THE INFLUENCE OF DIETARY AND HORMONAL FACTORS ON GROWTH AND DEVELOPMENT IN THE RUMINANT

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ABSTRACT

THE INFLUENCE OF DIETARY AND HORMONAL FACTORS ON GROWTH AND DEVELOPMENT IN THE RUMINANT

By

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Increasing the efficiency of production in ruminant animals is of utmost importance. Hormonal regulation of growth would seem to be a very promising method of accomplishing this but as yet no one is sure of how hormones actually control growth. This study was designed to determine normal relationships between hormones and growth and development in the ruminant animal.

Forty-two lambs, half male and half female, at birth, 30, 60, 90 and 120 days of age were sacrificed for this experiment. All lambs were maintained on the ewe until slaughter or weaning. Six lambs were used in each of the first three age groups. Remaining lambs were weaned at 60 days and placed on either a 7 or 15 percent crude protein diet. The dietary protein levels were used to establish two rates of growth. Measurements were taken on tissue weights, plasma glucose and urea nitrogen, plasma and tissue free amino acids, tissue protein and nucleic acids and serum and glandular hormone levels.

Liver, muscle and pituitary weights were increased on an absolute basis by increasing dietary protein. Adrenal gland weights were not

influenced on an absolute basis but as a percent of body weight were greatly increased by feeding the low protein diet.

As expected, plasma glucose decreased and plasma urea nitrogen increased with age. Increasing dietary protein had no influence on plasma glucose but increased plasma urea nitrogen.

Plasma total free amino acids increased with age due to an increase in the essential amino acids. Decreasing the dietary protein decreased both essential and nonessential amino acids in plasma.

Neither age nor diet influenced essential or nonessential amino acids in liver.

Muscle essential and nonessential amino acids increased with age while decreasing the dietary protein decreased essential amino acids only.

Liver and muscle RNA and DNA content increased until 120 days of age when they decreased. Liver protein content followed the same pattern while muscle protein continued to increase. Increasing the dietary protein led to increases in each of the parameters.

Serum growth hormone decreased with age while serum insulin increased. Dietary protein level had no influence on growth hormone but increasing protein led to increased serum insulin.

Neither diet nor age had any influence on serum or adrenal glucocorticoid content or concentration.

Total pituitary growth hormone content increased with age but was not influenced by diet. Neither diet nor age had any effect on pituitary growth hormone concentration.

Correlation coefficients indicate that growth is highly related to muscle nucleic acid content and that hormones may exert their influence on growth through nucleic acids.

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INTRODUCTION

As the world population becomes more affluent, the demand for meat in the diet increases accordingly. The increased demand for beef has meant a diversion of feedstuffs from the human population into beef production. This has met with much resistance from advocates claiming that feedstuffs are utilized much more efficiently by direct human use. The human competition has greatly increased the cost of feed to the beef producer. Still people want and demand red meat for their diet. The solution to the problem would seem to be an increase in the efficiency of beef production. To produce a beef animal acceptable to the general population and to be able to decrease competition for human foods is a goal that must be attained in animal agriculture.

Many experimental methods have been tried in an effort to reach this goal. The most promising category would appear to be hormonal regulation of growth. The use of synthetic hormones that act as growth stimulants has greatly increased the efficiency of beef production; however, this has met with resistance from human health advocates. A method to regulate hormonal control of growth without risking a human health hazard is needed.

This study was designed to elucidate the relationships between normal hormone patterns and growth rates so as to provide a basis for further research into the area of hormonal regulation of growth.

LITERATURE REVIEW

Growth and Development in Animals

Growth and development are perhaps the most universal phenomena that occur on earth. From the simplest microorganism to the most complex individual, each has the ability to grow and develop. For people in the livestock production, growth and development are the most important aspects of their industry. The parameters of growth and development are so complex that they cannot adequately be, and perhaps should not be, distinguished from each other.

Many definitions and descriptions of growth and development have been put forward, some more adequate than others. Schloss (1911) defined growth as a correlated increased in the mass of the body in definite intervals of time in a way characteristic of the species. This definition is good in that it implies a characteristic rate of growth that is species dependent and includes development as an integral part of growth. It does not, however, make any distinction between muscle and fat accretion. Brody (1945) has defined growth as the production of new biochemical units brought about by cell division, cell enlargement or incorporation of materials from the environment. This definition does not necessarily imply an increase in physical magnitude as it could be applied to only a maintenance situation.

Hammond (1952) and McMeekan (1959) have defined growth as merely an increase in weight until a mature size is reached. Again this makes no distinction between muscle and fat accretion and ignores development as a part of growth. Maynard and Loosli (1969) have perhaps suggested the most widely preferred description of growth. They indicate that true growth involves an increase in the structural tissues such as muscles and bone and also in the organs. They distinguished between fat deposition in reserve tissues and characterized true growth primarily as an increase in protein, mineral matter and water.

It does not appear important as to whether growth is defined to include development or, as some have done, define development to include growth as long as the two are closely related. Lewis (1939) has described development as a process involving growth, cellular differentiation and/or development of form.

Palsson and Verges (1952) have reported that the order of tissue growth and development follows an outward trend starting with the central nervous system and progressing through bone, tendon, muscle, intermuscular fat and subutaneous fat. Growth and development of the central nervous system is essentially complete at birth; therefore, postnatal growth is concerned primarily with increases in bone, muscle and fat.

Berg and Butterfield (1968) have shown that bone, muscle and fat growth occur in three overlapping phases. Bone growth is the earliest developing while muscle tissue shows intermediate development and fat deposition occurs later than both. These workers found that

factors such as level of nutrition, sex and breed differences could affect the rate of growth of the tissue but not the order of development.

Rate of growth of bone, muscle and fat is not the same as order of development. Zinn (1967) has reported that over a 270 day feeding period fat exhibited the greatest rate of growth, muscle an intermediate rate and bone was the slowest growing tissue.

From a weight and economic viewpoint, muscle is the most important body tissue. For this reason, some detail of muscle growth and development will be presented. Stromer et al. (1974) have described the characteristics of mature skeletal muscle cells. These cells are more elongated than normal, averaging 1 to 2 centimeters in length. Each cell is multinucleated, containing from 100 to 200 nuclei. These nuclei all lie immediately under the outer cell membrane. Each of the muscle cells or fibers contain elongated protein threads with their long axis oriented parallel with the long axis of the cell. These threads serve as the contractile structure of the cell and are called myofibrils. The myofibrils compose over 50 percent of the total protein in a mature skeletal muscle cell and are composed principally of myosin or actin.

Ashmore et al. (1972) and Ashmore (1974) use a slightly different terminology. These workers describe the myofibrils as either α or β fibers. They further classify the α or β fibers into red, white or intermediate categories.

Regardless of terminology used, the sequence and timing of events in skeletal muscle differentiation and development is known.

Mesoderm gives rise to a presumptive myoblast which yields a myoblast. The myoblasts will fuse to form myotubes and through maturation and differentiation myotubes will form mature myofibers (Stromer et al., 1974). Myoblast fusion ceases about the time of birth in domestic animals; therefore, a newborn contains approximately all of the skeletal muscle cells that it ever will. Postnatal muscle growth is then accomplished by enlargement of existing cells. Ashmore et al. (1972) and Ashmore (1974) have described this enlargement beginning in late fetal stages as a biphasic development. Beta fibers develop first and α fibers later develop and expand around them. The number of nuclei do increase during this enlargement but not because of mitosis. They apparently result from the fusion of satellite cells to the end of existing myofibrils (Stromer et al., 1974).

A differentiation in fiber type was observed (Ashmore et al., 1972) postnatally. Both α and β fibers are classed as red and are adapted for aerobic metabolism in the newborn. Alpha fibers are capable of changing phenotype to white and function to anerobic metabolism depending upon muscle activity. Red fibers predominate in active muscle while white fibers are generally in inactive muscles. Fiber type is important in meat animal production as white fibers are larger than red, thus influencing meat quantity but red fibers metabolize and store more lipid than white, thus influencing meat quality.

The Influence of Age on Body Changes

Blood Metabolites

A decrease in blood glucose in the ruminant with increasing age is well documented. Kennedy et al. (1939) reported a decrease in blood

glucose with age in calves from two days past partum to about one year. The physiological factor triggering this decrease is still unknown. Murley et al. (1952) measured a decrease in blood glucose values in calves from birth to five weeks of age. Type of diet fed had no effect on this response. Lambert et al. (1955) also found no relationship of diet to a decrease in blood glucose measured from birth to eight weeks of age in calves. Sterm et al. (1971) also found a decrease in serum glucose with age when comparing suckling, weanling and mature ruminants.

Lupien et al. (1962) studied the effects of the digestive tract in calves on an observed decrease in blood glucose from birth to eight weeks of age. Removal of the rumen, reticulum, omasum and a portion of the abomasum did not affect the observed decrease. Nicolai and Stewart (1965) observed a decrease in blood glucose in calves from 92 mg% at one week to 67 mg% at ninety days of age. These workers concluded that the drop was not related to forestomach development or absorption of volatile fatty acids. Young et al. (1970), using milk fed calves, measured an increase in blood glucose from birth to two days and then a decrease to 100 days. Purser and Bergen (1969) and Ponto and Bergen (1974) have concluded that the decrease in blood glucose may be a constitutive change natural to the ruminant species as diet, rumen development and volatile fatty acid absorption have no apparent relationship to the decrease.

Only a few workers have studied the relationship of age with the nitrogen components of blood. Leibholz (1965) reported a decrease in total α-amino nitrogen concentration in calves from birth to 24 weeks

of age. She observed significant decreases with age in the individual amino acids serine, proline, glutamine, methionine, leucine, lysine and histidine. Suprisingly, she also reported that plasma urea was significantly higher at one week of age and decreased thereafter. Oltjen et al. (1969) found no relationship of age with blood amino acids in bulls and heifers except for an increase in asparagine, glutamine, glutamic acid and citrulline. Bergen et al. (1973) also reported no effect of age on individual amino acids in the growing lamb from 60 to 120 days except for lysine and leucine, and the response of the two to increasing age was dietary dependent.

Tissue and Blood Hormone Levels

Curl et al. (1968) reported that total content of growth hormone in the pituitary of the bovine increased with age. Growth hormone concentration, expressed either per unit of gland or per unit of body weight, decreased with age. A positive correlation between pituitary growth hormone per unit of body weight and rate of gain was observed. Baker et al. (1956) were not able to find any effect of age on pituitary growth hormone content in holstein heifers. They did report a decrease in growth hormone concentration with increasing age and a positive correlation between growth rate and pituitary growth hormone concentration. Purchas et al. (1970) measured pituitary and plasma growth hormone levels in bulls from birth to one year of age. Pituitary growth hormone content and concentration, either as μg/mg of gland or per unit of body weight, increased until four months of age and then decreased to levels measured at birth and then remained

constant. Siers and Swiger (1971) reported a decrease in serum growth hormone with age but concluded that the decrease was related to body size instead of age. They found that pigs of similar size regardless of age had similar serum growth hormone levels. A negative correlation between growth rate and serum growth hormone was also observed.

Regardless of the nature of the change in pituitary growth hormone, it is apparent that pituitary growth hormone content and concentration and plasma levels do change with age and are related in some manner to growth rate.

Changes in levels of other hormones with age have been studied. Macmillan and Hafs (1968) reported a decrease in pituitary luteinizing hormone concentration from one month to a year. Pituitary total content as well as plasma concentration increased with age. Rawlings et al. (1972) reported an increase in levels of blood and testicular testosterone with age in holstein bulls. Dvorak (1972) reported a decrease in plasma cortisol and corticosterone in swine from one day postnatally to maturity.

Tissue Nucleic Acid Content

Devi et al. (1963) measured an increase in rat liver DNA and RNA concentration immediately after birth but a decrease shortly thereafter. Enesco and Puddy (1964) measured a 2-4 fold increase in muscle DNA content in rats from suckling to young adults. Gordon et al. (1966) reported an increase in total DNA of rat quadriceps from birth to 90 days but no further increase after this point. Robinson and Lambourne (1970) also reported a decrease in muscle DNA and RNA concentration in

the mouse. Buchanan and Pritchard (1970) found an increase in total DNA content of tibialis anterior muscle of rats from birth to puberty. Females increase were slightly less than those in males. Howarth and Baldwin (1971) have reported the same trends in the gastrocnemius of rats. Gilbreath and Trout (1973) have also reported a decrease in muscle DNA and RNA concentration with age in swine. Similar findings have also been reported for cattle (LaFlamme et al., 1973).

The Influence of Diet on Body Changes

Blood Metabolites

Many contrasting results concerning the effects of dietary changes on blood glucose levels in ruminants have been reported. In beef heifers Stufflebeam et al. (1969) could find no effect of level of energy intake on blood glucose. Likewise Memon et al. (1969) reported no change in plasma glucose of mature ewes with changes in dietary protein or energy level. Howland et al. (1966) found increased levels of plasma glucose in ewes when dietary energy and protein were increased. Jugular blood glucose in sheep increased significantly when the level of corn in the ration fed was increased (Clary et al., 1967). Preston and Burroughs (1958) found a decrease in serum glucose levels in lambs with increasing levels of dietary protein even though feed consumption was increased at higher protein levels. Although the differences may be species related, Tumbleson et al. (1969) using Hormel miniature swine reported increased blood glucose when feeding a 16 percent versus 4 percent crude protein diet. Although several contrasting reports have been presented, it seems reasonable to believe that blood glucose levels

in ruminants nearing mature weight may be influenced by ration composition as well as total feed consumption.

Much work exists concerning the relationship of diet and the nitrogen components of blood. Decreased protein intake, either by starving (Leibholz and Cook, 1967) or decreasing the crude protein content of the ration (Leibholz, 1969), lowered plasma urea nitrogen in sheep.

Tagari et al. (1964) suggested that plotting the change in blood urea nitrogen with time after feeding may be a useful method for assessing protein utilization in ruminants. They observed an increase in blood urea nitrogen of sheep as ration protein was increased but the time of maximum blood levels was delayed with increased protein. Preston et al. (1965) found that blood urea nitrogen could be quantified with protein intake per unit of metabolic body weight ($BW^{\cdot 75}$) and therefore proposed that the protein status of the animal could be partially assessed by the blood urea nitrogen concentrations. Nimrick et al. (1971) also observed an increase in plasma urea nitrogen of lambs as the amount of soy in a corn-soy ration increased.

Abou Akkada and El Sayed Osman (1967) found that although blood urea nitrogen increased as ration crude protein increased, it was more closely related to changes in rumen ammonia. These workers stated that total nitrogen intake was probably not a major factor in controlling blood urea nitrogen levels because of differing rumen solubilities of the protein sources. Boling et al. (1972) observed an increase in plasma urea nitrogen of cattle when increasing ration protein from 6

to 16 percent for either soy or urea. However, the magnitude of increase was higher for urea. Little et al. (1968) fed sheep the same amount of nitrogen but varied the source. They reported lower plasma urea nitrogen values when the protein source was soy or zein than casein or gelatin.

Changes in the microflora and microfauna population of the rumen can also affect blood urea nitrogen values. Defaunation will result in a significantly increased rumen bacterial population (Klopfenstein et al., 1966). Males and Purser (1970) have suggested that the lower blood urea values of defaunated sheep are a result of a greater rumen ammonia utilization by the increased bacterial numbers.

It would appear that level of dietary crude protein does affect blood urea nitrogen values. However, it seems likely that other factors such as source of protein, ration energy density and rumen microbial population may be as important in determining blood urea nitrogen levels as nitrogen intake alone.

Changes in ration protein may also affect blood amino acid levels. Nimrick et al. (1971) have reported significantly increased levels of branched chain amino acids and a trend for all amino acids to increase as dietary protein and feed consumption increased. Likewise, Weston (1971) and Hogan et al. (1968) have reported a decrease in both plasma total essential and nonessential amino acids in sheep fed purified diets ranging from 6 to 15 percent crude protein.

The source of dietary nitrogen has also been thought to influence blood amino acid levels. Oltjen et al. (1969) reported a decrease in blood essential amino acids of cattle fed a urea diet compared to

cattle fed an isolated soy diet. Boling et al. (1972) reported no change in plasma amino acid concentration of cattle fed a corn silage ration supplemented with either soy or urea. Bergen et al. (1973) reported increased ration protein raised plasma total essential amino acids with no change in total nonessential amino acids. Source of nitrogen also influenced plasma amino acid patterns. Sheep fed fish protein concentrate as the major protein had higher plasma amino acid levels than sheep on other rations. Sheep fed a low protein basal ration or an NPN containing ration tended to have lower branched chain amino acids and phenylalanine than sheep on other rations. The low lysine, high leucine content of zein was reflected in plasma levels of sheep fed a corn protein ration. Schelling et al. (1967) and Nimrick et al. (1970 A, B) have speculated that in ruminants total essential amino acids may be limiting when urea or a highly soluble nitrogen source is fed. These workers have reported changes in the blood amino acid patterns by either supplementing the ration or abomasally infusing certain essential amino acids. It seems likely that plasma amino acids are controlled more by the amount of absorbable amino acid reaching the lower gut than protein source per se. The amount of absorbable amino acid reaching the lower gut will be influenced by ration energy level and protein solubility in the rumen.

Tissue Weights

Tumbleson et al. (1969) and Elsley (1963) reported significantly lighter adrenals, liver and gastrocnemius muscle in swine fed 4

versus 16 percent crude protein. Significantly heavier thyroid, kidney, liver and pituitary weights were found in sheep when ration protein was increased (Preston and Burroughs, 1958). Pituitary and adrenal weights were increased in ewes receiving increased levels of energy and protein (Howland et al., 1966); however, Bellows et al. (1966) found no difference in pituitary weight of rats fed two levels of dietary energy. Clarke (1969) reported an increase in rat adrenals on either an absolute basis or as a percent of body weight when dietary crude protein was lowered to 4 percent. Memon et al. (1969) showed an increase in pituitary weight of ewes when ration protein was increased. The increased weight was apparently due to an increase in the size of pituitary cells as measured by pituitary protein to DNA ratios.

Dietary protein intake apparently affects tissue weights more than does dietary energy. The response in tissue weight to dietary changes is variable and may be species dependent.

Tissue and Blood Hormone Levels

Armstrong and Hansel (1956) found no difference in pituitary growth hormone content or concentration of holstein heifers grown on a high and low plane of nutrition, although animals on the high plane of nutrition grew much faster than on the low level. Stephan et al. (1971) malnourished rat pups by underfeeding of dams during gestation and lactation and found that growth hormone activity was greatly reduced in the malnourished compared to the well-nourished pups. Total growth hormone content of the gland in malnourished rats was less than 25 percent of that in well-nourished rats. Sinha et al. (1973)

also malnourished rats by placing 16 pups on a female compared to 4 pups for controls. Pituitary growth hormone concentration was significantly decreased by malnourishment. Rate of growth hormone synthesis was checked by label incorporation and found to be lower in the malnourished animals. Plane of nutrition would appear to be an important factor in controlling pituitary growth hormone levels in at least some species. The true relationship of pituitary growth hormone content and growth rate in maximally growing animals has yet to be elucidated.

Results of dietary alterations on blood growth hormone levels have been variable. Stephan et al. (1971) and Sinha et al. (1973) have reported decreased blood levels of growth hormone in malnourished rat pups. Trenkle (1970) reported no effect of feeding high energy rations to cattle on plasma growth hormone levels. McAtee and Trenkle (1971), using cattle, and Trenkle (1971), using sheep, found no effect of feeding, fasting or nutrient intake on plasma growth hormone levels. Bassett et al. (1971) reported that plasma growth hormone in sheep was negatively related to digestable organic matter intake and the amount of protein passing to the lower gut. The majority of the evidence would indicate less than a direct influence of diet on plasma growth hormone levels although there may be species differences.

Direct effects of dietary changes on blood insulin levels have been reported. Trenkle (1966, 1970) found that plasma insulin increased in cattle fed high energy finishing rations and appeared to be related to consumption of the grain and supplement portion of the ration. Trenkle (1966) also reported higher plasma insulin in sheep fed rations in which the energy density was increased. In sheep

Bassett et al. (1971) found high positive correlations between plasma insulin and daily digestable organic matter intake as well as amount of protein in the intestines. Borger et al. (1973 A) reported significantly lower plasma insulin in steers fed a low protein ration than in steers fed normally. It would appear that dietary changes in energy or protein influence blood insulin levels. An elevation of blood sugar in ruminants increases insulin secretion (Manns and Boda, 1967) and increased VFA from rumen fermentation is also thought to influence insulin levels (Manns et al., 1967). Amino acids also stimulate insulin secretion (Frohman, 1969).

Tissue Nucleic Acid Content

Much work on the relationships of dietary change and tissue nucleic acid content has been reported. Borger et al. (1973 B) found no change in muscle DNA or RNA of finishing cattle due to level of protein fed. Umana (1965) reported an increase in DNA content and concentration in rat liver when either 5 percent protein or protein free diets were compared to diets adequate in protein. Gilbreath and Trout (1973) reported a significant decrease in muscle DNA and RNA content of swine fed 5 percent protein. The low protein fed pigs had significantly increased muscle DNA concentrations and decreased RNA concentrations. Apparently, cellular DNA content did not decrease as much as other cellular constituents. Young and Alexis (1968) reported an increase in skeletal muscle RNA content but a decrease in concentration when rats were changed from a 3 to 18 percent protein diet. Young et al. (1971) reported a decrease in skeletal muscle DNA and RNA content but

an increase in DNA concentration in rats fed a low protein diet. Ashley and Fisher (1967) found similar results with protein depleted cocks. Howarth and Bladwin (1971) reported a decrease in the rate of RNA and DNA synthesis in rat muscle when food intake was decreased. Protein as well as energy intake seems to affect both RNA and DNA content of tissues, but RNA is more sensitive to nutrient changes.

The Mechanism of Protein Synthesis

Before one can discuss the influence of hormones on growth and protein synthesis, an understanding of the mechanisms of protein synthesis is essential. Therefore, a brief review will be presented.

The synthesis of all protein is related to and controlled by the genetic information contained in cellular DNA. The information may be thought of as flowing from DNA to RNA to proteins. Two major processes are involved in the transmission of this genetic information for protein synthesis (Lehninger, 1971). The first process is transcription, in which the genetic message contained in DNA is transcribed into messenger RNA. The second is translation, the process in which the genetic message is decoded and proteins are synthesized. Before any protein can be synthesized these processes must occur.

Lehninger (1971) described the protein synthesis process *in vivo* as occurring in four major stages: (1) Amino acid activation, (2) Initiation, (3) Elongation and (4) Termination.

Amino acid activation requires the proper amino acids, transfer RNAs (tRNA), aminoacyl-tRNA synthetases, ATP and Mg^{++}. Amino acids are enzymatically esterified to the respective tRNA utilizing energy from ATP. The charged tRNA is now ready for later use in protein synthesis.

According to Lucas-Lenard and Lipmann (1971) initiation of the protein chain requires the initiating charged tRNA, messenger RNA (mRNA), GTP, Mg^{++}, three initiation factors, 40S ribosomal subunit and a 60S ribosomal subunit. The initiating tRNA in bacteria is formylmethionyl-tRNA and the initiating tRNA in mammalian cells is thought to be methionyl-tRNA also (Lucas-Lenard and Lipmann, 1971). Two forms of methionyl-tRNA have been isolated from eukaryotic cells. One form has been found to supply only the N terminal methionine while the other functions only internally in the growing peptide chain (Lucas-Lenard and Lipmann, 1971). Messenger RNA is produced from DNA by transcription and acts as a template for protein synthesis. Energy is supplied from GTP. An initiation complex is formed by the binding of mRNA, the 40S ribosomal subunit and the initiating charged tRNA. Initiation factors and GTP are utilized. The 60S ribosomal subunit can now join the complex to complete the ribosome formation.

Elongation of the protein chain requires specific charged tRNAs, Mg^{++}, GTP and two elongation factors (Haselkorn and Rothman-Denes, 1973). Elongation is carried out by the sequential addition of new aminoacyl residues transferred from charged tRNAs specified by a code located in the mRNA. After peptide bond formation, the mRNA and peptidyl-tRNA chain are moved along the ribosome to bring the next mRNA code into position. The processes require elongation factors and energy from GTP (Lehninger, 1971).

Termination is accompanied by release of the protein chain from the ribosome complex and requires the termination code in mRNA, apparently two releasing factors and GTP (Haselkorn and Rothman-Denes, 1973).

When the termination code is encountered and the releasing factors are available, the protein chain is released from the complex. Messenger RNA is now released, the intact ribosome dissociates into subunits and associates randomly at initiation steps for continued protein synthesis.

Hormonal Influences on Growth and Protein Synthesis

Normal growth and development in domestic animals is dependent, at least in part, on hormone action. As a human food source, we are primarily interested only in the muscle component of the animal carcass. This portion of the review will then be concerned with the effects of hormones on protein synthesis. Before actions of individual hormones are considered, possible general modes of action for all hormones should be briefly mentioned.

It is generally thought that growth and developmental hormones exert their effects on protein synthesis via RNA metabolism. Wannemacher and McCoy (1966) and Howarth (1972) have shown significant and positive correlations between cellular RNA content and rates of protein synthesis. Manchester (1970) suggested three possible modes of action of hormones in RNA metabolism. (1) Hormones affect protein synthesis by stimulating production of specific messenger RNA's. (2) Hormones affect protein synthesis through a general increase in all forms of cellular RNA due to a hormone induced increase in RNA polymerase activity. (3) Hormones affect protein synthesis by influencing the integrity and/or functional capacity of polysomes. These are possibilities, individual hormones may function in any one or a combination of the above methods. They may also function in yet a different manner.

Growth Hormone

It is well established that hypophysectomy lessens and treatment with growth hormone stimulates growth and protein synthesis in animals. Manchester (1970) indicated that protein and RNA content and rate of synthesis decreases in a hypophsectomized rat. Treatment with growth hormone reversed these findings. Tissue DNA content and rate of synthesis is also decreased by pituitary gland removal, but returned to normal by injections of growth hormone (Snipes, 1968; Cheek and Hill, 1970; Trenkle, 1974).

A lag period between tissue contact with growth hormone and the observable increase in protein synthesis indicates that some metabolism of the hormone is necessary (Rillema and Kostyo, 1971). Regardless of the nature of the hormone metabolism, the increase in tissue protein content is due to an increase in synthesis and not a decrease in degradation rate (Goldberg, 1969).

Other factors affecting protein synthesis have also been studied for their relationship with growth hormone. If amino acid levels are limiting, protein synthesis rates will be decreased. Riggs and Walker (1960) observed that growth hormone treatment of hypophysectomized rats increased the tissue uptake of a synthetic amino acid almost immediately. Snipes (1967) reported a decrease in histidine uptake by rat diaphragm from animals with the pituitary removed due to lack of growth hormone. Snipes and Kostyo (1962) reported similar results for alanine as well as histidine. When growth hormone was administered, amino acid transport returned to normal.

Although it is apparent that growth hormone can stimulate amino acid transport, this does not appear to be its main effect in the cell (Kostyo, 1968) and appears unnecessary even for short term stimulation of protein synthesis (Kostyo, 1964; Reeds et al., 1971). However, enhanced amino acid transport would seem necessary for a long term general increase in protein synthesis.

A hormone-induced increase in mRNA or a general increase in all species of RNA has been mentioned previously. Jefferson and Korner (1967) reported an increase in labeling of all nucleic acids from [^{3}H] orotic acid due to growth hormone stimulation. Sells and Takahashi (1967), studying the labeling pattern of liver RNA in hypophysectomized rats, reported the initial range of label incorporation to be 4S to 18S. This range is characteristic of messenger RNA. Additional time, however, revealed an increase label in ribosomal RNA as well; so, no conclusion as to which action was most important for stimulating protein synthesis was reached. Salaman et al. (1972) reported the earliest observable effect of growth hormone at the cellular basis was an increase in 45-S ribosomal precursor RNA in the nucleolus from rat liver. This appeared to be a secondary effect due to a hormone induced increase in the activity of the enzyme RNA polymerase. Widnell and Tata (1966) and Korner (1967) have also reported a growth hormone induced increase in the activity of RNA polymerase from rat liver nuclei. Growth hormone thus seems capable of stimulating general RNA production and possibly that of specific messenger RNA.

Some actions of growth hormone occur so rapidly as to suggest a mode of action other than increased RNA synthesis. Martin and

Young (1965), using diaphragm from hypophysectomized rats, and Korner (1967) found that use of actinomycin D with growth hormone did not block the hormone-stimulated increase in protein synthesis; therefore, synthesis of new RNA is not necessary for initial growth hormone action. It would seem reasonable, however, that for a long term increase in protein synthesis, additional RNA would be necessary.

Considering that growth hormone may stimulate protein synthesis without new synthesis of RNA, additional work has been done in an attempt to elucidate the relationship of protein synthesis and growth hormone. Korner (1967) found that the decrease in protein synthesis in hypophysectomized rats was due to a decrease in the ability of the liver microsome fraction to incorporate amino acids rather than a decrease in the activation process itself or any defect in tRNA. The change in the microsomal fraction was found in the ribosome itself. Comparison of polysome profiles from hypophysectomized and normal rats have not revealed any differences (Garren et al., 1967; Kostyo and Rillema, 1971). It would seem then that some hormone sensitive factor controlling ribosome function or efficiency is the control point (Garren et al., 1967). Kostyo and Rillema (1971) have suggested that growth hormone stimulates the ability of the ribosome to promote peptide bond synthesis (elongation) possibly due to an increased activity of peptidyl transferase.

Other work has indicated that initiation rather than elongation is the hormone control mechanism (Korner, 1968). This worker has concluded that an attachment factor needed for combination of the ribosome

and messenger RNA either is not present or has impaired function. Barden and Korner (1969), using hybridization studies have shown a defect in the 40S ribosome from hypophysectomized rats. Tata (1968) showed that growth hormone treatment increased the appearance of a 40S ribosomal precursor-messenger RNA particle just before the observed increase in protein synthesis. It would appear that growth hormone can affect initiation via either initiation factor competency or by a direct effect on the ribosome itself.

Growth hormone can cause rapid short term increases in protein synthesis as well as more general long term effects. Several modes of action for control have been discussed but it seems most reasonable to believe that several factors serve as true controlling mechanisms for the hormone-induced increase in protein synthesis and body growth.

Insulin

Carbohydrate metabolism usually comes to mind when metabolic actions of insulin are discussed. However, insulin also can exert profound influences on protein synthesis. Snipes (1968) reported that the presence of insulin is necessary for the maximal response of hypophysectomized rats to treatment with growth hormone. Many different modes of action for insulin stimulation of protein synthesis have been suggested and many are similar to those discussed above for growth hormone. The more important ones will be discussed briefly.

Without an adequate supply of amino acids for substrate, protein synthesis rates would be greatly decreased. One action of insulin that has been reported is the stimulation of tissue amino acid transport.

Guidotti et al. (1968) reported that insulin administration stimulated glycine and leucine transport in chick embryo heart. Manchester (1970) reported an enhanced accumulation of alanine, histidine and methionine by rat diaphragm following insulin administration. Hider et al. (1971) reported an increase in glycine transport in rat skeletal muscle due to insulin stimulation. Reeds et al. (1971) reported that insulin stimulated the transport of leucine, arginine, valine, lysine and histidine into rabbit muscle. Growth hormone also enhanced the uptake of these amino acids and the effects with insulin were more than additive, suggesting that insulin may stimulate amino acid transport in a different manner than growth hormone (Reeds et al., 1971).

Wool and Moyer (1964) reported a stimulation of amino acid uptake by rat diaphragm due to insulin even in the presence of actinomycin. Therefore, new RNA synthesis is not necessary for the insulin enhancement of amino acid transport. Goldstein and Reddy (1970) have suggested that the major mode of action for insulin stimulation of protein synthesis is through an enhanced amino acid transport. However, Manchester (1970) reported that puromycin, an inhibitor of protein synthesis did not inhibit the insulin stimulation of amino acid transport in diaphragm muscle. Thus, enhancement of substrate supply would be important for long term increases in protein synthesis but may not be a point of control for rapid adjustment of protein synthesis rates.

Protein synthesis rates are decreased in diabetes mellitus and Tragl and Reaven (1971) have proposed that it is due to a decreased

amount of messenger RNA resulting from the insulin deficiency. Manchester (1970) and Pilkis and Salaman (1972) have reported increased RNA polymerase following insulin treatment. However, Eboue-Bonis et al. (1963) and Wool and Cavicchi (1966) reported that RNA synthesis is not necessary for insulin stimulation of protein synthesis. Protein synthesis was necessary as both puromycin and cycloheximide prevented any response to insulin addition (Wool and Cavicchi, 1966).

If synthesis of new RNA is not needed for an initial stimulus of protein synthesis by insulin then the rapid control point must be some factor in translation. Wool et al. (1966) and Leader et al. (1971) have suggested that the difference in protein synthesis between diabetic and control animals is due to a decreased capacity to initiate synthesis via a factor in the cell sap. Tragl and Reaven (1972) reported a change in the polysome profile from heavy polysomes to free ribosomes in an insulin deficiency, indicating less binding of messenger RNA. This may be due to a decreased amount or an impaired function of binding factors in the cell. Wool and Kurihara (1967) also observed a change in the polysome profile from heavy to light with insulin deficiency and formulated a hypothesis of action for the hormones. They hypothesize that insulin first stimulates translation of an existing messenger for a specific protein. This protein associates with the ribosome and makes it more competent to bind messenger and form polysomes. Martin and Wool (1968) using hybridization studies and Castles et al. (1971) have reported that the 60S ribosome of diabetic animals carries a defect not allowing proper formation of polysomes.

Other factors may also be important in the insulin stimulation of protein synthesis. Wool et al. (1968) reported a decrease in the activity of aminoacyl-tRNA synthetase in diabetic animals that could be corrected with insulin additions. Davey and Manchester (1969) reported that insulin increased labeling of leucyl and tyrosyl-tRNA in vitro, indicating that the hormone could increase charging. Other work has indicated that uncharged tRNA may be able to actively inhibit protein synthesis, thus the ratio of uncharged to charged tRNA may be a regulator of protein synthesis (Seeds and Conway, 1966; Levin and Nirenberg, 1968).

Insulin can have many effects on protein synthesis, ranging from charging to messenger binding to a general increase in RNA synthesis to an increase in amino acid transport. Perhaps all of these factors in combination and others as yet unknown are needed for the long term increase in protein synthesis due to insulin.

Glucocorticoids

The class of hormones synthesized by the adrenal gland known as glucocorticoids can have profound and varied effects on tissue protein synthesis. Palmer (1966) has indicated that glucocorticoids have catabolic effects on skeletal muscle and anabolic effects on liver. Bellamy (1964) reported a cessation of growth in rats given daily injections of cortisol and Hafs et al. (1971) reported that adrenal and plasma levels of glucocorticoids were negatively related to rate of growth in beef cattle. Adrenalectomy has been shown to increase muscle amino acid uptake and rate of protein synthesis while treatment

with glucocorticoid has reversed this finding (Manchester, 1970). Manchester (1970) also reported a decrease in thymus RNA polymerase activity and ribosome function following glucocorticoid treatment. Thus it would seem that glucocorticoids inhibit protein synthesis more than stimulate it. However, these hormones are known to have anabolic effects in liver at least. It is as yet unclear how glucocorticoids decrease muscle protein synthesis in some tissues, but have the opposite effect on the liver. The evidence for mode of action which does exist will be briefly reviewed.

Korner (1967) reported an increase in liver glutamic alanine transaminase synthesis following corticosteroid treatment. This was apparently due to new messenger synthesis as an increase in label incorporation into liver RNA was observed before the increase in enzyme synthesis began. Kenney (1970) has supported the above findings by suggesting that glucocorticoids induce liver enzyme synthesis by promoting specific transcriptions of DNA. Litwack and Singer (1972) have found labeled cortisol complexed to rat liver nuclear histones following injection of the hormone in vivo. These workers suggested that this interaction would allow more gene transcription. They also reported an increase in the activity of DNA dependent RNA polymerase activity which would also allow more messenger synthesis (Litwack and Singer, 1972). In contrast to these findings, Tata (1968) reported an increased appearance in liver of a 40S ribosomal precursor-messenger RNA particle just before a hydrocortisone induced increase in protein synthesis. Tata (1968) felt that the hormone was influencing ribosome competency through an influence on messenger binding.

Although all of the evidence does not agree, the predominant portion suggests that glucocorticoids influence liver protein synthesis by stimulation of specific messengers. The exact manner of increased messenger synthesis and how one gene can be selected over another for stimulation of transcription have yet to be elucidated.

Androgens

Androgens, as the male sex hormones, are usually thought of in relation to sex organ development; however, they apparently play an important role in general body development and protein synthesis. Korner (1967) observed that castration decreased the protein synthetic capacity of thigh muscle by decreasing the activity of ribosomes. Treatment with testosterone restored the activity but administration of actinomycin blocked the hormone response, indicating synthesis of new RNA was necessary. Widnell and Tata (1966) reported an increase in RNA polymerase activity of nuclei isolated from castrated rat liver following testosterone treatment. Autoradiographic studies have shown administered testosterone to be located with the chromosomes (Manchester, 1970; Liao and Stumpf, 1968). The studies of Liao and Stumf (1968) showed an enhanced nucleolar RNA synthesis that was inhibited by actinomycin representing new RNA synthesis. Breuer and Florini (1966) reported that treatment of castrate rats with testosterone propionate increased RNA synthesis by increasing the priming efficiency of DNA, leading to an increase synthesis of specific messenger RNA molecules. Liao et al. (1966) also suggested that testosterone acted through the chromatin to increase RNA synthesis.

Fujii and Villee (1968) also reported increase in RNA synthesis in young rats following testosterone treatment; however, they suggested another action for the hormone. They propose that testosterone may increase the transport of nuclear RNA to the cytoplasm, making more available for protein synthesis. Recently, Palmiter and Haines (1973) reported an increase in number of ribosomes and amount of messenger RNA per cell in chick oviduct following dihydrotestosterone treatment. They concluded that the increased messenger results from hormone stimulation of RNA polymerase initiation on estrogen activated genes.

Although clear evidence for the method of action of testosterone is not known, it appears that the hormone stimulates new messenger RNA synthesis by interaction with nuclear chromatin.

Estrogens

The estrogens, in accordance with their role in sexual differentiation, influence protein synthesis in several tissues. Luck and Hamilton (1972) overiectomized rats and reported a decrease in ribosomal RNA synthesis. Treatment with estrogen returned the rate to normal and increased the rate or efficiency of processing ribosome precursors. Hamilton et al. (1968) reported a decreased rate of synthesis of nucleolar RNA in overiectomized rats. Treatment with estradiol 17 β increased the rate of synthesis within 20 minutes. Use of actinomycin D abolished this response, leading the workers to conclude that estrogen acts by stimulating all forms of RNA synthesis.

Other work (Hamilton, 1968) has led to the conclusion that the mode of action of estrogen on protein synthesis is more indirect, reacting first with the nuclear chromatin to promote transcription. Hamilton (1968) also suggested that estrogen accelerates the rate of formation of ribosomal precursor particles and the transport of particles with attached messenger to the cytoplasm. Palmiter (1972) administered estradiol to immature chicks and observed an increase in oviduct protein synthesis. He concluded that the hormone mediated protein synthesis primarily by influences on chain initiation and increasing the amount of messenger RNA available.

Most workers have not found direct influences on the protein synthetic process but have confined their conclusions to increased RNA synthesis. Moore and Hamilton (1964) and Teng and Hamilton (1967) concluded the initial effect of estrogen on the overiectomized rat uterus is gene activation allowing synthesis of new RNA leading to increased polysome function and protein synthesis. Gorski and Axman (1964), using cycloheximide, reported that protein synthesis was necessary before the estrogen stimulation in RNA and protein synthesis is seen. Gorski (1964) suggested that estrogen stimulates synthesis of a specific protein that can stimulate RNA polymerase activity thus explaining the observed increase in RNA synthesis. Notides and Gorski (1966) added evidence to this when they showed induction of a specific protein within 30 minutes after estrogen treatment and prior to the increase in protein synthesis. They did not, however, show a relation between the synthesized protein and RNA polymerase. Knowler and Smellie (1971) concluded that estrogen first

stimulates production of a new messenger coding for a specific protein that in turn leads to the stimulation of ribosomal RNA and protein synthesis.

The protein synthesis stimulating effects of estrogen are apparently indirect ones. It is suggested that the hormone acts at the DNA level mediating synthesis of an intermediate compound which then stimulates RNA and protein synthesis.

MATERIALS AND METHODS

Design of Experiment

A total of 42 lambs of the following ages: birth, 30, 60, 90 and 120 days were sacrificed in this experiment. Six lambs, half male and half female, were taken from the ewe for slaughter in each of the first three age groups. The 24 remaining lambs were weaned at 60 days and placed on the experimental rations (Table 1). The rations were designed to be isocaloric and 7 and 15 percent crude protein for the low and high protein rations respectively. The 24 lambs were grouped by weight and divided evenly between the two diets. Six high protein and 6 low protein fed lambs were sacrified at both 90 and 120 days of age.

Pre-Slaughter and Slaughter Procedures

New born lambs were removed from the ewe immediately after birth and not allowed to suckle. All newborns were slaughtered within 12 hours of birth with a blood sample taken only at slaughter. Pre-slaughter blood samples were not taken as it was assumed that the stress of birth would mask any stress associated with slaughter procedures. Pre-slaughter blood samples were taken from lambs of the remaining age groups via jugular puncture. Heparin was used as an

TABLE 1

Rations

Ingredient	7% C.P. % of Total	15% C.P. % of Total
Corn, Dent, Yellow, grain, gr 2 US mm wt 54 (4) 4-02-931	40	40
Oats, grain (4) 4-03-309	10	10
Cerelose	10	6
Starch	17	10
Sugarcane molasses, (5) 5-04-604	10	10
Fish Protein Concentrate (80% Crude Protein)	--	8
Soybean, Seeds, Solv-extd, grnd, mx 7% fiber (5) 5-04-604	--	3
Alfalfa, hay, S-C, mature 1-00-071	7	7
Mineral-Vitamin Mix	3[a]	3[b]
Bed-O-Cobs[c]	3	3
	100	100

[a] MSU Vitamin Mineral Mix P1, Composition in Appendix Table 6

[b] MSU Vitamin Mineral Mix P2, Composition in Appendix Table 7

[c] Andersons' No. 4 fines, The Andersons, Maumee, Ohio

anticoagulant and sodium fluoride at a concentration of 1 mg per ml of blood was used to prevent glycolysis. After bleeding, lambs were trucked approximately 3 miles to the abbatoir and killed by exsanuiation without stunning.

Blood Sample Collection and Preparation

Plasma

Pre-slaughter blood samples were collected into heparinized tubes via jugular puncture. Slaughter samples were taken by collecting trunk blood into heparinized beakers. Both samples were centrifuged at 4,080 x g for 10 minutes to separate plasma from red cells. The plasma was transferred into small test tubes with disposable pasteur pipettes and frozen (-70°) for later analysis of glucose and urea nitrogen. A protein free filtrate was prepared (as described later) from a portion of the plasma and frozen for amino acid analysis.

Serum

Pre-slaughter and slaughter blood samples were collected as described above except that anticoagulant was not used. Blood was allowed to stand at room temperature for 1 hour and then overnight in a coldroom at about 5°. The clot was rimmed and spun down at 2,200 x g for 30 minutes. The serum was transferred into small vials with disposable pasteur pipettes and frozen (-70°) for hormone analysis.

Plasma Urea Nitrogen Determination

Plasma urea nitrogen was determined by the microdiffusion method of Conway (1960). All plates were prepared by placing 1 ml of glycerol in the outer well, 1 ml boric acid solution (.04N) in the inner

well, 0.5 ml of plasma and 0.5 ml of distilled water in one side of the middle well and 0.5 ml urease solution (20 mg/ml) in the other side of the middle well in order to hydrolyze the plasma urea to ammonia. The lid was placed on the plate and rotated in the glycerol to provide a seal and prevent ammonia escape. The plate was swirled gently to mix the sample and enzyme and then placed on a rotator for one hour to allow completion of the enzyme reaction. At completion of urea hydrolysis 1 ml of potassium carbonate (K_2CO_3) solution (100% w/v) was added to the middle well, the lid replaced and sealed and the plates returned to the rotator for an additional hour. A water blank was prepared in a similar manner for each group of samples. Each sample was run in duplicate. At the end of the ammonia diffusion period the content of the inner well of plates containing plasma appears green in color due to the trapped nitrogen. This content was titrated with a standard solution of 0.04 N HCl until the color matched that of the water blank (light pinkish red) and burette readings were recorded. The grams of urea nitrogen per 100 ml of plasma were calculated by the following equation: Grams of urea nitrogen per 100 ml plasma = $\frac{(A)(B)(.014)(100)}{C}$ where A = ml of acid used to titrate, B = normality of the acid, C = ml of sample used.

All of the nitrogen detected was assumed to be in the form of urea as previous experiments have shown the ammonia level in blood of normal animals to be undetectable by this method.

Plasma Glucose Determination

Plasma glucose was determined by the glucose oxidase method of Hugget and Nixon (1953). Plasma was diluted with distilled water so that 1 ml of diluted plasma contained 10-75 µgrams of glucose. One ml of the diluted plasma was mixed with 2 ml of glucose oxidase reagent (Appendix Table 1) and incubated at 37° for 30 minutes. The incubation was ended with the addition of 4 ml of 5 N HCl to each tube. After mixing, the tubes were allowed to stand 20 minutes for maximum color development. Optical density was read at a wavelength of 525 nm on the Coleman Spectrophotometer model 620. A reagent blank containing distilled water instead of diluted plasma was run in the same manner as above. Aqueous glucose standard solutions containing glucose in concentrations of 10, 20, 40, 60, 80 and 100 µgrams per ml were also run as above for the construction of a standard curve. Glucose concentrations of the unknown samples were calculated from the standard curve.

Plasma Free Amino Acid Determination

One mM norleucine was added to plasma used for free amino acid analysis to act as an internal standard. Norleucine was added at the rate of 0.1 ml per ml of plasma. This was followed by the addition of 50% (w/v) sulfosalicylic acid (SSA) at the rate of 0.1 ml SSA per ml of plasma to precipitate plasma proteins. After placing in ice for 30-60 minutes the mixture was centrifuged at 35,000 x g for 15 minutes. The supernatant (protein free filtrate) was removed with a pasteur pipette and stored at -70° until a complete amino acid analysis could be run (Bergen et al., 1973; Bergen and Potter, 1971).

Serum Glucocorticoid Determination

Extraction

Trimethylpentane (nanograde) was added to serum in a ratio of 1:5 and vortexed vigorously to wash out progestogens. The mixture is frozen and stored at -20° for 1 hour and with caution to avoid thawing the serum, the trimethylpentane layer containing progestogens is decanted and discarded. For glucocorticoid extraction, the washed serum was thawed, mixed with 2 ml of methylene chloride (reagent grade) and vortexed vigorously for 1 minute. Two phases formed with the methylene chloride glucocorticoid containing phase being the lower one. This phase was transferred with a disposable pipette to a culture tube and the methylene chloride was evaporated. The tube walls were rinsed 3 times drying between each rinse, with redistilled chloroform: methanol (99:1) saturated with distilled water.

Competitive Protein Binding Assay

The isolated glucocorticoids were resuspended in redistilled chloroform:methanol (99:1) saturated with distilled water. Aliquots of 50 and 100 µl were transferred to disposable culture tubes and the solvent evaporated. One ml of 1.25% dog plasma (Colorado Serum Company) containing about 20,000 cpm/ml of ^{3}H-cortisol was added to each tube, vortexed and incubated for 12-16 hours at 5°. Bound and free glucocorticoid were separated with the addition, while stirring, of 0.5 ml of 0.05% dextran 150 (Pharmacia) and 0.5% carbon decolorizing neutral norit (Fisher Scientific Company) to each tube at a temperature of 5°. Total time from addition of dextran-coated charcoal

to the first tube until addition to the last tube should not exceed 10 minutes. The tubes were vortexed and centrifuged at 2,500 x g for 10 minutes. Radioactivity was determined by the addition of 0.5 ml of supernatant to 10.0 ml of PCS scintillation fluid (Amersham Searle) in a glass vial and counting in a Nuclear-Chicago liquid scintillation counter model 6848. Glucocorticoid standards of concentrations 0.0, 0.1, 0.25, 0.5, 1.0, 1.5, 2.5, 5.0 and 10.0 ng/ml were treated as described above for construction of a standard curve and calculation of unknown glucocorticoid concentrations. Final concentrations were corrected for procedural losses. Approximately 2,000 cpm of ^{3}H-glucocorticoid were placed in disposable culture tubes and unknown serum (0.1 or 0.2 ml) added and allowed to equilibrate with the tracer for 20 minutes. These samples were extracted and assayed as described above and a recovery figure was calculated.

Insulin Determination

Serum insulin was determined by using the two antidoby radioimmunoassay system of Grigsby (1973) modified from the prolactin assay of Koprowski and Tucker (1971). The assay used guinea pig antibovine insulin serum (GPABI) and sheep antiguinea pig gamma globulin (SAGPGG) to form an isoluble complex with mass great enough to be precipitated when centrifuged at 2,500 x g for 30 minutes. Compositions of all reagents used are shown in Appendix Table 2. The assay has been validated by Grigsby (1973) and further validation was not considered necessary. Standards were prepared from purified bovine insulin (Eli Lilly and Company, Indianapolis, Indiana, lot 795372, 24.2 units per mg)

with 100 µl of each standard containing 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 3.0, 4.0, or 5.0 ng of insulin.

In preparation for the insulin assay either 250 or 350 µl of 0.05 m phosphate buffered saline-1% bovine serum albumin, pH 7.4, were added to all tubes prepared for serum samples. Four hundred µl of the above buffer were added to the tubes for the bovine insulin standards. This was done before volume was brought to 500 µl with serum samples (150 or 250 µl) or standards (100 µl) to prevent any binding of the hormone to tube walls.

On day zero, 200 µl of GPABI diluted 1:105,000 in normal guinea pig serum (NGPS) were added to each tube (except total counts), vortexed and incubated for 24 hours at 4°.

On day one, 100 µl of ^{125}I-insulin containing about 15,000 cpm were added to each tube, vortexed and incubated for 24 hours at 4°.

Two hundred µl of SAGPGG were added to each tube except total counts on day two, vortexed and incubated for 96 hours at 4°.

At the completion of incubation on day six, 3 ml of 0.05 M phosphate buffered saline were added to each tube except total counts and all tubes were centrifuged at 2,500 x g for 30 minutes in a refrigerated centrifuge with a swinging bucket rotor (Sorval Model RC-3, Ivan Sorval, Inc., Norwalk, Connecticut). The supernatant was decanted and the tubes inverted on absorbent paper for 30 minutes. The tubes were then wiped dry and counted for 10 minutes or 10,000 counts, whichever came first, in a Nuclear-Chicago Model 4230

autogamma scintillation counter. The tube number and counting time was simultaneously punched onto a paper tape (Teletype Corp., Skokie, Illinois) which was later used in calculating unknown insulin concentrations. The insulin standards were used to construct a standard curve based on the percent of labeled insulin bound. Regression coefficients for the standard curve were calculated on the C.D.C. 3,600 and entered into an Olivetti calculator (Programma 101, Olivetti Underwood, New York, New York) which corrected for dilution and automatically calculated hormone concentrations of unknown sera as counting time and tube number were entered via the punched tape editor (Beckman Model 6912 Tape Editor, Beckman Instruments, Inc., Fullerton, California).

Growth Hormone Determination

The assay used for growth hormone (GH) was the double antibody radioimmunoassay of Purchas (1969). The assay used guinea pig anti-bovine growth hormone serum (GPABGH) and sheep antiguinea pig gamma globulin (SAGPGG) to form an insoluble complex that would precipitate when centrifuged at 2,500 x g for 30 minutes. Standards were prepared from NIH-GH-B with 100 µl of each standard containing 0.1, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 ng of GH.

In preparation for the GH assay either 250 or 350 µl of 0.05 M phosphate buffered saline-1% bovine serum albumin, pH 7.4, were added to all tubes prepared for serum. Both standards and serum samples were handled in the manner described previously for the insulin assay. This was considered as day zero.

On day one, 200 µl of GPABGH diluted 1:3200 were added to all tubes except total counts, vortexed and incubated for 24 hours at 4°.

On day two, 100 µl of ^{125}I-GH containing about 30,000 cpm were added to each tube, vortexed and incubated for 24 hours at 4°.

Two hundred µl of SAGPGG were added to each tube except total counts on day three, vortexed and incubated for 72 hours at 4°.

At the completion of the incubation on day six, additions and handling procedures were identical with those described earlier for the insulin assay. The calculation of results were also identical to the method described for the insulin assay.

Tissue Collection and Analysis Procedures

Liver

Following slaughter the liver was immediately removed from the animal and weighed. Subsamples were taken from each lobe and frozen in liquid nitrogen and stored at -70° for later analysis.

Samples of liver were taken from the freezer and allowed to thaw. Approximately 2 grams of liver were weighed and homogenized in ice cold distilled water. The homogenate was made up to a volume of 40 ml with cold distilled water. Two ml of the homogenate were analyzed for total nitrogen by the Kjeldahl method using copper as a catalyst. Eighteen ml of the homogenate were mixed with 2 ml of 50% (w/v) SSA, vortexed, placed in ice for 30-60 minutes and centrifuged at 35,000 x g for 15 minutes. The supernatant was made up to a volume of 20 ml and a 5 ml sample taken for Kjeldahl nitrogen analysis. This fraction

represents the soluble or non protein nitrogen fraction. The remainder of the homogenate was frozen for DNA-RNA analysis.

Liver was also analyzed for free amino acids. Approximately 0.5 grams of liver was homogenized in 5 ml of 5% (w/v) SSA with 0.7 ml of norleucine (1 mM) added to act as an internal standard. The homogenate was placed in ice for 30-60 minutes and centrifuged at 35,000 x g for 15 minutes. The supernatant was evaporated to near dryness and then resuspended in 2 ml of a pH 2.0 buffer. The samples were frozen and stored at -70° until a complete amino acid analysis could be run.

A modification of the method of Munro and Fleck (1969) was used to determine RNA and DNA. Two ml of the original liver homogenate were pipetted into glass centrifuge tubes and 10 ml of 2:1 methanol-chloroform (v/v) were added. The tubes were vortexed, stoppered and agitated on a rotator for 18 hours. At the end of agitation, the tubes were vortexed and centrifuged at 39,000 x g for 15 minutes. The supernatant was decanted and discarded and the tubes drained upside down on absorbent paper under the hood to allow evaporation of solvent fumes. The pellet was broken up with a small wooden applicator stick and 5 ml of cold 2.5% (w/v) perchloric acid (PCA) were added. Tubes were vortexed, placed in ice for 10 minutes, vortexed and centrifuged at 39,000 x g for 15 minutes. The supernatant was decanted and discarded. The pellet was broken up as before and 5 ml of cold 1.0% (w/v) PCA were added. Tubes were vortexed and centrifuged at 39,000 x g for 15 minutes and the supernatants discarded. The pellet was broken up and 4 ml of 0.3 N potassium hydroxide were added and the

tubes vortexed. Tubes were incubated at 37° in a water bath for 3 hours, being agitated several times during the incubation. After incubation, tubes were vortexed and placed in ice until cold. Five ml of cold 5.0% (w/v) PCA were added and the tubes vortexed and placed in ice for 15 minutes after which the tubes were vortexed and centrifuged at 39,000 x g for 10 minutes. The supernatant was decanted into 25 ml graduated tubes and saved. The pellet was washed twice with 5 ml of cold 5.0% (w/v) PCA, vortexing and centrifuging at 39,000 x g for 10 minutes each time. The washings were added to the 25 ml graduated tubes and total volume was brought to 20 ml with 5.0% (w/v) PCA. This fraction represented RNA and was saved. The pellets were saved for DNA extraction and analysis.

DNA extraction was begun by breaking up the pellets as described above and adding 5.0 ml of 10% (w/v) PCA to each tube. The samples were vortexed and marbles placed on top to act as condensers. The samples were then digested in a 70° water bath for 25 minutes. The samples were agitated frequently during the digestion. After digestion, the samples were vortexed, placed in ice until cold and centrifuged at 39,000 x g for 10 minutes. The supernatant was decanted into tubes calibrated at 10 ml. The pellet was washed with 4.85 ml of cold 10.0% (w/v) PCA, vortexed and centrifuged as above with the supernatant added to the 10 ml calibrated tubes. The total volume was made up to 10 ml with 10.0% (w/v) PCA and saved. This fraction represented total DNA.

RNA concentration was determined by a colorimetric procedure utilizing orcinol. Two ml of the RNA fraction were pipetted into a

test tube and 2 ml of a 1.0% (w/v) orcinol reagent (Appendix Table 3) were added and mixed. Marbles were placed on top to act as a condenser and the tubes were placed in a boiling water bath for 30 minutes. A reagent blank of 2 ml of 5.0% (w/v) PCA instead of sample and RNA standards of 12.5, 25.0, 37.5 and 50.0 mg per ml were treated in the same manner. After boiling, the tubes were cooled in running cold water, allowed to reach room temperature and read immediately in the Coleman Spectrophotometer model 620 at a wavelength of 680 nm.

DNA concentration was determined by a colorimetric procedure utilizing diphenylamine and acetaldehyde. Two ml of the DNA fraction were pipetted into a test tube and 2 ml of 4.0% (w/v) diphenylamine in glacial acetic acid (Appendix Table 4) and 0.1 ml of acetaldehyde solution (Appendix Table 5) were added and mixed. Marbles were added to the top to act as condensers and the samples incubated overnight at 30° in a water bath. A reagent blank containing 2 ml of 10.0% (w/v) PCA instead of sample and DNA standards of 12.5, 25.0, 37.5, and 50.0 mg per ml were treated in the same manner. The samples were removed from the water bath and cooled to room temperature and read in the Coleman Spectrophotometer model 620 at a wavelength of 595 nm.

Muscle

The gastrocnemius muscle was removed immediately after slaughter, weighed, frozen whole in liquid nitrogen and stored at -70° for later analysis.

Muscle samples weighing 2 grams were homogenized and analyzed for nitrogen as described previously for liver. Muscle was also

analyzed for free amino acids. Approximately 0.5 grams of muscle were homogenized in 20.0 ml of 5.0% (w/v) SSA. The homogenate was placed in ice for 30-60 minutes and centrifuged at 35,000 x g for 15 minutes. Five ml of the supernatant were mixed with 0.7 ml of 1 mM norleucine as an internal standard and evaporated to near dryness. The sample was resuspended in 1.0 ml of a pH 2.0 buffer and frozen until a complete amino acid analysis could be run.

Muscle DNA and RNA were analyzed as described for liver with one exception. The length of the potassium hydroxide digestion was decreased from 3 to 2 hours duration. The remainder of the analysis was as previously described.

Adrenal Glands

Both adrenal glands were removed immediately after slaughter, weighed, frozen whole in liquid nitrogen and stored at -70° for later hormone analysis.

The glands were homogenized for approximately 15 seconds with a Polytron (Brinkmann Instruments, Inc., Westbury, New York). Total volume of the homogenate was made up to 10.0 ml with distilled water. The extraction and analysis of glucocorticoids was as described previously for serum.

Anterior Pituitary

The head was severed from the animal and the anterior pituitary removed, weighed, frozen whole in liquid nitrogen and stored at -70° for later analysis of growth hormone content.

The pituitary was homogenized for approximately 15 seconds in sufficient volume of 0.05 M phosphate buffered saline, pH 7.4, to give a concentration of 10.0 mg pituitary per ml of buffer. Growth hormone was determined as previously described for serum.

RESULTS

Average daily gain, daily feed intake and feed to gain ratios for rams and ewes are presented in Table 2. Increasing the percent crude protein in the diet significantly ($P<.05$) increased average daily gain and daily feed intake for rams and ewes at both 90 and 120 days of age. Feed to gain ratios were significantly ($P<.05$) decreased in all cases by the increase in dietary protein except for rams at 120 days of age. Feed conversion was excellent in all cases for the 15% crude protein diet, always being less than 3.0.

The effects of diet on adrenal, pituitary, liver and gastrocnemius muscle are presented in Table 3. Organ weights would normally increase with age; therefore, the statistical analysis for effects of age was not performed. Increasing the dietary protein had no significant ($P>.05$) effect on adrenal weight, all glands averaging between 0.8 and 0.9 grams.

Pituitary weights were significantly ($P<.05$) increased by the increase in dietary protein. Glands from lambs fed the high protein diet averaged 0.49 grams vs 0.21 grams for glands from lambs on the low protein diet.

Increasing the dietary crude protein led to a large and highly significant ($P<.01$) increase in liver weights. Lambs fed the high

TABLE 2

Average Daily Gain and Feed Intake

	Rams			Ewes		
	ADG[1]	Intake[2]	F/G[3]	ADG[1]	Intake[2]	F/G[3]
90 da 7%	41[A]	513[A]	12.5[A]	36[A]	409[A]	11.2[A]
90 da 15%	309[B]	781[B]	2.5[B]	318[B]	649[B]	2.0[B]
120 da 7%	95[A]	463[A]	4.9[A]	27[A]	373[A]	13.7[A]
120 da 15%	340[B]	1003[B]	2.9[A]	254[B]	722[B]	2.8[B]

1 Grams per sheep, mean of 6 animals per group.

2 Grams per day per sheep, mean of 6 animals per group.

3 Grams of feed/gram of gain.

A,B Means differing in superscripts differ significantly between diets $P<.05$.

TABLE 3

Effect of Age and Diet on Tissue Weights[1]

Age	Left Adrenal	Right Adrenal	Pituitary	Liver	Muscle
Birth	.46	.41	.11	96	20
30	.66	.66	.17	280	56
60	.58	.50	.39	455	102
90	.84	.82	.27	924	156
120	.96	.92	.71	941	172
SEM	.02	.03	.03	16	2
Diet	**Left Adrenal**	**Right Adrenal**	**Pituitary**	**Liver**	**Muscle**
Low Protein	$.88^{A}$	$.80^{A}$	$.21^{A}$	532^{a}	122^{a}
High Protein	$.90^{A}$	$.87^{A}$	$.49^{B}$	933^{b}	164^{b}
SEM	.03	.03	.04	19	3

[1] Least Square Means in grams, 12 animals/mean for diet.

a,b Means in columns differing in superscripts differ significantly, $P<.01$; $^{A,B}P<.05$.

protein diet yielded livers averaging 933 grams while livers of low protein fed lambs averaged only 532 grams.

Gastrocnemius muscle weights also increased significantly ($P<.01$) with the additional dietary crude protein. Muscles from lambs fed the high protein diet averaged 164 grams while lambs on the low protein diet yielded muscles averaging only 122 grams.

Age, diet and slaughter stress effects on plasma glucose and urea nitrogen are presented in Table 4. The stress associated with handling procedures prior to slaughter led to a significant ($P<.01$) increase in plasma glucose levels in 90 and 120 day old lambs. Plasma glucose levels increased from 65 to 75 mg/100 ml with slaughter in 90 day old lambs and from 58 to 90 mg/100 ml in 120 day old lambs. No effect of stress on glucose values was observed in either 30 or 60 day old lambs. As stated previously, pre-slaughter blood samples were not taken from lambs at birth; therefore, no assessment of stress for this age group could be made.

Pre-slaughter plasma glucose values decreased significantly ($P<.01$) with age at 30, 60, and 90 days, averaging 100 to 85 to 67 mg/100 ml respectively. The pre-slaughter value of 58 mg/100 ml taken at 120 days was numerically but not significantly smaller than the value at 90 days. Slaughter glucose values exhibited the same general trends in decreasing with age as the pre-slaughter samples except from birth to 30 days of age. A significant ($P<.01$) increase from 73 to 108 mg/100 ml was observed from birth to 30 days.

The increase in dietary protein exerted no effect on pre-slaughter plasma glucose levels, being 60 and 62 mg/100 ml for low and high protein

TABLE 4

Effects of Age, Diet and Slaughter Stress on Plasma Glucose and Urea Nitrogen[1]

	Glucose		Urea Nitrogen	
Age	Preslaughter	Slaughter	Preslaughter	Slaughter
Birth	--------	73 ± 10^{B}	------	15 ± 1^{A}
30	100 ± 2^{A2}	108 ± 4^{A2}	15 ± 1^{A2}	17 ± 1^{A2}
60	85 ± 4^{B2}	80 ± 4^{B2}	22 ± 2^{B2}	22 ± 1^{B2}
90	67 ± 2^{C2}	75 ± 4^{B3}	21 ± 2^{B2}	24 ± 2^{B2}
120	58 ± 2^{C2}	90 ± 8^{B3}	20 ± 1^{B2}	24 ± 1^{B2}
Diet				
Low Protein	60 ± 3^{a}	70 ± 3^{a}	$4\pm.7^{a}$	$5\pm.7^{a}$
High Protein	62 ± 2^{a}	82 ± 5^{b}	20 ± 1^{b}	24 ± 1^{b}

[1] Means ± Standard Error, mg/100 ml, 6 animals/mean for age and 12/mean for diet.

Glucose
Means differing in superscripts differ significantly, ^{A,B}age $P<.01$; ^{2,3}slaughter stress $P<.01$; ^{a,b}diet $P<.05$.

Urea Nitrogen
Means differing in superscripts differ significantly, ^{A,B}age $P<.01$; ^{2}slaughter stress $P>.05$; ^{a,b}diet $P<.01$.

fed lambs respectively. A significant ($P<.05$) increase from 70 mg/100 ml for low protein fed lambs to 82 mg/100 ml for high protein fed lambs was observed in samples taken at slaughter.

The stress of pre-slaughter handling procedures had no effect on levels of plasma urea nitrogen of any age group.

Pre-slaughter urea nitrogen levels increased significantly ($P<.01$) from 15 mg/100 ml at 30 days to 22 mg/100 ml at 60 days. No further increases were observed as levels were 21 and 20 mg/100 ml for 90 and 120 days respectively.

Plasma urea nitrogen from samples taken at slaughter exhibited the same trends as pre-slaughter samples, being significantly ($P<.01$) lower at birth and 30 days than any other age group.

Decreasing the dietary protein led to a significant ($P<.01$) decrease in plasma urea nitrogen levels for both pre-slaughter and slaughter samples. Lambs fed the low protein diet averaged 4 to 5 mg/100 ml while those fed the high protein diet averaged 20 to 24 mg/100 ml plasma urea nitrogen.

The changes in muscle free amino acid pools with increases in age are presented in Table 5. Total essential amino acid (TEAA) levels decreased numerically but not significantly from birth to 60 days. A significant ($P<.01$) increase in TEAA levels was observed at 90 days and again ($P<.01$) at 120 days of age. The same pattern of change was observed with total nonessential amino acids (TNEAA). Ratios of nonessential to essential (N/E) amino acids were significantly ($P<.01$) greater at 60 days than at any other age. The N/E

TABLE 5

Effects of Age on Muscle Free Amino Acid Pools[1]

Amino Acids	Birth	30	60	90	120	SEM
Lysine	$.04^{A}$	$.06^{A}$	$.03^{A}$	$.05^{A}$	$.04^{A}$	.01
Histidine	$.10^{A}$	$.09^{A}$	$.09^{A}$	$.09^{A}$	$.07^{A}$	.03
Arginine	$.15^{a}$	$.08^{b}$	$.08^{b}$	$.05^{c}$	$.06^{bc}$	.02
Threonine	$.48^{a}$	$.06^{b}$	$.07^{b}$	$.04^{b}$	$.04^{b}$	.02
Valine	$.04^{a}$	$.06^{b}$	$.08^{c}$	$.07^{bc}$	$.07^{bc}$	.02
Methionine	$.03^{A}$	$.03^{A}$	$.03^{A}$	$.03^{A}$	$.03^{A}$	.008
Isoleucine	$.04^{a}$	$.05^{bcd}$	$.06^{bd}$	$.04^{cd}$	$.05^{d}$	.01
Leucine	$.05^{a}$	$.07^{b}$	$.07^{b}$	$.07^{b}$	$.08^{e}$	.02
Phenylalanine	$.03^{ab}$	$.03^{a}$	$.03^{b}$	$.03^{c}$	$.02^{d}$	.006
TEAA	$.96^{a}$	$.58^{a}$	$.53^{a}$	1.68^{b}	4.36^{c}	.15
TNEAA	3.03^{ab}	1.77^{a}	2.24^{a}	4.84^{b}	10.81^{c}	.47
N/E	3.30^{a}	3.09^{ac}	4.18^{b}	2.65^{cd}	2.48^{d}	.13

[1] µmoles of amino acid per gram of wet tissue, Least Square Means.

a,b,c,d,e
Means in a row differing in superscripts differ significantly between age groups $P<.01$; $^{A}P<.05$.

ratio decreased at both 90 and 120 days, reaching a value of 2.48 at 120 days which was significantly ($P<.01$) less than any other ratio obtained.

Dietary effects on muscle free amino acid pools are presented in Table 6. TEAA levels of muscle from lambs fed the high protein diet were significantly ($P<.05$) greater than levels measured in muscle of lambs consuming the low protein diet. No significant ($P>.05$) dietary effect on TNEAA was observed. N/E ratios were significantly ($P<.01$) increased in muscle from lambs on the low protein diet, reaching a value of 4.35.

Dietary effects on individual essential amino acids were not constant. No change was observed in the level of lysine, phenylalanine and histidine. Methionine significantly ($P<.01$) increased with the increase in dietary crude protein. The branched chain amino acids (valine, leucine and isoleucine) also exhibited a significant ($P<.01$) increase when dietary crude protein was increased.

Changes in liver free amino acid pools with changes in age are presented in Table 7. Liver TEAA levels were significantly ($P<.01$) greater at 60 days than at any other age. Levels at all other ages were not statistically different. Liver TNEAA significantly ($P<.01$) increased at 30 and again ($P<.01$) at 60 days. A significant ($P<.01$) decrease to levels measured at birth was observed at 90 and 120 days. No significant ($P>.05$) change in liver N/E ratios was found with changes in age.

TABLE 6

Effects of Diet on Muscle Free Amino Acid Pools[1]

Amino Acids	High Protein	Low Protein	SEM
Lysine	.04^{A}	.04^{A}	.04
Histidine	.08^{A}	.11^{A}	.05
Arginine	.06^{A}	.05^{A}	.05
Threonine	.04^{a}	.03^{b}	.01
Valine	.07^{a}	.04^{b}	.02
Methionine	.03^{a}	.02^{b}	.01
Isoleucine	.05^{a}	.04^{b}	.01
Leucine	.07^{a}	.05^{b}	.02
Phenylalanine	.02^{A}	.02^{A}	.008
TEAA	3.02^{A}	2.06^{B}	.19
TNEAA	7.82^{A}	8.45^{A}	.71
N/E	2.56^{a}	4.35^{b}	.15

[1] µmoles of amino acid per gram of wet tissue, Least Square Means.

a,b Means in a row differing in superscripts differ significantly between diets $P<.01$; $^{A,B}P<.05$.

TABLE 7

Effects of Age on Liver Free Amino Acid Pools[1]

Amino Acids	Birth	30	60	90	120	SEM
Lysine	.28^{a}	.33^{a}	.51^{b}	.30^{a}	.31^{a}	.01
Histidine	.37^{a}	.57^{b}	.65^{c}	.68^{c}	.58^{b}	.01
Arginine	.00^{a}	.06^{c}	.14^{b}	.08^{c}	.06^{c}	.005
Threonine	1.97^{A}	1.63^{A}	2.52^{A}	1.00^{A}	.90^{A}	.19
Valine	.32^{a}	.44^{d}	.84^{b}	.52^{e}	.64^{c}	.02
Methionine	.23^{A}	.15^{C}	.23^{A}	.20^{B}	.21^{B}	.008
Isoleucine	.19^{a}	.24^{d}	.43^{b}	.26^{d}	.32^{c}	.008
Leucine	.50^{a}	.64^{b}	1.05^{d}	.69^{b}	.84^{c}	.02
Phenylalanine	.15^{a}	.16^{a}	.25^{b}	.15^{a}	.22^{b}	.008
TEAA	4.02^{a}	4.23^{a}	6.63^{b}	3.87^{a}	4.10^{a}	.19
TNEAA	15.95^{a}	20.44^{b}	23.63^{c}	15.07^{a}	16.84^{a}	.58
N/E	3.99^{A}	4.90^{A}	3.76^{A}	3.93^{A}	4.11^{A}	.17

[1] µmoles of amino acid per gram of wet tissue, Least Square Means.

a,b,c,d,e
Means in a row differing in superscripts differ significantly between age groups $P<.01$; $^{A,B,C}P<.05$.

The effects of changing dietary crude protein level on liver free amino acid pools are presented in Table 8. Increasing the dietary crude protein had no effect on liver TEAA. Liver TNEAA increased numerically but not significantly (P>.05) in lambs consuming the low protein diet. A nonsignificant (P>.05) increase in the liver N/E ratio of lambs eating the low protein diet was also found. As was the case in muscle, lysine and phenylalamine levels did not change and histidine significantly (P<.01) decreased with the increase in dietary crude protein. Methionine levels also remained constant with the increasing level of crude protein in the diet. Unlike muscle, only one of the branched chain amino acids, valine, significantly (P<.05) increased with increasing levels of dietary crude protein. Isoleucine and leucine levels remained constant.

Plasma free amino acid pool changes with increases in age are shown in Table 9. Plasma TEAA levels measured at birth were significantly (P<.01) lower than levels measured at any other age. Levels measured at all other ages were relatively constant. Plasma levels of TNEAA decreased numerically but nonsignificantly (P>.05) with each increase in age. The plasma N/E ratio was significantly (P<.01) higher at birth than any other time, with all other ages being statistically identical.

Changes in plasma free amino acid pools with increased dietary crude protein are presented in Table 10. Lambs consuming the high protein diet exhibited significantly (P<.01) higher TEAA than lambs consuming low protein. Plasma TNEAA levels were the reverse, being

TABLE 8

Effects of Diet on Liver Free Amino Acid Pools[1]

Amino Acids	High Protein	Low Protein	SEM
Lysine	.30^{A}	.30^{A}	.02
Histidine	.63^{a}	.81^{b}	.02
Arginine	.07^{a}	.12^{b}	.006
Threonine	.95^{A}	.80^{A}	.04
Valine	.58^{A}	.49^{B}	.02
Methionine	.20^{A}	.19^{A}	.01
Isoleucine	.29^{A}	.27^{A}	.01
Leucine	.77^{A}	.78^{A}	.03
Phenylalanine	.18^{A}	.20^{A}	.01
TEAA	3.98^{A}	3.96^{A}	.11
TNEAA	15.96^{A}	21.20^{A}	.98
N/E	4.02^{A}	5.45^{A}	.28

[1] µmoles of amino acid per gram of wet tissue, Least Square Means.

a,b Means in a row differing in superscripts differ significantly between diets $P<.01$; $^{A,B}P<.05$.

TABLE 9

Effects of Age on Plasma Free Amino Acid Pools[1]

Amino Acids	Birth	30	60	90	120	SEM
Lysine	4.83^{a}	16.00^{b}	13.17^{b}	15.50^{b}	15.67^{b}	.006
Histidine	8.50^{A}	7.00^{B}	5.67^{C}	4.83^{C}	7.83^{AB}	.004
Arginine	6.83^{a}	15.50^{b}	21.33^{c}	9.67^{a}	15.33^{b}	.009
Threonine	30.33^{a}	26.33^{b}	21.83^{c}	16.33^{d}	14.33^{d}	.009
Valine	13.17^{a}	34.83^{bc}	35.17^{bc}	33.17^{b}	40.17^{e}	.020
Methionine	1.00^{a}	4.17^{b}	4.33^{b}	6.00^{c}	7.67^{d}	.002
Isoleucine	4.83^{a}	17.83^{b}	12.67^{c}	18.33^{b}	12.33^{c}	.008
Leucine	8.00^{a}	17.67^{b}	23.17^{c}	20.83^{bc}	28.50^{d}	.009
Phenylalanine	5.67^{A}	8.67^{A}	6.83^{A}	8.00^{A}	9.17^{A}	.004
TEAA	83.17^{a}	148.00^{bc}	144.17^{bc}	132.67^{b}	151.00^{c}	.040
TNEAA	178.33^{A}	167.33^{A}	164.67^{A}	151.33^{A}	151.00^{A}	.050
N/E	2.20^{a}	1.15^{b}	1.14^{b}	1.15^{b}	1.00^{b}	.040

[1] µmoles of amino acid per 100 ml of plasma, Least Square Means.

a,b,c,d,e Means in a row differing in superscripts differ significantly between age groups $P<.01$; $^{A,B,C}P<.05$.

TABLE 10

Effects of Diet on Plasma Free Amino Acid Pools[1]

Amino Acids	High Protein	Low Protein	SEM
Lysine	15.58[a]	8.42[b]	.005
Histidine	6.33[a]	8.58[b]	.004
Arginine	12.50[a]	6.50[b]	.008
Threonine	15.33[a]	8.17[b]	.006
Valine	36.67[a]	19.25[b]	.010
Methionine	6.83[a]	3.00[b]	.002
Isoleucine	15.33[a]	10.25[b]	.004
Leucine	24.67[a]	16.33[b]	.009
Phenylalanine	8.58[a]	5.33[b]	.003
TEAA	141.83[a]	85.83[b]	.020
TNEAA	151.58[A]	167.50[B]	.030
N/E	1.08[a]	1.97[b]	.050

[1] µmoles of amino acid per 100 ml of plasma, Least Square Means.

[a,b] Means in a row differing in superscripts differ significantly between diets $P<.01$; [A,B] $P<.05$.

significantly (P<.05) higher in lambs consuming the low protein diet. Hence, the plasma N/E ratio was significantly (P<.01) increased in lambs consuming the low protein diet. Individually, all essential amino acids measured significantly (P<.01) increased except histidine which significantly (P<.01) decreased when lambs were fed the high protein diet.

The effects of age and diet on liver and muscle nucleic acid concentrations are presented in Table 11. Liver RNA concentrations decreased significantly (P<.01) from birth and 30 days to 60 and 90 days. A further significant (P<.01) decrease to the lowest value measured of 6.3 mg/gram was found at 120 days of age. Increasing the dietary crude protein led to a significant (P<.01) decrease in liver RNA concentration.

Liver DNA concentrations decreased significantly (P<.01) with each increase in age. Values dropped from 6.88 mg/gram at birth to 2.7 mg/gram at 120 days. Liver DNA concentrations also decreased significantly (P<.01) with the increase in dietary crude protein.

Muscle RNA concentrations also decreased significantly (P<.01) with age, dropping from 8.98 mg/gram at birth to 3.47 mg/gram at 120 days. The increased dietary crude protein exerted no significant (P>.05) effect on muscle RNA concentrations.

Muscle DNA concentrations followed the same trends as RNA, decreasing significantly (P<.01) from 3.64 mg/gram at birth to 1.16 mg/gram at 120 days. No significant (P>.05) effect on muscle DNA concentration was observed when increasing dietary crude protein content.

TABLE 11

Effects of Age and Diet on Liver and Muscle Nucleic Acid Concentration[1]

Age	Liver RNA	Liver DNA	Muscle RNA	Muscle DNA
Birth	24.56[a]	6.88[a]	8.98[a]	3.64[a]
30	24.21[a]	5.64[b]	5.49[b]	2.03[b]
60	17.53[b]	4.26[c]	4.19[c]	1.54[c]
90	18.54[b]	3.62[d]	4.52[c]	1.53[c]
120	6.30[c]	2.70[e]	3.47[d]	1.16[d]
SEM	.68	.16	.17	.07
Diet	**Liver RNA**	**Liver DNA**	**Muscle RNA**	**Muscle DNA**
Low Protein	17.08[a]	4.12[a]	3.66[A]	1.49[A]
High Protein	12.42[b]	3.16[b]	3.99[A]	1.34[A]
SEM	.36	.10	.24	.06

[1] Least Square Means, 6 animals/mean for age, 12/mean for diet; mg/gram fresh weight.

[a,b] Means differing in superscripts differ significantly $P<.01$; [A,B] $P<.05$.

Age and dietary effects on total nucleic acid content of liver and muscle are presented in Table 12. Liver RNA content was significantly ($P<.01$) increased over levels measured at birth for both 30 and 60 day old lambs. A further significant ($P<.01$) increase was also measured at 90 days. Liver RNA content at 120 days was significantly ($P<.01$) decreased compared to levels obtained at 30 and 60 days. Liver RNA content was numerically but not statistically decreased by the decrease in dietary protein.

Liver DNA content tended to follow the same pattern as liver RNA. Levels at 30 and 60 days were significantly ($P<.01$) higher than at birth and a further increase ($P<.01$) was shown at 90 days, followed by a drop at 120 days ($P<.01$). Unlike RNA, liver DNA content was significantly ($P<.01$) raised by the increase in dietary crude protein.

Muscle RNA content followed the trend observed for liver. Total content significantly ($P<.01$) increased with age up to 90 days, with a decrease at 120 days. Total content of muscle RNA was increased significantly ($P<.01$) by the increase in dietary crude protein.

Muscle DNA content followed the same pattern as RNA with significant ($P<.01$) increases up to 90 days and then a decrease at 120 days. No significant ($P>.05$) effect of diet on muscle DNA content was found.

The effects of age and diet on liver and muscle RNA/DNA ratios are presented in Table 13. Liver ratios increased from birth to 90 days ($P<.01$) but decreased at 120 days ($P<.01$). The increased dietary crude protein had no significant ($P>.05$) effect on liver RNA/DNA ratios.

TABLE 12

Effects of Age and Diet on Total Nucleic Content of Liver and Muscle[1]

Age	Liver RNA	Liver DNA	Muscle RNA	Muscle DNA
Birth	2.34±.26^{a}	.65±.08^{a}	.18±.02^{a}	.07±.006^{a}
30	6.71±.62^{b}	1.57±.09^{b}	.30±.03^{ab}	.11±.010^{ab}
60	8.10±.97^{b}	1.97±.25^{bc}	.42±.04^{bc}	.16±.008^{bc}
90	17.17±1.27^{c}	3.30±.16^{d}	.70±.05^{d}	.24±.009^{d}
120	5.93±.51^{b}	2.52±.15^{c}	.61±.10^{cd}	.20±.030^{cd}
Diet	**Liver RNA**	**Liver DNA**	**Muscle RNA**	**Muscle DNA**
Low Protein	9.32±1.56^{A}	2.19±.19^{a}	.44±.05^{a}	.18±.01^{A}
High Protein	11.55±1.81^{A}	2.91±.16^{b}	.65±.06^{b}	.22±.02^{A}

[1] Means ± Standard Error, 6 animals/mean for age, 12/mean for diet, Grams/organ.

^{a,b}Means differing in superscripts differ significantly, $P<.01$; $^{A,B}P<.05$.

TABLE 13

Effect of Age and Diet on RNA/DNA Ratios of Liver and Muscle[1]

Age	Liver	Muscle
Birth	$3.69 \pm .24^{aA}$	$2.52 \pm .12^{A}$
30	$4.33 \pm .30^{abA}$	$2.82 \pm .21^{A}$
60	$4.22 \pm .30^{aA}$	$2.74 \pm .14^{A}$
90	$5.22 \pm .23^{bB}$	$2.98 \pm .17^{A}$
120	$2.34 \pm .06^{c}$	$3.08 \pm .21^{A}$
Diet	**Liver**	**Muscle**
Low Protein	$4.10 \pm .34^{A}$	$2.51 \pm .17^{A}$
High Protein	$3.78 \pm .32^{A}$	$3.04 \pm .13^{B}$

[1] Mean ± Standard Error, 6 animals/mean for age and 12 animals/mean for diet.

^{a,b,c}Means in each column differing in superscripts differ significantly, $P<.01$; $^{A,B}P<.05$.

Muscle RNA/DNA ratios tended to increase with age but no significant ($P>.05$) effect was found. Increasing the level of dietary crude protein significantly ($P<.05$) increased the muscle RNA/DNA ratio.

Age and dietary effects on levels of serum growth hormone and insulin are presented in Table 14. Levels of growth hormone were not significantly ($P>.05$) different from birth to 90 days, although the trend was to increase from birth to 60 days and decrease thereafter. A significant ($P<.05$) decrease, resulting in the lowest value measured, occurred at 120 days. Changing the level of dietary protein had no significant ($P>.05$) effect on serum growth hormone levels.

Serum insulin was somewhat erratic but tended to increase with age to 90 days and then decrease. The level of 86 μunits/ml measured at 90 days was significantly ($P<.05$) larger than values obtained at any other time. The increase in dietary protein levels led to a significant ($P<.01$) increase in insulin from 34 μunits to 69 μunits/ml.

The effects of changes in age and diet on total content and concentration of adrenal glucocorticoids are presented in Table 15. Although both total content and concentration tended to decrease with age and increase with increasing levels of dietary crude protein, no significant ($P>.05$) difference was found.

Changes in serum glucocorticoids as related to age, diet and slaughter stress are presented in Table 16. No significant ($P>.05$) effect was recorded in either pre-slaughter or slaughter samples for any change in age or dietary crude protein level. The stress of

TABLE 14

Effects of Age and Diet on Serum Growth Hormone and Insulin[1]

Age	Growth Hormone[2]	Insulin[3]
Birth	5.4±1.7^{A}	27±3^{A}
30	7.5±1.4^{A}	41±5^{B}
60	8.8±2.0^{A}	28±4^{A}
90	4.8± .6^{A}	86±18^{C}
120	2.4± .2^{B}	52±6^{B}
Diet		
Low Protein	4.3± .7^{a}	34±3^{a}
High Protein	3.8± .4^{a}	69±10^{b}

[1] Means ± Standard Error, 6 animals/mean for age and 12 animals/mean for diet.

[2] Growth Hormone, Ng/ml
Means differing in superscripts differ significantly, ^{A,B}age $P<.05$; ^{a}diet $P>.05$.

[3] Insulin, μunits/ml.
Means differing in superscripts differ significantly, ^{A,B,C}age $P<.05$; ^{a,b}diet $P<.01$.

TABLE 15

Effect of Age and Diet on Adrenal Glucocorticoids[1]

Age	Ng/total gland	Ng/100 mg wet weight
Birth	2175[A]	549[A]
30	2068[A]	329[A]
60	1004[A]	238[A]
90	1525[A]	201[A]
120	1029[A]	111[A]
SEM	260	50
Diet		
Low Protein	881[A]	112[A]
High Protein	1277[A]	156[A]
SEM	202	27

[1] Least Square Means, 6 animals/mean for age, 12/mean for diet.

[A] Means in each column differing in superscripts differ significantly, $P<.05$.

TABLE 16

Effect of Age, Diet and Slaughter Stress on Serum Glucocorticoids[1]

Age	Preslaughter	Slaughter
Birth	-----	64±21
30	15±3[a]	20±5[a]
60	12±2[a]	11±2[a]
90	6±2[a]	19±5[b]
120	13±2[a]	14±3[a]
Diet		
Low Protein	7±1	12±1
High Protein	9±2	16±3

[1] Means ± Standard Error, Ng/ml, 6 animals/mean for age and stress, 12/mean for diet.

[a,b] Means differing in superscripts differ significantly due to slaughter stress $P<.01$; no significant effect of age and diet $P>.05$.

pre-slaughter handling procedures had no effect except at 90 days when a significant ($P<.01$) increase in hormone level was found in blood samples taken at slaughter.

Table 17 shows the relationship of age and diet to pituitary gland growth hormone content and concentration. Growth hormone concentration expressed per mg of pituitary or on a body weight basis exhibited no significant change ($P>.05$) with age or level of dietary protein. However, growth hormone concentration per mg of pituitary was lowest for new born lambs. Total growth hormone content per gland increased significantly ($P<.01$) with age after birth with maximum values at 90 and 120 days of age. No significant ($P>.05$) effect of dietary protein level on total growth hormone content was found although a large numerical increase was observed at the higher protein level.

The effects of age and diet on liver protein content and concentration expressed as grams per gram of liver are presented in Table 18. Total liver protein increased significantly ($P<.01$) with each increase in age and with the increase in dietary protein level. The concentration of liver protein increased significantly ($P<.01$) from birth to 60 days and then decreased significantly ($P<.01$) to 120 days, reaching values obtained in the newborn. Level of dietary protein had no influence on liver protein concentration.

Table 19 presents the effects of age and diet on muscle protein content and concentration. Changes in muscle total protein were identical to changes observed with liver, a significant increase with

TABLE 17

Effect of Age and Diet on Pituitary Growth Hormone[1]

Age	µg/mg wet weight		µg/total gland		µg/100 lbs body weight
Birth	1.32[A]		180[a]		----
30	5.22[A]		899[b]		3849[A]
60	3.74[A]		1293[b]		4013[A]
90	6.34[A]		3200[c]		2306[A]
120	5.04[A]		3682[c]		3484[A]
Diet	.56	SEM	206	SEM	490
Low Protein	6.26[A]		2025[A]		2814[A]
High Protein	5.08[A]		3596[A]		3298[A]
	.71	SEM	410	SEM	532

[1] Least Square Means, 6 animals/mean for age, 12/mean for diet.

[A]Means in each column differing in superscripts differ significantly, P<.05; [a,b]P<.01.

TABLE 18

Effect of Age and Diet on Liver Protein[1]

Age	Total Content[2]		Concentration[3]
Birth	12.8[a]		.13[a]
30	52.5[b]		.19[b]
60	97.2[c]		.22[c]
90	184.8[e]		.20[b]
120	133.0[d]		.14[a]
Diet	2.95	SEM	.004
Low Protein	92.3[a]		.17[A]
High Protein	158.9[b]		.17[A]
	4.5	SEM	.005

[1] Least Square Means, 6 animals/mean for age, 12/mean for diet.

[2] Grams.

[3] Grams/Grams.

[A] Means in a column differing in superscripts differ significantly, P<.05; [a,b] P<.01.

TABLE 19

Effect of Age and Diet on Muscle Protein[1]

Age	Total Content[2]		Concentration[3]
Birth	1.8^{a}		$.09^{a}$
30	9.8^{b}		$.17^{b}$
60	19.1^{c}		$.18^{b}$
90	24.7^{d}		$.16^{b}$
120	27.5^{e}		$.16^{b}$
Diet	.58	SEM	.005
Low Protein	18.6^{a}		$.16^{A}$
High Protein	26.1^{b}		$.16^{A}$
	.68	SEM	.003

[1] Least Square Means, 6 animals/mean for age, 12/mean for diet.

[2] Grams.

[3] Grams/Grams.

[A] Means in a column differing in superscripts differ significantly, [a,b] $P<.05$; $P<.01$.

age and level of dietary protein. Muscle protein concentrations increased significantly ($P<.01$) from birth to 30 days and remained constant thereafter. Level of dietary protein had no effect on muscle protein concentrations.

The correlation coefficients of some selected parameters are presented in Table 20. Growth, measured in this study as average daily gain, was positively correlated ($P<.01$) with muscle DNA and RNA content. Pituitary growth hormone content showed only a small positive correlation ($P>.05$) with average daily gain. However, pituitary growth hormone content was positively correlated ($P<.01$) with muscle DNA and RNA content. Serum insulin was positively correlated ($P<.01$) with average daily gain as well as muscle DNA ($P<.05$) and RNA ($P<.01$) contents. Pituitary growth hormone concentration expressed per mg of gland was negatively correlated ($P>.05$) with average daily gain. A small positive correlation ($P>.05$) with muscle DNA content was observed. A positive correlation ($P<.05$) was established between pituitary growth hormone concentration and muscle RNA content. A small negative correlation ($P>.05$) between serum growth hormone and muscle DNA and RNA was noted.

TABLE 20

Correlation Coefficients

Parameter	ADG[1]	PGH[2] Content	Serum Insulin	PGH Concentration[3]	Serum Growth Hormone
ADG	----	.13	.64[a]	-.19	.09
MDNA[4] Content	.55[a]	.53[a]	.37[A]	.24	-.26
MRNA[5] Content	.54[a]	.62[a]	.52[a]	.33[A]	-.19

1 Average Daily Gain

2 Pituitary Growth Hormone

3 µg/mg of Gland

4 Muscle DNA

5 Muscle RNA

A P<.05

a P<.01

DISCUSSION

An objective of this study was to elucidate hormonal relationships with observed growth rates in order to learn more of hormonal influences that control growth. Two growth rates, normal and reduced, were needed in order to determine which hormone-growth rate relationships were important in normal growth. For this reason sheep were maintained on a ration permitting normal growth (high protein) and on a ration only sufficient in protein for maintenance of body weight.

Voluntary food intake in cattle (Elliott, 1967 A) and sheep (Elliott, 1967 B) has been shown to be influenced by level of dietary protein. Therefore, decreasing the level of dietary protein from 15 percent to 7 percent was chosen as the method to control growth in this study. The method was effective (Table 2) as both rams and ewes at 90 and 120 days of age fed the low protein diet gained and ate significantly less than those on high protein. Feed efficiency was excellent on the 15 percent ration with a feed ratio conversion of less than 3 to 1. It must be emphasized that in the remaining discussion, results reflecting dietary changes are a function not only of decreased protein intake but also of decreased energy intake as well.

Blood glucose (Table 4) values increased from birth to 30 days and then decreased to 120 days. The peak increase occurs before 30 days in calves (Murley et al., 1952; Kennedy et al., 1939; Young et al., 1970) and was probably missed in this experiment. Factors causing this change are as yet still unclear. The beginning of the decrease tends to coincide with the time when the ruminant is changing metabolism from carbohydrate-lipid (milk) to volatile fatty acid dependent. Jarrett et al., 1964 and Webb et al., 1969 reported a decrease in the glucose utilization rate as ruminants increased in age, thus it would appear that the change in energy dependence from glucose to fatty acid would precipitate the decrease in blood sugar. However, other workers have shown the decrease in blood glucose to occur regardless of diet or rumen function (Lupien et al., 1962; Nicolai and Stewart, 1965; Lambert et al., 1955). Ponto and Bergen (1974) studied changes in blood glucose in both germfree and conventional ruminants and found a decrease in blood glucose regardless of diet or germfree status. They concluded the decrease was constitutive to the ruminant animal and apparently unrelated to rumen function or volatile fatty acid production. It can be concluded that the change in blood glucose reported in the current study is a normal occurrence in ruminants but the cause is as yet unknown.

In an effort to measure the effects of pre-slaughter handling procedures on levels of blood metabolites, blood samples were taken via jugular puncture with the animals in a resting state and trunk blood was collected at the slaughter house. At 30 and 60 days there

was no effect of stress on blood glucose (Table 4); however, at 90 and 120 days glucose was significantly increased by stress. The 90 and 120 day old lambs were much larger and more difficult to load and handle than younger groups which probably accounts for the additional stress. The increase may have been mediated through increased glucocorticoids. Edwards (1969) has indicated that increased glucocorticoids will lead to increased blood glucose in lambs. Bassett (1963) observed an increase in blood glucose as plasma cortisol increased in sheep. Serum glucocorticoids (Table 16) were significantly ($P<.01$) elevated at 90 days and although not significantly different were still high at 120 days in this study. It is possible that the increased difficulty in handling was manifested by an increased secretion of glucocorticoid which in turn mediated the rise in blood glucose.

Increasing the level of dietary protein in this study did not influence plasma glucose values in pre-slaughter blood samples but significantly increased glucose values in samples obtained at slaughter. The reason for the difference in glucose levels due to time of sampling is not readily apparent although it could represent an interaction of stress and diet.

Alterations in diet have been shown to influence glucose values in the ruminant. Clary et al. (1967) reported increased blood glucose in sheep when corn in the ration was increased. Likewise, Howland et al. (1966) reported increased blood glucose in ewes when dietary protein and energy were increased. Bassett et al. (1971) reported a positive and significant correlation between blood glucose and digestable organic matter intake in sheep. Other reports have

indicated either no effect (Stufflebeam et al., 1969; Memon et al., 1969) or a decrease (Preston and Burroughs, 1958) in plasma glucose of ruminants following an increase in dietary energy or protein. As pointed out previously (Clary et al., 1967), increased carbohydrate in the diet can lead to increased blood glucose in ruminants probably through rumen bypass; therefore, it might be expected tht if the increased feed intake was sufficient to provide rumen bypass of carbohydrate, an increased plasma glucose should have been detected. The results unfortunately are not sufficient to allow a definitive conclusion concerning plasma glucose and dietary relationships in the ruminant.

Blood urea nitrogen (Table 4) exhibited significant increases from birth and 30 days to older ages. As the animals increased in age and size, feed intake (nitrogen intake) increased. Preston et al. (1965) and Nimrick et al. (1971) have reported an increase in plasma urea nitrogen as amount of protein being consumed increased. This is a reasonable explanation for the increase in urea nitrogen with increasing age reported in this study.

Differences in urea nitrogen values in the low and high protein fed animals reflect differences in ration protein content and animal protein intake. Others have shown that blood urea nitrogen increases in the ruminant as protein intake increases (Preston et al., 1965; Nimrick et al., 1971; Tagari et al., 1964). However, factors other than ration protein content could have influenced blood urea values in this experiment and must be considered. Dietary nitrogen, due to differences in rumen solubility, will influence the amount of

ammonia escaping the rumen and consequently the blood urea levels (Little et al., 1968; Ely et al., 1969; Abou Akkada and Osman, 1967). Ration energy availability through the effects on rumen microbial protein synthesis will also influence blood urea values; consequently, low TDN containing rations can lead to increases in blood urea (Leibholz, 1969; Dror et al., 1969). Although ration protein sources varied, the rations were isocaloric and the low protein sheep apparently used the small amount of nitrogen received very efficiently. Thus, it seems reasonable to assume that differences in plasma urea nitrogen values were a direct result of the differences in nitrogen intake.

Tables 5, 7 and 9 show individual and total amino acid responses in muscle, liver and plasma to increases in age. With the exception of threonine, individual plasma amino acids increased with age. Bergen et al. (1973), with a ration corresponding closely to the one used in this study, reported no time dependent changes in plasma amino acids other than an increase in leucine and lysine. Examination of Table 9 will show significant differences as early as 30 days in the present study. The sheep used by Bergen et al. (1973) were not sampled before weaning which may account for the contrasting results. Leibholz (1965) reported that in calves from birth to 24 weeks of age plasma methionine, leucine, lysine and histidine decrease with age while other amino acids did not change.

Similar to this study Bergen et al. (1973) also reported no change in liver free amino acids.

A differential effect with age in levels of essential amino acids was noted in muscle, liver and plasma. Total essential amino

acids decreased from birth to 60 days and increased from 60 to 120 days in muscle; increased from birth to 120 days in plasma; increased to 60 days in liver and then decreased. This pattern of change in the three tissues might indicate a less than optimal supply of energy and/or amino acids for protein synthesis. The muscle N/E ratios (Table 5) which increased until 60 days and decreased thereafter also suggest that nutrient intake was not sufficient at 60 days of age (Bergen et al., 1973).

The low protein ration significantly decreased muscle (Table 6) and plasma (Table 10) total essential amino acids but caused no change in liver (Table 8) essential amino acids. In contrast with our data Boling et al. (1972) reported no change in plasma amino acids of steers when dietary crude protein changed from 6 to 16 percent. However, most workers have reported an increase in plasma essential amino acids when protein intake increased (Weston, 1971; Hogan et al., 1968; Schelling et al., 1967; Cecyre et al., 1973). Feed intake also affects plasma amino acids (Weston, 1971; Nimrick et al., 1971). Hence, the decreased plasma pools observed on low dietary protein could be partially attributed to feed intake.

Muscle total essential amino acids decreased and the N/E ratio increased when dietary protein was decreased (Table 6). No change in liver free pools with diet changes were measured (Table 8). In a protein-energy deficiency, muscle will be degraded to supply amino acids. The bulk of the resulting amino acids would be taken up by the liver for catabolism and could account for the lack of change in liver free amino acid pools observed in our study.

When protein sources having low rumen solubility are used in feeding studies or before the rumen becomes totally functional, the amino acid balance of the protein source may be reflected in plasma (Leibholz, 1965; Bergen et al., 1973). Fish protein is limiting in methionine and histidine (Makdani et al., 1971) but this balance was not reflected in free amino acid pools in our study possibly because protein sources in addition to fish protein concentrate were also used in the ration (Table 1).

Organ and gland and body weights increased with age (Table 3). The rate of increase was highest at younger ages, decreasing as the animals neared a market age of 120 days. Purchas et al. (1970) reported a similar pattern for body weights in bulls.

Liver and muscle were smaller on the low protein diet as was previously reported in studies with swine (Elsley, 1963; Tumbleson et al., 1969). Pituitary glands were smaller on the low protein diet, but adrenal weights were not altered. Bellows et al. (1966) reported no change in pituitary weight of rats fed an energy restricted diet. Energy intake was also decreased in the present study because of decreased feed intake (Table 2). As a percent of body weight, there was no difference in pituitary weight, which agrees with the work of Bellows et al. (1966).

On an absolute basis, no difference in adrenal weight due to diet changes were measured; however, when measured as a percent of body weight adrenals from low protein lambs were much larger than those from high protein fed lambs, which supports the work of Clarke (1969) who reported adrenal hypertrophy in rats fed a 4 percent crude

protein diet. The adrenal hypertrophy is most readily explained as the animal response to a stress situation even though there was no increase in adrenal (Table 15) or serum (Table 16) glucocorticoid content due to diet. It is possible for adrenal hypertrophy to occur without an increase in glucocorticoid synthesis (Clarke, 1969).

An interest in domestic meat animal production forces one to ask why a lower protein diet resulted in decreased liver and muscle weights. Robinson (1971) proposed that cell size may be determined by either the protein or RNA to DNA ratio. An increase in either ratio would indicate an increase in cell size. The muscle RNA/DNA ratio (Table 13) was significantly lower in low protein lambs suggesting a smaller cell size in these muscles. Liver RNA/DNA ratios did not change with diet; however, the liver protein to DNA ratio of 42 for low protein and 55 for high protein groups indicate a smaller liver cell in lambs fed the low protein ration.

The decreased tissue weight is due to a decrease in cell size (Robinson, 1971) and cell number. Tissue DNA content is an indicator of cell number in mononucleate cells. Muscles apparently do not increase in cell number after birth (Hedrick, 1968; Stromer et al., 1974; Enesco and Puddy, 1964; Rowe and Goldspink, 1969); therefore, any increase in muscle size is probably due to a larger cell size because of increased cellular constituents. Protein is the major cellular dry matter constituent thus any factor influencing protein synthesis will influence mature muscle size.

Nucleic acids are essential for protein synthesis to occur. Protein synthesis is known to be highly correlated with cellular RNA content (Howarth, 1972; Wannemacher and McCoy, 1966). Feeding a low protein diet will decrease protein synthesis (Waterlow and Stephen, 1966; Young and Alexis, 1968; Young et al., 1971) possibly by decreasing the RNA content (Young et al., 1971; Gilbreath and Trout, 1973; Trenkle, 1974). Total tissue protein was decreased in this study and the decreased cell size discussed earlier indicates less protein per cell. Total RNA content, as well as DNA, was also decreased in the low protein animals (Table 12), thus it would appear that a decrease in protein synthesis occurred in the low protein lambs and resulted in decreased tissue size.

As discussed in the literature review, hormones influencing growth and development can have a significant effect on protein synthesis. It now becomes important in domestic meat animal production to determine which hormone-protein synthesis relationships are most important and how these relationships are mediated.

The hormones chosen for study in this experiment were growth hormone, insulin and glucocorticoids. Growth hormone and insulin are known to have positive influences on muscle protein synthesis (Manchester, 1970; Goldberg, 1969; Wool et al., 1968) possibly at the ribosome level (Manchester, 1970; Wool and Cavicchi, 1966). Although glucocorticoids have a catabolic effect on skeletal muscle, they stimulate liver protein synthesis (Palmer, 1966) probably through some influence on RNA (Tata, 1968). Positive correlations between pituitary growth hormone concentration and growth rate have been

observed in the bovine (Curl et al., 1968; Armstrong and Hansel, 1956) consequently measurement of glandular levels of hormones were also made in this study.

Serum growth hormone concentration decreased with age (Table 14) while pituitary content and concentration (Table 17) increased. If plasma clearance rate of growth hormone does not change with age (Trenkle, 1971) secretion rate must have decreased. At 120 days of age serum growth hormone concentration, liver protein, RNA and DNA and muscle RNA and DNA content were all decreasing, even though liver and muscle weights were still increasing. Ribosome activity decreases with age (Breuer and Florini, 1965), and so does protein synthesis. If degradation rates remained constant and synthesis decreased, then a decrease in cellular constituents would occur. However, tissue weight could have been maintained or increased by an increase in fat content. As discussed above, growth hormone positively influences protein synthesis; therefore, the decrease in growth hormone may have caused the decrease in cellular constituents. It has been suggested that the cessation of rapid growth in animals is due to a dilution of growth hormone per unit of body weight (Curl et al., 1968; Baird et al., 1952; Baker et al., 1956).

The association of growth and growth hormone becomes less clear when the effects of diet are examined. Feeding the low protein diet did not significantly alter serum or pituitary growth hormone levels although liver and muscle RNA, DNA and protein levels were decreased. Although other work has established a positive relationship between serum and pituitary growth hormone and induced growth rates

(Stephan et al., 1971; Sinha et al., 1973), no relationship could be established in the present study.

A relationship between plasma insulin and induced growth rate can be established. Feeding the high protein diet increased liver and muscle protein, RNA and DNA content and also increased serum insulin (Table 14). Insulin would seem to more directly influence growth rate in this study than growth hormone.

No clear relationship of serum or adrenal glucocorticoids and growth rate could be established as there was no significant effect of diet on adrenal (Table 15) or serum (Table 16) glucocorticoids.

The correlation coefficients presented in Table 20 emphasize previous points. No clear relationship of pituitary or serum growth hormone and growth measured as average daily gain were established. Average daily gain and serum insulin were significantly correlated with muscle nucleic acid content. Serum insulin also was directly correlated with average daily gain. Thus, it can be postulated that growth measured as average daily gain is influenced by muscle RNA and DNA content and that hormonal influences on growth may be mediated through a relationship with nucleic acids.

GENERAL CONCLUSIONS

1. Feeding a low protein diet to growing lambs will create a stress situation resulting in decreased liver and muscle weights but in adrenal hypertrophy.

2. A decrease in plasma glucose and increase in plasma urea nitrogen with increasing age are normal responses in ruminants. A clear relationship of dietary protein level and plasma glucose is not known but plasma urea nitrogen increases as dietary protein intake increases.

3. Muscle total free amino acids, both essential and non-essential, increase with age. Decreasing dietary protein does not influence total amino acids but essential amino acids are decreased.

4. Liver total free amino acids, both essential and nonessential, are not influenced by age or diet.

5. Plasma total free amino acids increased with age due to an increase in the essential amino acids. Feeding the low protein diet decreased total amino acids, both essential and nonessential.

6. Low protein intake will lead to decreased tissue protein and nucleic acid content. No clear relationship of diet and growth hormone can be established but insulin varies directly with dietary protein intake.

7. Growth measured as average daily gain is directly influenced by muscle nucleic acid content and this may be the route of hormonal influence on growth. Insulin apparently also has another more direct relationship with growth rate and needs further investigation.

APPENDIX

APPENDIX

TABLE 1

Glucose Oxidase Reagent

Tris-phosphate-glycerol buffer	100 ml
Glucose oxidase (Boehringer, New York, N.Y.)	30 mg
Horseradish perosidase (Boehringer, New York, N.Y.)	3 mg
O-dianisidine dihydrochloride (Sigma, St. Louis, Mo.)	10 mg

Dissolve and store at 4°C

Tris-phosphate-glycerol buffer

Tris	36.3g
$NaH_2PO_4 \cdot H_2O$	50.0g
Glycerol	400 ml

Add water to 1 liter and adjust pH to 7.0 by addition of solid $NaH_2PO_4 \cdot H_2O$

APPENDIX

TABLE 2

Composition of Reagents for Radioimmunoassays

A. 0.05 M PBS-1% BSA pH 7.4

NaCl---------- 9.0 g
Dissolve with 1 liter of Buffer A_1

B. Buffer A_1

$NaH_2PO_4 \cdot 2H_2O$---------- 6.2 g
Merthiolate---------- 0.25g
BSA (Fraction V, Sterils, 35% solution serological, NBC, Cleveland, Ohio)---------- 14.6 ml
Add 950 ml distilled water
pH to 7.5 with 5 N NaOH
Dilute to 1 liter

C. Guinea Pig Anti-bovine Insulin and Guinea Pig Anti-bovine Growth Hormone

Antisera diluted 1:400 with 0.05 m
PBS-EDTA, pH 7.0. On day of use, dilute 1:400 antisera to required concentration using 1:400 NGPS as diluent

D. 0.05 M PBS-EDTA pH 7.0

Disodium EDTA---------- 18.612 g
Add about 950 ml PBS
Adjust pH to 7.0 with 5 N NaOH
Dilute to 1 liter

E. 0.01 M phosphate buffered saline, pH 7.0 (PBS)

NaCl----------143 g
Monobasic phosphate----------120 ml
Dibasic phosphate----------240 ml
Merthiolate---------- 1.75 g
Dissolve in distilled water and transfer to large container
Dilute to 17.5 liters with distilled water
Adjust pH to 7.0 with NaOH if necessary

APPENDIX TABLE 2 (cont'd.)

F. Monobasic phosphate (0.5m)

$NaH_2PO_4 \cdot H_2O$---------- 69.05 g
Dissolve in distilled water and dilute to 1 liter

G. Dibasic phosphate (0.5m)
Na_2HPO_4---------- 70.98 g
Dissolve in distilled water, heat to dissolve and dilute to 1 liter

H. 1:400 Normal Guinea Pig Serum (NGPS)

Obtain blood from guinea pigs not used for antibody production
Clot the blood, recover serum and store
Add 2.5 ml of serum to 1 liter volumetric flask and dilute to 1 liter with 0.05 m PBS-EDTA and store

I. Sheep Anti-Guinea Pig Gamma Globulin Antibody (SAGPGG)

Dissolve 50 mg guinea pig gamma globulin (Pentex, Kankakee, Illinois, Fraction II) in 5 ml of .85% sterile saline
Emulsify in 5 ml Freund's complete adjuvant by continous flux through an 18 guage needle. (Emulsified when a droplet retains a bead form when dropped on a water surface)
Antigen is injected subcutaneously in 6-8 sites on side of animal
Injections repeated every two weeks with Freund's incomplete adjuvant substituted for the complete
Antisera is collected about six weeks after first injection (collect about 600 ml blood from a 70 Kg sheep)

J. Guinea Pig Anti-Bovine Growth Hormone Antibody (GPABGH)

Two mg of bovine GH is dissolved in 0.5 ml saline and emulsified with Freund's complete adjuvant as described above
Injections were started as above with subsequent injections of 0.5 mg emulsified in Freund's incomplete adjuvant made every two weeks for up to seven injections
Blood is collected by heart puncture and serum recovered

APPENDIX

Table 3

Orcinol Reagent

Make a stock solution of 0.1% $FeCl_2 \cdot 6H_2O$ in concentrated HCl. Before each use, prepare a 1.0% orcinol solution using the stock solution.

APPENDIX

TABLE 4

Diphenylamine Reagent

Prepare a 4.0% solution of diphenylamine in glacial acetic acid. Store at 4°C.

APPENDIX

TABLE 5

Acetaldehyde Solution

Add 0.4 ml of acetaldehyde to a 250 ml volumetric flask. Finish filling with distilled water and store at 4°C.

APPENDIX

TABLE 6

MSU Vitamin and Mineral Mix P1

Ingredient	% of Total
Dicalcium Phosphate	47.38 (26.5% Ca and 20.5% P)
Trace Mineral Salt (High Zn)	47.42
Na_2SO_4	4.78 (22.5% S)
Vitamin A (10,000 IU/g)	.32
Vitamin D (9,000 IU/g)	.10

APPENDIX

TABLE 7

MSU Vitamin and Mineral Mix P2

Ingredient	% of Total
Dicalcium Phosphate	42.29 (26.5% Ca and 20.5% P)
Trace Mineral Salt (High Zn)	42.29
Na_2SO_4	15.00 (22.5% S)
Vitamin A (10,000 IU/g)	.32
Vitamin D (9,000 IU/g)	.10

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