion was precipitated with trichloroacetic acid. Greenwood (1967) pointed out that chromatoelectrophoresis was the only method that enabled assessment of hormone damage by iodination or by plasma components, but that many of the other methods yielded more rapid results. The

advantages and disadvantages of various separation methods were extensively discussed at a recent symposium on protein and polypeptide hormones (Margoulies, 1969).

The principle advantage of radioimmunoassay over other methods used to assay GH is its sensitivity, while the main disadvantage is probably the fact that it measures immunological rather than biological properties (Garcia and Geschwind, 1968). Greenwood (1967) noted that radioimmunoassays are inhibition techniques and that if any protein present in the unknown plasma ". . . inhibits the reaction between labelled GH and its antiserum, then the plasma contains a protein which is immunologically indistinguishable from standard GH as isolated from the pituitary."

Hunter (1969) in a discussion on control of specificity in the radioimmunoassay stated that in principle specificity is determined only by the homogeneity of the protein that is labelled and the specificity of the antibody for the homogeneous protein. He indicated, however, that in practice the overall specificity of the method may also be influenced by: (1) the presence of factors in the solution being assayed that affect the antigen-antibody reaction; (2) incubation damage to components of the assay; and (3) iodination damage to the labelled hormone. Although evidence for specificity is given by: (1) demonstrations of parallel dose-response curves; (2) the absence of any cross reaction with other pituitary hormones; and (3) the absence of any response to plasma from hypophysectomized animals or from

noncrossreacting species (Yalow and Berson, 1968), such evidence does not prove specificity. According to Greenwood (1967) proof that the biological activity of GH is being measured will not be acquired until a sensitive biological assay is devised, or GH from plasma is quantitatively isolated and characterized.

Control of Growth Hormone Secretion

Recent reviews concerning the control of GH synthesis and release (Pecile and Müller, 1966a; Schally et al., 1968; McCann and Porter, 1969) indicate that the exact mechanisms involved are far from clear. There is considerable evidence supporting the statement by Schally et al. (1968) that "Hypothalamic control of the secretion of pituitary growth hormone is exercised through the hypophysial portal blood supply and mediated by a neurohumor designated growth hormone-releasing factor." There is also some evidence for the existence of a hypothalamic GH inhibiting factor, which has led McCann and Porter (1969) to suggest that GH may be under dual control by the hypothalamus. Although non-hypothalamic factors are known to affect GH secretion, their importance relative to hypothalamic factors is not known. McCann and Porter (1969) have reviewed evidence suggesting that GH and corticosteroids may act at the pituitary level to suppress GH release. Also the relative importance of neural and systemic factors in controlling the release of GH releasing factor is not clear (Pecile and Müller, 1966a).

Hales (1968) has reviewed the most important factors that are known to affect plasma GH levels in man. They may be summarized generally as: (1) fasting, which causes a rise in concentration; (2) hypoglycemia, which causes a rise; (3) oral glucose, which causes a fall; (4) circulating amino acids, which cause a rise in most cases; (5) exercise, which causes a rise; (6) stress, which causes an increase; and (7) sex, with females generally having higher levels. In discussing the mechanisms that have been postulated to control GH in man, Baylis et al. (1968) suggested that the three basic factors, which increase GH secretion are stress, a decreased energy supply and an increased amino nitrogen pool. They pointed out, however, that none of these factors give consistently clear cut effects and that much more work will need to be done to clarify the situation. Most of the work on factors affecting plasma GH levels has been done in man and the results obtained may not apply in other species. For example, Garcia and Geschwind (1968) and Schalch and Reichlin (1968) indicated that some of the stimuli which most reliably increase plasma GH levels in man have no apparent effect on radioimmunoassayable GH in the pituitaries or plasma of rats or rabbits.

Growth Hormone and Growth

The basic metabolic actions of GH which suggest it should influence the composition of growth have been discussed in previous sections. Other evidence for the

GH-growth relationship may be direct or indirect. In order to obtain direct evidence both the growth characteristics and the endogenous GH status of an animal must be measured, while indirect evidence includes experiments involving administration of GH or indirect assessments of GH status. Knobil (1961) has reviewed early work with laboratory animals which indicated administration of exogenous GH increased both growth rates and the lean to fat ratio of the carcass. Emerson and Emerson (1969), in a more detailed study on the effect of GH showed that administration of 3.2 mg GH/day to fully grown rats (260 g) resulted in rapid growth with a decline in growth rate with time. On stopping the treatment after 136 days, the rats lost weight but grew rapidly again on recommencing treatment 20 days later. The same sequence was repeated three times with almost identical patterns. Each time the GH treatment was started, the initial growth rate was approximately the same regardless of body weight, suggesting that the decreased effectiveness of GH with time was not due to body size. They also discussed previous work that had indicated the decreased effectiveness with time was not due to aging, diabetes, altered steroid metabolism, increased muscle fiber diameter or a change in cardiac index.

A comparison of the mechanisms involved in muscular growth induced by work and by GH administration has been made by Goldberg (1969a). By injecting radioactive amino

acids into rats and comparing the quantity of label retained and the specific activity of the muscles of the treated and control rats, he showed that work hypertrophy caused an increase in synthesis and a decrease in degradation, while GH caused an increase in synthesis only.

Kaplan et al. (1968) studied the effects of intramuscular administration of anterior pituitary GH in 134 children with growth retardation and noted that increased growth rate occurred in children with demonstrable growth hormone deficiency. However, no sustained change in growth rate was discernible in children who had a normal growth hormone response to hypoglycemia. These and other similar findings (Hunter, 1968) suggest that although endogenous GH may account for some of the genetic variation in growth rates, there are also other important factors.

GH administration to domestic animals has not been widely practiced even on an experimental basis, mainly because the costs of administering it commercially are probably prohibitive (Casida et al., 1959). Turman and Andrews (1955) studied the effect of GH (2.5 to 10 mg/15 kg/day) on seven pigs. They showed that the treated pigs had significantly better feed conversion, a greater daily feed consumption, a lower dressing percent, less backfat thickness and more protein and water but less fat in the carcass. However, the responses in these animals were not proportional to the doses for all parameters.

A dose of 5 mg GH/100 lb/day was administered subcutaneously to Jersey heifers for 12 weeks by Brumby (1959). He reported a significant ($P < 0.05$) increase in adjusted weight at 12 weeks for the GH treated group, but there was no change in blood glucose or nonprotein nitrogen and the increase in growth rate disappeared upon stopping treatment. Yousef and Johnson (1966) investigated the effect of single doses of 200 or 300 mg GH to Holstein heifers and demonstrated an increased calorigenesis and a decreased respiratory quotient, which persisted for 40-50 hours. These results suggested an increased dependence on fat metabolism in the treated animals. The work of Shamberov et al. (1968) indicated that a combination of GH together with insulin and a corticosteroid administered subcutaneously to bulls weighing from 220 to 240 kg increased 86 day weight gains by 17%. Struempler and Burroughs (1959) administered 12.5 mg GH/day to lambs by intramuscular injection and showed that the nitrogen balance, and therefore probably protein synthesis, was increased.

Wheatley et al. (1966) likewise demonstrated increased nitrogen retention in sheep treated with GH (5 mg/day for 4 weeks). They also reported a slight rise in plasma glucose but no changes in plasma free fatty acids or ketone bodies. Body weight was not affected in this experiment, which may have been because adult sheep were used. However, wool growth, although it decreased initially, showed a

secondary rise and remained at a higher level for 20 weeks following the experiment which suggests that protein stores had been built up during the experiment. In a similar experiment Manns and Boda (1967) administered GH to sheep at the rate of 1 mg/kg/day and showed an increased circulating level of nonesterified fatty acids and a reduced circulating level of amino acid nitrogen. Their comment that the dose used should have elevated GH levels far in excess of normal values (based on work with humans) has been borne out by the work of Hertelendy et al. (1969).

In experiments involving GH administration to normal and hypophysectomized lambs and rabbits, Vezinhet (1967) showed that administration of 0.5, 1 or 3 mg GH/kg three times a week had no effect on growth of normal rabbits and lambs during the first 26 weeks or 100 days of life, respectively. For hypophysectomized animals, similar administrations resulted in no distinct effects after one month in the rabbits, while in the lambs, increased growth was obtained only with male lambs for the highest dosage.

Arginine is known to be an effective stimulant for GH release in humans, and on this basis Lind et al. (1969) investigated the effects of arginine, GH (3 mg/15 kg/day) and GH antisera on certain growth characteristics of pigs. They pointed out that arginine would be a much more economical feed additive than GH. However, none of the treatments significantly affected growth rates, total lean cut percent or proximate composition of the right side. These few

experiments which have involved the administration of GH to animals of several species do not offer a clear pattern, and in no instances, except perhaps in rats, have the effects been particularly striking. It appears, then, that levels of GH are frequently not the limiting factor in the improvement of growth characteristics.

An example of a measurement made in hopes of obtaining an indirect indication of GH status is that of pituitary DNA and RNA. Martin and Lamming (1958) showed that sheep subcutaneously implanted with hexestrol had more pituitary DNA and RNA than controls. Consequently, they suggested that the increased growth rate was obtained through stimulation of the anterior pituitary. In a study involving male and female intact and castrate sheep, Bradfield (1968) found that the ranking of the four sex groups was the same for growth rate as for pituitary RNA:DNA ratio. Within each group the simple correlation between these two variables ranged from 0.66 to 0.78. He concluded from these studies and from other work in the literature that estrogens generally stimulate growth through their effect on the anterior pituitary, but that androgens act directly at the tissue level.

A number of studies have related measures of GH status to various growth characteristics. Generally only a single measure of GH has been made--usually either pituitary or plasma GH levels. One of the earliest and most promising studies was that of Baird et al. (1952), in which

pituitary GH concentrations were measured in pigs from two lines selected for increased and decreased growth rates over 9 generations. Pituitary GH content per unit body weight, but not pituitary GH concentration, was higher in the rapidly gaining group at 5 different ages from 56 days to maturity. Pituitary GH content per unit body weight reached a peak and then decreased with age, with the changes quite closely paralleling those of weight gains. These results suggested that both genetic and age effects on growth rates may be mediated through GH, although the rates of gain at slaughter were not given. In a similar study, Baker et al. (1956) measured pituitary GH levels in fetal and pregnant pigs as well as in growing pigs. In agreement with the work of Baird et al. (1952) they showed that although pituitary GH concentration remained fairly constant, the content increased with age due to increases in pituitary weights. Likewise, the content per unit body weight declined with increasing age due to the decreased ratio of pituitary weight to body weight.

Armstrong and Hansel (1956) assayed for GH in the pituitaries of Holstein heifers at ages from 1 to 80 weeks and showed that the quantity of GH per unit weight of pituitary and per 100 lb of body weight was positively correlated with the percent of total growth acquired during the 16 weeks prior to slaughter. Human pituitary GH concentration does not appear to change with age either (Gershberg, 1957). On the basis of these and similar studies, Nalbandov

(1963) suggested that the reduction in growth rate with age is primarily due to a decreased amount of available GH per unit body weight. Gershberg (1957) suggested that cessation of growth in man is not due to a deficiency of GH but to a change in the responsiveness of the target cells. Nalbandov (1963) rejected this suggestion, however, on the basis of studies such as that of Simpson et al. (1950) which indicated that rats, after ceasing to respond to a particular dose of GH, will respond if the dose is increased. Such results do not rule out the possibility of changes in tissue responsiveness, however, and the results of Emerson and Emerson (1969) are not entirely compatible with Nalbandov's "dilution theory," since in their experiment the degree of response to GH appeared to depend more on how long the GH had been administered than on body size.

The results of Purchas et al. (1969) also showed discordance with the "dilution theory." They measured pituitary GH levels in bulls at monthly intervals from birth to one year of age using both the tibia test bioassay (Greenspan et al, 1949) and a radioimmunoassay. In this study, changes in pituitary GH content and in pituitary content per unit body weight were largely determined by changes in pituitary GH concentration, which reached a peak at three to four months of age. Although there was a positive correlation between pituitary GH content per unit body weight and a measure of specific growth rate

$$\left(\frac{\text{weight gain for last month before slaughter}}{\text{slaughter weight}}\right),$$

this appeared to be due to the decrease in pituitary GH concentration after 4 months of age rather than a change in the ratio of pituitary weight to total body weight as reported in other studies (Baird et al., 1952; Baker et al., 1956; Armstrong and Hansel, 1956). Purchas et al. (1969) also measured plasma GH levels in samples collected at slaughter using a rdioimmunoassay, but no close relationships with measures of growth were shown.

Studies of changes in pituitary GH concentration with age have been made in most detail with the rat, but results of different studies have not been consistent. Using bioassay techniques, Solomon and Greep (1958) and Bowman (1961) studied the changes in pituitary GH concentration in female and male rats, respectively, from 10 to approximately 600 days of age. Bowman (1961) reported results comparable to those discussed earlier herein for pigs and cattle in that pituitary GH per unit body weight, but not pituitary GH concentration, decreased with age. However, Solomon and Greep (1958) indicated that neither of these parameters showed any particular pattern of change with age. Using more sensitive radioimmunoassays, Birge et al. (1967a) and Garcia and Gerschwind (1968) showed that there was an increase in pituitary GH concentration with age up to 8 weeks and 75 days, respectively. Changes in pituitary GH content per unit body weight were not given in either study, but in both cases there was a marked sex

effect with the male rats having higher pituitary GH concentrations after 50 days.

Gerrits (1968) compared pituitary GH levels for two lines of pigs selected for increased and decreased backfat thickness with that of an unselected control line. He showed that the control line had significantly higher anterior pituitary weights than either of the selected lines, but there were no differences in the GH content of the pituitary per unit body weight. In a study involving four cattle at each of three ages, Curl et al. (1968) indicated that pituitary GH content was the greatest in the oldest group (feedlot cattle), while pituitary GH content per unit body weight was greatest in calves, which is in agreement with the work of Armstrong and Hansel (1956).

Estrogenic steroids, which have been widely administered as growth promoters in the cattle industry, are examples of external factors that may affect growth characteristics through an effect on GH production. Clegg and Cole (1954) showed that although stilbestrol implantations produced greater growth responses in steers, a significant increase in pituitary GH level was found only in heifers. On the other hand, Struempler and Burroughs (1959) demonstrated that stilbestrol implanted into steers increased anterior pituitary weight, pituitary GH content and GH per unit body weight. Shroder and Hansard (1958) obtained similar results with sheep.

In a study of the effects of estradiol benzoate on male rats, Birge et al. (1967a) indicated that the treated rats grew more slowly and had lower pituitary GH levels than ad lib. fed controls. When compared with pair fed controls, however, the estrogen treated rats had higher pituitary GH contents and similar growth rates. From these results, the authors concluded that estrogen created a caloric deficiency, possibly through an effect on appetite, and that the effect of this on pituitary GH content was greater than any direct effect of estrogen. Generally, these results support the claim that estrogens affect growth through pituitary GH content, although there were no data to indicate whether the GH response was a cause or an effect.

Since immunoassays have been developed for the measurement of GH, a number of studies have provided information on the relationship between growth characteristics and measures of GH, especially circulating levels. Kaplan et al. (1968) indicated that circulating GH levels in children responded less to insulin-induced hypoglycemia than in adults. Hunter (1968), however, reviewed some work with humans which indicated that after an overnight fast, plasma GH levels in children increased more than those of adults. Similar decreased responses to periods of fasting with increased age have been demonstrated in sheep (Hertelendy et al., 1969) and pigs (Machlin et al., 1967).

In a study of circulating GH levels in pigs, Siers (1968) found low correlations with indices of carcass quality and most of these were negative. No significant relationships between plasma GH and growth rates were found for Hereford cattle by Dev and Lasley (1969). They used an assay based on complement fixation, however, and the fact that the values obtained were approximately ten times greater than those obtained by radioimmunoassays would suggest that factors other than GH were influencing complement fixation. Eaton et al. (1968) and Trenkle and Burroughs (1967) measured circulating levels of GH in cattle. Although their experiments were not designed to investigate the relationship between GH and growth, there was some pertinent data, but it did not suggest a close relationship.

On the other hand, some experiments with rats do suggest that such a relationship exists. The two rat experiments below, however, differ from experiments with other species discussed previously in that the level of GH was experimentally manipulated rather than the growth characteristics. Frohman and Bernardis (1968) showed that the decreased plasma and pituitary levels of GH resulting from lesions of the ventromedial hypothalamus were paralleled by a decrease in linear growth and an increase in body fat content, despite a decreased food intake. In another experiment, Peake et al. (1968) found increased plasma GH levels and decreased pituitary levels when a GH producing tumor was transplanted into rats. They noted that the

resulting increases in weight gain corresponded closely to increased plasma GH levels.

Other Endocrinological Aspects

Work reviewed in the previous section indicated that groups of similar animals raised under similar conditions frequently do not demonstrate close relationships between their GH status and growth rates or body composition. Bradfield (1968) has pointed out that the impetus for growth has generally been considered to be provided by the endocrine system as a whole, with the relative importance of different components of the system possibly varying with age. Thyroid activity and adrenal cortical activity are two physiological parameters that are known to influence growth. Evidence giving some indication of the extent to which these influences are expressed through an effect on GH is discussed below.

Thyroid Activity

The importance of thyroid hormones to growth and development in mammals is well recognized and is effectively demonstrated in the reduced growth of hypothyroid and of thyroidectomized subjects (Tata, 1964). Attempts to restore growth in such subjects by administration of thyroactive substances have generally been successful, but attempts to increase the growth of normally growing animals by the use of the same substances have not produced consistent results

(Reineke, 1946; Casida et al., 1959). This appears to be due to the fact that thyroid hormones at low doses have an anabolic effect on protein and lipid metabolism, but at higher levels their catabolic effects become more pronounced, resulting in an overall detrimental effect on growth. Thus, an anabolic dose in one animal may be catabolic in another due to differences in the endogenous production of thyroxine (Tata, 1964). Because thyroid hormones increase the basic metabolic rate, it has been suggested that decreasing thyroid activity should reduce maintenance costs and increase growth efficiency. However, the results of experiments attempting to exploit this effect have not been consistent (Blaxter et al., 1949; Casida et al., 1959).

The relationships between endogenous thyroid activity and growth characteristics of animals have not been widely investigated, but the results of studies that have been made are generally compatible with the known actions of thyroid hormones. Draper et al. (1968), for example, measured thyroid secretion rate in rapidly growing lambs and demonstrated a highly significant curvilinear correlation with growth. They concluded that thyroid activities considerably above or below the optimum have an adverse effect on growth, which agrees with the effects of administration of thyroactive and goitrogenic substances to animals. In reviewing the changes in thyroid activity in cattle, Kossila (1967) stated that ". . . the results of several studies

have demonstrated that the thyroid activity in relation to body size of the animals generally decreases with age. . . ."

Mixner et al. (1966) investigated the relationship between thyroid secretion rate and body weight in Holstein heifers from birth to 2 years of age by using the allometric equation. They showed that, on the average, a 10% increase in body weight was accompanied by only a 6.4% increase in thyroid secretion rate. These results suggest that the decreases in growth rate and in specific growth rate with age may be partially due to changes in the availability of thyroid hormones. Significant correlations have also been demonstrated between thyroid secretion rates and growth rates of cattle of similar ages in the same environment (Post, 1965), which suggests some genetic differences in growth may also be mediated through an effect on the thyroid gland. The fact that these correlations were positive in the summer and negative in the winter was attributed to food shortage in the winter. Thus, in winter the food supply replaced thyroid activity as the limiting factor in growth so that animals with more active thyroids grew more slowly, because a larger portion of their limited intake was used for maintenance.

Early work on the relationship between thyroxine and GH and growth, which has been summarized by Tata (1964), indicated that these hormones act synergistically in their effect on the growth of hypophysectomized rats, but their effects on growth were not identical, particularly with

respect to bone growth and maturation. The fact that thyroxine induces growth in hypophysectomized rats indicates that it does not affect growth entirely through GH. There is some evidence, however, which suggests that thyroid hormones may affect the GH status of an animal. Contopoulos et al. (1958) showed that the pituitaries of thyroidectomized rats contained less GH than controls, and that thyroxine administration led to the restoration of normal levels. In humans, it has been demonstrated that hypothyroidism is associated with a reduced response of plasma GH to insulin-induced hypoglycemia and also to arginine infusion. Iwatsubo et al. (1967) indicated that the response to insulin-induced hypoglycemia was restored by treatment with desiccated thyroid, but MacGillvray et al. (1968) found that administration of sufficient thyroxine to overcome the growth inhibiting effects of hypothyroidism did not invariably restore GH responses to normal. Thus, it seems possible that thyroid hormones influence growth both independently of GH and also through an affect on GH production, but the relative importance of these two mechanisms is not clear.

Adrenal Cortical Activity

Only the corticosteroid hormones with glucocorticoid activity will be discussed in this section. These include primarily cortisol, cortisone and corticosterone, although it is recognized that all steroids of the adrenal cortex probably have some mineralocorticoid and some glucocorticoid

activity (Gorbman and Bern, 1962). In a review of the metabolic effects of adrenal glucocorticoid hormones on carbohydrate, protein, lipid and nucleic acid metabolism, Ashmore and Morgan (1967) stated that ". . . their action may be described primarily as catabolic since their most pronounced metabolic effects are to increase protein breakdown and nitrogen excretion." This effect is mainly on the proteins of the skeletal muscles, while the protein content of the liver may actually increase (Silber and Porter, 1953) due to specific induction of key enzymes of gluconeogenesis by the glucocorticoids (Weber, 1968). The mechanism of corticosteroid induced protein catabolism in skeletal muscle is not well understood, but it has been shown in rats to involve both an increased degradation and a decreased synthesis of both myofibrillar and sarcoplasmic proteins (Goldberg, 1969b).

Although corticosteroids have the opposite effect from GH on peripheral protein, there are stimuli, such as hypoglycemia and various forms of stress, which bring about the release of both of these hormones (Basset and Hinks, 1969). Cushing's syndrome, which is the result of bilateral adrenal hyperplasia with a resulting excessive production of glucocorticoids, is characterized by high levels of circulating corticosteroids and two of the important symptoms of this syndrome are stunted growth and obesity (Dixon et al., 1967). A review of investigations into the relationship between corticosteroids and obesity has been made by

Dixon et al. (1967). They concluded that ". . . in the majority of obese subjects there is an increased adrenocortical synthesis of cortisol, but that this is secondary to some change in cortisol disappearance." Basset (1968) suggested that the lower levels of free fatty acids and ketone bodies in the blood of fasted sheep treated with cortisol was not due directly to the cortisol, but rather to an increased insulin output. Insulin output was not measured in these experiments, but the authors indicated its release would have been expected from the hyperglycemia, and from the decreased ability of insulin to stimulate peripheral glucose utilization in the presence of cortisol. Basset (1968) supported his suggestion with data showing increases in plasma free fatty acids and ketones in the cortisol treated sheep when hypoglycemia was induced by phloridzin treatment. Other studies (Kyle et al., 1963; Kekwick and Pawan, 1965) have indicated that the increased adiposity frequently associated with high levels of corticosteroids is dependent on maintenance of positive nitrogen balances. Thus, it seems that glucocorticoids generally have effects on protein and lipid metabolism that are opposite to those of GH and appear to be independent of GH. As was the case with thyroxine, however, there is evidence that glucocorticoids may influence GH production and possibly also the action of GH at the tissue level.

Early work with rats indicated that adrenalectomy and restoration of growth with corticosteroids had no effects on pituitary GH content (Reichlin and Brown, 1960). Although pituitary GH is unchanged in cortisol treated rats, however, the activity of the hypothalamic GH releasing factor, as well as the response of pituitary GH content to insulin-induced hypoglycemia is markedly reduced according to Pecile and Müller (1966b). Even though these results suggest that any corticosteroid effect on GH is mediated through GH releasing factor, this does not seem to be a complete explanation, as it has been shown by Birge et al. (1967b) that cortisol decreases the rate of GH release from the isolated pituitary in vitro. After showing that a single subcutaneous injection of cortisol into one day old rats resulted in very low pituitary GH levels and undetectable hypothalamic GH releasing factor activity five to six weeks later, Sawano et al. (1969) also concluded that the growth retarding effect of cortisol was at least partially due to an effect on GH.

The mechanism whereby corticosteroids retard growth in man has received considerable attention as corticosteroids are used widely in the treatment of certain diseases (Morris et al., 1968), and decreased growth is frequently an undesirable side effect in children. Several workers have indicated that both Cushing's syndrome and corticosteroid administration may be accompanied by a reduced response of plasma GH to insulin-induced hypoglycemia (Hartog et al., 1964;

James et al., 1968). However, the response to other factors which usually increase plasma GH, such as arginine infusion, may not be affected (Strauch et al., 1969; Nakagawa, 1969). Sadeghi and Senor (1969) indicated that the response of GH to hypoglycemia and also normal growth were regained in children on corticosteroid treatment by substituting a single dose on alternate days for the previous daily dosage. Stempfel et al. (1968) showed that the normal GH response to hypoglycemia was regained upon replacing biweekly intramuscular injections of a long acting corticosteroid with daily administrations of cortisone acetate. If the retarded growth of children on corticosteroid treatment is due to decreased GH production, then administration of human GH should overcome it. Morris et al. (1968) investigated this possibility, but found that it was not the case, and concluded that the dwarfism in corticosteroid treated children results from the antagonism of GH at the peripheral tissue level. It should be noted, however, that the children in their study did not show decreased responses in plasma GH to fasting or insulin-induced hypoglycemia.

MATERIALS AND METHODS

Experimental Design

The data discussed herein were collected from animals involved in an experiment designed to investigate the effects of MGA (registered trade mark for melengestrol acetate, Upjohn Company, Kalamazoo, Michigan) upon growth and subsequent lactational performance of Holstein-Friesian heifers. The experiment involved 14 groups with ten heifers in each, but since groups 1 through 4 were not slaughtered, data from only 100 heifers in ten groups are included. The animals were acquired as calves in four separate lots over a 12 mo period with lots 1 and 2 each containing half of the animals in groups 1 to 8, and lots 3 and 4 each containing half the animals in groups 9 to 14. Each group may be classified according to level of nutrition from 2.5 mo of age, MGA treatment, and age at slaughter as outlined in Table 2.

The normal level of nutrition involved ad lib. corn silage and alfalfa hay plus 0.9 kg of grain per animal per day, while the high level of nutrition involved ad lib. corn silage, alfalfa hay and 4.5 kg of grain per animal per day. MGA at a dose of 0.45 mg per animal per day was administered

Table 2. Classification of groups according to treatments

Group number	Number of animals	Level of nutrition	MGA administration	Slaughter criteria
5	10	Normal	None	Breeding size
6	10	High	None	Breeding size
7	10	High	From 2.5 mo	Breeding size
8	10	High	From first estrus	Breeding size
9	10	Normal	From 2.5 mo	Breeding size
10	10	Normal	None	First estrus
11	10	High	None	First estrus
12	10	High	From 2.5 mo	First estrus
13	10	High	From first estrus	First estrus
14[a]	10	--	--	2.5 mo of age

[a]Group 14 was slaughtered at the beginning of the experiment (2.5 mo of age).

orally as a premix together with the grain. The time of estrus was determined by observation twice daily. Breeding age was defined as the age at which a withers height of 120 cm was attained. Since MGA suppresses estrous cycles in cattle, those animals on the MGA treatment which were to be slaughtered at first estrus were paired with comparable animals not on MGA treatment. All animals except those on MGA treatment and those in group 14 were slaughtered during the late diestrous period (15 to 17 days after estrus) which

most closely followed the slaughter criteria given in Table 2. All animals were weighed at monthly intervals. At the time of weighing, blood samples were taken from the jugular vein of animals in groups 5, 6, 7 and 9. More details regarding the source and the treatment of these animals have been given by Pritchard (1970).

Slaughter Procedure

Animals to be slaughtered were transported by truck approximately 5 miles to the abattoir, where they were weighed, stunned with a captive bolt pistol and bled within three hours after being removed from their pens. At the time of slaughter approximately three liters of blood were collected in a heparinized glass jar (ca. 5 ml of 1% sodium heparin) and were stored immediately at approximately 6^{o}C. The right fore cannon bone (metacarpal) was removed and skinned. The anterior pituitary gland was removed, weighed and stored in a plastic bag (Whirl Pak, Nasco, Fort Atkinson, Wisconsin) on dry ice (solid CO_2).

The thyroid glands were removed, trimmed free of fat and weighed. The glands from animals in groups 5, 6, 7 and 9 were stored in Bouin's fluid in a plastic bag and frozen for histology. The adrenal glands were trimmed free of fat and weighed. A cross-sectional slice approximately 4 mm thick was cut from the left adrenal. This was stored in Bouin's fluid in a plastic bag and frozen for histological studies. The remainder of the left adrenal and the entire

right adrenal were stored in 0.25 M sucrose in a plastic bag and frozen for steroid assays. The right hind quarter of the carcass was labelled and delivered to the Michigan State University Meat Laboratory (approximately 4 miles) within 4 days of slaughter.

Body Composition Measurements

Cannon Bone

The right fore cannon bone was scraped free of connective tissue using a boning knife. Then after drying at 100-110°C for 24 hr, it was weighed to the nearest 0.1 g and its length was measured to the nearest 1.0 mm. The drying step was included in order to reduce the effects of variation in the length of time following slaughter and in the thoroughness of scraping.

Physical Analysis

The right hind quarter was stored at approximately 6°C. All dissection work was carried out at this temperature within 6 days of slaughter. After removal of the perirenal fat from the hindquarter, the round was separated by cutting between the fourth and fifth sacral vertebrae through a point 2 cm anterior to the exposed portion of the pelvic bone. The flank was removed by making a cut along a line parallel to the plane of the exposed lumbar vertebrae, starting at a distance below the *Longissimus* muscle equal to the long axis of its exposed surface. This cut was

extended to meet a second cut made tangential to the ventral surface of the Rectus femoris muscle.

The flanks from all animals (groups 5-14) and the rounds of animals in groups 5, 6, 7 and 9 were dissected into fat, muscle and bone plus tendon. No attempt was made to dissect the distal 15 cm of the rounds which consisted mainly of tendon and bone.

Specific Gravity

The specific gravity of all rounds was calculated by hydrostatic weighing at 6°C. Specific gravity has been shown to be indicative of carcass composition (Garrett, 1968).

Eye Muscle Area

Tracings of the exposed surface of the Longissimus muscles on the right hindquarter were made on acetate paper, and the areas were measured using a compensating polar planimeter.

Chemical Analysis

Immediately following dissection of the rounds from the animals in groups 5, 6, 7 and 9, the fat and muscle portions were mixed and passed through a meat grinder using first a plate with 9.5 mm holes and then a plate with 3 mm openings. The ground meat was thoroughly mixed after the initial grinding. As it was passed through the second plate, samples consisting of about 20% of the total were collected at regular intervals. This was repeated twice using the

same plate to give a final sample of approximately 100 g which was transferred to an air-tight glass jar and frozen.

The ground samples were analyzed in triplicate for moisture and petroleum ether extract. After thawing, samples of between 5 and 10 g were weighed to the nearest mg into tared Soxhlet thimbles containing sheets of aluminum foil. The moisture content was calculated from the weight loss after drying for 24 hr at 100-110°C. The petroleum ether extract was calculated from the additional weight loss during 8 hr of extraction with petroleum ether (B. Pt. 30-60°C) in a Soxhlet fat extractor. The thimbles were heated at 100-110°C for 12 hr and then cooled in a desiccator before weighing, both prior to use and following extraction.

Meat Quality Measurement

Color Assessment

When the hindquarter was separated into cuts, a single steak approximately 2.5 cm thick was removed from the anterior end of the loin of all animals in groups 5, 6, 7 and 9. Between one and three hours after cutting, the freshly cut surface was subjectively assessed for color on a seven point scale using three color cards prepared from oil paints.

Tenderness Measurement

Tenderness was measured on the same steaks used for color assessment. After color assessment, the steaks were

wrapped in aluminum foil and stored at 6°C until the following day. They were then roasted to an internal temperature of 63°C in an electric oven preheated to 150°C. Shear measurements were taken 24-36 hr later on six 2.2 cm cores with a Warner-Bratzler shear device.

Growth Hormone Measurement

Plasma Preparation

Within 12 hours of slaughter, the blood from all animals was centrifuged (ca. 15,000 x g for 40 min). The resulting plasma was frozen in plastic bags for hormone assays, with separate bags for each assay.

Preparation of Pituitary Extract

Anterior pituitaries were thawed, weighed and homogenized in 10 ml of 0.85% saline in a Sorvall Omnimixer. The resulting homogenate, after being made up to a concentration of 50 mg/ml, was centrifuged at approximately 1200 x g for 6 min. The supernatant obtained was frozen in glass vials.

Growth Hormone Radioimmunoassay Development

Antibody Production and Evaluation

Antibodies to bovine GH (NIH-GH-B12[a]) were produced in guinea pigs by an initial subcutaneous injection of 2 mg

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[a]Supplied by the National Institutes of Health, Endocrinology Study Section, Bethesda, Maryland.

of GH in 0.5 ml of saline plus 0.5 ml of Freund's complete adjuvent. Subsequent injections were made at intervals of two weeks and differed from the first only in that Freund's incomplete adjuvant replaced the complete adjuvant. A maximum of seven injections was given. Blood was collected by heart puncture under ether anaesthesia using a 10 ml syringe and a 1.5 inch 18 gauge needle. Blood was collected the day before the first injection as a control and the next bleeding was made on the day prior to the fourth injection (six weeks after the first injection). Subsequent bleedings were made on the day prior to the injections of GH and at two week intervals following the final injection. Serum was obtained from all blood samples by centrifugation (ca. 15,000 x g for 30 min) after coagulation.

An estimation of a titer of the antiserum was obtained from micro-Ouchterlony plates. From 2.2 to 2.5 ml of a 0.85% agar solution in buffered (0.01 M phosphate, pH 8.4) physiological saline were pipetted onto a microscope slide. After cooling, two patterns, each consisting of one central and four peripheral holes, were cut. Antisera at dilutions of 1 in 1 and 1 in 10 were run against doubling antigen (GH) dilutions from 1 in 5000 up to 1 in 80,000. All the serum collected on a particular day was pooled. The titers of the sera from the first three bleedings were found to be from 20,000 to 40,000. All of this serum was combined and will subsequently be referred to as anti-GH. If the

sera from each bleeding of each guinea pig had been tested separately for both titer and avidity (strength of binding to the antigen), then it may have been possible to obtain a better antisera in terms of these two parameters (Yalow and Berson, 1968).

Anti-guinea pig gamma globulin was either obtained commercially (Nutritional Biochemicals Corp., Cleveland, Ohio) or produced by immunization of a sheep. Immunization involved the initial subcutaneous injection of 50 mg of guinea pig gamma globulin (Pentex, Kankakee, Illinois, Fraction 11) in Freund's complete adjuvant, followed at approximately monthly intervals by comparable injections but with Freund's incomplete adjuvant. Serum was prepared from blood collected prior to the fourth and subsequent injections.

Preparation and Purification of Iodinated Growth Hormone

The iodination procedure used was based on the method of Greenwood et al. (1963) and involved the following steps:

(1) A microsyringe (Hamilton Co., Whittier, California) was used to transfer 25 µl of 0.5 M phosphate buffer (pH 7.5) to a 1 ml glass vial.

(2) Then 5 µl of a solution containing 1 µg GH (NIH-GH-B12) per µl of buffer (pH 8.5, 0.05 M phosphate, 0.85% NaCl) were added.

(3) Approximately one millicurie of a solution of $Na^{125}I$ in NaOH with a specific activity greater than 50 mc/ml (Isoserve Division of Cambridge Nuclear Corp., Cambridge, Massachusetts) was transferred to the vial by microsyringe.

(4) After adding 60 μg of chloramine T in 20 μl of 0.05 M phosphate buffer, pH 7.5, the vial was shaken for exactly two minutes. Then 125 μg of sodium metabisulfite in 50 μl of 0.05 M phosphate buffer, pH 7.5, were added in order to reduce any excess chloramine T and to convert residual iodine to iodate.

(5) Approximately 100 μl of a solution containing 1% KI, 0.01% bromphenol blue and 16% sucrose were added to the vial. The contents were then layered under buffer onto the top of a 20 x 1.2 cm Biogel P60, 50-100 mesh (Biorad Labs., Richmond, California) column, using a disposable 1 ml syringe and a 1.5 inch 27 gauge needle. The vial was immediately rinsed with 70 μl of a solution containing 1% KI, 0.01% bromphenol blue and 8% sucrose, and this was applied to the column in a similar way. The column was pretreated with approximately 50 mg of bovine serum albumin in order to saturate any protein binding sites.

(6) Elution was carried out at a rate of approximately 0.3 ml/min with phosphate buffer (0.05 M phosphate, pH 7.5). Aliquants of 1 ml were collected into 1 ml volumes of phosphate buffered saline (0.01 M phosphate, pH 7.0, 0.85% NaCl) containing 5% bovine serum albumin (PBS-5% BSA).

(7) A graph of counts per minute versus tube number was drawn after counting 5 μl from each tube plus 5 ml of Bray's solution (Bray, 1960) in a Nuclear Chicago Mark I liquid scintillation counter. Figure 1 shows an example of such a graph for GH. The first peak represents the iodinated hormone. The contents of the tube represented by the highest point on this peak were frozen in aliquants large enough to last for one or two assays.

A number of workers have made comparisons betwen ^{131}I and ^{125}I with respect to their usefulness in providing a radioactive label for protein molecules (Hunter et al., 1966; Lambert et al., 1967; Yalow and Berson, 1968; Freedlander, 1969). Because it has a shorter half life (8 days vs 60 days), ^{131}I has an expected specific activity approximately eight times as great as ^{125}I. However, it is possible to obtain ^{125}I in virtually a carrier-free state, as opposed to ^{131}I, which seldom had an isotopic abundance of more than 30%. This fact, together with the two- or three-fold greater counting efficiency of ^{125}I means that the measured specific activity of protein iodinated with ^{125}I is frequently as high or higher than that iodinated with ^{131}I. Freelander (1969) discussed the effects of specific activity, counting efficiency and half life and concluded that ^{125}I as a label for radioimmunoassay methods provides at least a two-fold advantage over ^{131}I. The longer half life of ^{125}I means that the iodinated hormone produced in

Figure 1. The elution pattern from Biogel P60 after iodination of GH. The first peak represents iodinated GH and the second free iodine.

Figure 2. The elution pattern from Sephadex G100 showing the purification of iodinated GH. The iodinated GH in this run was 44 days old.

Figure 3. The effect of anti-GH dilution on the percent of GH bound to anti-GH.

Figure 4. The effect of anti-gamma globulin dilution on the percent of GH bound to anti-GH, expressed as a percent of the amount bound when a dilution of 1 in 1 was used.

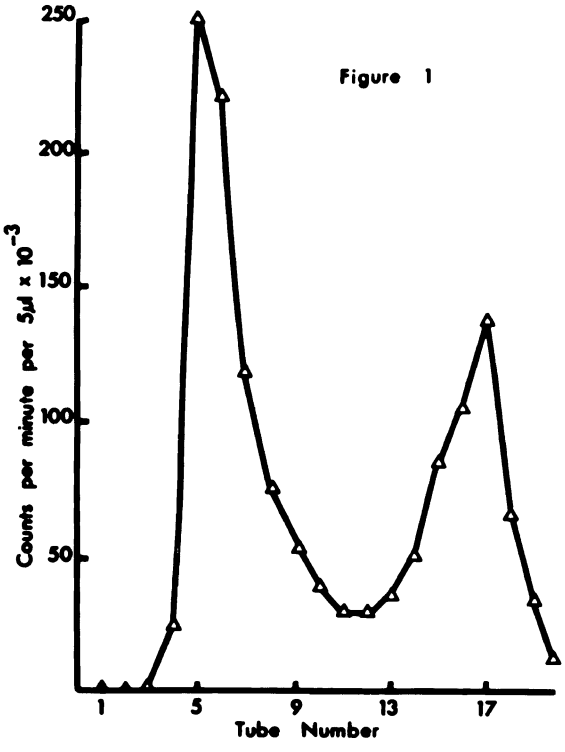

Figure 1

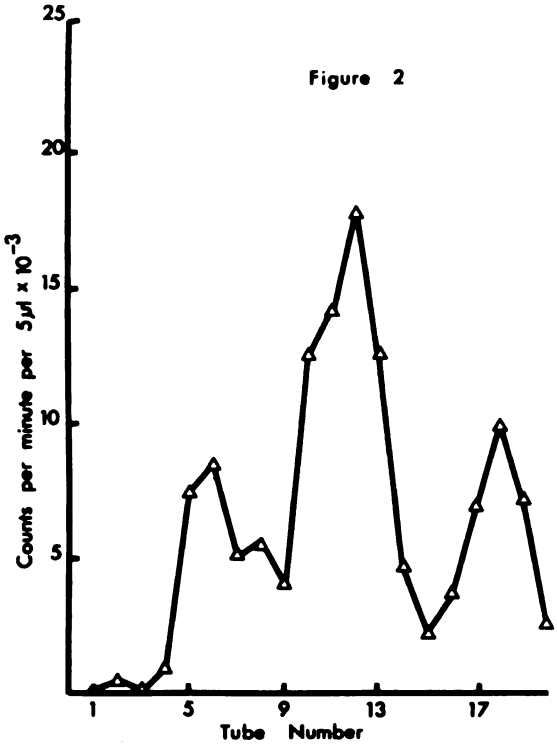

Figure 2

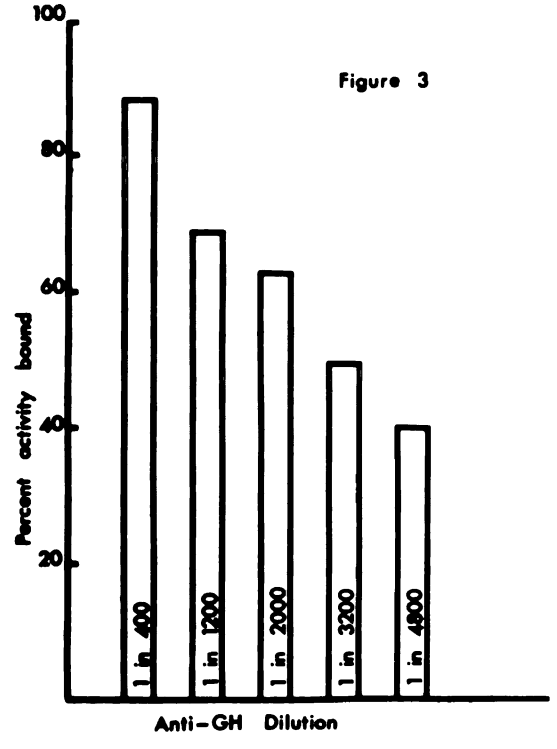

Figure 3

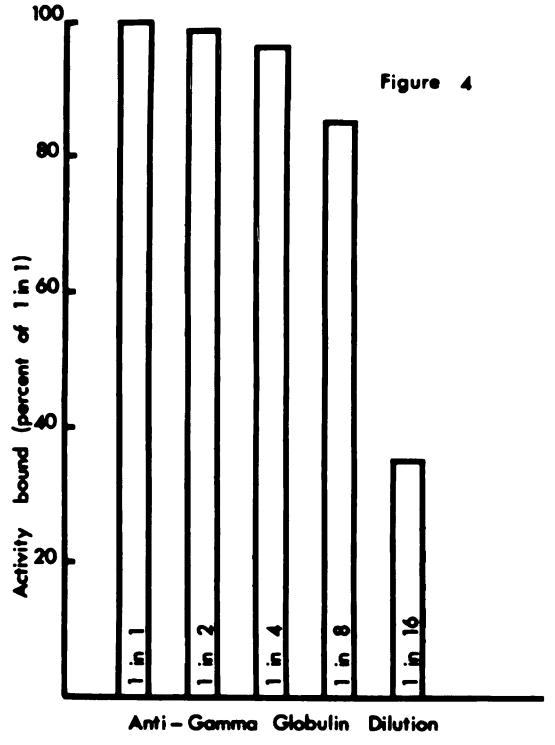

Figure 4

a particular iodination can be used longer. Extra precautions must be taken, however, since Yalow and Berson (1968) have shown that aging of iodinated proteins is accompanied by more damage than aging of the proteins alone. They also indicated that iodination with ^{125}I results in more damage than iodination with ^{127}I.

In order to reduce the content of damaged hormone in the iodinated GH preparation (GH-^{125}I), GH iodinated for more than ten days was repurified by passing it through a 20 x 1.2 cm Sephadex G100, medium (Pharmacia Fine Chemicals Inc., New Market, New Jersey) column. Procedures for elution, fraction collection and counting were the same as described above. A typical elution pattern is shown in Figure 2. The third peak was assumed to represent free iodine. The first peak appeared to represent damaged material, as indicated by the fact that when an equal number of counts from peaks one and two were incubated with the same quantity of anti-GH, more than twice as much activity from peak two was bound. Figures 3 and 4 are explained in a later section.

Radioimmunoassay Procedure

The diluents, volumes and times of incubation were patterned after those used by Midgley and co-workers at the University of Michigan (see Niswender et al., 1969). Hamilton microsyringes were used to measure any volume less than one milliliter. The procedure was as follows:

(1) On day zero, PBS-1% BSA and the standard GH solutions or the material to be assayed were added to 12 x 75 mm disposable glass culture tubes to make a total volume of 500 µl. Each lot of 48 tubes included ten tubes containing 0, 0.1, 0.3, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 or 5.0 ng of standard GH (NIH-GH-B12). Also on day zero, 200 µl of anti-GH at an appropriate dilution in PBS-0.05 M EDTA were added.

(2) On day one, 100 µl of iodinated GH in PBS-1% BSA were added. This solution contained from 20,000 to 30,000 cpm/100 µl.

(3) On day two, 200 µl of anti-gamma globulin at an appropriate dilution in PBS-0.05 M EDTA were added. After adding the various components on day zero and days one and two, the tubes were shaken, stoppered and stored at 4°C.

(4) After addition of 3 ml of PBS on day five, each tube was centrifuged at approximately 1800 x g for 20 minutes. The supernatant was then decanted, and the tubes were left in an inverted position in contact with absorbent paper for several minutes. The inside of each tube was then wiped with a strip of filter paper and 0.1 ml of NCS reagent (Amersham Searle Corp., Des Plaines, Illinois) was added in order to solubilize the precipitate. The tubes were shaken vigorously and then incubated at 37°C until complete solubilization was attained. The solutions in the tubes were transferred to scintillation vials with two 2.5 ml portions

Figure 5. Dose response curves for standard GH and for plasma.

Figure 6. The effect of GH standards and bovine prolactin on the percent of GH bound to the anti-GH.

Figure 7. A least squares curve for GH standards and dose response effects of two anterior pituitary extracts.

Figure 8. The effect of plasma on the GH standard curve.

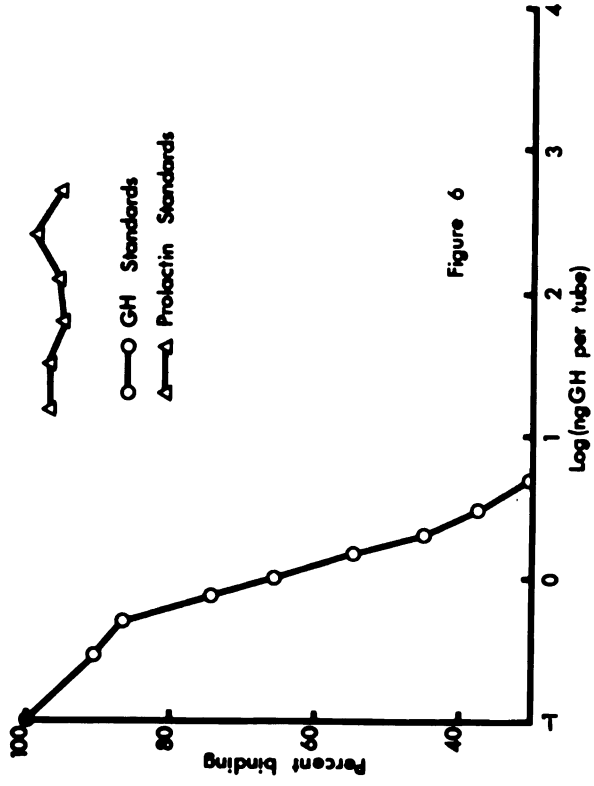

Figure 6

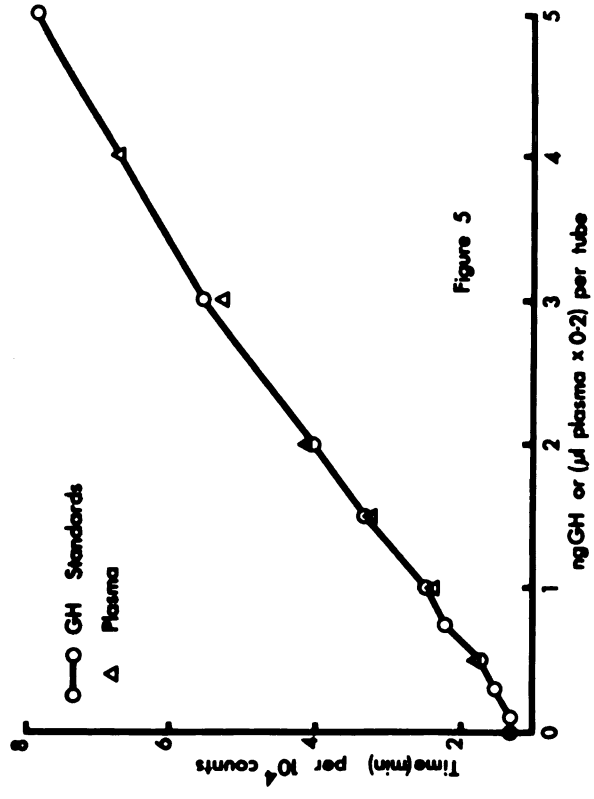

Figure 5

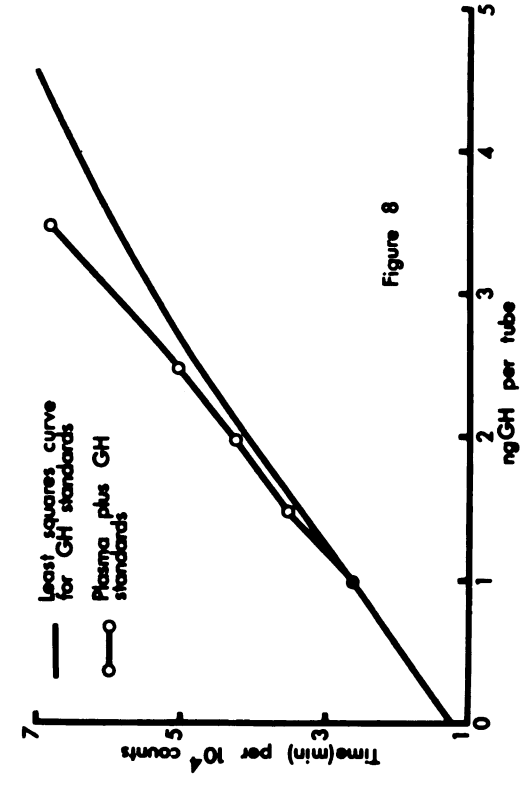

Figure 8

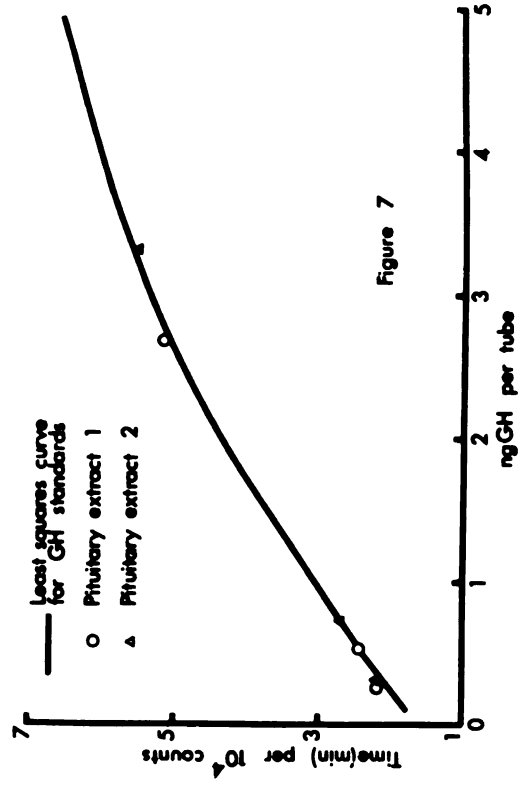

Figure 7

of Bray's solution. The resulting 5 ml quantities were counted for ten minutes or until 10,000 counts had been collected. The data from the tubes containing standard GH constituted the standard curve. These were plotted either as time to collect 10,000 counts against ng GH/tube (Figure 5) or as percent of radioactivity bound relative to the percent bound with no unlabelled GH versus log ng GH/tube (Figure 6). Least squares equations with linear, quadratic and cubic components were calculated from the standard curve data. These equations, examples of which are plotted in Figures 7 and 8, were used to estimate the GH concentration of unknown solutions.

Selection of Appropriate Conditions

A suitable dilution of anti-gamma globulin was determined by running an assay with no unlabelled GH, with anti-GH at a dilution of 1 in 400 and with varying dilutions of anti-gamma globulin. Changes in quantity of GH bound with changes in anti-gamma globulin dilution (Figure 4) indicated that a dilution of 1 in 2 should be satisfactory. Each new source of anti-gamma globulin was tested in this way.

An appropriate anti-GH dilution was found by running an assay with no unlabelled GH, with anti-gamma globulin at a dilution of 1 in 2 and with varying dilutions of anti-GH. Results (Figure 3) indicated that a dilution of 1 in 3200 resulted in approximately 50% binding.

For the assay of plasma or serum, duplicate 250 μl samples were used. For the assay of the 50 mg/ml pituitary extracts, a 1 to 12,500 dilution was made to give a final concentration of 4 μg/ml. This was accomplished by an initial 1 to 100 dilution with PBS and a second 1 to 125 dilution using PBS-1% BSA as a diluent. Duplicate 10 μl aliquants of the 4 μg/ml solution were assayed. When the counts of duplicates in any assay differed by more than 10%, they were reassayed.

Validation of the Assay

Cross reactivity with bovine luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and prolactin was tested in triplicate at levels of 50 and 100 ng per tube. There were negligible decreases in the percent of labelled GH bound in all cases except for prolactin, where there was a small but consistent decrease in all replicates. In a more detailed investigation of the cross reactivity of bovine prolactin at six concentrations from 15 to 500 ng per tube, however, no dose response effect could be detected (Figure 6). Prolactin consistently decreased the percent binding in this experiment as well (Figure 6), but this was considered negligible.

Figure 5 indicates that the variation in time to collect 10,000 counts with changes in plasma volume and with changes in ng of standard GH are very similar. This suggests that GH is the plasma component that is being measured.

However, Yalow and Berson (1968) pointed out that ". . . proper behavior on dilution is a necessary but not sufficient condition to prove immunologic identity." Figure 7 shows comparable dose response curves for two pituitary extracts. In this case, the positions of the center two points were computed from the least squares equation, and then the expected GH content of the other dilutions was plotted against time to collect 10,000 counts.

The results of an experiment in which varying quantities of standard GH were added to plasma (Figure 8) indicated that at all levels tested there was more than 100% recovery. However, these recoveries did not exceed 110% except at GH levels of 3 ng/tube and greater, and such levels were seldom encountered. Thus, the effect was probably not important, particularly for relative values. It may be important, however, if it varied widely for plasma samples from different animals. It appears that this deviation from the expected curve is due to components in the plasma which influence the reaction between gamma globulin and anti-gamma globulin, i.e., the second antibody-antigen reaction. When the experiment involving different dilutions of anti-gamma globulin (Figure 4) was repeated with plasma present, the maximum binding of GH was found to take place at an anti-gamma globulin dilution of 1 in 6. This did not indicate whether or not the proportion of GH bound at an anti-gamma globulin dilution of 1 in 2 (the dilution used in assays) was reduced by the presence of plasma. However,

the results shown in Figure 8 indicate that in the presence of plasma, a particular quantity of GH was associated with a longer time to collect 10,000 counts, and therefore, gave a lower percent binding than expected. It seems then that in the presence of plasma, the percent binding at an anti-gamma globulin dilution of 1 in 2 was reduced, and that this was due to a shift in the position of the maximum percent binding to a higher dilution (approximately 1 in 6).

Other workers have also found that the presence of plasma may influence the standard curve in radioimmunoassays. Thorell (1968) prepared an antigen free plasma by the use of an immunosorbent and showed that when this was used as a diluent for standard antigen, the standard curve was shifted so that a particular level of antigen was associated with a higher percent binding. This was not a double antibody radioimmunoassay which suggested that the plasma affected the antigen-antibody reaction involving the hormone being assayed. Della Casa (1968) discussed factors which influenced a double antibody radioimmunoassay of human GH in plasma and showed that the change in percent binding of labelled hormone with time of incubation was different if plasma was present. He could offer no explanation for this effect and concluded that ". . . radioimmunological assay procedures for polypeptide hormones in plasma give only relative and not absolute estimates of these hormones. . . ." Similar findings were made by Burr et al. (1969) in a study

of several different systems utilizing sheep anti-rabbit gamma globulin. They showed that normal human serum and EDTA acted additively in enhancing precipitation but that heat treated human plasma did not increase enhancement by EDTA. They suggested that a complement component, which was not inactivated by EDTA, was responsible and that the effect would become unimportant if the same quantity of serum was present in all unknowns and standards. This is contrary to the recommendation of Hunter (1967) that all unknown plasmas should be run at different dilutions in order to check for dose-response parallelism.

Yalow and Berson (1968) have indicated that the concentration of labelled hormone in an assay should be "vanishingly small," since an increase in this concentration would result in the loss of the portion of the standard curve with the greatest slope, and therefore, the greatest sensitivity. In the radioimmunoassay reported herein, however, a six-fold difference in the concentration of labelled GH resulted in no systematic changes in percent binding. The range in percent binding for four dilutions (1, 1/2, 1/4, 1/6) in triplicate was from 49.8 to 51.4. Further, the percent reduction in these values on adding 1.0 ng of unlabelled GH did not vary systematically and ranged from 23.4 to 27.1. If it is assumed that the antibody-antigen reaction complies with the law of mass action (Yalow and Berson, 1968), then it follows that the percent of the antigen bound to the antibody will be determined by the size of the equilibrium

constant relative to the concentrations of antigen and antibody, and by the relative concentrations of antigen and antibody. It can be shown that if the equilibrium constant is fixed, then the changes in percent binding with the same proportional changes in hormone concentration will decrease as the ratio of hormone concentration to antibody concentration decreases below unity. Thus, the absence of any detectable change in percent binding with a six-fold change in hormone concentration would suggest that the concentration of hormone present was much less than the concentration of antibody. The similar effect of 1 ng of unlabelled hormone at these different concentrations further suggests that the quantity of labelled hormone present at all dilutions was much less than 1 ng.

The relationships between GH values of 13 pituitary extracts as obtained by the radioimmunoassay reported herein and the tibia test bioassay (Greenspan et al., 1949) has been discussed by Purchas et al. (1969).

Corticosteroid Assay

The corticosteroids measured were corticosterone (Compound B, 11β:21-dihydroxypregn-4-en-3:20 dione) and cortisol (hydrocortisone, compound F, 11β:17α:trihydroxypregn-4-en-3:20 dione). It has been shown that these two compounds are the principle steroids in bovine adrenal extracts (Hechter et al., 1951; Cavino and Giocoli, 1968), and that they are the principle steroids released from

perfused bovine adrenals *in vitro* (Hechter *et al.*, 1951). They have also been shown to be present in the peripheral plasma of cows (Estergreen and Venkataseshu, 1967).

Assay procedures used in the present study were based on the method of Riegle and Nellor (1967), with the main differences being that a fluorimetric method was used to quantify the purified steroids (Silber, 1966) and thin layer chromatography was used in place of paper chromatography.

Blood Plasma

The procedures used for the purification, separation and quantification of blood plasma cortisol and corticosterone are outlined below:

(1) An exact amount of ^{14}C-cortisol and ^{14}C-corticosterone (Nuclear Chicago Corp., Des Plaines, Illinois) in 100% ethanol was added by micro-syringe to 37.07 ml plasma in a separatory funnel. Usually from 10-15,000 cpm were added in 10 to 30 µl of ethanol. The labelled steroids were used within eight weeks after purification by the thin layer chromatography steps described below (steps 5 and 6).

(2) After several hours equilibration, the plasma was extracted gently for eight minutes with three 50 ml portions of glass distilled dichloromethane (CH_2Cl_2).

(3) The dichloromethane layers were transferred to a second separatory funnel and were then extracted gently for two minutes with 50 ml of chilled 0.1 N NaOH to

neutralize the acidic components (Eik-Nes, 1968) and reduce estradiol (Matsumura, 1967).

(4) The dichloromethane was run into a round bottom flask and was reduced to less than 1 ml in a rotary vacuum evaporator (40-45^{o}C).

(5) The contents of the flask, after being transferred to a 15 ml conical tube with four 3 ml portions of redistilled acetone, were evaporated to approximately 1 ml. This was spotted onto a 20 x 20 cm thin layer chromatographic plate (350 μ, Silica Gel GF^{254} acc. to Stahl, Brinkman Instruments Inc., Westbury, New York) at a point approximately 3 cm from the bottom and 7 cm from the side. Two successive 1 ml acetone washes were then applied at the same spot. Approximately 10 μl of a solution containing 1 mg/ml of standard cortisol and corticosterone were spotted 3.5 cm on either side of the sample spot.

(6) The plate was run for 45 minutes in hexane:ethyl acetate, (5:2, v/v), in a direction parallel to the line through the three spots. Then after drying, it was run at right angles to the above line for 90 min in chloroform: methanol:water (90:10:1, v/v/v). The corticosteroids did not migrate in the first solvent system, but many other substances did making this step a purification rather than a separation. In the second solvent system, corticosterone had an R_f of approximately 0.4 and cortisol had an R_f about half this. These results agree well with those of Cavino and Vicari (1964).

(7) The sample spots were located by interpolation between the standard spots, which could be seen under ultraviolet light. The cortisol and corticosterone spots plus one control spot were scraped into 12 ml graduated conical tubes.

(8) Elution was accomplished by first adding 1.5 ml distilled water to each tube and then extracting the resulting mixture with a 3 ml and then with three 1.5 ml portions of dichloromethane. Pearson Murphy (1968) indicated that the combination of water and organic solvent during elution of steroids from silica gel resulted in both purification and higher recoveries.

(9) One tenth of the corticosterone and control eluants and one-half of the cortisol eluant were transferred to scintillation vials, dried and counted in a Nuclear Chicago Mark 1 scintillation counter with 10 ml of scintillation fluid. This fluid was prepared from 7.5 g PPO (2,5-diphenyloxazole), 75 mg POPOP (p-bis-(2-(5-phenyloxazolyl))-benzene), 120 g naphthalene, 500 ml xylene, and 500 ml dioxane.

(10) The remainder of the eluant was dried down to 2 ml and in some cases the tube was sealed and stored for up to six days before quantification. Quantification involved addition of 4 ml of fluor reagent (Conc. H_2SO_4:100% ethanol, 3:1, v/v) to each tube followed by vigorous mixing. Between 10 and 15 minutes later, the dichloromethane layer was

removed, and fluorescence of the remaining material was measured on a Turner Model III fluorometer fitted with filters to give an excitation wave length of 470 nm and an emission wave length of 530 nm. Each time the fluorometer was used standards of cortisol (0.3, 0.6 and 0.9 μg) and corticosterone (0.1, 0.2 and 0.3 μg) were run in duplicate. For each steroid, the standard curve was a straight line through the origin after corrections had been made for the blank. Thus, mean values for fluorometer units per μg were determined and used to calculate the steroid content of the unknowns in μg/100 ml. Corrections for extraction losses were made on the basis of recovery of radioactivity.

Adrenal Homogenates

Procedures for the purification, separation and quantification of cortisol and corticosterone in adrenal homogenates are given below:

(1) The right adrenal was thawed, weighed, diced into a 50 ml glass homogenizer and then homogenized to produce a final concentration of 0.67 g adrenal tissue per 5 ml homogenizing fluid (20% ethanol, 0.85% NaCl).

(2) Five ml of adrenal homogenate were added to 50 ml round bottom glass stoppered centrifuge tubes. After heating the tubes in a water bath at 60°C for 30 minutes, labelled cortisol and corticosterone were added in the way described for plasma.

(3) After several hours equilibration, the homogenate was extracted vigorously for five minutes with three 20 ml portions of glass distilled dichloromethane. The phases were separated by centrifugation (ca. 800 x g for 5 min) and the lower dichloromethane layer was transferred to a separatory funnel.

(4) The total of approximately 60 ml of dichloromethane was extracted for one minute with 10 ml of chilled 0.1 N NaOH, transferred to a round bottom flask and taken to dryness in a vacuum evaporator (40-45^{o}C).

(5) The flask was washed first with 40 ml hexane, which was transferred to a separatory funnel, and then with two 30 ml portions of 70% glass distilled methanol. Each methanol wash was transferred to the same funnel and extracted separately with the hexane for three minutes. The 70% methanol was transferred to a round bottom flask, reduced to 10 to 15 ml in a vacuum evaporator (45-50^{o}C) and transferred to a 50 ml conical tube. Three 12 ml portions of glass distilled dichloromethane were then used to first wash the flask and then to extract the 70% methanol for approximately 3 min each.

(6) The dichloromethane layer was evaporated to dryness, and the residue was redissolved in 1 ml of dichloromethane. This 1 ml plus two 1 ml acetone washes were spotted onto a thin layer chromatographic plate similar to that described for plasma. Two sample spots and three standard spots were evenly spaced along a line about 3 cm from

the bottom of the plate. This plate was run in chloroform: methanol:water (85:15:1, v/v/v) for approximately 90 minutes.

(7) Elution from the plate and quantification of cortisol and corticosterone were the same as previously described for plasma, except that half of the eluant of each steroid was used for counting.

Discussion of Corticosteroid Assays

Although the procedures outlined above for plasma and adrenal homogenates have apparently not been previously reported, many of the individual steps have been widely used and have been discussed by Eik-Nes (1968).

Both plasma and adrenal homogenates were run in duplicate. A measure of the closeness of duplicates was obtained when the steroid levels were analyzed for treatment effects using one way analyses of variance with subsampling. The ratios of the mean squares due to animals within treatment to the mean squares due to duplicates within animals were 29.9 for plasma cortisol, 102.3 for plasma corticosterone, 159.2 for adrenal cortisol and 403.3 for adrenal corticosterone. The value required for significance at the 1% level was 2.38.

The difference between the concentration of either steroid in a pair of right and left adrenal glands was found to be small compared with differences between animals. This is in agreement with the highly significant correlations ($P < 0.01$) shown between left and right adrenal glands for

cortisol (r = 0.886) and progesterone (r = 0.857) by Wagner et al. (1969). The mean percent recoveries in the study reported herein were 69% for plasma cortisol, 68% for plasma corticosterone, 60% for adrenal cortisol and 79% for adrenal corticosterone.

Because of the greater lipid content of the adrenal homogenates, the 70% methanol-hexane partition was used rather than the first thin layer chromatography step used with plasma. The change in the chromatographic solvent system from chloroform:methanol: water in the proportions 90:10:1 (v/v/v), to the proportions of 85:15:1 (v/v/v) was made because the latter system increased the distance that the steroids migrated from the origin, although it did not increase the separation appreciably.

The heat treatment of the adrenal homogenate prior to adding the labelled hormone was included because without it a third radioactive spot was obtained on the thin layer chromatographic plate. This spot, which had a higher R_f than corticosterone, appeared to be due to breakdown of the radioactive corticosterone rather than the cortisol. The fact that the spot did not appear when the homogenate was heated at 60°C for 30 minutes suggests that an enzyme may have been involved. Steroids are known to be heat-sensitive, but the above treatment did not appear to affect those being measured as it had no apparent effect on the standard curves of either cortisol or corticosterone.

Some support for the specificity of these methods was acquired by using an Aminco Bowman Spectrophotofluorometer with an X-Y recorder to obtain scans for emission and excitation spectra of cortisol and corticosterone. These were obtained both on standard solutions and on extracts from adrenal homogenates prepared by the procedure previously described. For an emission scan, the excitation wavelength was set at 470 nm. For an excitation scan, the emission wavelength was set at 530 nm. No differences were observed in the patterns of the scans for the standards and the extracts. The changes in peak height with changes in time after adding the fluor reagent (up to 20 minutes) did differ slightly but consistently between the extracts and standards, however, with the extracts reaching a peak later. This may have resulted from the fact that the standards in 20 µl of ethanol were mixed with the fluor reagent, while the extracts in 2 ml of dichloromethane were extracted into the fluor reagent.

Assessment of Thyroid Activity

Protein Bound Iodine

Plasma protein bound iodine (PBI) was estimated using the technique outlined by Reineke and Lorscheider (1967) for thyroidal iodine analysis. Preparation of 1 ml plasma samples for ashing followed a procedure of Reineke (unpublished method), which was essentially the same as that of Faulkner *et al.* (1961). The standard curve was from 0.02

to 0.10 μg iodine, and a recovery of 90% was assumed in the calculation of PBI values.

Thyroid Histology

Part of the thyroid gland was mounted in paraffin and sections 10 μ in thickness were cut, mounted, and stained with Harris's hemotoxylin and eosin. Ten measurements of follicular cell height were made on each gland using an ocular micrometer at a magnification of 1000 times.

Statistical Methods

Table 3 classifies the nine groups of heifers described previously in a 2 x 2 x 3 factorial arrangement. It is apparent from this table that the 2 x 2 x 3 factorial design is incomplete, but that some smaller factorial combinations are possible. Factorial and non-factorial statistical models that were considered in this study are as follows:

(1) Age at slaughter versus MGA treatment within the high level of nutrition treatment (groups 6, 7, 8, 11, 12 and 13).

(2) Age at slaughter versus level of nutrition within the "No MGA" group (groups 5, 6, 10 and 11).

(3) Plane of nutrition versus two MGA treatments within the group slaughtered at breeding size (groups 5, 6, 7 and 9).

Table 3. Factorial arrangement of treatment groups by group number

Age at slaughter	First estrus		Breeding	
Nutrition level	High	Normal	High	Normal
MGA Treatment				
No MGA	11	10	6	5
MGA from 2.5 mo.	12	--	7	9
MGA from first estrus	13	--	8	-

(4) Groups 5, 6 and 7 were also combined in some non-factorial analyses of variance since certain measurements were made only on these animals.

With the data available, relationships between endocrine parameters and growth or meat quality were investigated in several ways. Correlation and/or regression analyses were run (Snedecor, 1956). Within each of the above four models, the effects of age at slaughter, nutrition or MGA treatment on carcass weight were tested using general least-squares analyses (Harvey, 1960). The endocrine parameters were analyzed in the same way as carcass weight, except that carcass weight was included as a covariate. Growth and meat quality parameters were also analyzed in the same way except that carcass weight and a selection of endocrine parameters were included as covariates. General least squares computer programs developed by Michigan State University Agricultural Experiment Station were used to make most of these analyses.

An attempt was made to get an indication of whether age, nutrition, MGA treatment or carcass weight affected meat quality measurements through an effect on endocrine parameters. First, the percent of the variation accounted for by all components was calculated. Then, the reduction in this value upon removal of two or more variables separately was compared with the reduction upon removing the same variables simultaneously. For example, in the case of age effects, if the sum of the percents variation in a meat quality parameter that was accounted for by age and by endocrine parameters separately was more than the percent variation accounted for when these were together, then the effect of age on the meat quality parameter would appear to be at least partially mediated through an effect on the endocrine characteristics measured. Comparable arguments can be made for the effects of MGA treatment and nutrition, but in the case of carcass weight, there is no way to tell whether carcass weight effects are being mediated through endocrine effects or vice versa.

RESULTS AND DISCUSSION

The raw data together with the means and standard deviations for each treatment group are given in Appendix 1. Data on color are not included, as variation was not detected with the scoring system used. There was no evidence that MGA administration caused any increased incidence of dark cutting beef. This is in agreement with some work (Anon., 1968), although it has been suggested that MGA administration may be associated with increased numbers of dark cutters.

The means and standard deviations of derived variables for each treatment group are given in Table 4. The variable codes and numbers used in all tables are given in Table 5.

Effectiveness of Measurements

Carcass Composition

Table 6 shows correlation coefficients between measures of carcass composition for all animals. On the basis of other work, it is assumed that the best indicator of carcass lean in this table is percent lean of the flank, while the best indicators of carcass fat and carcass bone are percentage of fat in the flank and cannon bone weight

Table 4. Means and standard deviations of some derived variables

Variable and code number[b]	Group[a] 5	6	7	8	9	10	11	12	13
DR-% (31)	54.3 ± 1.5	55.9 ± 1.2	56.3 ± 1.2	56.4 ± 1.5	51.3 ± 0.2	50.6 ± 1.6	51.9 ± 2.1	51.6 ± 2.3	52.2 ± 2.5
CB-% (32)	0.657 ± 0.072	0.621 ± 0.034	0.564 ± 0.057	0.591 ± 0.074	0.629 ± 0.067	0.760 ± 0.052	0.761 ± 0.063	0.733 ± 0.066	0.759 ± 0.077
CBW/L (33)	12.79 ± 0.68	12.70 ± 0.74	12.83 ± 0.59	12.91 ± 0.81	11.76 ± 0.81	10.45 ± 0.77	9.99 ± 0.71	9.85 ± 0.40	10.92 ± 0.99
RD-% (34)	13.09 ± 0.70	12.67 ± 0.44	12.00 ± 0.49	12.25 ± 0.70	12.80 ± 0.42	14.18 ± 0.46	14.05 ± 0.73	13.83 ± 0.74	13.76 ± 0.61
FL-% (35)	2.41 ± 0.14	2.58 ± 0.35	2.76 ± 0.45	2.50 ± 0.28	2.42 ± 0.25	2.28 ± 0.17	2.12 ± 0.23	2.21 ± 0.25	2.16 ± 0.21
F-FT % (36)	37.40 ± 5.55	45.10 ± 3.32	46.27 ± 4.79	41.54 ± 4.58	36.23 ± 5.19	32.10 ± 3.71	35.33 ± 3.14	32.70 ± 5.39	35.52 ± 4.99
F-LN % (37)	61.00 ± 5.65	53.03 ± 3.34	52.10 ± 5.31	57.40 ± 4.82	62.04 ± 5.38	67.90 ± 3.89	63.77 ± 3.04	65.83 ± 5.35	62.92 ± 5.40
EMA-V (38)	30.43 ± 4.09	32.64 ± 7.56	36.59 ± 7.84	33.40 ± 4.61	26.15 ± 4.34	22.38 ± 3.99	24.22 ± 3.59	24.56 ± 3.64	24.58 ± 5.15
ADG (39)	1.09 ± 0.10	1.24 ± 0.15	1.41 ± 0.14	1.40 ± 0.07	1.08 ± 0.06	1.12 ± 0.11	1.24 ± 0.19	1.29 ± 0.15	1.30 ± 0.14
ADR % (40)	3.22 ± 0.39	3.58 ± 0.52	3.17 ± 0.48	3.00 ± 0.32	3.53 ± 0.47	4.02 ± 0.32	4.32 ± 0.70	3.72 ± 0.24	3.93 ± 0.87
THY % (41)	4.07 ± 0.57	6.12 ± 3.86	4.95 ± 2.60	6.06 ± 4.50	5.05 ± 1.77	12.51 ± 14.97	7.47 ± 2.72	8.53 ± 4.09	6.88 ± 2.33
PT-CT (42)	19.80 ± 5.16	18.56 ± 4.46	19.34 ± 5.00	17.18 ± 3.05	24.44 ± 3.82	22.26 ± 7.17	17.18 ± 6.07	20.20 ± 6.86	19.83 ± 3.67
PL-CT (43)[c]	8.13 ± 5.62	12.57 ± 6.77	8.24 ± 5.03	9.12 ± 7.47	11.55 ± 4.99	12.04 ± 9.26	8.13 ± 6.27	8.40 ± 5.70	7.33 ± 2.79
PL/PT (44)	0.152 ± 0.076	0.235 ± 0.121	0.160 ± 0.098	0.192 ± 0.121	0.206 ± 0.100	0.303 ± 0.218	0.243 ± 0.183	0.230 ± 0.174	0.175 ± 0.080
GH/CC (45)	4.63 ± 1.18	4.15 ± 0.81	3.94 ± 0.97	3.63 ± 0.68	6.13 ± 1.35	7.72 ± 2.34	6.36 ± 2.28	7.37 ± 2.80	6.67 ± 1.73
R-FT % (46)	11.92 ± 2.06	14.63 ± 2.37	14.75 ± 2.28		11.74 ± 1.18				
R-LN % (47)	68.02 ± 2.43	65.16 ± 1.24	66.49 ± 2.04		68.96 ± 1.75				
R-BN % (48)	19.71 ± 0.95	19.67 ± 1.51	18.53 ± 1.19		19.52 ± 1.42				
R-L/B (49)	3.46 ± 0.23	3.33 ± 0.25	3.60 ± 0.25		3.55 ± 0.32				
R-F/L (50)	0.176 ± 0.037	0.225 ± 0.039	0.223 ± 0.041		0.170 ± 0.018				
C-P/A (51)	4.24 ± 0.92	4.87 ± 2.30	9.11 ± 7.47						
AC-CN (52)	36.83 ± 9.24	41.62 ± 30.35	15.41 ± 13.14						
PC-CN (53)[c]	375.6 ± 72.3	325.0 ± 120.1	214.4 ± 139.7						
C/CC (54)	8.68 ± 2.50	9.17 ± 6.63	3.09 ± 2.68						
CSP/A (55)	1.169 ± 0.986	1.170 ± 1.088	2.068 ± 1.717						
ACSCN (56)[c]	24.67 ± 17.68	25.10 ± 25.28	5.45 ± 4.38						
PCSCN (57)	44.64 ± 16.20	28.92 ± 16.79	20.47 ± 13.63						
CS/CC (58)	5.95 ± 4.84	5.50 ± 5.45	1.13 ± 0.95						

[a] See Table 2 for explanation of group numbers.

[b] See Table 5 for variable definitions.

[c] Plasma volume in liters was taken to be 1.75% of carcass weight in kg.

Table 5. Key to variable numbers and codes

Variable code and number	Variable
AN-NO (1)	Animal number
CC-WT (2)	Cold carcass weight (lb)
SL-WT (3)	Live slaughter weight (lb)
RD-WT (4)	Round weight (lb)
SP-GR (5)	Round specific gravity
FL-WT (6)	Flank weight (lb)
FL-FT (7)	Flank dissectable fat (lb)
FL-LN (8)	Flank dissectable lean (lb)
AGE (9)	Age at slaughter (days)
TH-WT (10)	Thyroid weight (g)
AD-WT (11)	Adrenals weight (g)
AP-WT (12)	Anterior pituitary weight (g)
PL-GH (13)	Plasma GH concentration (ng/ml) at slaughter
PT-GH (14)	Anterior pituitary GH concentration (μg/10 μg)
EMA (15)	Eye muscle area (sq. in.)
CB-WT (16)	Cannon bone weight (g)
CB-LE (17)	Cannon bone length (cm)
RD-FT (18)	Round dissectable fat (lb)
RD-LN (19)	Round dissectable lean (lb)
RD-BN (20)	Round dissectable bone (lb)
RD-WA (21)	Water in round lean + fat (%)
RD-EE (22)	Petroleum ether extract of round lean + fat (%)
TEND (23)	Tenderness shear value
JU-GH (24)	Mean plasma GH (ng/ml) in jugular samples taken at 4, 5, 6, 7, 8, 9 and 10 mo. of age
TH-CH (25)	Mean of 10 thyroid cell heights (μ)
PBI (26)	Protein bound iodine (μg/100 ml)
PL-CO (27)	Plasma cortisol (μg/100 ml)
PL-CS (28)	Plasma corticosterone (μg/100 ml)
AD-CO (29)	Adrenal cortisol (μg/g)
AD-CS (30)	Adrenal corticosterone (μg/g)
DR-% (31)	Dressing %
CB-% (32)	Cannon bone weight (g)/carcass weight (lb)
CBW/L (33)	Cannon bone weight (g)/cannon bone length (cm)
RD-% (34)	Round weight (lb)/carcass weight (lb)
FL-% (35)	Flank weight (lb)/carcass weight (lb)
F-FT % (36)	Flank fat %
F-LN % (37)	Flank lean %
EMA-V (38)	$(\text{Eye Muscle Area})^{3/2}$
ADG (39)	Carcass weight (lb)/Age (days)
ADR % (40)	Adrenal glands weight (g) x 100/carcass weight (lb)
THY % (41)	Thyroid gland weight (g) x 100/carcass weight (lb)
PT-CT (42)	Total anterior pituitary GH content (mg)
PL-CT (43)	Plasma GH content (μg)
PL-PT (44)	Plasma GH conc. (ng/ml)/Anterior pituitary GH conc. (μg/mg)
GH/CC (45)	Anterior pituitary GH content (mg) x 100/carcass weight (lb)
R-FT % (46)	Round fat %
R-LN % (47)	Round lean %
R-BN % (48)	Round bone %
R-L/B (49)	Round lean to bone ratio
R-F/L (50)	Round fat to lean ratio
C-P/A (51)	Plasma cortisol (μg/100 ml)/Adrenal cortisol (μg/g)
AC-CN (52)	Total adrenal cortisol content (μg)
PC-CN (53)	Total plasma cortisol content (μg)
C/CC (54)	Total adrenal cortisol content (μg) x 100/Carcass weight (lb)
CSP/A (55)	Plasma corticosterone (μg/100 ml)/Adrenal corticosterone (μg/g)
ACSCN (56)	Total adrenal corticosterone content (μg)
PCSCN (57)	Total plasma corticosterone content (μg)
CS/CC (58)	Total adrenal corticosterone content (μg) x 100/Carcass weight (lb)
CT-WD (59)	$(\text{Adrenal cortex width (mm)})^3$
CT/AW (60)	$(\text{Adrenal cortex width (mm)})^3$/Adrenal weight (g)

Table 6. Correlations between measurements of carcass composition made on animals in groups 5 to 13[a]

Variable code and number[b]	F-LN % (37)	SP-GR (5)	CB-% (32)	EMA-V (38)
F-FT % (36)	-0.99	-0.48	-0.68	0.51
SP-GR (5)	--	--	0.38	-0.38
CB-% (32)	--	--	--	0.70

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

per unit carcass weight, respectively. If these were the best indicators, then the correlations suggest that specific gravity of the round and eye muscle area were not good indicators of carcass composition. None of the correlations between these two characteristics and the other carcass measurements in Table 6 were high enough to be of predictive value. Correlations within individual treatment groups varied considerably and, in general, were lower in the groups slaughtered at puberty and in the groups on normal rather than the high level of nutrition. This was particularly the case with correlations involving specific gravity of the round and was probably due to the low fat content in those groups. Kelly et al. (1968) have also shown that the relationship between specific gravity and carcass composition is less close in groups of animals with a low carcass fat content.

Relationships between measures of carcass composition for groups 5, 6, 7 and 9 (Table 7) indicate that flank and round composition were not very closely correlated. There is no way to assess the relative usefulness of the flank and the round components as indicators of carcass composition in this study, but based on size relative to the whole carcass, the round should be superior. A similar situation exists in comparing the percent cannon bone and the percent bone in the round as indicators of total bone. Again, correlations within groups tended to be higher in those groups on a high plane of nutrition (6 and 7), especially when measures of fatness were involved. Percent of lean in the flank was not included in Table 7 because it was almost perfectly correlated with percent flank fat (Table 6). The lower correlation between water content and lean than between ether extract and dissectable fat appears to be due to the greater variability of the fat measurements (Table 4).

Growth Hormone

Table 8 gives correlation coefficients between various measures of GH. One value for pituitary GH concentration was missing for groups 5, 6, 8, 10 and 12 so that the correlations for "Groups 5 to 13," which involved this parameter, included 85 animals while those in "Groups 5, 6, 7 and 9" included 38 animals. For all other parameters there were ten observations per group. Table 8 indicates that none of the measurements were very closely correlated

Table 7. Correlations between measurements of carcass composition made on animals in groups 5, 6, 7 and 9[a]

Variable code and number[b]	RD-WA (21)	R-FT % (46)	R-LN % (47)	R-BN % (48)	F-FT % (36)	CB-% (32)	CBW/L (33)
RD-EE (22)	-0.99	0.92	-0.73	-0.42	0.76	-0.58	0.21
RD-WA (21)	--	-0.91	0.72	0.43	-0.76	0.58	0.23
R-FT % (46)	--	--	-0.77	-0.49	0.76	-0.55	0.24
R-LN % (47)	--	--	--	-0.11	-0.66	0.19	-0.03
R-BN % (48)	--	--	--	--	-0.31	0.66	-0.28
F-FT % (36)	--	--	--	--	--	-0.56	0.22
CB-% (32)	--	--	--	--	--	--	-0.11

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

Table 8. Correlations between various measures of growth hormone

Variable number and code[a]	PT-GH (14) / PL-GH (13)	PT-GH (14) / PT-CT (42)	PT-GH (14) / JU-GH (24)	PL-GH (13) / PT-CT (42)	PL-GH (13) / JU-GH (24)	PT-CT (42) / JU-GH (24)	PT-CT (42) / AP-WT (12)
Groups 5 to 13[b]	0.35	0.69	--	0.21	--	--	0.38
Groups 5, 6, 7 and 9	0.37	0.66	-0.07	0.29	0.09	0.01	0.58

[a]See Table 5 for variable definitions.

[b]See Table 2 for group classification.

with any of the others. Correlations within individual groups were quite variable with no apparent patterns. A low correlation between GH concentrations of plasma and pituitary samples collected at slaughter has also been shown by Purchas et al. (1969) for 65 Holstein bulls slaughtered at ages ranging from birth to 12 months. In contrast, Peake et al. (1968) reported a significant negative correlation ($P < 0.001$, $r = -0.80$) between pituitary and plasma GH concentration in rats with GH producing tumors, but the plasma GH levels were much greater than normal in their animals.

Adrenal Activity

Correlation coefficients between various measures of adrenal cortical activity are given for groups 5, 6 and 7 in Table 9. In general, this table shows that the correlations between chemical determinations of steroid concentrations in plasma and in adrenal glands are quite high relative to the correlations between chemical determinations and weight or volume estimates. Procedures used for measuring the width of the adrenal cortex are given by Pritchard (1970). These values were cubed in order to convert them from linear units to the equivalent of volume units. It can be concluded from the data in Table 9 that adrenal weights and widths of the adrenal cortex do not give a good indication of adrenal cortical activity.

Table 9. Correlations between different measures of adrenal cortical activity in groups 5, 6 and 7[a]

Variable code and number[b]	PL-CS (28)	AD-CO (29)	AC-CN (52)	AD-CS (30)	ACSCN (56)	CT/AW (60)
PL-CO (27)	0.82	0.59	0.57	--	--	--
PL-CS (28)	--	--	--	0.69	0.68	--
AD-CO (29)	--	--	0.98	--	--	0.33
AD-CS (30)	--	0.85	--	--	0.99	0.27
CT-WD (59)	--	--	0.36	--	0.31	--
AD-WT (11)	--	--	-0.12	--	-0.16	--

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

Since corticosteroids are known to be affected by stress (Dixon et al., 1967), the values reported in this study, which represent levels at slaughter, may be high. An example of the response of corticosteroids to stress in ruminants has been given by Basset and Hinks (1969). They showed that 15 minutes after venipuncture, sheep that were unaccustomed to the bleeding procedures had significantly higher plasma cortisol levels (approx. 3.9 vs. 2.5 μg/100 ml) than trained sheep.

Thyroid Activity

Table 10 shows correlations between measures of thyroid activity made on animals in groups 5, 6, 7 and 9.

Table 10. Correlations between different measures of thyroid activity in groups 5, 6, 7 and 9[a]

Variable code and number[b]	PBI (26)	TH-WT (10)
TH-CH (25)	-0.01	-0.36
PBI (26)	--	0.10

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

The absence of any high correlations was supported by the wide variation in the correlation coefficients within each group. Kossila (1967) reported that the estimated weight of thyroid epithelium per 100 kg of body weight in 75 cows was positively correlated with the mean of five PBI levels made on each cow at two-month intervals ($r = 0.27$, $P < 0.025$). The essentially zero correlation between PBI and thyroid cell height (Table 10) may have been due to inaccuracy in measurement of cell height. The sizes of the cells appeared to differ as much within animals as between animals, and the heights were frequently too small to measure accurately. Consequently, the PBI values are considered to be more accurate measurements than the thyroid cell heights. This does not necessarily mean, however, that they will be better indicators of thyroid activity. Falconer and Draper (1968) pointed out that PBI levels represent a balance between secretion and tissue utilization, and thus, may not be

related to secretion rate. They reported a correlation of 0.053 between the PBI and thyroxine secretion rate for 25 adult sheep. A very low correlation between thyroxine secretion rate and PBI in cattle (r = -0.03) has also been reported by Johnson et al. (1959).

On the other hand, Post (1965) concluded that PBI values effectively reflected seasonal changes in the thyroid secretion rate of cattle. On the basis of correlations with thyroid cell height, Hoersch et al. (1961) indicated that thyroid secretion rate was a better measure of thyroid activity than thyroid output half-time and percent uptake. Likewise, Johnson et al (1959) showed that thyroid secretion rate was more closely correlated to BMR (r = 0.9) than was PBI or thyroxine half-life. Thus, measurements of thyroid cell heights are probably not as accurate as measurements of PBI in the study reported herein, but they may give a better indication of thyroid activity, if they are more closely related to thyroxine secretion rate.

Least Squares Models

Results obtained by the use of the four least squares models, which were outlined in the statistical methods, will be given separately. Because many of the data are used in more than one of these models, the results can not be considered independent and will, therefore, be discussed together.

Age at Slaughter vs. MGA Treatment

These analyses included data from animals in groups 6, 7, 8, 11, 12 and 13 (Tables 2 and 3). All animals were on the high plane of nutrition. They were slaughtered either at first estrus or breeding size, and MGA was administered either from 2.5 months of age, from first estrus or not at all.

Table 11 shows the analysis of variance of log weight of lean in the flank as an example of the type of analysis that was used. The logs of the weight of lean in the flank and of carcass weight were used rather than absolute weights, as it was considered that the relationship between these two variables would be more effectively represented in this way. By making log transformations on both variables, the allometric equation of Huxley (1932) was used. Other work, which has enabled an assessment of this approach, has been reviewed previously herein. Log values of hormone levels have also generally been used (Table 12). This is because the biological effectiveness of hormones is often more closely related linearly to their log than to their absolute values (Sokal and Rohlf, 1969). The deviations of the distributions of the transformed variables from normality as shown by measures of skewness and kurtosis did not appear to differ appreciably from those for the raw variables.

Table 11. Analysis of variance for log flank lean weight in groups 6, 7, 8, 11, 12 and 13[a]

Source of variation	Sum of squares	Degrees of freedom	Mean square	F ratio	Significance
Age at slaughter	0.0004	1	0.0004	0.178	0.675
MGA treatment	0.0016	2	0.0008	0.391	0.678
Age x MGA	0.0025	2	0.0012	0.602	0.552
Log carcass wt.	0.0587	1	0.0587	28.348	<0.0005**
Endocrine parameters	0.0169	5	0.0034	1.629	0.170
Error	0.0993	48	0.0021		

[a]See Table 2 for group classification.

*P < 0.05

**P < 0.01

For all variables except log weight of lean in the flank, only the values in the "significance" column in Table 11 will be given. Table 12 gives these values for some measurements made on animals in groups 6, 7, 8, 11, 12 and 13. The variables included as covariates under the heading of endocrine parameters were log plasma GH concentration at slaughter, log pituitary GH concentration, log pituitary GH content, log adrenal weight and log thyroid weight. This group of parameters and comparable groups in analyses discussed later were considered only as a group, except when the significance level attributed to it was less than 0.05.

Table 12. Significance levels for the effects of various sources of variation on some endocrine and carcass quality measurements in groups 6, 7, 8, 11, 12 and 13[a]

Source of variation	Age at slaughter	MGA treatment	Interaction	Log carcass weight	Endocrine parameters
Variable code and number[b]					
CC-WT (2)	<0.0005**	0.048*	0.241		
Log (PL-GH (13))	0.873	0.306	0.302	0.461	
Log (PT-GH (14))	0.570	0.893	0.147	0.305	
Log (PT-CT (42))	0.100	0.689	0.391	0.086	
Log (AD-WT (11))	0.590	0.028*	0.375	<0.0005**	
Log (TH-WT (10))	0.913	0.990	0.616	0.464	
DR-% (31)	0.735	0.992	0.982	<0.0005**	<0.0005**
Log (RD-WT (4))	0.580	0.245	0.684	<0.0005**	0.596
Log (FL-WT (6))	0.816	0.076	0.631	<0.0005**	0.105
Log (FL-FT (7))	0.899	0.005**	0.105	<0.0005**	0.074
Log (FL-LN (8))	0.675	0.678	0.552	<0.0005**	0.170
Log (CB-WT (16))	0.047*	0.017*	0.339	0.001**	0.588
CB-% (32)	0.045*	0.015*	0.383	<0.0005**	0.478
CBW/L (33)	0.041*	0.030*	0.405	0.002**	0.440
RD-% (34)	0.551	0.284	0.822	<0.0005**	0.608
FL-% (35)	0.943	0.075	0.601	0.051	0.238
F-FT % (36)	0.743	0.008**	0.033*	<0.0005**	0.195
F-LN % (37)	0.718	0.005**	0.014*	<0.0005**	0.086
EMA-V (38)	0.206	0.317	0.702	0.002**	0.771
ADG (39)	0.077	0.027*	0.145	0.027*	0.399

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

Then a measure of the significance of each component was considered based on F statistics for each regression coefficient.

Dressing percent was significantly affected by the endocrine parameters in the model being discussed (Table 12). This was primarily due to a significant (P = 0.009) positive effect of log plasma GH concentration at slaughter.

Table 13 lists percentages of the total variation in some carcass quality parameters that can be attributed to various factors. The first column (Total R^2) indicates the percent variation accounted for by the complete model. The second column (Endocrine ΔR^2) indicates the change in R^2 when the endocrine parameters were removed from the model. Under "Age ΔR^2" the first column shows the change in R^2 when the age effects are removed. The second column indicates the difference between the sum of reductions due to the removal of age and endocrine parameters separately, and the reduction due to removing them simultaneously. No attempt was made to compute significance levels or confidence intervals for these values. Comparable pairs of columns are given for MGA ΔR^2 and log carcass weight ΔR^2. The r^2 column under log carcass weight contains the squares of the appropriate simple correlation coefficients. The value in this column is consistently greater than the sum of the previous two columns because of the significant age and MGA effects on carcass weight (Table 12). No attempt was made to ascertain whether or not carcass quality was indirectly altered

Table 13. The percent of variation in some carcass quality traits attributed to effects of age at slaughter, MGA treatment and log carcass weight, both directly and through an effect on endocrine parameters--groups 6, 7, 8, 11, 12 and 13[a]

Variable code and number[b]	Total R^2	Endocrine ΔR^2	Age ΔR^2		MGA ΔR^2		Log carcass wt. ΔR^2		
			Alone	Through endocrines	Alone	Through endocrines	Alone	Through endocrines	r^2
Log (RD-WT (4))	97	0	0	0	0	0	7**	8	98
Log (FL-WT (6))	94	1	0	0	0	0	7**	9	92
Log (FL-FT (7))	93	1	0	0	1**	0	9**	7	90
Log (FL-LN (8))	91	2	0	0	1	-1	6**	9	88
Log (CB-WT (16))	88	1	1*	0	3*	0	4**	4	83
CB-% (32)	87	2	2*	-1	3*	-1	13**	7	81
CBW/L (33)	89	2	1*	-1	2*	-1	3**	5	85
DR-% (31)	82	12**	1	-1	1	-1	7**	3	69
RD-% (34)	79	1	0	0	1	0	8**	5	76
FL-% (35)	56	6	0	0	5	-1	3	7	45
F-FT % (36)	75	4	0	0	6**	-2	10**	1	64
F-LN % (37)	75	5	0	0	6**	-2	11**	0	62
EMA-V (38)	67	2	2	0	2	0	8**	10	61
ADG (39)	38	6	4	0	10*	-4	6*	5	17

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

by MGA or nutrition through their effects on carcass weight and then the influence of carcass weight upon the endocrine parameters.

Age at Slaughter vs. Level of Nutrition

Groups 5, 6, 10 and 11 were included in these analyses. None of the animals received any MGA treatment. They were slaughtered either at first estrus or breeding size and were either on the high or normal level of nutrition. Table 14 lists significance levels for various sources of variation. The group of endocrine parameters was the same as for the previous model (log plasma GH concentration at slaughter, log pituitary GH concentration, log pituitary GH content, log adrenal weight and log thyroid weight). Table 15 lists the percent variation in various carcass quality traits attributable to different sources.

Level of Nutrition vs. MGA Treatment

These analyses involved groups 5, 6, 7 and 9. All the animals were slaughtered at breeding size (120 cm withers height). They were raised on a high or a normal level of nutrition and either received no MGA or MGA from 2.5 months of age. Table 16 lists significance levels for various sources of variation. The endocrine parameters included as covariates in this model were log thyroid weight, log adrenal weight, log anterior pituitary weight, log of the mean GH concentration of seven jugular plasma samples,

Table 14. Significance levels for the effects of various sources of variation on some endocrine and carcass quality measurements in groups 5, 6, 10 and 11[a]

Source of variation	Age of slaughter	Plane of nutrition	Interaction	Log carcass weight	Endocrine parameters
Variable code and number[b]					
CC-WT (2)	<0.0005**	0.967	0.147		
Log (PL-GH (13))	0.947	0.815	0.029*	0.364	
Log (PT-GH (14))	0.182	0.747	0.906	0.337	
Log (PT-CT (42))	0.032*	0.140	0.699	0.019*	
Log (AD-WT (11))	0.419	0.050*	0.438	0.002**	
Log (TH-WT (10))	0.913	0.914	0.048*	0.920	
DR-% (31)	0.691	0.053	0.759	0.005**	0.171
Log (RD-WT (4))	0.365	0.163	0.694	<0.0005**	0.645
Log (FL-WT (6))	0.829	0.419	0.099	<0.0005**	0.440
Log (FL-FT (7))	0.884	0.005**	0.103	<0.0005**	0.802
Log (FL-LN (8))	0.915	0.079	0.486	<0.0005**	0.584
Log (CB-WT (16))	0.214	0.105	0.723	0.008**	0.699
CB-% (32)	0.228	0.114	0.737	0.001**	0.701
CBW/L (33)	0.566	0.187	0.465	<0.0005**	0.771
RD-% (34)	0.336	0.192	0.699	0.003**	0.633
FL-% (35)	0.913	0.295	0.124	0.249	0.506
F-FT % (36)	0.872	0.001**	0.270	0.159	0.950
F-LN % (37)	0.744	0.001**	0.320	0.125	0.983
EMA-V (38)	0.220	0.117	0.919	0.003**	0.679
ADG (39)	0.139	0.048*	0.884	0.242	0.914

[a]See Table 2 for group classifications.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

Table 15. The percent of variation in some carcass quality traits attributed to effects of age at slaughter, plane of nutrition and log carcass weight, both directly and through an effect on endocrine parameters--groups 5, 6, 10 and 11[a]

Variable code and number[b]	Total R^2	Endocrine ΔR^2	Age ΔR^2		Nutrition ΔR^2		Log carcass wt. ΔR^2		
			Alone	Through endocrines	Alone	Through endocrines	Alone	Through endocrines	r^2
Log (RD-WT (4))	97	0	0	0	0	1	7**	6	96
Log (FL-WT (6))	94	1	0	0	0	0	7**	7	92
Log (FL-FT (7))	92	1	0	0	2**	0	6**	7	88
Log (FL-LN (8))	89	2	0	0	2	1	8**	6	83
Log (CB-WT (16)	87	1	1	0	1	0	3**	5	85
Log (CB-% (32)	75	2	1	0	2	0	11**	5	71
CBW/L (33)	89	1	0	0	1	-1	6**	5	88
DR-% (31)	78	6	0	1	3	7	7**	0	61
RD-% (34)	72	3	1	0	2	2	10**	4	66
FL-% (35)	51	7	0	1	2	-1	3	6	37
F-FT % (36)	68	2	0	0	14**	-3	3	2	44
F-LN % (37)	70	1	0	0	14**	6	2	3	48
EMA-V (38)	62	4	2	-1	4	0	13**	1	53
ADG (39)	29	4	6	-2	11*	11	4	0	2

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

Table 16. Significance levels for the effects of various sources of variation on some endocrine and carcass quality measurements in groups 5, 6, 7 and 9[a]

Source of variation	Plane of nutrition	MGA treatment	Interaction	Log carcass weight	Endocrine parameters
Variable code and number[b]					
CC-WT (2)	0.001**	0.388	0.016*		
Log (TH-WT (10))	0.006**	0.702	0.821	0.094	
Log (AD-WT (11))	0.482	0.844	0.097	0.004**	
Log (AP-WT (12))	0.095	0.102	0.906	0.002**	
Log (JU-GH (24))	0.340	0.032*	0.476	0.905	
Log (PL-GH (13))	0.923	0.872	0.008**	0.524	
Log (PT-GH (14))	0.121	0.455	0.059	0.123	
Log (PT-CT (42))	0.033*	0.092	0.116	0.281	
TH-CH (25)	0.349	0.934	0.571	0.752	
PBI (26)	0.270	0.665	0.309	0.262	
TEND (23)	0.755	0.391	0.195	0.199	0.462
ADG (39)	0.010**	0.382	0.030*	0.190	0.023*
DR-% (31)	<0.0005**	0.012*	0.003**	<0.0005**	0.072
RD-% (34)	0.285	0.020*	0.404	<0.0005**	0.178
R-FT % (46)	0.008**	0.934	0.676	0.012*	0.322
R-LN % (47)	<0.0005**	0.085	0.419	0.460	0.016*
R-BN % (48)	0.808	0.143	0.219	0.004**	0.616
FL-% (35)	0.450	0.936	0.881	0.004**	0.587
F-FT % (36)	<0.0005**	0.978	0.596	0.192	0.176
F-LN % (37)	<0.0005**	0.903	0.540	0.125	0.163
R-L/B (49)	0.052	0.042*	0.162	0.028*	0.229
R-F/L (50)	0.004**	0.825	0.780	0.019*	0.235
RD-WA (21)	0.210	0.669	0.659	0.005**	0.506
RD-EE (22)	0.199	0.867	0.640	0.005**	0.441
CB-% (32)	0.593	0.045*	0.178	<0.0005**	0.574
CBW/L (33)	0.611	0.032*	0.535	0.010**	0.155
Log (RD-WT (4))	0.243	0.016*	0.318	<0.0005**	0.173
Log (RD-FT (18))	0.011*	0.695	0.560	<0.0005**	0.354
Log (RD-LN (19))	0.003*	0.383	0.254	<0.0005**	0.013*
Log (RD-BN (20))	0.685	0.013*	0.582	0.011*	0.972
Log (FL-WT (6))	0.582	0.999	0.852	<0.0005**	0.601
Log (FL-FT (7))	0.006**	0.923	0.618	<0.0005**	0.419
Log (FL-LN (8))	0.014*	0.979	0.812	<0.0005**	0.370
Log (CB-WT (16))	0.605	0.025*	0.291	0.083	0.431
Log (EMA-V (38))	0.548	0.491	0.598	0.021*	0.599

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

log plasma GH concentration at slaughter, log anterior pituitary GH concentration, mean thyroid cell height and log plasma protein bound iodine concentration. The significant effect of the endocrine parameters on average daily carcass weight gain (Table 16) was due to the effects of log thyroid weight ($P = 0.023$, $b = 0.35$) and log mean jugular GH concentration ($P = 0.010$, $b = -0.27$). The significant effects of endocrine parameters on log round lean weight and on round lean percent (Table 16) were due to the effects of log plasma GH concentration ($P = 0.006$, $b = -0.05$ and $P = 0.011$, $b = -0.04$, respectively) and log plasma protein bound iodine concentration ($P = 0.005$, $b = 0.09$ and $P = 0.01$, $b = 0.07$, respectively).

Table 17 lists the percentage of variation in different carcass quality traits attributable to different sources of variation.

Groups 5, 6 and 7

These three groups were analyzed separately, since adrenal and plasma corticosteroid data were only available for animals in these groups. The analyses did not involve factorial arrangements of treatments as did the other three models. However, groups 5, 6 and 7 each consisted of five animals in lot 1 and five in lot 2, so that a factorial of lot against treatment (normal plane of nutrition with no MGA--group 5, high plane of nutrition with no MGA--group 6, and high plane of nutrition with MGA from 2.5 months--group 7) was run. An effect of lot could not have been tested for

Table 17. The percent of variation in some carcass quality traits attributed to effects of plane of nutrition, MGA treatment and log carcass weight, both directly and through an effect on endocrine parameters--groups 5, 6, 7 and 9[a]

Variable code and number[b]	Total R^2	Endocrine ΔR^2	MGA ΔR^2		Nutrition ΔR^2		Log carcass wt. ΔR^2		
			Alone	Through endocrines	Alone	Through endocrines	Alone	Through endocrines	r^2
ADG (39)	80	17*	1	4	7*	19	3	0	32
DR-% (31)	89	7	3*	6	9**	9	8**	4	52
RD-% (34)	73	13	6*	4	2	2	21**	0	46
R-FT % (46)	60	14	0	0	14**	-2	13*	-3	30
R-LN % (47)	68	28*	4	1	28**	2	5	-4	2
R-BN % (48)	61	10	4	-1	2	0	16**	21	45
FL-% (35)	51	12	0	1	1	0	17**	3	37
F-FT % (36)	69	14	0	0	25**	-1	6	0	30
F-LN % (37)	69	15	0	1	23**	-1	6	1	30
R-L/B (49)	63	16	7	1	10	3	12*	21	26
R-F/L (50)	61	17	1	0	17**	-2	12*	-5	26
CB-% (32)	80	4	3	3	0	0	31**	18	67
CBW/L (33)	70	15	6*	5	2	-2	11**	14	41
RD-EE (22)	52	14	0	1	5	1	18**	-6	29
RD-WA (21)	53	13	0	1	6	0	20**	-7	31
Log (RD-WT (4))	92	4	2*	1	0	1	30**	33	85
Log (RD-FT (18))	78	8	0	1	7*	-1	23**	8	62
Log (RD-LN (19))	87	12*	0	0	6**	2	25**	37	66
Log (RD-BN (20))	53	5	13*	4	1	-1	13*	8	30
Log (FL-WT (6))	84	4	0	0	0	0	33**	16	79
Log (FL-FT (7))	82	6	0	0	7**	-1	19**	12	69
Log (FL-LN (8))	77	8	0	0	8*	-2	41**	15	62
Log (CB-WT (16))	58	13	9*	7	1	-1	5	7	23
TEND (23)	31	20	2	4	3	-3	7	-6	0.8
Log (EMA-V (38))	66	8	0	1	0	2	7*	20	55

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

easily in the previous models as the lots were generally not arranged factorially with the experimental treatments.

Table 18 lists significance levels for various sources of variation. Endocrine parameters, which had been analyzed in the previous model (groups 5, 6, 7 and 9), were not analyzed in this model as the only differences were the removal of group 9 and the inclusion of a lot effect, which had no significant effect on any variable except the ratio of plasma to adrenal corticosterone concentration (Table 18). Carcass quality measurements were all analyzed again in order to test for effects of the measures of adrenal cortex activity. The endocrine parameters as covariates in these analyses included mean thyroid cell height and the logs of mean jugular plasma GH level, plasma GH concentration at slaughter, pituitary GH concentration, plasma protein bound iodine concentration, plasma cortisol concentration, plasma corticosterone concentration, adrenal cortisol concentration and adrenal corticosterone concentration. In general, the significance levels for the endocrine parameters in Table 18 are lower than comparable values in Table 16. This appears to be mainly due to the decreased total degrees of freedom (29 vs. 39), and the increased degrees of freedom for endocrine parameters (8 vs. 6) in the analyses shown in Table 18. An example of the effect of these differences is shown in the analysis of log weight of lean in the round. Although the endocrine parameters did not significantly affect log

Table 18. Significance levels for the effects of various sources of variation on some endocrine and carcass quality measurements in groups 5, 6 and 7[a]

Source of variation	Treatment	Lot	Interaction	Log carcass weight	Endocrine parameters
Variable code and number[b]					
Log (PL-CO (27))	0.008**	0.869	0.514	0.896	
Log (PL-CS (28))	0.006**	0.906	0.257	0.757	
Log (AD-CO (29))	0.002**	0.726	0.665	0.244	
Log (AD-CS (30))	0.069	0.286	0.636	0.611	
AC-CN (52)	0.013*	0.878	0.679	0.229	
PC-CN (53)	0.025*	0.845	0.713	0.446	
ACSCN (56)	0.134	0.615	0.501	0.857	
PCSCN (57)	0.012*	0.529	0.338	0.557	
PL/PT (44)	0.118	0.626	0.499	0.294	
C-P/A (51)	0.012*	0.532	0.245	0.102	
CSP/A (55)	0.825	0.042*	0.406	0.084	
ADG (39)	0.355	0.210	0.298	0.168	0.005**
DR-% (31)	0.242	0.696	0.751	0.058	0.512
RD-% (34)	0.108	0.201	0.336	0.007**	0.396
R-FT % (46)	0.167	0.562	0.879	0.127	0.580
R-LN % (47)	0.156	0.942	0.419	0.823	0.380
R-BN % (48)	0.965	0.381	0.614	0.030*	0.948
FL-% (35)	0.096	0.918	0.478	0.648	0.317
F-FT % (36)	0.011*	0.566	0.793	0.515	0.740
F-LN % (37)	0.013*	0.541	0.710	0.532	0.766
R-L/B (49)	0.814	0.473	0.323	0.068	0.868
R-F/L (50)	0.152	0.584	0.811	0.155	0.509
RD-EE (22)	0.557	0.627	0.232	0.233	0.733
RD-WA (21)	0.613	0.569	0.211	0.216	0.793
CB-% (32)	0.826	0.690	0.331	<0.0005**	0.385
CBW/L (33)	0.513	0.822	0.189	0.040*	0.380
Log (RD-WT (4))	0.077	0.202	0.372	<0.0005**	0.364
Log (RD-FT (18))	0.316	0.831	0.920	0.018*	0.790
Log (RD-LN (19))	0.053	0.318	0.257	0.001**	0.179
Log (RD-BN (20))	0.328	0.132	0.907	0.240	0.816
Log (FL-WT (6))	0.112	0.913	0.419	0.003**	0.341
Log (FL-FT (7))	0.017*	0.627	0.660	0.031*	0.577
Log (FL-LN (8))	0.234	0.708	0.363	0.005**	0.444
Log (CB-WT (16))	0.625	0.795	0.419	0.342	0.474
Log (EMA-V (38))	0.248	0.716	0.092	0.001**	0.180
TEND (23)	0.958	0.257	0.467	0.209	0.914

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

round lean weight in this model (Table 18), they accounted for more of the variation in that parameter (18%) than they did in the previous model (12%), where they were statistically significant (Table 16).

Table 19 lists the percentage of variation in various carcass quality traits attributable to different sources of variation. The significant effect of the endocrine parameters on average daily carcass weight gain (Table 18) was due mainly to the effect of log mean jugular GH concentration ($P = 0.032$, $b = -0.26$) and log adrenal corticosterone concentration ($P = 0.043$, $b = -0.16$). The absence of any effects of the other corticosteroid measurements is probably a result of the positive correlations between these measurements and adrenal corticosterone concentration (Table 9). Although not statistically significant, the endocrine parameters with the greatest effects on log round lean weight were the same as those for the previous model (log plasma GH at slaughter--$P = 0.058$, $b = -0.06$, and log plasma protein bound iodine concentration--$P = 0.095$, $b = 0.087$).

Discussion of Least Squares Models

Because the four models discussed are not independent, they will all be considered together. Results of a particular model are not considered significant unless they are in agreement with the results of the others.

Table 19. The percent of variation in some carcass quality traits attributed to effects of treatment and log carcass weight, both directly and through an effect on endocrine parameters--groups 5, 6 and 7[a]

Variable code and number[b]	Total R^2	Endocrine ΔR^2	Treatment ΔR^2		Log carcass wt. ΔR^2		
			Alone	Through endocrines	Alone	Through endocrines	r^2
ADG (39)	89	32**	1	23	1	2	27
DR-% (31)	75	15	6	9	8	5	41
RD-% (34)	78	15	8	1	15**	2	50
R-FT % (46)	64	19	10	4	6	7	29
R-LN % (47)	64	27	11	15	0	0	3
R-BN % (48)	63	8	0	1	16**	15	46
FL-% (35)	67	28	13	-12	1	15	32
F-FT % (36)	68	13	29*	-2	1	4	24
F-LN % (37)	68	13	28*	-3	1	4	24
R-L/B (49)	62	11	1	8	10	13	29
R-F/L (50)	64	22	11	4	5	6	25
CB-% (32)	89	9	1	0	25**	9	79
CBW/L (33)	70	23	3	4	11*	13	20
RD-EE (22)	55	20	4	3	5	8	21
RD-WA (21)	54	19	4	4	6	8	20
Log (RD-WT (4))	90	9	5	-1	28**	26	76
Log (RD-FT (18))	74	10	5	4	4*	14	55
Log (RD-LN (19))	84	18	9*	2	22**	21	52
Log (RD-BN (20))	49	18	9	-1	6	5	12
Log (FL-WT (6))	87	10	5	-4	11**	28	74
Log (FL-FT (7))	83	10	14*	-5	8*	17	62
Log (FL-LN (8))	83	12	4	1	14	29	61
Log (CB-WT (16))	56	28	3	4	3	5	5
Log (EMA-V (38))	74	28	6	-6	36**	-8	38
TEND (23)	44	14	0	2	6	8	12

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

One way in which information on relationships between endocrine parameters and carcass quality parameters may be obtained from Tables 12 to 19 is to compare the effects of treatments on each of these groups of parameters. Thus, the significant effect of MGA treatment on adrenal weight and flank composition in the first model (Table 12) would suggest that these parameters may be correlated, but the fact that no such effects were shown in the third model (Table 16) suggests that this may not be a true effect. On the other hand, the significant interaction between age and MGA treatment in the first model (Table 12) indicates that MGA affects flank composition differently at different ages. In this case, the significant effect may have been at the early age only, with the animals receiving MGA having less flank fat. However, the biggest difference between the MGA treatment groups for log flank fat weight was between the group receiving no MGA and that receiving MGA from first estrus. As these two groups were treated similarly for those animals slaughtered at first estrus, the effect of MGA on flank composition, and therefore, the relationship between adrenal weight and flank composition, are probably non-existent.

The significant effect of age on pituitary GH content in the second model (Table 14) was not supported by the results of the first model (Table 12). Neither was the significant effect of nutrition on pituitary GH content that was found in the third model (Table 16) repeated in the

second model (Table 14). The effect of age on cannon bone weight was not consistent either. The significant treatment effects on log thyroid weight (Tables 14 and 16) are not considered important, since measures of skewness and kurtosis (1.90 and 7.40, respectively) indicated that this parameter was not normally distributed.

The results in Table 18 suggest a relationship between corticosteroids and composition, as treatment effects on measures of both these characteristics were significant. Furthermore, re-analysis of the carcass quality parameters without any endocrine parameters in the model revealed significant treatment effects on average daily gain and log round lean weight. Results of more detailed analyses on corticosteroids, daily gain and log round lean weight are given in Table 20. Flank composition showed essentially the same pattern as round composition with the primary effect being nutritional as shown by a comparison of group 5 with groups 6 and 7 (Table 20) rather than an MGA treatment effect, which is shown by comparing group 6 with group 7 (Table 20). In general, the results in Table 20 suggest that there may be a relationship between plasma corticosteroid levels and rate of gain as well as composition, with high levels being related to low growth rates and less fat.

Additional information on the relationships between carcass quality parameters and endocrine parameters is provided by significance estimates for the effects of endocrine parameters on carcass quality parameters and the estimates

Table 20. A comparison of treatment effects on measures of adrenal cortical activity and on average daily gain or log round lean weight in groups 5, 6 and 7[a]

Group comparison	Means			Significance levels		
	5	6	7	Overall treatment	5 vs 6 and 7	6 vs 7
Variable code and number[b]						
PL-CO (27)	5.61	4.59	2.70	**	**	*
PL-CS (28)	0.67	0.40	0.26	**	**	-
AD-CO (29)	2.68	2.73	0.04	*	-	*
AD-CS (30)	1.78	1.67	0.37	*	-	*
	Corrected Means					
ADG (39)	1.090	1.243	1.414	<0.0005**	**	*
Log (RD-LN (19))	1.597	1.569	1.567	0.042*	**	-

[a]See Table 2 for group classification.

[b]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

of the percent variation in the latter parameters that are accounted for by the former (Tables 12 to 18). The effect of plasma GH concentration on dressing percent shown in the first model (Table 12) did not show up in any of the other models and is, therefore, not considered important.

The significant negative relationship between mean jugular GH concentration and average daily gain in the third and fourth models is in agreement with the negative but non-significant correlations, which were reported between plasma GH concentration and growth rates of pigs by Siers (1968). It may be that low plasma GH levels reflect higher rates of GH utilization.

Although log plasma GH concentration significantly affected log round lean weight in the third and fourth models, it did not significantly affect measures of carcass composition in other models. It is difficult to explain why plasma GH concentration at slaughter should be related to round composition but not average daily gain, while mean jugular GH concentration was related to average daily gain but not to round composition. If one of these measures of GH is a good indicator of GH utilization, then the other is apparently not, as they are not well correlated ($r = 0.09$).

The significant negative relationship between adrenal corticosterone concentration and average daily gain in the fourth model supports the results in Table 20 that were discussed previously.

Estimates of the degree to which treatments influence carcass quality traits through effects on endocrine parameters (Tables 13, 15, 17 and 19) are difficult to interpret, but some are high enough that they merit discussion. Any effect of MGA treatment on the parameters considered did not appear to be through effects on the endocrine parameters, except perhaps in the case of dressing percent in the third model (Table 17). Here, MGA treatment had a significant direct effect by accounting for 3% of the variation. Thus the 9% of variation that was accounted for indirectly through the endocrine parameters may be important. No attempt was made to identify the most important individual endocrine parameters in this analysis. The data in Tables 13 and 15 suggest that age at slaughter did not affect carcass quality parameters through an effect on endocrine parameters.

The effect of nutrition on carcass quality measurements, however, does appear to be mediated through endocrine parameters in some cases. In the second and third models (Tables 15 and 17), an equal or greater proportion of the variation in dressing percent was accounted for by an indirect effect of nutrition than was accounted for directly. Also, the 19% of variation in average daily gain, which was accounted for by an indirect effect of nutrition in the third model (Table 17), probably explains why there was a significant nutritional effect on this measurement in the second model (Table 15) but not in the third (Table 17).

The greatest indirect effects are shown in the "Through Endocrine" columns under "Log Carcass Weight ΔR^2" in Tables 17 and 19. These estimates may be a measure of the degree to which carcass weight effects are expressed through endocrine effects or of the degree to which endocrine effects are expressed through carcass weight effects. It may be appropriate to consider them as resulting from a relationship between the aspects of carcass weight and endocrine parameters that affect the particular carcass quality trait in question. Thus, the percent variation in log flank weight that may be accounted for by the relationship between carcass weight and the endocrine parameters is 9, 7, 16 and 28% in the four models, respectively. In the case of the first two models, these effects may be partly due to the direct effects of carcass weight on some of the endocrine parameters, such as adrenal and thyroid weights (Tables 12 and 14). However, in the fourth model none of the endocrine parameters used as covariates were significantly influenced by carcass weight (Tables 16 and 18). Apart from log flank weight, other variables that had an appreciable percent of their variation accounted for by the relationship between log carcass weight and the endocrine parameters in both the third and fourth models were the percent bone of the round, the lean to bone ratio of the round, the ratio of weight to length of cannon bone, the log of round weight, the log of round lean weight and the log of the lean weight in the flank.

In order to study the effect of this relationship between log carcass weight and the endocrine parameters in more detail, log round lean weight in the fourth model was taken as an example and least squares models, each containing only one of the endocrine parameters, were run separately. In no case was the total R^2 greater than 72%. The greatest amount of variation that could be attributed to a relationship with log carcass weight was 9% for log plasma protein bound iodine concentration. The sum of the indirect or interaction effects between log carcass weight and each of the endocrine parameters individually was 9%. This suggests that part of the 18% variation in log weight of lean in the round, that was accounted for by an interaction between log carcass weight and all the endocrine parameters simultaneously (Table 19) was due to some form of interaction between the different endocrine parameters.

Correlation and Regression Analyses

Coefficients of correlations between some measures of carcass quality and some endocrine measurements are given in Table 21. Data from 90 animals are included except for round composition measures (40 animals), pituitary GH measurements (85 animals), mean jugular GH concentration (40 animals), tenderness (40 animals), thyroid activity (40 animals), and corticosteroid measurements (30 animals). Also, data from only 38 animals were involved in correlations between pituitary GH and round composition or tenderness.

Table 21. Simple correlation coefficients between some measures of carcass quality and some endocrine parameters

Code and number of carcass quality variable[a]	ADG (39)	RD-% (34)	R-FT % (46)	R-LN % (47)	R-BN % (48)	R-L/B (49)	F-FT % (36)	CB-% (32)	TEND (23)	DR-% (31)
Code and number of endocrine variable[a]										
JU-GH (24)	-0.37*	0.08	-0.08	-0.01	0.09	-0.08	-0.02	0.23	0.05	0.04
PL-GH (13)	-0.08	0.22	0.03	-0.24	0.22	-0.28**	-0.25*	0.28**	-0.07	-0.21
PT-GH (14)	-0.26*	0.45**	-0.34*	0.23	0.31	-0.19	-0.51**	0.48**	-0.02	-0.59**
PL-CT (43)	-0.02	-0.11	0.18	-0.29	0.05	-0.15	0.04	-0.07	-0.05	0.06
PT-CT (42)	-0.20	0.07	-0.25	0.34*	-0.05	0.18	-0.13	0.03	-0.17	-0.36**
GH/CC (45)	-0.29**	0.60**	-0.44**	0.36*	0.24	-0.06	-0.52**	0.57**	-0.12	-0.74**
PL/PT (44)	0.02	0.04	0.15	-0.33*	0.10	-0.20	-0.07	0.10	-0.07	0.04
PL-CO (27)	-0.64**	0.41*	-0.15	-0.05	0.30	-0.31	-0.26	0.46**	0.39*	-0.42*
AD-CO (29)	-0.69**	0.17	0.15	-0.19	0.00	-0.11	-0.01	0.14	-0.11	-0.41*
PC-CN (53)	-0.53**	0.20	0.02	-0.14	0.14	-0.20	-0.11	0.23	0.29	-0.24
AC-CN (52)	-0.64**	0.10	0.20	0.20	-0.05	-0.07	0.05	0.08	-0.11	-0.32
C-P/A (51)	0.47**	-0.15	-0.17	0.15	0.17	-0.08	-0.04	0.06	0.21	0.18
C/CC (54)	-0.70**	0.21	0.11	-0.15	0.03	0.12	-0.04	0.20	-0.03	-0.41*
PL-CS (28)	-0.68**	0.34	-0.15	0.07	0.12	-0.10	-0.27	0.37*	0.37*	-0.37*
AD-CS (30)	-0.69**	0.24	0.01	-0.02	0.01	-0.05	-0.15	0.19	0.07	-0.39*
PCSCN (57)	-0.61**	0.17	-0.02	-0.01	0.01	-0.03	-0.16	0.20	0.27	-0.24
ACSCN (56)	-0.66**	0.18	0.03	-0.04	-0.01	-0.04	-0.11	0.15	0.05	-0.33
CSP/A (55)	0.47**	-0.06	0.09	0.08	-0.21	0.24	0.11	-0.07	0.02	0.28
CS/CC (58)	-0.68**	0.27	-0.03	0.01	0.03	-0.05	-0.16	0.23	0.14	-0.40*
TH-CH (25)	-0.35*	0.13	0.03	-0.07	0.04	-0.08	0.09	0.14	0.11	0.05
PBI (26)	0.35*	-0.14	0.05	0.17	-0.33*	0.36*	0.09	-0.22	0.05	-0.14

[a]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

Some of the relationships suggested in Table 21 were analyzed in more detail and will be discussed separately for the corticosteroids, GH and thyroid activity.

Corticosteroids, Daily Gain and Tenderness

Measurements of the concentration or content of corticosteroids either in the plasma or the pituitary were consistently, significantly and negatively related to average daily carcass weight gain (Table 21). The relatively high correlations are to some extent attributable to the wide variation in all the variables involving corticosteroids. For the appropriate variables in Table 4, for example, the coefficients of variation are frequently greater than 50%. The only parameters involving corticosteroids that were not negatively related to average daily gain were the ratios of plasma levels to adrenal levels for both cortisol and corticosterone. In both of these instances, the relationships were highly significant and positive. Some possible implications of these correlations are discussed in a subsequent section (General Discussion).

Results in Table 21 also suggest that high circulating levels of corticosteroids are detrimental to cooked meat tenderness. The relationships between plasma corticosteroids and growth rates or tenderness were analyzed in more detail within groups and by the use of linear and curvilinear regression techniques (Table 22). These analyses

Table 22. Correlation and regression analyses of the relationship between plasma corticosteroid levels and growth rates or tenderness

Variable code and number[a]		PL-CO (27)		PL-CS (28)	
		ADG (39)	TEND (23)	ADG (39)	TEND (23)
Statistic	Group[b]				
r	5	-0.52*	0.83**	-0.83**	0.81**
r	6	-0.50*	0.12	-0.40	-0.07
r	7	-0.04	0.005	-0.05	-0.21
	Regression with linear component only				
r^2 (%)	5, 6 and 7	40.8	15.3	45.6	13.9
	Regression with linear and quadratic components				
R^2 (%)	5, 6 and 7	42.4	32.3	46.8	33.6
	Regression with linear, quadratic and cubic components				
R^2 (%)	5, 6 and 7	42.4	36.8	46.8	33.6

[a]See Table 5 for variable definitions.

[b]See Table 2 for group classification.

*P < 0.05

**P < 0.01

indicated that the correlation coefficients were lowest in the group which received the high level of nutrition plus MGA (group 7). Since this group had the lowest levels of corticosteroids (Table 20), it would appear that the hormones may only be important at high concentrations. If this were the case, then the relationships between tenderness or average daily gain and the steroid concentrations would be expected to be curvilinear rather than linear. Results in Table 22 indicate that this probably is the case for tenderness, as including a quadratic component in the regression analysis increased the percentage of variation accounted for from approximately 14 to approximately 33%. However, the percent of variation in average daily gain was not increased by including a quadratic component.

A possible alternative explanation for the very low correlations between corticosteroids and average daily gain in the group fed MGA (group 7, Table 22) is that the MGA accounted for much of the variation in daily gain that was normally accounted for by corticosteroids. This would suggest that MGA influences growth in ways other than through an effect on corticosteroid levels, although it was shown previously in this study (Table 20) that corticosteroid levels were significantly reduced in the group fed MGA.

Zimbelman (1966) suggested that MGA administration stimulates growth in heifers by the inhibition of LH release from the anterior pituitary. This lack of LH would prevent

ovulation and result in the maintenance of large estrogen-producing follicles. Thus, any MGA suppression of adrenal gland activity may be mediated through an effect on circulating estrogen levels, although data from humans (O'Connell and Welsh, 1969; Doe et al., 1969) indicate that increased estrogen levels give rise to increased plasma corticosteroid levels. Even though such a mechanism may account for the fact that MGA is effective only in females, it would not account for the low correlations between average daily gain and corticosteroids in the group fed MGA.

The relationships between corticosteroids and tenderness shown in Table 22 are not supported by any significant effect in Table 18, although the endocrine parameters did account for 14% of the total variance in tenderness (Table 19). This lack of significance can probably be attributed to the low percent of variation in tenderness that was accounted for by the fourth model as a whole (44%), and to the curvilinearity of the relationship (Table 22). The reason why tenderness should tend to be higher when corticosteroid levels are low is not clear. It would not appear to be through a quantitative effect on connective tissue, as corticosteroids generally inhibit the synthesis of connective tissue components (Grant, 1967). This does not rule out a qualitative effect, however. Corticosteroids generally increase the ratio of sodium to potassium in the body, but it is not known if this would affect tenderness. It is known,

however, that adding sodium chloride to meat will increase tenderness (Deatherage, 1963), apparently through an effect on water holding capacity.

Grant (1967) reviewed evidence indicating that corticosteroids may induce hypocalcemia. This may give rise to decreased tenderness as Huffman et al. (1969) found that blood calcium concentration in hogs was significantly correlated with taste panel assessments ($r = 0.37$), although they were not related to shear values ($r = -0.01$). On the other hand, it has also been shown that low calcium levels in meat increase tenderness, apparently by decreasing the degree of shortening associated with rigor mortis (Weiner, 1967).

A further action of corticosteroids, which may affect meat tenderness, is their tendency to stabilize lysosomal membranes (Grant, 1967). Since lysosomes contain proteolytic enzymes, it seems possible that increasing their stability may decrease meat tenderness. Finally, the gluconeogenic actions of corticosteroids on skeletal muscle (Ashmore and Morgan, 1967) may result in changes in the proportions of different muscle proteins in such a way as to affect the tenderness of cooked meat.

Growth Hormone and Composition

The low but significant correlation between mean jugular GH levels and average daily gain supports the results of the least squares models (Tables 16 and 18), which have been discussed previously.

The significant relationships between pituitary GH concentration or pituitary GH content per unit carcass weight and various measures of growth and composition (Table 21) were examined in more detail by the use of multiple regression analyses with linear and quadratic components. Analyses were made within the two age groups and also over all animals in groups 5 to 13 (Table 23). In most cases the reduction in R^2 on removal of either the linear or the quadratic component was not significant, indicating that the variation could be accounted for equally well by either of these components. However, in the case of average daily gain for animals slaughtered at first estrus, the quadratic component was significant ($P = 0.045$), suggesting that the relationship was curvilinear. Because pituitary GH content per unit carcass weight is a function of carcass weight, it is, as expected, more closely related to other parameters that are functions of carcass weight than is pituitary GH concentration.

In general, the relationships are much lower in the group slaughtered at first estrus. The animals slaughtered at breeding size were selected on the basis of withers height. Thus, the negative relationship between pituitary GH concentration and carcass weight in those animals implies that a high pituitary GH concentration gives rise to a lower carcass weight per unit withers height. In other words, the animals with high pituitary GH concentrations tended to have

Table 23. Results of multiple regression analyses containing linear and quadratic components to evaluate the relationships between measures of pituitary GH and some carcass quality parameters

Age at slaughter		First estrus (groups 10 to 13)		Breeding age (groups 5 to 9)		Either age (groups 5 to 13)	
		R^2	Sig.	R^2	Sig.	R^2	Sig.
Independent variable[a]	Dependent variable[a]						
PT-GH (14)	CC-WT (2)	6.5	0.306	27.0	0.001**	24.0	<0.0005**
	ADG (39)	11.7	0.114	24.1	0.002**	6.6	0.061
	DR-% (31)	20.4	0.019*	36.1	<0.0005**	34.6	<0.0005**
	RD-% (34)	6.5	0.310	13.4	0.042*	21.2	<0.0005**
	F-FT (36)	2.0	0.702	24.3	0.002**	16.6	0.001**
	CB-% (32)	11.4	0.119	16.9	0.017*	23.8	<0.0005**
GH/CC (45)	CC-WT (2)	11.2	0.126	27.3	0.001**	45.2	<0.0005**
	ADG (39)	5.4	0.378	23.4	0.003**	9.3	0.018*
	DR-% (31)	32.0	0.001**	50.8	<0.0005**	56.2	<0.0005**
	RD-% (34)	8.6	0.207	13.1	0.045*	37.8	<0.0005**
	F-FT (36)	5.4	0.382	17.7	0.014*	29.7	0.0005**
	CB-% (32)	4.3	0.467	16.8	0.018*	35.0	<0.0005**

[a]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

lower carcass weights at the same skeletal size. The effects of fat percent (negative), round percent (positive), bone percent (positive) and dressing percent (negative) shown in Tables 21 and 23 would be expected to accompany such an effect on the ratio of carcass weight to carcass size. This collection of effects seems most likely to have resulted from either a specific stimulation of bone growth or a specific inhibition of fat synthesis. Both of these actions have been attributed to GH in other species, but in this case, the negative relationship between pituitary GH concentration and average daily gain implies that the dominant effect was an inhibition of fat synthesis.

The relationships between measures of pituitary GH and carcass quality traits summarized in Table 23 are not supported by any significant results in any of the least squares models reported previously. This may be due to the fact that corrections were made for carcass weight in all these models. The relationships in Table 23 may, in fact, be concerned with some of the variation in several carcass quality parameters that was attributed to a relationship between carcass weight and the endocrine parameters (Table 19).

Thyroid Activity and Daily Gain

Although low, the correlations between measures of thyroid activity and average daily gain were significant. Regression analyses indicated that the inclusion of a quadratic component did not influence the percent variation in

average daily gain accounted for by protein bound iodine, but it did increase the amount accounted for by thyroid cell height by 7% (P = 0.090 for quadratic component). Thus, according to the regression equation (R^2 = 19%), a thyroid cell height of 9.32 microns would give rise to the lowest average daily gain. Other workers have found no clear-cut relationship between protein bound iodine and growth rate in beef cattle (Kunkel et al., 1957); while Gawienowski et al. (1955) showed a significant negative correlation (r = -0.60) between growth rate and protein bound iodine in swine. Not much importance is attached to the relationships between average daily gain and thyroid cell height or protein bound iodine shown in Table 21, because they are low, opposite in sign and not supported by other work. The results in Table 21 do not give any support for the significant effect of log protein bound iodine on log lean weight of the round shown in the third least squares model. The positive correlation between protein bound iodine and lean to bone ratio of the round, however, is supported by the work of Scow (1959) with rats. He showed that thyroxine administration preferentially stimulated the growth of muscle.

Correlations Between Endocrine Parameters

Correlations between measures of GH, corticosteroids and thyroid activity are given in Table 24. Measurements of any two hormones may be correlated either because the level

Table 24. Correlations between measures of the activity of different endocrine glands

Variable code and number[a]	PL-GH (13)	PT-GH (14)	JU-GH (24)	TH-CH (25)	PBI (26)
PL-CO (27)	0.42*	0.41*	0.31	0.20	-0.16
PL-CS (28)	0.35	0.42*	0.43*	0.17	-0.29
AD-CO (29)	0.23	0.28	0.33	0.11	-0.04
AD-CS (30)	0.23	0.40*	0.33	0.01	-0.04
TH-CH (25)	-0.12	-0.02	0.05		
PBI (26)	-0.26	-0.25	-0.14		

[a]See Table 5 for variable definitions.

*P < 0.05

**P < 0.01

of one is affected by the level of the other, or because they are both affected by some other factor. The fact that two hormones act additively on the same physiological process does not mean that their levels will be correlated. The results in Table 24 suggest no close relationships, but there does appear to be a consistently positive relationship between levels of corticosteroids and of GH. Work reviewed in an earlier section suggested that corticosteroids inhibit GH production, but that there are stimuli such as various forms of stress and hypoglycemia, which bring about production of both glucocorticoids and GH. The results in Table 24 suggest that the effect of common stimuli may have overridden other effects in the animals of this study. All hormone data discussed, except the jugular levels of GH, represent values

at slaughter, so that stress may have acted as a common stimulus for GH and corticosteroids.

Age Effects on Plasma Growth Hormone

Figure 9 shows the changes in GH concentration of jugular samples taken at monthly intervals from heifers in groups 5, 6, 7 and 9. Each point on each curve represents the mean of ten values. For the statistical analysis of these data, only groups 5, 6 and 7 were considered since these groups each contained animals from the same two lots, while group 9 was made up of animals from two different lots. Thus, the effect of treatment would have been completely confounded with lot effects had group 9 been included in the analysis.

A split plot design was used to analyze the data with group-lot combinations representing the whole plots, and ages representing the sub-plots. Table 25 summarizes the analyses of variance for GH concentrations in jugular plasma and for the logs of these values.

Because of sequential non-random sampling from the same animals, it is possible that the variance-covariance matrix among ages taken over all groups and lots is non-homogeneous. If this is so, the significance of age effects will be over-estimated, and the probability estimates given in Table 25 will be underestimated. The application of a conservative F test, whereby the degrees of freedom for the

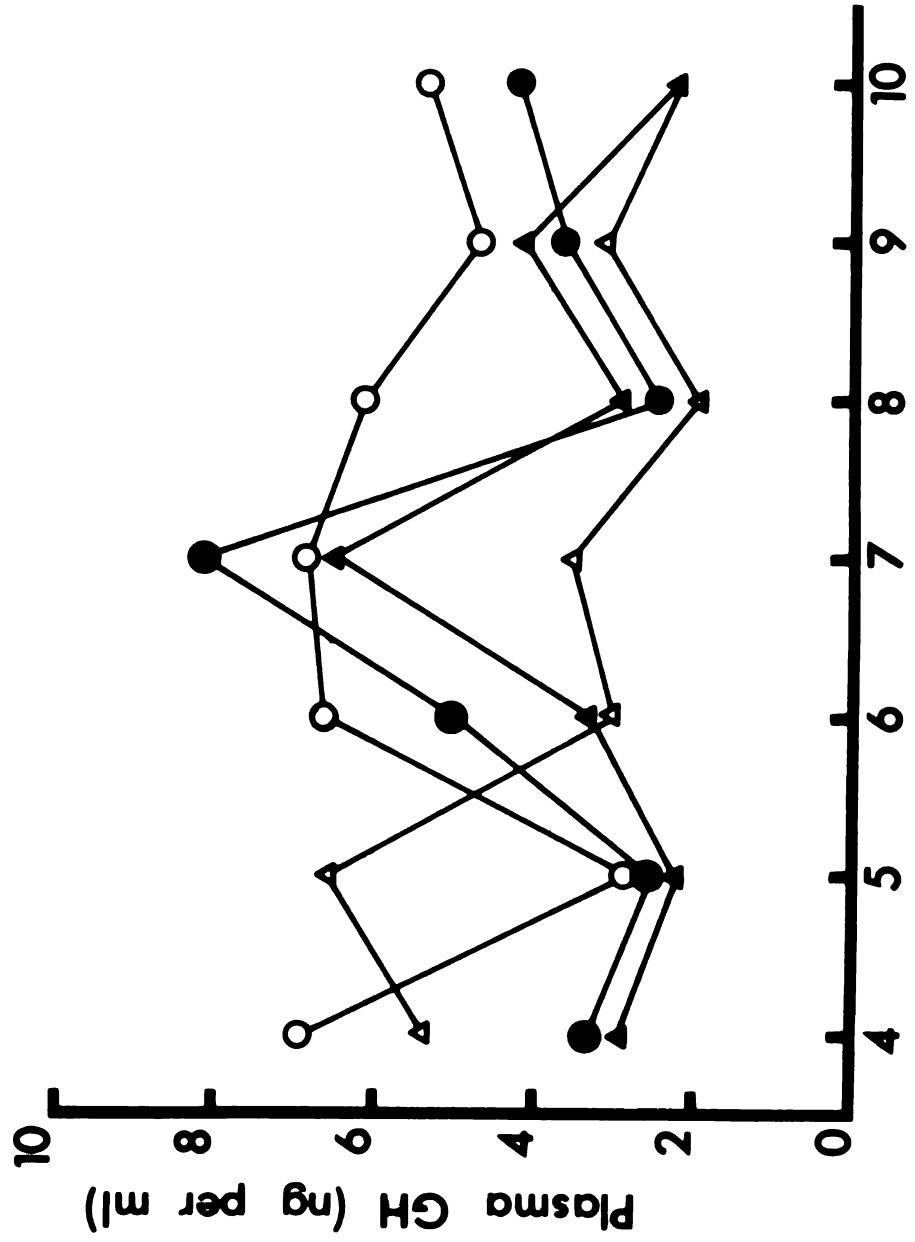

Figure 9. Changes in the concentration of plasma growth hormone with age in Holstein heifers. High plane of nutrition (●—●), normal plane of nutrition (○—○), high plane of nutrition with MGA (▲—▲), normal plane of nutrition with MGA (△—△).

Table 25. The effects of treatments, lots and age on jugular GH levels in groups 5, 6 and 7[a]

Variable	Plasma GH concentration		Log (Plasma GH concentration)	
Statistic	F	Sig.	F	Sig.
Source of variation				
Treatment	2.57	NS[b]	3.70	<0.05
Lot	1.49	NS	0.40	NS
Lot x Treatment	0.32	NS	0.12	NS
Age	2.95	0.009	3.14	0.006
Age x Trt	0.58	NS	0.89	NS
Age x Lot	1.66	NS	2.79	0.013

[a]See Table 2 for group classification.

*P > 0.05

two mean squares in the F ratio are divided by the smaller degree of freedom, indicated that the age effects were not statistically significant. Thus, the significance of age on the GH level of jugular plasma samples is questionable. Also the pattern of change with age shown in Figure 9 for groups 5, 6 and 7 has no apparent explanation, and the changes shown for group 9 animals do not follow the pattern set by the other groups.

The fact that there is a significant treatment effect on log GH concentration, but not on the absolute values of GH concentration, emphasizes the fact that the

use of log transformations of hormone measurements may be appropriate. Orthogonal contrasts indicated that the treatment effect was due to a nutritional rather than an MGA effect, with the animals on the normal level of nutrition having higher GH concentrations. This is in agreement with the correlation analyses (Table 21) and the least squares models (Tables 17 and 19) discussed previously. The relatively large error terms associated with the analyses in Table 25 may be partly due to short term changes in plasma GH concentrations, induced by factors such as the bleeding procedure. A supplemental study revealed that the concentration of GH in the jugular vein does change over a short period of time when samples are drawn by syringe. This was shown by bleeding three cows four times during a period of 24 hours (Table 26). Table 26 indicates that there were no consistent diurnal changes. Neither was there a consistent relationship between the concentrations of samples taken at slaughter and those taken prior to slaughter. Comparable results were obtained by Eaton et al. (1968), who showed that bleeding procedures may affect the plasma GH levels of cows.

Table 26. Plasma GH levels (ng/ml) of samples taken by syringe from the jugular vein and of samples collected at slaughter for three Holstein heifers

Animal number	Sampling time (hrs before slaughter)				
	21.5-22.0	17.5-18.0	13.5-14.0	0.5-0.8	At slaughter
344	4.42	1.69	1.88	2.70	3.05
357	1.83	1.64	5.16	2.71	1.83
368	2.52	1.76	0.97	2.28	4.86

General Discussion

The data from this study suggest that only a few relationships between endocrine parameters and carcass quality or growth parameters are close enough to be valuable. However, the null hypothesis that endocrine parameters do not affect meat quality has not been proved for any combination of a particular hormone and a particular meat quality parameter. The fact that no relationship is shown in one situation does not preclude the existence of such a relationship in some other situation, where variation in the quality attribute is due to other factors, and/or where the hormone status is assessed in another way.

Variation in carcass quality parameters can generally be attributed to either genetic, environmental or age and/or size effects. In this study the variation due to age at slaughter can be attributed to age or size effects. The

variation due to plane of nutrition and to MGA administration can be classified as effects of the external environment, while variation within each treatment group can be attributed to genetic effects. When considering a particular hormone, the effects of the other hormones can be classified as an aspect of the internal environment. Thus, the internal environment will also be influenced by genetic, environmental and age or size effects.

The objective of this study was to investigate possible relationships between endocrine and carcass quality parameters. Therefore, the total effects of the above variables on the carcass quality parameters are not of as much interest as the degree to which these factors act through the endocrine characteristics.

Whether or not the status of an animal with regard to a particular hormone is effectively estimated by a particular measure of that hormone will depend on a number of factors. Some of these factors and an assessment of the usefulness of the measures made in this study will be discussed.

If the hormone status of an animal is defined as the influence that the hormone has at the site(s) of action, then hormone status will be a function of effectiveness per unit weight and of quantity of hormone. The effectiveness per unit weight of a hormone at the site of action is frequently assumed to be constant, although it will probably be

affected by a number of factors including other hormones. The fact that measures of different hormones were included as covariates in the least squares analyses discussed previously should account to some extent for hormone interactions at the tissue level. If hormone effectiveness per unit weight is fairly constant, then variation in the quantity of hormone at the site of action will account for most of the variation in hormone status. The quantity of hormone present will, in turn, be a function of the turnover rate of the hormone in the tissue and of the rate of uptake from the blood stream. If it is assumed that the turnover rate is constant, then factors affecting rate of uptake will be the principle determinants of hormone status. Thus, plasma hormone level should provide a good indication of hormone status, when it is the main variable influencing rate of hormone uptake from the circulation. The results of this study indicated that plasma levels of GH at slaughter were not closely related to carcass quality parameters. Also plasma GH at slaughter was poorly related to the mean of seven GH determinations on jugular plasma collected at monthly intervals. The mean jugular GH level was significantly and negatively related to growth rates, but was not related to other carcass quality parameters. This would suggest that either the measurements made were not good indicators of GH status or that GH status is not related to carcass quality. It is also possible that the single

measurements made, and the way in which the samples were collected, resulted in non-representative values for plasma GH.

Hunter (1968) discussed the diurnal and day to day changes in plasma GH concentrations that have been demonstrated in man and suggested that in order to investigate relationships between plasma GH levels and growth velocity, it may be necessary to develop standardized tests. He suggested three possibilities: (1) measurement following a standardized period of exercise, (2) measurement of the secondary rise in GH during an extended glucose tolerance test, and (3) a standardized insulin sensitivity test.

The consistent correlations between measures of corticosteroids and growth rate and the curvilinear relationship between both plasma cortisol and corticosterone levels and tenderness suggested that the measurements made were related to glucocorticoid status, and that the glucocorticoids play a role in determining carcass quality. Corticosteroid levels are also known to vary diurnally, at least in humans (Dixon et al., 1967), but the changes appear to follow a more definite pattern than do GH changes. Thus, the fact that all the animals were slaughtered at the same time of the day may have decreased diurnal effects.

If the rate of uptake of a hormone from the circulation is primarily determined by factors other than plasma levels, then the turnover rate of the hormone in the plasma

may be more closely related than plasma levels to the hormone status. The plasma turnover rates were not measured for any hormones in this study. No reference could be found to any work which has attempted to relate turnover rates of GH or corticosteroids to carcass quality. Work concerning thyroxine turnover rates has been discussed previously herein.

Another measurement, which should be at least as good an indicator of rate of uptake under most conditions as either plasma levels or plasma turnover rate, is the rate of release of the hormone from the endocrine gland. Again, direct measures of rate of release were not made in this study. However, concentrations of GH and corticosteroids in the pituitary gland and adrenal glands, respectively, were measured in hopes that these values would be indicative of rates of release.

Although very little direct evidence is available concerning the relationship between pituitary GH levels and the rate of release of GH from the pituitary, some information may be acquired from what is known about the mechanism of action of GH releasing factor (GRF). If GRF stimulates GH release indirectly by stimulating GH synthesis, then the relationship between level and rate of release should be close. If GRF stimulates release directly, however, with any effect on synthesis being indirect, then the above relationship is less likely to be close. McCann and Porter (1969) in reference to releasing factors in general suggested

that "a reasonable explanation would be that an effect on release is primary, but that effects on synthesis also occur and that they may be required for appreciable release of hormone in some cases and not in others." This suggests that the relationship may not be close. Also, the fact that the total pituitary content of GH is much greater than the content of GH in the plasma (Table 4) would suggest that even if a close relationship does exist it may be difficult to detect, since an appreciable change in the rate of release may result in an undetectable change in pituitary GH concentration.

In the case of the corticosteroids, however, the quantity in the adrenal and in the plasma appears to be approximately the same (Table 4). Furthermore there is some evidence that adrenal steroid levels are a good indication of secretion rates. Holzbauer (1957) using rats, showed that under a number of different situations the ratio of corticosterone concentration in the adrenal glands to the rate of release of corticosterone from the adrenals was quite constant.

If this relationship is close, then it may be expressed in the following way:

$$\left(\frac{dQ}{dt}\right)_{\text{release}} = kA$$

where, $\left(\frac{dQ}{dt}\right)_{release}$ = rate of release of corticosteroids from the adrenals

k = constant

A = corticosteroid concentration in the adrenals.

Also, we know that metabolic clearance rate (MCR), which is a measure of turnover rate, is related to plasma hormone levels (P) in the following way (Tait and Burstein, 1964):

$$MCR = \frac{\left(\frac{dQ}{dt}\right)_{remove}}{P}$$

where $\left(\frac{dQ}{dt}\right)_{remove}$ = rate of removal of corticosteroids from the circulation.

Thus, under steady state conditions the rate of release will be equal to the rate of removal and:

$$MCR = k\,\frac{A}{P}\ .$$

If this equality holds, then an increase in the ratio of plasma concentration to adrenal concentration of corticosteroids will indicate a decreased MCR, or plasma corticosteroid turnover rate. Thus, the significant positive correlations between the ratio of plasma and adrenal concentrations of both cortisol and corticosterone, and average daily gain (Table 21) would suggest that faster gaining animals had a lower turnover rate of cortisol and corticosterone. This seems to fit in with other correlations in Table 21, which suggest that corticosteroids in the plasma and in the adrenal are lower in faster growing animals.

It has been suggested (Baird et al., 1952; Nalbandov, 1963) that the quantity of GH available per unit tissue is an important determinant of growth, and that the decrease in growth with increasing age and size results from tissue weight increasing at a faster rate than the total quantity of GH. In investigating this possibility, an appropriate measure of growth is the proportional increase in size or the specific growth rate. This is equal to the growth rate at a particular time divided by the weight at that time:

$$\text{Specific growth} = \frac{d\ (\text{weight})}{d\ (\text{time})} \times \frac{1}{\text{weight}} .$$

The growth rates of the heifers in this study were fairly constant for the duration of the experiment (Pritchard, 1970), so that:

$$\text{Specific growth} = \frac{\text{weight - birth weight}}{\text{age}} \times \frac{1}{\text{weight}} .$$

If it is assumed that birth weight is zero, then the reciprocal of age will provide a measure of specific growth rate.

When all groups in this study were considered, plasma GH level was positively related to the above measure of specific growth rate ($r = 0.24$, $P < 0.05$), as was pituitary GH content per unit carcass weight ($r = 0.50$, $P < 0.01$). However, within the two age groups the comparable correlations were essentially zero. This suggests that the availability of GH may play a role in determining changes in specific growth rate with age, although it probably does not

account for variation in specific growth rate in a group of animals of similar age and size. Other work pertaining to Nalbanov's "dilution theory" has been discussed previously.

Although the endocrine measurements made in this study have generally accounted for quite a small proportion of the variation in carcass quality parameters, the relationships may be of some practical usefulness, if the variation accounted for is largely due to additive genetic effects. Heritability estimates for carcass quality and growth characteristics in beef cattle are not particularly high (Warwick, 1968), so that estimates of additive genetic effects would be particularly valuable.

SUMMARY AND CONCLUSIONS

Relationships between some carcass quality or growth parameters and growth hormone and other endocrine measurements were investigated using 90 Holstein heifers in nine treatment groups. Two levels of nutrition, three MGA treatments and two ages at slaughter were involved.

Growth rates, carcass composition and meat tenderness were determined on some or all of the animals. A double antibody radioimmunoassay was developed for measuring bovine growth hormone (GH). This assay did not cross react with bovine prolactin, LH, FSH, or TSH. The dose response curves for anterior pituitary extracts and plasma paralleled that for standard bovine GH. Radioimmunoassay values agreed satisfactorily with bioassay values.

Modifications of existing procedures for estimation of cortisol and corticosterone in bovine blood plasma and adrenal homogenates were utilized to assess the adrenal corticol activity of some animals. Plasma protein bound iodine concentrations and thyroid follicular cell heights were determined as indices of thyroid activity in some of the animals.

Several statistical approaches were used in attempts to characterize the relationships being investigated. Simple

correlation coefficients were calculated and a number of regression analyses containing linear and quadratic components were made. General least squares models were employed to assess the effects of endocrine parameters on carcass quality parameters after corrections had been made for the effects of nutrition, age at slaughter, MGA treatment and carcass weight. A similar influence of any of these factors (nutrition, age, MGA) on both a carcass quality and an endocrine parameter was considered indicative of a relationship between those two parameters. Estimates were also made of the degree to which age, nutrition, MGA and carcass weight influenced carcass quality indirectly through endocrine effects.

Levels of jugular GH from four to ten months of age were significantly higher in a group of heifers on a normal level of nutrition than in similar animals on a high level of nutrition or on a high level of nutrition plus MGA ($P < 0.05$). The mean of seven jugular GH levels was significantly and negatively related to average daily carcass weight gain, both on the basis of a simple correlation coefficient ($r = -0.37$) and a least squares model ($P = 0.01$). Plasma GH level at slaughter was not related to the mean jugular GH level ($r = -0.07$), and was not consistently related to any carcass quality traits. Growth hormone concentration in anterior pituitary extracts was significantly correlated with carcass weight in animals which were slaughtered upon reaching a withers height of 120 cm ($r = -0.51$).

This suggests that animals with high pituitary GH levels had less carcass weight per unit withers height. Support for such a suggestion was given by significant correlations showing that high levels of pituitary GH were associated with decreased fatness, decreased dressing percent and an increased percentage round. Since pituitary GH levels were significantly and negatively related to average daily carcass weight gain in the same animals ($r = -0.49$), it seems likely that the dominant effect of GH may have been to decrease fat deposition. The absence of any significant effects of pituitary GH levels in the least squares models may have been because carcass weight was included as a covariate. Thus, pituitary GH levels were more closely related to carcass quality and growth than levels of GH in plasma collected at slaughter or by syringe. Although supported by some other work, the negative relationships between GH and growth were unexpected.

Measures of both total content and concentration of cortisol and corticosterone in blood plasma and adrenal homogenates of 30 animals were significantly ($P < 0.01$) and negatively correlated with average daily carcass weight gain. Only adrenal corticosterone had a signficant negative effect on average daily gain in the least squares models, possibly because of the moderately high correlations between cortisteroid measurements. The fact that average daily gain was more closely related to cortisteroids than GH in this study

may have been because the measurements of corticosteroids more closely reflected the true status of these hormones.

Plasma levels of cortisol and corticosterone accounted for 32.3 and 33.6 percent of the variation in tenderness, respectively, when quadratic components were included in the regression equations. It is not clear why high levels of corticosteroids tended to decrease tenderness.

Plasma protein bound iodine concentration was significantly and positively related to the weight of lean in the round according to a least squares equation, but it was not significantly correlated with percent lean in the round ($r = 0.17$). No clear relationships between measures of thyroid activity and growth rates were shown.

Correlations between measures of GH and corticosteroids were not all significant but they were consistently positive, suggesting that stress at slaughter may have affected these measurements similarly.

None of the relationships investigated in this study were particularly close, but they may be of practical utility, if the variation in carcass quality accounted for by endocrine parameters was primarily due to additive genetic effects.

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APPENDIX

Appendix 1. Raw data with means and standard deviations.

Variable code and number[a]							
AN-NO (1)	CC-WT (2)	SL-WT (3)	RD-WT (4)	SP-GR (5)	FL-WT (6)	FL-FT (7)	FL-LN (8)
			Group 8				
271	485	855	58.65	1.095	12.90	5.44	7.28
277	560	990	65.45	1.083	17.80	6.92	10.60
276	525	895	59.40	1.095	13.20	5.94	7.05
233	515	900	62.50	1.087	11.90	5.67	6.09
243	485	850	60.80	1.099	11.60	4.90	6.60
246	475	850	60.30	1.092	11.50	4.30	7.10
234	425	760	54.50	1.090	10.80	4.30	6.40
201	508	880	56.50	1.093	12.70	6.25	6.31
290	405	760	53.50	1.089	9.30	3.50	5.70
289	385	700	49.60	1.101	8.30	2.94	5.36
Mean	477	844	58.12	1.092	12.00	5.02	6.85
Std. Dev.	56	84	4.67	0.005	2.57	1.26	1.45
			Group 10				
300	314	600	43.55	1.102	6.70	2.21	4.42
373	240	500	36.60	1.100	5.09	1.60	3.48
354	295	590	40.70	1.099	6.70	2.65	4.00
311	315	630	43.90	1.095	8.25	2.84	5.30
320	305	575	43.30	1.098	7.10	2.10	4.93
355	310	605	43.00	1.107	7.10	2.37	4.63
292	266	515	37.50	1.090	6.60	1.74	4.87
293	265	525	36.60	1.091	5.60	1.59	3.92
366	270	555	39.60	1.100	6.35	1.94	4.39
363	290	575	41.35	1.098	6.15	2.11	4.00
Mean	287	576	40.61	1.098	6.56	2.12	4.39
Std. Dev.	26	42	2.90	0.005	0.87	0.42	0.55
			Group 11				
347	260	535	36.55	1.089	6.20	2.25	3.90
352	310	585	43.35	1.096	6.35	2.48	3.86
350	300	587	40.15	1.097	7.85	3.01	4.73
371	260	510	38.00	1.099	5.00	1.59	3.32
358	275	550	42.40	1.095	5.85	2.00	3.77
309	255	500	36.10	1.094	5.58	1.66	3.86
308	310	570	40.50	1.095	6.45	2.46	3.94
301	261	470	34.10	1.101	4.80	1.79	3.00
315	260	490	37.50	1.087	5.30	1.90	3.30
298	215	415	30.80	1.104	4.30	1.40	2.90
Mean	271	521	37.95	1.096	5.77	2.05	3.66
Std. Dev.	29	55	3.83	0.005	1.01	0.49	0.54

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]							
AN-NO (1)	CC-WT (2)	SL-WT (3)	RD-WT (4)	SP-GR (5)	FL-WT (6)	FL-FT (7)	FL-LN (8)
				Group 12			
360	260	530	35.00	1.099	6.60	2.28	4.28
365	310	595	42.25	1.091	6.95	2.40	4.45
359	325	635	40.80	1.093	8.15	3.57	4.50
362	240	500	36.85	1.094	5.60	1.48	4.00
297	295	540	39.90	1.100	5.60	1.60	3.93
291	236	435	32.80	1.096	4.30	1.25	2.97
295	260	500	37.50	1.100	5.30	1.47	3.81
307	275	535	38.20	1.095	6.70	2.32	4.25
302	330	610	44.00	1.092	6.85	2.59	4.12
353	245	495	34.85	1.098	5.45	1.63	3.71
Mean	278	538	38.22	1.096	6.15	2.06	4.00
Std. Dev.	35	61	3.54	0.003	1.10	0.71	0.45
				Group 13			
351	370	700	49.60	1.087	7.63	2.78	4.78
367	255	520	36.20	1.093	4.35	1.41	2.90
321	300	540	40.05	1.102	6.20	1.60	4.52
303	405	755	52.75	1.101	10.05	3.86	6.13
356	270	550	40.80	1.095	6.23	2.04	4.12
361	260	525	36.35	1.097	5.60	2.29	3.25
319	285	520	37.90	1.092	6.30	2.43	3.68
317	310	580	41.90	1.096	6.80	2.83	3.74
299	320	595	43.50	1.097	6.70	2.52	4.07
349	270	535	38.00	1.098	6.25	1.92	4.23
Mean	304	582	41.71	1.096	6.61	2.37	4.14
Std. Dev.	15	82	5.56	0.004	1.48	0.70	0.90
				Group 5			
242	405	720	49.80	1.090	9.75	4.47	5.05
221	425	805	57.40	1.097	10.35	3.28	6.87
229	460	840	60.00	1.089	10.80	4.57	5.97
238	470	840	60.80	1.096	12.50	4.38	7.81
226	445	805	56.85	1.098	11.50	4.99	6.42
266	395	750	54.70	1.095	9.80	3.32	6.28
264	345	665	50.40	1.100	7.65	2.70	4.89
263	440	795	54.90	1.090	10.30	3.68	6.56
278	440	815	56.45	1.102	10.35	3.01	7.19
251	465	865	58.50	1.100	10.35	4.32	5.91
Mean	429	790	55.98	1.096	10.34	3.87	6.29
Std. Dev.	38	61	3.66	0.005	1.25	0.77	0.90

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]							
AN-NO (1)	CC-WT (2)	SL-WT (3)	RD-WT (4)	SP-GR (5)	FL-WT (6)	FL-FT (7)	FL-LN (8)
			Group 6				
223	495	860	62.40	1.090	13.40	5.80	7.30
204	440	795	58.90	1.097	9.30	3.80	5.40
241	465	830	57.80	1.089	12.70	5.76	6.68
217	435	765	53.60	1.095	11.30	4.90	6.10
214	500	890	59.30	1.088	16.55	8.51	7.81
254	440	820	55.95	1.094	12.28	5.73	6.35
255	380	695	49.30	1.089	9.90	4.61	5.00
262	410	740	51.10	1.094	9.30	3.75	5.40
279	480	840	61.50	1.085	11.05	5.28	5.64
285	400	710	52.70	1.100	9.30	4.22	4.90
Mean	444	794	56.26	1.092	11.51	5.24	6.06
Std. Dev.	41	65	4.46	0.005	2.32	1.39	0.98
			Group 7				
230	535	935	62.40	1.090	13.00	6.05	6.80
237	465	855	55.00	1.063	10.70	4.90	5.80
219	450	805	58.30	1.081	10.10	4.60	5.40
202	510	890	63.00	1.087	16.10	7.40	8.30
240	595	1040	66.90	1.080	20.25	10.69	8.89
253	485	855	56.65	1.083	16.80	9.37	7.09
256	515	925	63.50	1.094	15.25	6.82	8.09
260	450	810	53.10	1.095	12.15	5.41	6.51
265	480	825	59.20	1.094	11.70	4.67	6.89
282	434	785	51.10	1.097	11.00	4.52	6.36
Mean	492	873	58.92	1.086	13.71	6.44	7.01
Std. Dev.	48	77	5.05	0.010	3.28	2.15	1.11
			Group 9				
322	430	815	53.20	1.101	11.00	4.00	6.81
364	330	665	42.50	1.088	7.20	2.44	4.64
305	430	840	55.25	1.099	11.55	4.14	6.89
294	450	890	57.05	1.096	12.55	3.90	8.51
312	470	870	58.00	1.096	11.80	4.73	6.88
368	405	795	51.00	1.101	10.70	4.62	5.91
318	440	835	55.15	1.100	10.08	3.99	6.07
357	400	765	53.20	1.102	8.35	2.57	5.62
344	375	755	48.00	1.100	8.65	3.72	4.75
369	330	670	45.20	1.098	6.95	1.98	4.91
Mean	406	790	51.86	1.098	9.88	3.61	6.10
Std. Dev.	48	77	5.14	0.004	1.98	0.95	1.21

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]							
AN-NO (1)	CC-WT (2)	SL-WT (3)	RD-WT (4)	SP-GR (5)	FL-WT (6)	FL-FT (7)	FL-LN (8)
			Group 14				
296	98	191	14.90	1.088	1.69	0.42	1.25
310	106	196	16.75	1.084	1.37	0.31	1.04
313	110	203	16.00	1.088	1.82	0.45	1.35
314	117	224	17.70	1.084	1.85	0.56	1.27
316	124	205	17.60	1.084	2.47	0.57	1.87
346	89	175	13.55	1.071	1.65	0.33	1.29
348	102	190	15.21	1.084	1.38	0.33	1.04
345	127	235	18.07	1.077	2.11	0.47	1.60
343	110	205	16.71	1.084	1.57	0.33	1.21
342	157	275	23.69	1.091	2.50	0.62	1.84
Mean	114	210	17.02	1.084	1.84	0.44	1.38
Std. Dev.	19	29	2.74	0.000	0.40	0.11	0.30

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]									
AN-NO (1)	AGE (9)	TH-WT (10)	AD-WT (11)	AP-WT (12)	PL-GH (13)	PT-GH (14)	EMA (15)	CB-WT (16)	CB-LE (17)
					Group 8				
271	330	24.6	18.04	1.15	1.47	0.146	10.62	247.8	21.3
277	399	42.6	16.34	1.48	1.62	0.110	11.67	304.7	21.7
276	405	93.3	15.98	1.50	2.02	0.099	11.76	268.5	21.0
233	363	41.5	14.87	1.76	7.38	0.141	11.12	288.5	21.0
243	339	18.8	14.55	1.42	1.56	0.123	10.18	267.9	22.2
246	330	23.5	13.56	1.62	1.49	0.110	10.50	289.6	21.3
234	330	14.1	12.41	1.41	2.04	0.094	9.40	260.4	21.3
201	369	13.7	12.83	1.61	1.60		9.75	295.9	21.8
290	267	17.3	11.28	1.15	2.67	0.158	9.37	279.7	22.2
289	279	11.5	12.72	1.12	2.04	0.144	9.14	282.8	22.0
Mean	341	30.1	14.26	1.42	2.39	0.125	10.35	278.6	21.6
Std.Dev.	45	24.8	2.09	0.22	1.79	0.022	0.95	17.4	0.5
					Group 10				
300	276	39.1	12.22	1.13	4.75	0.122	8.86	251.0	21.5
373	240	19.0	9.58	0.95	3.26	0.129	7.65	208.4	20.9
354	300	14.4	13.18	1.30	3.52	0.222	6.46	216.0	21.0
311	285	17.5	12.95	2.32	1.88	0.120	8.84	217.9	20.8
320	270	13.9	10.70	1.32	3.06	0.171	8.98	221.8	20.3
355	273	167.8	14.05	1.05	14.56	0.275	7.28	234.7	20.5
292	210	14.3	10.32	1.26	2.44	0.228	6.46	192.9	20.2
293	201	37.5	11.14	1.13	7.95		7.70	191.5	20.5
366	249	22.7	10.25	1.10	6.86	0.210	8.50	209.4	21.0
363	291	22.3	10.96	0.85	4.16	0.298	8.43	233.4	21.6
Mean	260	36.8	11.54	1.24	5.24	0.197	7.92	217.7	20.8
Std.Dev.	34	46.9	1.48	0.41	3.79	0.066	0.96	18.6	0.5
					Group 11				
347	216	28.8	10.68	1.04	1.35	0.216	8.25	197.9	21.2
352	234	17.7	15.32	1.07	2.72	0.104	8.20	236.6	21.4
350	213	12.7	13.35	1.03	1.62	0.186	7.85	217.9	21.3
371	201	18.6	10.41	0.89	12.06	0.271	8.24	225.2	21.0
358	225	35.0	10.52	1.15	3.52	0.228	9.20	222.8	21.1
309	337	23.0	10.41	1.11	2.59	0.141	7.90	194.3	20.1
308	231	16.8	12.16	0.99	2.43	0.145	10.21	198.4	19.2
301	195	16.7	10.67	0.85	4.79	0.201	8.43	182.1	19.8
315	189	14.0	9.61	0.95	2.62	0.162	7.89	200.5	20.1
298	195	16.2	13.01	0.80	5.20	0.078	7.38	174.8	19.7
Mean	224	20.0	11.61	0.99	3.89	0.173	8.36	205.1	20.5
Std.Dev.	43	7.0	1.79	0.11	3.12	0.059	0.81	19.9	0.8

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]									
AN-NO (1)	AGE (9)	TH-WT (10)	AD-WT (11)	AP-WT (12)	PL-GH (13)	PT-GH (14)	EMA (15)	CB-WT (16)	CB-LE (17)
				Group 12					
360	213	22.0	10.09	1.19	2.93	0.281	6.95	199.8	20.2
365	240	21.4	11.29	1.01	1.56	0.232	9.18	219.0	21.4
359	234	62.7	13.27	1.36	1.80	0.144	9.10	208.6	20.4
362	225	19.8	8.12	0.95	2.42	0.265	7.60	195.2	20.1
297	189	15.7	11.22	1.09	8.26	0.148	8.50	204.9	20.8
291	195	13.5	7.94	0.97	3.26		8.58	179.3	20.1
295	189	20.1	10.07	0.89	8.30	0.173	8.82	206.5	20.7
307	237	26.5	9.83	1.09	1.53	0.123	8.00	205.5	20.3
302	231	16.8	12.16	1.38	4.40	0.158	9.75	203.4	20.2
353	204	21.9	9.67	0.91	3.82	0.264	7.82	191.8	20.1
Mean	216	24.0	10.37	1.08	3.83	0.199	8.43	201.4	20.4
Std.Dev.	20	14.1	1.67	0.18	2.53	0.061	0.84	10.8	0.4
				Group 13					
351	252	16.9	12.92	1.00	1.18	0.222	10.91	263.7	22.2
367	258	21.3	13.40	0.78	2.21	0.197	7.09	231.7	20.6
321	240	14.9	15.20	1.28	1.62	0.134	8.96	201.7	19.2
303	321	17.9	12.64	1.52	3.24	0.127	9.05	278.2	21.6
356	204	29.4	13.76	1.15	2.48	0.182	7.48	231.4	20.8
361	225	15.6	9.07	1.00	3.55	0.246	7.58	202.2	20.8
319	210	26.0	10.48	1.20	4.48	0.136	7.24	202.3	19.7
317	210	17.7	9.15	0.81	3.95	0.212	8.81	218.9	21.1
299	231	30.0	11.02	1.16	3.60	0.162	8.84	248.5	21.8
349	210	14.5	10.00	1.16	4.40	0.228	8.23	210.4	21.5
Mean	236	20.4	11.76	1.11	3.07	0.185	8.42	228.9	20.9
Std.Dev.	35	6.0	2.11	0.22	1.15	0.043	1.15	27.2	0.9
				Group 5					
242	405	17.3	15.02	1.37	2.46		8.34	257.8	21.7
221	411	17.3	11.83	1.43	1.14	0.127	10.74	285.5	22.2
229	399	16.8	11.56	1.14	2.87	0.139	9.70	288.5	21.8
238	420	18.4	13.71	1.06	1.72	0.193	10.40	276.2	21.8
226	414	19.4	14.97	1.23	1.83	0.119	9.66	288.5	22.0
266	330	16.1	12.82	1.21	2.21	0.178	8.83	314.4	22.5
264	360	13.7	13.00	1.10	1.96	0.162	8.49	264.9	22.1
263	345	23.8	13.86	1.22	1.55	0.104	10.27	250.5	20.9
278	450	14.1	14.94	1.34	6.67	0.197	10.28	280.1	21.8
251	420	17.5	15.65	1.78	1.41	0.165	10.57	293.2	21.9
Mean	395	17.4	13.74	1.29	2.38	0.154	9.73	280.0	21.9
Std.Dev.	38	2.8	1.42	0.21	1.59	0.032	0.89	18.7	0.4

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]									
AN-NO (1)	AGE (9)	TH-WT (10)	AD-WT (11)	AP-WT (12)	PL-GH (13)	PT-GH (14)	EMA (15)	CB-WT (16)	CB-LE (17)
					Group 6				
223	354	15.6	22.52	1.39	2.12	0.155	10.31	294.6	22.1
204	336	16.0	17.28	1.25	2.02	0.167	11.10	292.8	22.2
241	375	22.3	15.41	1.25	2.52	0.160	9.32	278.8	20.9
217	381	20.4	14.52	1.05	6.91	0.210	9.09	283.0	21.1
214	429	20.2	16.48	1.47	5.94	0.176	9.90	293.3	21.8
254	495	25.1	13.37	1.13	2.60	0.141	10.41	264.1	21.2
255	300	32.0	11.88	1.14	3.35		8.92	244.2	22.0
262	306	26.1	13.59	1.03	5.81	0.115	9.26	265.0	21.8
279	363	20.3	16.80	1.28	2.00	0.141	13.94	284.1	21.6
285	294	64.8	17.42	1.07	2.49	0.112	9.40	271.5	22.0
Mean	363	26.3	15.93	1.21	3.58	0.153	10.16	275.1	21.7
Std.Dev.	62	14.4	2.97	0.15	1.89	0.028	1.49	16.5	0.4
					Group 7				
230	360	22.2	14.98	1.25	2.77	0.124	12.50	265.4	21.0
237	360	16.6	14.85	1.33	0.98	0.146	11.05	271.6	21.8
219	354	17.3	15.21	1.06	1.72	0.139	10.70	269.9	21.5
202	339	15.6	13.25	1.19	1.59	0.117	9.65	272.7	22.4
240	405	20.2	18.86	2.06	4.43	0.110	13.14	270.3	20.7
253	345	24.0	12.99	1.29	1.53	0.122	8.52	269.5	21.2
256	345	18.0	16.88	2.15	1.37	0.141	10.65	282.2	20.8
260	396	22.7	12.64	1.31	1.86	0.137	9.35	278.6	21.4
265	315	29.7	17.96	1.81	2.60	0.127	13.14	297.8	21.3
282	279	51.4	17.75	1.12	1.59	0.180	11.00	272.3	22.4
Mean	350	23.8	15.54	1.46	2.04	0.134	10.97	275.0	21.5
Std.Dev.	36	10.6	2.23	0.40	1.00	0.020	1.57	9.3	0.6
					Group 9				
322	411	14.1	16.79	1.45	4.34	0.134	9.19	253.6	21.3
364	321	24.4	12.71	1.22	8.38	0.228	7.00	236.5	22.2
305	384	33.6	14.13	1.63	3.76	0.151	10.20	272.5	21.9
294	390	26.0	12.60	1.21	2.25	0.184	8.70	271.0	21.6
312	411	15.4	19.44	1.27	2.42	0.153	9.68	284.7	20.8
368	378	18.2	16.47	1.77	4.86	0.171	9.15	224.3	20.8
318	390	18.2	13.08	1.37	1.76	0.201	9.18	273.1	21.4
357	384	14.6	13.34	1.20	1.83	0.234	9.19	244.1	21.8
344	384	14.1	11.92	1.26	3.05	0.182	8.10	236.0	21.2
369	300	22.8	12.30	1.03	4.67	0.214	7.45	253.8	22.1
Mean	375	20.1	14.27	1.34	3.73	0.185	8.78	253.0	21.5
Std.Dev.	36	6.4	2.47	0.22	2.00	0.034	0.99	17.4	0.5

[a]See Table 5 for variable definitions.

Appendix 1. (Continued)

Variable code and number[a]									
AN-NO (1)	AGE (9)	TH-WT (10)	AD-WT (11)	AP-WT (12)	PL-GH (13)	PT-GH (14)	EMA (15)	CB-WT (16)	CB-LE (17)
				Group 14					
296	78	24.2	6.90	0.66	2.33	0.195		109.5	17.6
310	75	14.2	5.09	0.52	4.16	0.244		133.6	17.6
313	75	17.5	6.45	0.58	2.06	0.218		110.5	17.5
314	75	10.6	5.30	0.65	13.36	0.153		118.6	18.3
316	75	11.7	5.48	0.58	2.84	0.186		111.2	17.3
346	75	15.9	7.40	0.55	1.96	0.218		81.9	16.4
348	75	10.0	4.74	0.74	4.18	0.218		102.1	17.3
345	81	10.4	7.24	0.84	4.16	0.180		118.1	17.7
343	75	14.8	5.76	0.82	4.41	0.135		118.0	18.5
342	81	12.9	6.58	1.14	5.33	0.173		145.2	19.4
Mean	77	14.2	6.09	0.71	4.48	0.192		114.9	17.8
Std.Dev.	3	4.3	0.94	0.19	3.32	0.032		17.1	0.8

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]									
AN-NO (1)	RD-FT (18)	RD-LN (19)	RD-BN (20)	RD-WA (21)	RD-EE (22)	TEND (23)	JU-GH (24)	TH-CH (25)	PBI (26)
				Group 5					
242	6.90	32.40	10.00	65.80	13.89	18.4	11.39	8.00	2.37
221	5.60	39.80	11.90	70.22	8.80	16.5	4.53	9.19	4.61
229	9.45	37.90	12.00	62.04	18.34	16.0	3.90	10.23	4.16
238	6.95	42.20	11.65	66.23	12.60	13.2	3.73	6.21	3.68
226	6.65	38.65	11.50	64.68	14.81	16.4	4.06	8.72	5.68
266	6.50	36.90	11.60	68.26	10.49	16.1	7.06	9.91	5.45
264	5.12	35.20	9.87	69.12	9.30	24.0	4.13		5.49
263	7.37	37.30	9.80	64.47	15.18	14.8	2.85	7.88	6.89
278	6.95	38.55	10.80	68.33	10.55	16.4	9.08	8.12	4.99
251	5.20	41.95	11.20	70.42	7.85	14.7	5.59	9.55	7.53
Mean	6.67	38.08	11.03	66.96	12.18	16.6	5.63	8.64	5.01
Std.Dev.	1.25	2.94	0.86	2.76	3.35	2.9	2.74	1.25	1.50
				Group 6					
223	9.70	40.80	12.00	65.54	14.61	12.8	2.06	5.97	3.41
204	7.00	40.30	11.40	68.56	9.95	22.1	2.92	7.88	5.08
241	9.30	37.40	10.90	64.57	14.39	15.2	3.70	9.07	4.50
217	7.00	34.60	11.40	65.38	14.10	19.9	3.27	8.00	6.83
214	10.63	37.85	10.72	60.52	20.33	14.0	4.74	8.48	4.72
254	9.20	36.05	10.55	61.16	19.37	10.5	8.45	7.04	7.04
255	6.90	32.10	9.80	64.76	14.87	10.8	4.07	7.40	5.47
262	6.40	33.20	10.80	66.53	13.14	13.8	2.82	6.33	4.29
279	10.75	39.80	10.83	62.46	17.74	17.7	3.68	10.75	5.37
285	6.00	34.50	11.80	67.61	11.26	16.8	6.96	6.81	4.59
Mean	8.29	36.66	11.02	64.71	14.98	15.4	4.27	7.77	4.13
Std.Dev.	1.81	3.06	0.65	2.64	3.32	3.8	1.98	1.42	1.12
				Group 7					
230	9.40	40.80	11.80	64.41	15.96	13.9	4.64	6.09	5.49
237	7.70	36.10	10.90	66.29	12.61	14.6	1.65	9.79	4.73
219	9.20	38.30	10.80	64.53	15.83	14.1	2.85	10.75	6.14
202	9.20	43.15	10.60	65.68	14.05	14.3	1.84	6.57	5.55
240	11.60	43.60	11.20	62.54	17.50	16.4	3.93	7.28	5.92
253	10.85	35.30	10.35	58.73	23.15	13.0	4.14	9.07	5.77
256	9.25	42.80	11.37	64.10	15.18	14.9	2.06	7.40	9.46
260	7.00	36.00	10.00	66.29	12.76	13.6	5.47	10.75	4.61
265	6.90	41.00	11.20	68.42	11.35	12.5	6.09	6.69	7.23
282	6.20	34.60	10.50	65.48	13.48	20.2	2.23	5.97	6.15
Mean	8.73	39.17	10.87	64.65	15.19	14.8	3.49	8.04	6.12
Std.Dev.	1.75	3.51	0.53	2.61	3.35	2.2	1.59	1.88	1.39

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]									
AN-NO (1)	RD-FT (18)	RD-LN (19)	RD-BN (20)	RD-WA (21)	RD-EE (22)	TEND (23)	JU-GH (24)	TH-CH (25)	PBI (26)
				Group 9					
322	6.10	37.30	9.60	67.02	11.68	17.0	5.25	9.67	4.69
364	5.60	28.15	8.55	68.87	10.88	12.9	2.59	6.80	3.11
305	6.10	39.10	9.95	68.64	10.26	14.2	7.11	7.28	4.86
294	6.45	40.15	10.65	67.50	11.92	15.0	2.37	8.48	7.43
312	8.15	40.95	10.80	65.58	14.13	17.0	2.29	6.93	5.54
368	6.00	34.15	10.60	66.78	12.50	11.4	2.26	10.87	3.25
318	6.40	38.55	10.50	67.31	11.95	14.9	5.52	9.31	4.37
357	5.75	37.20	10.10	68.37	10.42	10.6	2.61	6.33	4.72
344	5.85	32.40	9.85	68.00	11.71	15.9	3.50	10.15	3.49
369	4.50	30.40	10.15	70.12	8.36	13.7	3.22	8.48	4.71
Mean	6.09	35.84	10.08	67.82	11.38	14.3	3.67	8.43	4.62
Std.Dev.	0.91	4.35	0.66	1.27	1.53	2.2	1.69	1.56	1.26

[a]See Table 5 for variable definitions.

Appendix 1. (continued)

Variable code and number[a]				
AN-NO (1)	PL-CO (27)	PL-CS (28)	AD-CO (29)	AD-CS (30)
		Group 5		
242	11.89	1.920	2.32	2.28
221	9.36	1.039	2.08	1.08
229	11.66	1.287	3.07	1.15
238	9.29	1.284	2.84	3.04
226	11.29	1.044	2.20	0.41
266	14.00	0.944	2.51	0.28
264	17.79	2.561	3.68	4.48
263	6.45	0.494	1.79	0.96
278	11.08	1.735	2.93	2.32
251	9.43	1.090	3.38	1.84
Mean	11.22	1.340	2.68	1.78
Std. Dev.	3.07	0.587	0.60	1.30
		Group 6		
223	8.34	0.764	1.72	0.58
204	7.33	0.330	1.14	0.22
241	8.20	1.075	3.96	4.02
217	16.76	1.321	3.16	1.92
214	9.25	1.589	4.26	3.22
254	11.17	0.789	7.93	4.80
255	6.85	0.380	0.91	0.30
262	10.94	0.822	2.20	1.22
279	8.86	0.592	1.50	0.15
285	4.01	0.346	0.50	0.25
Mean	9.17	0.801	2.73	1.67
Std. Dev.	3.36	0.424	2.23	1.75
		Group 7		
230	2.38	0.133	0.42	0.20
237	5.71	0.518	2.44	1.11
219	4.19	0.359	0.32	0.31
202	1.74	0.228	1.29	0.26
240	11.44	1.020	1.44	0.28
253	9.14	0.975	2.82	0.68
256	3.32	0.407	0.44	0.08
260	4.99	0.498	0.62	0.42
265	6.67	0.744	0.42	0.16
282	4.41	0.273	0.17	0.18
Mean	5.40	0.516	1.04	0.37
Std. Dev.	3.01	0.306	0.94	0.31

[a]See Table 5 for variable definitions.