SOME RELATIONSHIPS BETWEEN DIETARY REGIMES, GROWTH TRAITS AND PROTEIN SYNTHESIS IN RATS

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RALPH JAROLD LIPSEY

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ABSTRACT

SOME RELATIONSHIPS BETWEEN DIETARY REGIMES, GROWTH, CARCASS TRAITS AND PROTEIN SYNTHESIS IN RATS

by Ralph Jarold Lipsey

Three experiments involving 94 rats were conducted to study the relationship of nutritional regimes or growth hormone (GH) administration to hypophysectomized rats on growth, carcass, protein metabolism, insulin and GH traits. In Experiment I, 3 groups were fed a balanced high carbohydrate diet either ad libitum, 75% ad libitum or 50% ad libitum. The rats were bled every 2 weeks and at 6 weeks, 2 from each group were injected with ^{14}C-lysine and sacrificed. Livers and muscle samples were taken for lysine incorporation rate analysis. Three rats from each group were sacrificed for RNA and DNA analysis of muscle and liver and 5 from each group were sacrificed for carcass analysis.

Increasing dietary intake significantly increased average daily gains, percent carcass fat, and decreased carcass protein and moisture, and muscle and liver lysine incorporation rates. Serum insulin and GH levels did not show any significant relationships to the experimental treatment other than a general trend for insulin to be lowest for the 100% group, highest for the 75% group and intermediate for the 50% group.

In Experiment II, thirty weanling male rats were randomly divided into 2 groups and fed either a high fat or low fat diet ad libitum. At 3 and 10 weeks 6 rats from each group were injected with ^{14}C-lysine and sacrificed and blood samples, livers and muscle samples were taken for hormone assay, RNA, DNA analysis and lysine incorporation rate determination. Five rats at 3 weeks and 3 rats at 10 weeks from each group were sacrificed for carcass analysis.

The rats on the high fat diet consistently gained faster, had heavier carcasses, higher percent carcass fat, lower percent carcass protein and moisture, lower serum insulin and higher GH levels than those on low fat diets. There were no significant differences in either muscle or liver RNA, DNA and lysine incorporation rates into proteins between treatments.

In Experiment III, twenty-four hypophysectomized female rats were randomly assigned to 3 groups and injected for 4 days with either 50 μg or 25 μg of purified porcine GH (≈ 3 U.S.P. units/mg) while a control group was given isotonic saline. On the 5th day all rats were injected with ^{14}C-lysine, sacrificed and muscle and liver samples taken for RNA, DNA and lysine incorporation rate analysis. GH administration significantly increased muscle RNA and tended to increase serum insulin. Muscle DNA, liver RNA and DNA, and lysine incorporation rates into liver and muscle proteins were not significantly different between treatments.

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By

Ralph Jarold Lipsey

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INTRODUCTION

With increasing educational, economic and population resources, not only in America but throughout the world, the demand for meat and meat protein has increased overwhelmingly in the last decade. The most sought after trait in meat animal production today is rapid growth rate and efficiency. The need to more clearly understand the ability of meat animals to synthesize protein in the form of muscle is of major importance to the livestock industry.

Certain animals in all species have been identified, which are capable of converting feed to muscle in a more efficient manner than others. However, some of these animals are not without undesirable characteristics making them unsuitable to practical economic and management systems. Therefore, animal scientists are presented with the challenge to study the mechanisms of growth and establish the endocrine, nutritional, and genetic interrelationships.

Growth can be assessed by measuring weight gain and carcass composition; however, radioimmunoassay and radioisotope tracer methods may more clearly define regulation of growth metabolism. Ideally, cattle, swine or sheep, under their normal environmental conditions, would be the most appropriate models for assessing growth of muscle to be used as meat, but the contamination problems and expense of radioisotope tracer methods render large animals such as these less useful. For this reason, laboratory animals are generally used as models in growth studies.

The study reported herein involved 3 groups of rats in various nutritional and endocrine states. The objectives of this study were to find

the best laboratory animal model to examine the effects that nutrition have on protein synthesis and the modes through which nutritional endocrine factors act.

This study was designed to measure serum levels of insulin, growth hormone, tissue levels of nucleic acids, and the uptake of a radioisotope labeled amino acid and incorporation into tissue proteins in rats. The long term goals of this research were to develop models applicable to meat animals, define the nutritional and endocrine effects on protein metabolism, and study feasible methods of regulating the rate limiting step or steps of protein synthesis.

REVIEW OF LITERATURE

Growth and Development

From the time of conception to the time of death, animals have the ability to change their size, weight and composition. This phenomena of growth and development, is a very complicated biological process in which innumerable biochemical systems are involved. Growth and development is the most important aspect of animal agriculture. The specific phase of growth and development of major concern to the animal industry is that time period from birth to market weight.

There is no complete agreement among animal husbandmen in the distinction between growth and development. Growth may be described as an increase in the mass of a body in definite intervals of time (Schlose, 1911), production of new biochemical units by cell division; enlargement, or incorporation of materials from the environment (Brody, 1945) or simply an increase in weight until mature size is reached (Hammond, 1952; and McMeekan, 1959). At this time, no one definition of growth seems totally acceptable, but animal scientists and husbandmen generally prefer the description of Maynard and Loosli (1962). They indicated that "true" growth involves an increase in the structural tissues (primarily muscle and bone) and should be distinguished from the increase that results from fat deposition in the adipose tissues.

Development as generally defined includes growth, but also emphasizes differentiation or organization of a body. Hammond (1952) and McMeekan

(1959) stated that changes in body shape and/or conformation until the various parts reach maturity is defined as development. Needham (1931) indicated that differentiation involved both growth and cellular differentiation.

The order of growth and development follows an outward trend starting with and progressing exteriorally in order from the central nervous system, bone, tendon, muscle, intermuscular fat and finally to subcutaneous fat (Palsson and Verges, 1952; McMeekan, 1959). The mass and volume of these tissues increase in the order stated and they also attain mature size in this same order. Since the growth of the nervous system is nearly complete at birth, and tendon growth is small in terms of weight gain, postnatal growth is concerned primarily with increases in bone, muscle and fat.

McMeekan (1959) reported that bone completed a greater portion of its growth early in postnatal life. Zinn (1967) reported that bone growth of steers and heifers occurred at a near proportional rate to muscle for the first 150 days of a 270 day feeding period. However, from 180 to 270 days, bone increased less than muscle, indicating that bone growth rate declined. Sissons (1967) indicated that growth of the epiphyseal cartilage plate in the optimally growing male rat at 90 days of age was less than half (102 nm/day) that at 30 days of age (257 nm/day). Tulloh (1964) noted that bone increased with body weight in cattle, but at a decreasing rate, which caused percentage of bone to decline as weight increased.

Muscle is the major body tissue on a weight basis (Hedrick, 1968). Postnatal muscle growth is believed to occur primarily by an increase in the size of the muscle cells (Joubert, 1956). He also reported that muscle

fiber diameter was more closely related to muscle weight than to age of the animal. Relative differences in muscling whether expressed on a weight, percentage, individual muscle size or fiber size basis vary between animals even when weight or age are held constant (McMeekan, 1940; Joubert, 1956; Cole et al., 1964). Brody (1945) indicated that the growth of a particular muscle was almost entirely due to the increase of fiber diameter and length. Tuma et al. (1962) found that muscle fiber diameter was closely related to the longissimus area at the 12th rib in beef cattle.

The tissue which affects composition to the greatest extent is fat (Callow, 1947, 1948). Increases in fat can be accounted for by both adipocyte hypertrophy and hyperplasia (Anderson, 1972). Although fat deposition is a normal phase of the growth process, wide variation is observed due to both genetic and environmental effects. McMeekan (1940) observed that when genetically similar pigs were fed 4 planes of nutrition by adjusting feed intake (dietary energy) from birth to sixteen weeks and from sixteen weeks to slaughter at 91 kg, four different growth curves were obtained. The 4 planes of nutrition in terms of dietary energy content were high-high, high-low, low-high and low-low. He reported that pigs on the high-high plane of nutrition reached 91 kg at 150 days, high-low and low-high pigs at 200 days and those on the low-low plane at 350 days. Moreover, he observed that carcass composition was 62% muscle for the high-high pigs, 67% for high-low, 56% for low-high and 72% for those on the low-low plane. Schemmel, Mickelsen and Gill (1970) reported that various strains of laboratory rats performed differently when fed either a high or low fat diet even though their weaning weights and composition

at weaning were similar. Osborne-Mendel (O.M.) rats weighed 445 g on the low fat and 693 g on the high fat diet at 20 weeks post weaning, while S5B/PI rats weighed 304 g on the low fat and 346 g on the high fat diet. In addition, carcass composition varied from 13% and 10% fat (O.M. and S5B/PI on low fat) to 39% and 14% (O.M. and S5B/PI on high fat diets).

Mechanism of Protein Synthesis

Somatic cells replenish old and synthesize new proteins via phosphate energy compounds, nucleic acids, amino acids and ribosomal proteins. These compounds constitute a dynamic mass and are collectively referred to as polysomes. Mammalian polysome action is strikingly similar to that of bacteria; however, the nuclear membrane and endoplasmic reticulum in mammalian cells seemingly make this system more complex. Nuclear DNA functions as the storehouse of protein blueprints and is the ultimate source of all RNA. Jacob and Monod (1961) recognized that messenger RNA (mRNA) was transcribed from nuclear DNA, and contained a nucleotide sequence which coded for amino acids. Ribosomal RNA (rRNA) and ribosomal proteins from dense particles called ribosomes which exist in two subunits, one larger than the other (Spadoni and Gaetani, 1972). Brown and Gurdon (1964) indicated that the active site of ribosome organization (proteins combining with nucleic acids) was the nucleolus. The "mature" ribosome can be found "free" in the cytoplasm or "attached" to the endoplasmic reticulum. Transfer RNA (tRNA) is a small molecule made up of no more than 85 nucleotides, shaped in a clover leaf model (Spadoni and Gaetani,

1972). They also reported that every organism has at least 20 aminoacyl-tRNA synthetases which link the correct amino acid to the specific anti-codon on each tRNA molecule.

The protein synthetic process *in vivo* has three major steps, 1) initiation, 2) elongation and 3) termination. Initiation is the formation of a complex between the small subunit of the ribosome, the initiation site (codon) on the mRNA and formylmethionyl-tRNA (fMet-tRNA). In this process GTP and initiation factors are required, and subsequently the large and small ribosomal subunits associate and form the ribosome (Iwasaki *et al.*, 1968; Mukundan *et al.*, 1968; Gilbert, 1963). Elongation is the step by step addition of amino acids to the growing peptide chain by means of the peptidyl-tRNA chain alternating between the P (donor) and A (acceptor) sites on the larger ribosomal subunit, so that the next aminoacyl-tRNA can deliver its amino acid coded for by the mRNA (Trout and Monro, 1964). Termination is the release of the polypeptide chain linked to the tRNA and bound to the mRNA-ribosome complex. Termination apparently requires a termination codon on the mRNA and at least 3 releasing factors (Capecchi, 1967; Caskey *et al.*, 1968; Webster and Zinder, 1969; Capecchi and Klein, 1970; Beaudet and Caskey, 1970). After release of the mRNA by the intact ribosome, the ribosomal subunits dissociate and apparently reassociate randomly at subsequent initiation steps (Falvey and Staehelin, 1970).

Since there are distinct differences in the growth rates of animals, researchers have directed their attention toward measuring rates of protein metabolism in representative tissues. Before the widespread use of

radioactive traces, nitrogen balance index was the major method of measuring amino acid uptake into protein. Radioactive tracer methods, polysomal profiles and RNA and DNA tissue concentrations enabled more accurate measurement of *in vivo* and *in vitro* protein synthesis of liver and muscle. Fortunately, ribosomal RNA constitutes 80 to 90% of the total cellular RNA, and even during severe RNA depletion, this percentage remains fairly constant (Hirsch, 1967). Hirsch also reported that regardless of whether the ribosomes (rRNA) are free or membrane bound, at least 85% is combined with mRNA. With this in mind, measuring cellular RNA concentrations remains a popular method of expressing protein synthetic activity.

Since protein synthesis rates were shown to be modulated at different steps, determination of rate-limiting steps in protein synthesis was studied. Earl and Hindley (1971) studied the rate limiting steps of *in vivo* and *in vitro* protein synthesis by utilizing the action of puromycin at 0 C to observe the distribution of growing peptides between the donor and acceptor sites. The proportion of peptides released by puromycin was about 50% of the total growing peptides for both liver and muscle polyribosomes labeled *in vivo*, which suggested that neither the availability nor the binding of aminoacyl tRNA nor peptide bond synthesis nor translocation limited the rate of protein synthesis *in vivo*. They were also unsuccessful in altering this situation by starvation, hypophysectomy, partial hepatectomy, or growth hormone, alloxan or insulin administration. However, in their *in vitro* studies involving either a cell free system or tissue incubation, puromycin peptide release occurred largely from the donor site, which suggested that either the availability or binding of

aminoacyl tRNA, or peptide bond synthesis must be limiting. In addition, they reported that in neither study (*in vivo* vs *in vitro* tissue incubation) was there a correlation between the proportion of growing peptides in the donor site and changes in the rate of incorporation of radioactivity into protein. They concluded that the intracellular concentration of amino acids or aminoacyl-tRNA limited the rate of protein synthesis and that the increased incorporation resulted from increased but still suboptimal concentrations of either or both.

Henshaw *et al.* (1971) studied animals fed *ad libitum* but growing at different rates and reported that rates of protein synthesis per unit of ribosomes (RNA) in liver and muscle varied directly with growth rate. Liver ranged from 2.4 to 9.1 nmoles of lysine incorporated per mg of RNA per min. and muscle ranged from 1.1 to 3.9 nmoles. The variation in protein synthetic rates among animals was due entirely to changes in polyribosome activity. Their results showed that synthetic activity per unit of ribosomes is subject to modulation. They studied the mechanisms responsible for this modulation in some detail in liver. They concluded that the two general mechanisms which may alter the protein-synthesizing activity of ribosomes were: 1) controlling the proportion of ribosomes that are associated with mRNA and hence are active in synthesis; and 2) regulating the synthesizing activity of the mRNA associated polyribosomal ribosomes. During fasting, both mechanisms are important; however, in growing animals the only difference seemed to be that in the activity of the polyribosomal ribosomes.

Nutritional Effects on Protein Synthesis

Cuthbertson and Munro (1937) and Munro and Wikramayaki (1954) were among the researchers to show that energy intake has a greater influence (nitrogen retention) on protein metabolism and is more prolonged than the effects of dietary protein levels. Campbell and Kosterlitz (1948) were some of the earliest workers to report on the effects of various levels of dietary energy on protein deposition in certain tissues. They showed that energy intake in particular affected liver protein deposition.

Considerable research has been done on amino acid supply and balance in the diet and their effects on protein synthesis rates in liver and muscle. Reasons for measuring protein metabolism in muscle is obvious from a meat animal standpoint; however, the liver is known to play an important role in regulating the amino acid supply to the peripheral tissues. Elwyn, Parikh and Shoemaker (1968) showed that only about one-fifth of the absorbed amino acids reaching the liver is released to the peripheral tissues for utilization. Because the liver assumes this dominant role over muscle amino acid metabolism, it plays a role in protein metabolism of muscle.

Addis, Poo and Lew (1936) reported decreased liver protein content in rats fed a protein-free diet. Kosterlitz (1947) and Munro, Naismith and Wikramanayaki (1953) demonstrated that a protein-free diet quickly and drastically reduced liver RNA content. Hanking and Roberts (1965) reported that rat liver protein metabolism *in vitro* was sensitive to amino acid concentration of the medium. As they increased the concentration of ^{14}C

phenylalanine, threonine and valine in the medium, c.p.m. of TCA precipitable protein increased. Wannemacher, Cooper and Yatvin (1968) and Enwonwu and Screenby (1971) reported that there was a marked decrease in *in vitro* protein synthesis in rat liver during a protein deficient state. Pronczuk *et al*. (1968) were able to regulate rat liver polysome aggregation in a cell free system by varying the available supply of amino acids. They reported that by adding an incomplete mixture of amino acids, the system's polysomes broke down to monosomes. Munro (1968) pointed out that liver RNA and protein synthesis rates are directly related to dietary amino acid supply.

It should be pointed out that by measuring the RNA concentration in liver, one generally cannot determine if the animal is in a state of long-term or short-term protein deficiency. Noting that RNA content is a function of both nucleic acid synthesis and degradation working independently, Enwonwu and Munro (1970) stated that a deficient protein diet caused an abrupt increase in liver RNA turnover. In other words, the rate of RNA degradation is much higher than the rate of synthesis in early dietary protein deficiency. Continued feeding of a protein deficient diet decreases both degradation and synthesis, With prolonged protein deficiency of at least 12 to 15 days, RNA synthesis appears to increase (Shaw and Fillios, 1968; Quirin-Stricker, Gross and Mandel, 1968). At this point though, degradation rate is high, consequently increased concentrations of RNA are generally not observed.

Results of protein and nucleic acid metabolism of muscle have in general paralleled that of liver. Breur, Davies and Florini (1964) demon-

strated that ribosomes prepared from rat skeletal muscle catalyzed the incorporation of significant amounts of amino acids into protein when assayed *in vitro*. Young and Alexis (1968) reported that ribosomes of young rats fed adequate levels of dietary protein were more active for protein synthesis than ribosomes of young rats fed inadequate levels of protein. They also noted that the concentration of thigh muscle RNA decreased in the well nourished rats during growth, but total amount of RNA increased three-fold due to the increased muscle mass. However, Young and Alexis (1968) found a poorer relationship between muscle polysomal profiles and synthetic activity of the ribosomes than that observed for liver ribosomes by Pronczuk *et al*. (1968).

Waterlow and Stephen (1966) reported on incorporation of ^{14}C lysine into rat muscle proteins. They noted that when a labeled amino acid was given to a protein-depleted animal, the distribution of radioactivity in different tissues and organs of the body was not the same as in an animal which had received a normal protein intake. The low protein diet apparently did not cause any reduction in free lysine concentration in either muscle or liver. In muscle, there were no significant differences in free lysine content in different dietary groups. In liver, however, a low protein diet reduced the relative amounts of free lysine, but the differences were small. Their data also showed that the ratio of specific activity of protein bound lysine to the specific activity of protein free lysine (measurement of the amount of lysine incorporated) was reduced in protein depleted rats.

Van Venrooij, Henshaw and Hirsch (1970) studied the effect of changes

in composition of the media on the protein synthetic apparatus of Ehrlich ascites tumor cells in culture. They reported that essential amino acid supply and glucose concentration greatly affected synthesis on a cellular basis. The synthetic rate changes were directly related to a shifting of the polysomal profile. Their findings indicated that nutritional factors modulated the rate of synthesis through mechanisms other than substrate limitation operating at the translational level. The latter authors stated that amino acid supply and glucose concentration independently influenced peptide chain initiation and elongation.

Hormonal Effects on Protein Synthesis

Growth hormone. Since Simpson, Evans and Li (1949) revealed that growth hormone (GH) prevented decreases in the size and protein content of rat liver following hypophysectomy, a great deal of interest has evolved with attempts to specifically link the hormone with growth rate. Russell (1955) reported that GH stimulated growth by increasing the rate of protein biosynthesis rather than slowing down protein catabolism. Korner (1960) reported that hypophysectomy decreased protein synthesis in rat liver and injections of bovine GH partially restored amino acid incorporation rates to normal. Knobil (1966) established that GH added to the *in vitro* medium of incubating rat diaphragm increased both amino acid transport across the cell membrane and protein synthesis rates.

Korner (1964, 1967) demonstrated that the ability of ribosomes to incorporate amino acids into protein depended on circulating GH in the rat, and one of the events associated with GH action was increased RNA

synthesis. In addition, he established that GH stimulated protein synthesis and RNA synthesis in rat liver if it was injected into the rat, but not if added to a cell free system. Clemens and Korner (1970) concluded that GH could not stimulate protein synthesis *in vitro* unless amino acid levels were six times higher than normal plasma levels. However, Liberti, Wood and DuVall (1972) showed that elevated amino acid concentrations were not required to demonstrate GH action.

Since Korner (1967) was unable to show increased protein synthesis by adding GH to a cell free system, the GH effect on protein synthesis rates might possibly be a result of a secondary effect of the hormone in elevating plasma free fatty acids which in turn also elevates insulin. However, Jefferson and Korner (1967) subsequently showed that GH stimulated *in vitro* systems like perfused rat liver without the presence of insulin. They also pointed out that GH effects on protein synthesis rates were rapid; consequently its effect must be at the translational as well as the transcriptional steps.

Insulin Effects on GH Section

Roth *et al*. (1963) demonstrated that doses of insulin sufficiently large to lower blood glucose (to hypoglycemic levels), resulted in increased HGH (bioassay) in the plasma. However, Luft *et al*. (1966) reported that administration of insulin at levels too low to cause hypoglycemia also elicited GH release. Katz, Dhariwal and McCann (1967) demonstrated that hypoglycemia in the rat caused a significant depletion of pituitary GH and of hypothalamic GH-releasing factor. Schalch (1967) reported

contrary to the preceding workers that insulin-induced hypoglycemia diminished plasma GH in rats as measured by radioimmunoassay.

Thyroid Hormones Effect on GH Secretion

Thyroidectomy causes a decrease in pituitary GH content (Contopoulos et al., 1958; Meites and Fiel, 1967). Meites and Fiel (1967) showed that thyroxine therapy resulted in increased pituitary GH. Daughaday et al. (1968) confirmed these results using radioimmunoassay. Dickerman (1971) reported that the lack of thyroxine produced a dramatic decrease in GH synthesis as well as release, and these effects were reversed by thyroxine treatment.

Effects of Age and Sex on GH Secretion

Hormone concentrations at different ages vary as to whether data are determined by biological assay or RIA. Contopoulos and Simpson (1957) reported that GH (biological assay) was first detectable in the rat pituitary at day 19 of gestation. Solomon and Greep (1958) and Bowman (1961) reported that the total amount of GH in the rat pituitary increased with age, but the concentration of GH per mg of tissue remained constant in rats between 10 and 630 days of age.

Birge et al. (1967), using RIA methods, showed somewhat different results. They reported that pituitary GH concentration as well as total content increased with age. Male rats had higher GH concentrations than females after puberty. Garcia and Geschwind (1968) reported higher concentrations of GH with age, but they found no differences beteen males

and females. Dickerman (1971) reported that pituitary GH of male rats increased significantly with age to about 84 days, and plasma GH levels increased to about 64 days of age. On the other hand, the pituitaries of female rats showed greater total content as well as increased concentrations of GH up to 60 days of age. Plasma concentrations rose significantly to 60 days of age. He also showed no significant differences in plasma GH between males and females.

Josimovich, Mintz and Finster (1967) and Roth, Gordon and Bates (1968) reported that as plasma estrogen increased there was a decrease in plasma GH in the rat and human. Birge et al. (1967) reported that when rat pituitaries were incubated with DES a suppression of GH release was noted but there was no effect on pituitary concentration of GH.

Castrated male rats and adult female rats treated with testosterone propionate showed increased pituitary concentration of GH (Birge et al., 1967; Daughaday et al., 1968). Dickerman (1971) reported that thyroxine deficiency had a more profound influence on growth than the lack of testosterone.

Nutrition and Stress on GH Secretion

Pituitary and plasma GH activity and concentration decreased when rats were fed a protein free diet for extended periods of time (Srebnick, Nelson and Simpson, 1959; Meites and Fiel, 1965; Dickerman, Negro-Vilar and Meites, 1969). Dickerman et al. (1969) using bioassay and Trenkle (1970) using RIA showed that starved rats had decreased plasma GH activity.

Müller et al. (1967) noted that cold stress, high doses of vasopression, epinephrine and urecholine depleted pituitary GH. Schalch and Reichlin (1967) found decreases in plasma GH in rats subsequent to exercise, moderate or severe hypoglycemia and cold stress. Daughaday et al. (1968) and Garcia and Geschwind (1968) observed similar results.

Insulin

One of the principal functions of insulin is to enable the mammalian organism to rapidly and efficiently store the nutrients ingested. The tissues responsible for storing these nutrients are adipose tissue, muscle and liver, and they exhibit the most drastic metabolic alterations under conditions of insulin excess or deprivation (Krahl, 1961). Early work by Luck, Morrison and Wilbur (1928) showed that insulin caused a decrease in the concentration of plasma amino acids as well as plasma glucose. In in vitro studies insulin was shown to increase glucose uptake (Krahl, 1951), free fatty acid uptake (Raben and Hollenberg, 1960) by adipose tissue and it also stimulated the incorporation of amino acids into protein if some carbohydrate such as glucose was present (Krahl, 1959). In addition, insulin inhibited lipolysis (Randle and Morgan, 1962) and stimulated glucose metabolism (Kono and Barkam, 1971) in adipose tissue.

Although the membrane of liver cells is highly permeable to glucose under normal conditions, insulin stimulates glucose uptake (Hastings et al., 1952) and metabolism (Williams, Hill and Chaikoff, 1960) of livers of diabetic animals. Miller (1961) summarized data which showed that insulin increased protein metabolism only after mild insulin deficiency was estab-

lished on perfused normal rat livers. Krahl (1952, 1953, 1956) showed that no effect of insulin was noted on severely diabetic liver slices unless insulin was injected at least 10 hr. previously. However, he indicated that mildly diabetic rat liver responded to insulin if glucose was present. Penhos and Krahl (1961) observed similar findings with rat liver ribosomes.

Gemmill (1940, 1941) and Gemmill and Hamman (1941) demonstrated that glucose uptake and metabolism of rat diaphragm was stimulated by adding insulin to the *in vitro* medium. Wool and Krahl (1959) also found that insulin stimulated amino acid uptake and protein synthesis of rat diaphragm *in vitro*. In addition, they found that insulin stimulated protein synthesis over a 0 to 600 mg/100 ml range of glucose in the medium. They indicated that the effect of insulin on protein metabolism was independent of its known action of promoting glucose entry into the cell.

Following these findings, *in vitro* studies with muscle tissue were undertaken to clarify insulin's role in protein metabolism. Wool and Krahl (1959, 1964) pre-incubated rat diaphragm muscle with a labeled amino acid and then added insulin to the medium. He showed that insulin stimulated protein synthesis in the absence of added glucose. Manchester and Krahl (1959) used amino acid precursors like ^{14}C pyruvate, ketogluterate and bicarbonate in the medium and observed that added insulin stimulated ^{14}C labeled amino acids incorporation into protein. However, Wool (1964) did not explain why insulin stimulated accumulation of some amino acids and not others, or how insulin facilitated the membrane transport or intracellular accumulation.

Much interest was kindled in searching for the methods by which insulin stimulated amino acid incorporation into protein. Wool and Cavicchi (1967) reported that ribosomes from diabetic animals catalyzed protein synthesis far less efficiently than did ribosomes from normal animals. Ribosomes from diabetic animals treated with insulin before being sacrificed, were more efficient than non-insulin-treated ribosomes in an in vitro system. They also revealed that polyuredylic acid added to the preparation, restored diabetic ribosomes to normal efficiency and even increased the efficiency of normal ribosomes. Wool and Kurihara (1967) reported that by adding ^{3}H puromycin to ribosomal preparations of known weights of diabetic and normal ribosomes, they could calculate the number of active ribosome particles by the nacent ^{3}H peptidyl-puromycin particles formed. They indicated that 24.3% of the normal ribosomes were active and 8.7% of the diabetic preparations were active.

Wool and Krahl (1959), Manchester and Krahl (1959) and Wool (1965) showed that the stimulation of protein synthesis in muscle by insulin was not a secondary effect to the accumulation of amino acids. Thus, studies followed to determine effects of insulin on transcription. Ebové-Bonis et al. (1963) and Wool and Moyer (1964) revealed that although insulin increased RNA synthesis in muscle, this was a long term effect, and the process was not obligatory. Rampersad and Wool (1965) pointed out that insulin increased protein synthesis rates in an in vitro system with a constant amount of ribosomes and a constant amount of polyuredylic acid, thus, ruling out transcriptional effects on RNA. They concluded that the

locus of insulin action was ribosomal activity. Wool and Cavicchi (1966) later suggested that, in muscle, the initial action of insulin is to bring about the synthesis of a "specific protein" by modulating existing mRNA. They concluded this because insulin's effects on muscle protein synthesis were fast (5 min) and not affected by actinomycin.

Nutritional Effects on Insulin Secretion

Anderson and Long (1947) reported that the rate of insulin secretion from the perfused pancreas was most dependent on the level of glucose in the medium. Coore and Randle (1964) showed that insulin release from pancreatic preparations (tissue slices) increased with increasing glucose levels to a maximum glucose concentration of about 50 mM above which no additional release was observed. Gagliardino and Martin (1966) and Pallotta and Kennedy (1968) demonstrated that glucose released insulin from the β cells of the pancreas. They showed that in humans, plasma insulin rose to a peak value within 30 to 60 min. of glucose ingestion and gradually returned to fasting levels over the next 3 hours.

Coore and Randle (1964) reported that mannose was effective in stimulating insulin release. Fructose and galactose have been reported to be ineffective while some pentoses may be effective in altering plasma insulin levels in certain species (Taylor, 1972).

Floyd *et al.* (1966) showed that amino acids were effective in increasing insulin release. To date, amino acids which promote insulin release are arginine, lysine, phenylalanine, valine and leucine (Taylor, 1972). Milner (1970) observed that leucine may be the only essential amino acid which produces insulin release in the absence of glucose.

Starvation caused decreased plasma insulin in rats (Cahill et al., 1966). Malaisse, Malaisse-Lagae and Wright (1967) reported that starvation impaired insulin secretion and decreased pancreatic levels of insulin. Schalch and Kipnis (1965) indicated that feeding a high fat diet to rats caused a reduction of the carbohydrate tolerance and a concomitant hypersecretion of insulin. Consistent insulin hypersecretion is associated with obesity in humans as well as other animals (Perley and Kipnis, 1966). The amount of carbohydrate in the diet is still the primary factor in maintaining hyperinsulinism and alternatively reduction of dietary carbohydrate causes diminished secretion of insulin (Taylor, 1972). Elevated insulin release in vitro has been shown to be attributed to the addition (Goberna et al., 1971), of pancreozymin (Hinz et al., 1971) and gastrin (Taylor, 1972) to the medium.

Hormone Effects on Insulin Secretion

Circulating insulin levels become elevated following GH treatment (Randle and Young, 1956). The authors reported that GH appeared to sensitize the islet to secretagogues such as glucose.

Adrenalin inhibited insulin release in vitro (Coore and Randle, 1964) while ACTH and glucagon caused the release of insulin (Taylor, 1972). The stimulatory or inhibitory effect on insulin release is generally associated with increased or decreased cyclic AMP in the β cells (Kipnis, 1970).

METHODS AND MATERIALS

Experiment I: Limiting Dietary Intake

Thirty male weanling rats were obtained from Spartan Research Animals, Haslett, Michigan. The rats were randomly assorted into three groups, identified by an earnotching system, paired and housed in wire suspension type cages. Room temperature and light were controlled (temperature range 21 to 21.5 C and 12 hr. of both light and darkness). The diet fed is shown in table 1. The rats were fed once daily between 7:00 and 8:00 a m. The

TABLE 1. RATION COMPOSITION

Ingredient	%
Casein	21
Corn starch	40
Cerelose	28
Non-nutritive fiber[a]	2
Salt and mineral mix[b]	4
Vitamin fortification mix[c]	1
Corn oil	5

[a]Solka Floc, Brown Company, Chicago, Ill.
[b,c]General Biochemicals, Chagrin Falls, Ohio

first day all rats were fed ad libitum. Subsequently, throughout the feeding period, ten rats were continued ad libitum, ten received 75% of the previous day's ad libitum intake and ten received 50% of the ad libitum intake.

The feeding period continued for six weeks. Blood samples for hormone assays were taken on days 14, 28 and 42 of the feeding period. The rats were fed as usual on these sampling days and the bleeding began at 9:30 a m. The rats were anesthetized in an ether chamber and bled by eye-orbital puncture with a capillary tube.

Muscle and liver protein synthesis rates were assayed in vivo at six weeks. Two rats from each group (closest to the mean weight of the group) were selected and injected intraperitoneally with 8 microcuries (μc) of ^{14}C-lysine with specific activity 220 millicuries (mc) per m mole. The rats were sacrificed exactly 20 min. post injection and livers and muscle (longissimus) samples were rapidly removed, sealed in polyethylene bags and then frozen in a mixture of ethanol and dry ice. The samples were stored at -20 C.

Carcass composition of rats on the three dietary regimes were determined at the end of the six week feeding period. Five rats from each group were randomly selected, sacrificed and bled by decapitation, skinned and the feet removed at the carpus and the tarsus joints. The stomach and intestines were washed free of contents and included with the carcass for the composition sample. The carcasses were sealed in polytehylene bags and frozen as quickly as possible at -20 C. Three rats were randomly selected from each group for nucleic acid analysis of muscle and liver samples. The rats were anesthetized and killed by decapitation. The liver and longissimus muscles were rapidly removed, sealed in polyethylene bags and frozen in a mixture of dry ice and alcohol. The samples were stored at - 20 C until analyzed.

Experiment II: Effects of High Fat and Low Fat Diets

Forty weanling male Osborne Mendel rats were obtained courtesy of Dr. Rachel Schemmel, Food Science and Human Nutrition Department, Michigan State University. The rats were randomly assorted into two groups, housed and maintained on the same feeding schedule as outlined in experiment I. Feed was available ad libitum and one group received the diet described in table 1 and the other group received the high fat diet shown in table 2.

TABLE 2. HIGH FAT RATION COMPOSITION

Ingredient	%
Casein	25
Crisco	60
Non-nutritive fiber[a]	8
Vitamin fortification mix[b]	2
Salt and mineral mix[c]	5

[a]Solka Floc, Brown Co., Chicago, Ill.
[b],[c]General Biochemical, Chagrin Falls, Ohio

After 3 weeks of the feeding period, all rats were bled by eye-oribtal puncture for hormone assay. Two days later, 6 rats randomly selected from each group were injected intraperitoneally with 8 μc ^{14}C of lysine and sacrificed exactly 20 min. post injection. Livers and thigh muscles were removed as quickly as possible, sealed in a polyethylene bag and frozen in a mixture of dry ice and alcohol. Five rats from each group were also randomly selected at 3 weeks, sacrificed and prepared as in experiment I for carcass analysis. Nucleic acid analysis was performed on the muscle and liver samples collected for amino acid incorporation rate analyses.

After 10 weeks of the feeding period, all remaining rats were bled and 2 days later, 6 rats were randomly selected from each group and injected with 8 μc of ^{14}C-lysine and sacrificed 20 min. later. Livers and thigh muscles were collected for analysis. The three remaining rats in each group were sacrificed and prepared for carcass analysis as described in experiment I. Nucleic acid analysis was performed on the muscle and liver samples collected for amino acid incorporation rate analyses.

Experiment III: Effect of GH on Protein Synthesis in Hypophysectomized Rats.

Twenty-four female hypophysectomized rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. The rats were housed in wire suspension type cages, maintained at 29 C and fed a commercially prepared diet[a] ad libitum. The rats were "plateaued" for 12 days and then randomly assigned to 3 groups. One group received 50 μg purified porcine growth hormone[b] (pGH)(≈3 U.S.P. units/mg) per day, another group received 25 μg pGH, and one group served as a control and received isotonic saline. The pGH and saline were given intraperitoneally in 0.5 ml volumes once daily between 9:00 and 9:15 a.m. On the 5th day 4 rats were randomly selected from each group and injected intraperitoneally with 10 μc of ^{14}C-lysine with specific activity 220 mc/m mole and sacrificed 4 hr. later. The other 4 rats from each group were given 4 μc of ^{14}C-lysine and sacrificed 20 min. later. Livers and thigh muscle samples were sealed in polyethylene bags, frozen in a mixture of dry ice and alcohol and stored at -20 C.

[a]Wayne Feed Company, Chicago, Ill.
[b]Courtesy of Dr. L. J. Machlin, Life Sciences Dept., Monsanto Company, St. Louis, Mo.

Free Tissue Lysine Analysis

Free tissue lysine concentrations were determined as follows: liver and muscle free lysine samples were prepared essentially according to the method of Clark, Peng and Swendseid (1966) using norleucine as an internal standard. Approximately 0.5 g of tissue was homogenized in 5.0 ml of 5% sulfosalicylic acid (SSA) using a Brinkman Polytron at high speed for 10 to 20 seconds. The homogenates were centrifuged at 32,000 X g for 15 min. and decanted into a graduated test tube. The precipitate was washed with 5 ml of 5% SSA, centrifuged and again decanted into the test tube and the volume of the tube was adjusted to 10 ml with 5% SSA. Five ml of protein-free supernate were pipetted into a 100 ml round bottomed flask along with 0.25 ml of 1 mM norleucine for muscle samples and 0.50 ml of 1 mM norleucine for liver samples. The solution was concentrated in a rotary evaporator to near dryness. The residue was resuspended in 1 ml of lithium citrate buffer (pH 2.0) for muscle samples and 2.0 ml for liver samples. Amino acid analyses were performed on a 0.2 ml aliquot of the resultant solution with a Technicon TSM Amino Acid Analyzer.

^{14}C-Lysine Incorporation Rate Analyses

Samples for determining the ^{14}C incorporation rates were prepared as follows. Approximately 1 g of tissue was homogenized in 5 ml of 20% trichloroacetic acid (TCA) and centrifuged at 15,000 X g for 15 minutes. The supernatant was decanted into a graduated test tube and the precipitate washed twice with 5.0 ml of 20% TCA, centrifuged, decanted and adjusted to

a total volume of 20 ml with 20% TCA. The pellet was resuspended in 5 ml of hot 5% TCA for 15 min. at 90 C, then cooled, centrifuged and the supernate was discarded. The pellet was then dehydrated twice with acetone and once with ether and then air dried for 24 hours. Five ml of 1 N NaOH were used to dissolve the pellet and 0.2 ml of the protein-free solution and 0.2 ml of the solubilized protein fraction were counted in 10 ml of scintillation fluid on a Nuclear-Chicago Liquid Scintillation System Model 6488. The composition of the cocktail is given in table 3.

TABLE 3. LIQUID SCINTILLATION COCKTAIL COMPOSITION

Reagent	Quantity
Dioxane	385 ml
Xylene	385 ml
Ethanol (absolute)	230 ml
Naphthalene	80 g
Cabosil	25 g
PPO, Scinti Fluor	5 g
POPOP, Scinti Fluor	100 mg

Carcass Analyses

To get a homogeneous sample, the carcasses were chopped into small pieces and finely ground in a large stainless steel cup on a Waring Blendor with a small amount of dry ice to preserve the frozen state. The sample was returned to the polyethylene bag and kept frozen until analyzed.

Moisture determinations were performed by the A.O.A.C. (1970) method of drying 2.5 to 3.0 g of sample for 16 to 18 hr. at 100 to 105 C. Fat was determined according to the A.O.A.C. (1970) ether extraction (Goldfisch) method on the dried samples. Protein was determined according to The American Instrument Company (1961) micro-Kjeldahl technique.

Nucleic acid analyses of muscle and liver were performed on samples from rats randomly selected from each group. A modification of the Munro and Fleck (1962, 1963) method was followed. Approximately 1 g of muscle was homogenized in 10 ml of deionized water (approximately 1 g of liver was homogenized in 30 ml of deionized water) using a Brinkman Polytron at high speed for 10 to 20 seconds. Two ml samples of the homogenate were pipetted in duplicate into 15 ml Corex tubes and 5 ml of 2.5% cold perchloric acid (PCA) were added and the tubes were allowed to stand for 10 minutes. The sample was then centrifuged at 15,000 X g for 15 min. at 4 C and the supernate was discarded. The pellet was washed once with 5 ml of 1% PCA, centrifuged and the supernate was discarded. The tubes containing the precipitate were inverted and drained for 3 hours. Four ml of 0.3 N KOH at 37 C were used to digest the pellet and solubilize the RNA. The digestion was continued for at least 1 hr. after the pellet had dissolved; generally this required about 4 hours. The tubes were then cooled in ice and 5 ml of 5% PCA were added. After about 10 min. the samples were centrifuged and the supernatant was collected in graduated test tubes for RNA determinations. The pellet was washed twice with 5 ml of 5% PCA, centrifuged and the supernate was combined with the original solubilized fraction.

The combined volume of the supernatants was then adjusted to 20 ml with 5% PCA. Five ml of 10% PCA were then added to the remaining pellet, heated to 70 C for 25 min, then cooled in ice and centrifuged. The supernate was saved in a graduated test tube for DNA determination. The pellet was washed once with 4.5 ml of 10% PCA, centrifuged, and the supernatant added to the original soluble fraction. The combined volume was then adjusted to 10 ml with 10% PCA.

The RNA ribose was analyzed by the colorimetric orcinol procedure of Mejbaum (1939) as described by LePage (1957), while DNA was analyzed by the colorimetric diphenylamine method of Burton (1956) as modified by Giles and Meyers (1965).

Radioimmunoassays

Insulin

A double radioimmunoassay (RIA) procedure for rat insulin was followed using a porcine antibody system developed by Grigsby (1973), with modifications, according to the procedure reported by Koprowski (1972) and Koprowski and Tucker (1971).

Antibodies. The antibody to porcine insulin, guinea pig anti-porcine insulin gamma globulin (GPAPI) (Miles Laboratories, Inc., Research Division, Kankakee, Ill. 60901, lot no. 15) was diluted 1:400 with 0.05 M EDTA-PBS (pH 7.0) (Appendix I.A.4) and stored in small quantities at -20 C. On the day of use, it was diluted to 1:80,000 with 1:400 guinea pig control sera (GPCS) (Appendix I.A.6) so that 30 to 35% of the labeled insulin was bound.

The second antibody, sheep anti-guinea pig gamma globulin, was obtained courtesy of the Dairy Science Department, Michigan State University. This antibody was diluted to ensure maximal precipitation of the antibody bound labeled insulin with 0.05 M EDTA-PBS. Generally this dilution was 1:6 to 1:10 depending on the titer of the bleeding.

Labeled Insulin. ^{125}I labeled bovine insulin was obtained from Amersham Searle Corp., Arlington Hts., Ill. 60005. The ^{125}I insulin was diluted with 0.01 M phosphate buffer - 1% BSA. (IA_1) (Appendix I.A.1) so that 100 ul would have 15,000 to 20,000 cpm on the beginning day of the RIA.

Radioimmunoassay procedures

1. Tubes 1 and 2 contained 500 ul of PBS-1% BSA (IB_1) (Appendix I.A.5), 200 ul of GPCS (dil 1:400), 100 ul of labeled insulin, and 200 ul of SAGPGG (check for non-specific binding).

2. Tubes 3 and 4 contained 100 ul of labeled insulin (check total counts added to each tube).

3. Tubes 5 and 6 contained 500 ul of IB_1, 200 ul of GPAPI, 100 ul of labeled insulin, and 200 ul of SAGPGG (check for total precipitation).

4. Three groups of insulin standards were evenly distributed throughout the assay. These tubes contained 400 ul of IB_1, 100 ul of porcine insulin (purified preparation of porcine insulin obtained courtesy of Eli Lilly Co., Indianapolis, In. 46206, lot PJ-5682, 23.4 U/mg) diluted with IB_1, 200 ul GPAPI, 100 ul ^{125}I labeled insulin and 200 ul SAGPGG.

5. Each unknown was sampled at 100 and 150 ul and was diluted to 500 ul with IB_1. To each tube, 200 ul of GPAPI, 100 ul labeled insulin, and 200 ul of SAGPGG were added.

The order in which the above reagents were placed in tubes was as follows:

1. All tubes, except total count tubes, received IB_1.

2. The standards and unknowns were added to the appropriate tubes followed by GPCS and GPAPI. The contents of all tubes were stirred with a Vortex mixer following the addition of each reagent and then stored at 4 C for 24 hours.

3. Twenty-four hr. later, the SAGPGG was added and the tubes were stirred and stored at 4 C for 72 hours.

4. After 72 hr, 3 ml of PBS (Appendix I.A.2) were added to each tube (except total count tubes) and centrifuged at 2,500 X g for 27 min. in a refrigerated centrifuge.

5. The supernatant of all tubes was decanted and the precipitate counted on a Nuclear Chicago gamma counter.

Growth Hormone

A double RIA procedure for rat GH was followed according to Birge et al. (1967), as modified from Parker et al. (1965), Schalch and Parker (1964) and Schalch and Reichlin (1966).

Antibodies. The antibody to rat GH, monkey anti-rat GH gamma globulin (NIAMD-Anti-Rat GHS-1) was supplied by the National Institute of Arthritis and Metabolic Diseases Rat Pituitary Hormone Program through the courtesy of Dr. Albert Parlow (Department of Obstetrics and Gynecology, School of Medicine, Torrance, California). This antiserum was diluted 1:1000 with 0.025 M 1% BSA - 0.025 M EDTA-PBS buffer at pH 7.6 (Appendix I.B.2), and

stored at -20 C. On the day of use, it was diluted to 1:50,000 with 1:500 monkey control serum (MCS) (Appendix I.B) so that 40% of the labelled rat GH was precipitated in tubes containing only 1% BSA-EDTA-PBS, NIAMD-A-Rat GHS-1 (MARGH), labelled rat GH and the "second" antibody. It was found that a 1:50,000 dilution bound 35% of the radioiodinated GH.

The second antibody, goat anti-monkey gamma globulin (GAMGG) was obtained from CALBIOCHEM, San Diego, Cal. 92112. This antibody was diluted to ensure maximal precipitation of the antibody bound labeled rat GH. It was found that a 1:7 dilution with 0.025 M EDTA-PBS (Appendix I.B.1) was optimal for this assay.

Radioiodination of Rat GH. A highly purified rat GH preparation was obtained courtesy of Dr. Parlow. NIAMD-Rat GH-I-1 was dissolved in deionized water (pH 7.6) and diluted to a concentration of 1 mg/milliliter. Twenty μl (20 μg) of rat GH were aliquoted into 4 ml vials containing 100 μl of 0.5 M buffer (pH 7.6) (Appendix I.A.2) and stored at -20 C until iodinated. Prior to iodination, one vial was thawed and mixed well with 1 mc of ^{125}I with high specific activity (Amersham Searle Corp., Arlington Hts., Il. 60005). Twenty-five μl (87.5 μg) of Chloramine-T (Appendix I.C.1) were added and mixed while the reaction was allowed to proceed for 45 seconds. At the end of the reaction time, 125 μg of sodium metabisulfate ($Na_2S_2O_5$) were added (Appendix I.C.2) and mixed thoroughly. One hundred μl of transfer solution (Appendix I.C.3) were added and mixed thoroughly by withdrawing and expelling the solution from a plastic syringe. The solution was then transferred by the same syringe to a 0.9 x 20 cm column of Sephadex G-50 expanded in 0.05 M barbital buffer (Appendix I.C.5). The

reaction vial was rinsed with 100 µl of a rinse solution (Appendix I.C.4) and this solution was layered on the column with the same syringe. One ml aliquots were collected in tubes coated with 2 drops of 30% bovine serum albumin (BSA), as the column was eluted with 0.05 M barbital buffer (pH 8.6). Twenty µl from each tube were counted on a Nuclear Chicago gamma counter. Generally tubes 8 and 18 showed distinct peaks with the first peak being iodinated GH and the second "free" ^{125}I. Aliquots of 0.25 to 0.40 ml (20 to 40 x 10^6 CPM) were transferred from the peak iodinated GH tube along with 0.15 ml transfer solution to a 0.9 x 50 cm column for repurification. Two ml aliquots were collected in tubes coated with 1%-BSA-PBS at pH 7.6 during the column elution with barbital buffer. Twenty µl aliquots were again counted from each tube and the peak values determined. Three peaks were observed; peak one was aggregated GH; peak two was undamaged GH used in RIA; and peak three was digraded GH. Aliquots of tubes from peak two were diluted in 1% BSA-PBS at pH 7.2 until 100 µl gave 5,000 to 8,000 cpm for use in RIA. Dickerman (1971) reported that the diluted solution may be kept at 4 C for as long as 2 months without significant alterations in the immunological activity of the molecule.

Procedures for RIA. The assay was carried out in disposable culture tubes, 12 x 75 mm (Kimble Owens, Toledo, Ohio) and labeled in the following manner:

1. Tubes 1 and 2 contained 300 µl of 1% BSA + 0.025 M EDTA phosphosaline buffer + 0.01% thimersol (GHB_1), 100 µl of 1:500 normal monkey serum, 100 µl of labeled rat GH and 100 µl GAMGG (check for non-specific binding).

2. Tubes 3 and 4 contained 100 μl of labeled rat GH (check total "counts" added/tube).

3. Tubes 5 and 6 contained 300 μl of GHB_1, 100 μl of MARGH, 100 μl of labeled rat GH and 100 μl of GAMGG (check for total binding).

4. Three groups of GH standards (NIAMD-Rat GH-RP-1 supplied by NIH, courtesy of Dr. Parlow) were evenly distributed throughout the assay. These tubes contained 200 μl of GHB_1, 100 μl of rat GH (Appendix I.B.4) diluted with 1% BSA-PBS (pH 7.6). All tubes received 100 μl of MARGH, 100 μl labeled rat GH and 100 μl of GAMGG.

5. Each unknown was sampled at 25 and 50 μl and was diluted to 300 μl with GHB_1. To each tube, 100 μl of MARGH, 100 μl of labeled rat GH and 100 μl of GAMGG were added.

The order in which the above reagents were placed in tubes was as follows:

1. All tubes, except total count tubes, received GHB_1.

2. The standards and unknowns were added to the appropriate tubes followed by MCS and MARGH. The contents of all tubes were stirred with a Vortex mixer following the addition of each reagent and then stored at 4 C for 24 hours.

3. After 24 hr., the labeled GH was added and the tubes were stirred with a Vortex mixer. The tubes were stored at 4 C for 48 hours.

4. Forty-eight hr. later, the GAMGG was added and the tubes were stirred and stored at 4 C for 24 hours.

5. After 24 hr., the tubes were centrifuged at 2,500 X g for 20 min. in a refrigerated centrifuge. At the end of 20 min., 2.5 ml of PBS were added to each tube, then centrifuged again for 20 minutes.

6. The supernatant of all tubes was decanted and the precipitate counted on a Nuclear Chicago gamma counter.

Statistical Analyses

Data were analyzed on the 3600 Computer at the Michigan State University Computer Laboratory. Analysis of variance and simple correlation coefficients were determined and the correlation coefficients are listed in Appendix III. Analysis of variance data from Experiment I and III were analyzed by Duncan's New Multiple Range procedure (Steel and Torrie, 1960) to test for significant differences. Means and standard errors from Experiment II were computed on a Hewlett-Packard 9100A Calculator and analyzed by Student's t-test (Sokal and Rohlf, 1969) for significant differences.

RESULTS AND DISCUSSION

Growth Traits

Rat growth data for Experiments I and II are presented in tables 4 and 5. The initial weights of all treatment groups were not significantly ($P > .05$) different within experiments; however, in both studies the experimental treatment significantly affected average daily gain (ADG) and final weight. Since feed consumption was the basis of the experimental treatment in Experiment I, regulation of feed consumption would be expected to affect ADG. This observation is supported by the highly signficant correlation coefficient ($r = 0.96$) obtained between feed consumption and ADG. Feed consumption is not presented for Experiment II since both groups were fed *ad libitum* on diets greatly different in energy source and concentration. It is interesting to note that limiting feed intake in Experiment I, did not significantly affect the feed/gain ratio. These results are in partial agreement with those of Hines (1966) who showed that restricting swine to 75% of *ad libitum* intake resulted in no significant difference in feed utilization. However, he also reported that a level of 70% restriction or greater increased feed/gain ratio.

The ADG of the groups in Experiment II are significantly different when compared at constant age. These results paralleled those of Schemmel *et al.* (1970) for weight gains of Osborne-Mendel (O.M.) rats fed high fat or grain diets *ad libitum*, as they reported that male O.M. rats performed superior to six other strains and were capable of gaining approximately 7 g/day.

TABLE 4. MEANS AND STANDARD ERRORS OF WEIGHTS, GAINS AND FEED CONSUMPTION FOR EXPERIMENT I[a].

	Percent ad libitum intake			
Item	100[b]	75[b]	50[b]	S.E.M.
Initial wt, g	47.2	47.6	47.7	1.12
Final wt, g	324.0[c]	254.6[d]	188.7[e]	5.24
ADG, g	6.59[c]	4.93[d]	3.35[e]	0.12
Feed consumption, g	777.2[c]	583.0[d]	387.0[e]	3.72
Feed/gain	2.82	2.83	2.77	0.07

[a]Means having different superscripts are significantly different ($P < .05$).
[b]10 rats/group.

TABLE 5. MEANS AND STANDARD ERRORS OF WEIGHTS AND GAINS FOR EXPERIMENT II.

	3 weeks[a]		10 weeks[b]	
Item	Low fat[c]	High fat[c]	Low fat[d]	High fat[d]
Initial wt, g	46.0 + 2.3	44.5 ± 2.5	43.5 ± 2.5	45.0 ± 2.2
Final wt, g	185.4 ± 6.5[e]	200.0 ± 6.9[f]	328.9 ± 5.6[x]	465.5 ± 29.0[y]
ADG, g	6.76 ± 0.33[e]	7.33 ± 0.36[f]	4.10 ± 0.03[x]	6.10 ± 0.33[y]

[a]3 week means with different superscripts are significantly different ($P < .05$).
[b]10 week means with different superscripts are significantly different ($P < .05$).
[c]5 rats/group.
[d]3 rats/group.

Carcass Traits

Table 6 shows that level of feed intake significantly ($P < .05$) affected carcass weight. This is indicated by the highly significant relationship ($r = 0.92$) which was observed between carcass weight and feed intake. In Experiment II, carcass weights of rats on the high fat diet were significantly heavier than those on the low fat diet at 10 weeks, but not at 3 weeks (table 7). The mean differences for ADG were greater at 10 weeks (table 5) which would explain why carcass weights among high and low fat diets differed more at 10 weeks than at 3. Moisture and fat content of the carcasses showed the most dramatic changes when the rats were fed on the high fat diet (table 7). Even though carcass weights did not vary significantly at 3 weeks, the moisture and fat percentages were significantly different. The correlation coefficient ($r = -.97$) between carcass moisture and fat is in agreement with Reid *et al.*, 1967, who reported similar correlations ($r = -.99$) for sheep, cattle and swine carcasses. In both Experiments I and II, higher ADG was associated with increased carcass weight, carcass fat and decreased carcass moisture percentage. Data from Experiment I are consistent with the results of cattle fed 3 levels of dietary energy (Moulton, Trowbridge and Haigh, 1921). These authors indicated that percent fat was higher and percent lean lower in full-fed cattle, whereas, restricted-fed cattle showed the opposite trends. Results from Experiment II show the same general trends for percent carcass fat that were previously reported by Schemmel *et al.* (1970). They reported that O.M. rats fed low fat and high fat diets for 10 weeks had 12.8% and 34.0% carcass fat, respectively. Table 8 shows that rats of

TABLE 6. MEANS AND STANDARD ERRORS OF CARCASS DATA FOR EXPERIMENT I[a].

	Percent ad libitum intake			
Item	100[b]	75[b]	50[b]	S.E.M.
Carcass wt, g	203.7[c]	169.8[d]	118.1[e]	4.90
Moisture, %	67.0[c]	70.1[d]	73.1[e]	0.34
Protein, %	18.2[c]	18.4[d]	19.5[e]	0.12
Fat, %	11.4[c]	8.1[d]	3.9[e]	0.41

[a]Means having different superscripts are significantly different ($P < .05$).
[b]5 rats/group.

TABLE 7. MEANS AND STANDARD ERRORS OF CARCASS DATA FOR EXPERIMENT II.

	3 weeks[a]		10 weeks[b]	
Item	Low fat[c]	High fat[c]	Low fat[d]	High fat[d]
Carcass wt, g	118.4 ± 4.2	127.6 ± 4.4	213.7 ± 3.8[x]	301.0 ± 17.6[y]
Moisture, %	68.0 ± 0.8[e]	64.9 ± 0.9[f]	68.1 ± 0.3[x]	59.2 ± 1.4[y]
Protein, %	17.9 ± 0.4[e]	15.9 ± 0.2[f]	17.2 ± 0.1[x]	15.3 ± 0.6[y]
Fat, %	10.4 ± 0.9[e]	15.3 ± 0.8[f]	12.5 ± 0.3[x]	20.8 ± 2.1[y]

[a]3 week means with different superscripts are significantly different ($P < .05$).
[b]10 week means with different superscripts are significantly different ($P < .05$).
[c]5 rats/group.
[d]3 rats/group.

corresponding age (10 weeks) and diets had 12.5% and 21.0% carcass fat, respectively.

TABLE 8. CARCASS DATA MEANS FOR EXPERIMENT II GROUPED BY DIET.

	Low fat[a]		High fat[b]	
Item	3 weeks[c]	10 weeks[c]	3 weeks[d]	10 weeks[d]
Carcass wt, g	118.4[e]	213.7[f]	127.6	301.0
Moisture, %	68.0	68.1	64.9[x]	59.2[y]
Protein, %	17.9	17.2	15.9	15.3
Fat, %	10.4	12.5	15.3[x]	20.8[y]

[a]Low fat means with different superscripts are significantly different ($P < .05$).
[b]High fat means with different superscripts are significantly different ($P < .05$).
[c]5 rats/group.
[d]3 rats/group.

Results from both experiments show that as percent fat increased, percent protein decreased. This relationship is particularly evident when the comparisons are made between dietary treatment and when age is held constant (tables 6 and 7). Table 8 shows that percent protein was influenced to a greater extent by dietary effects on carcass fat than by age. Carcasses from rats on the low fat diet were not significantly different in percent fat or percent protein at either 3 or 10 weeks even though there were large differences in absolute carcass weight. Carcasses from the rats on the high fat diet at 3 and 10 weeks not only showed the expected differences in weight but they also had significant differences in percent fat while those fed the low fat diet were not significantly

different. Percent carcass protein was not different between the 3 and 10 week groups on either the high or low fat diets; however, percent carcass protein tended to decrease with age (table 8).

Although percent carcass protein tended to decrease with age in these experiments, it is important to point out that Goll, Bray and Hoekstra (1963) and Gann (1969) reported a small increase in muscle protein concentration expressed either on a wet weight or a dry, fat free basis with increasing age. These results observed by the latter authors, as well as the conclusions of McMeekan (1959), Zinn (1967) and Sissons (1967) indicate that bone development is completed early in terms of tissue maturity and consequently percent bone of the carcass tends to decrease throughout growth. Hence the major reason why percent carcass protein decreases from birth to maturity is explained by the fact that percent fat increases relatively faster during growth than muscle and bone. As a result, percent carcass protein tends to steadily decline. The carcass data in Experiments I and II agree with the conclusions of Palsson and Verges (1952) and McMeekan (1959) that there is obligatory order in tissue development, and if there is strict competition for nutrients, the bone and lean mass will take precedence over adipose tissue. Correlation coefficients between carcass traits, serum insulin and GH are presented in Appendix III.

Protein Metabolism

The means of the tissue nucleic acids and lysine incorporation rates for Experiments I and II are presented in tables 9 through 11. RNA content is a common method of estimating the protein synthetic ability of a given

tissue at the specific time of sampling. This is a reasonable assessment of protein synthetic capability since about 80% of the total cellular RNA has been reported to be engaged in protein synthesis (Hirsch, 1967). Certain drawbacks are apparent with this estimation; however, there is no measure of peptide elongation or initiation or the amount and quality of protein synthesized. Regardless of these drawbacks, RNA content is certainly a "course control" mechanism of protein metabolism.

Results of Experiment I (table 9) show that the ad libitum fed rats had significantly more RNA per gram of muscle and liver than the restricted fed groups, and the 75% group tended to show greater RNA concentration than the 50% group. In addition, feed consumption was significantly correlated with both muscle (r = 0.88) and liver (r = 0.66) RNA.

TABLE 9. MEANS AND STANDARD ERRORS OF NUCLEIC ACIDS AND ^{14}C-LYSINE INCORPORATION FOR EXPERIMENT I.

	Percent ad libitum intake[a]			
Item	100[d]	75[d]	50[d]	S.E.M.
Muscle RNA[b]	1.49[f]	0.63[g]	0.56[g]	0.03
Muscle DNA[b]	2.29[f]	2.10[f]	1.79[g]	0.11
Liver RNA[b]	12.68[f]	9.13[g]	7.54[g]	0.96
Liver DNA[b]	2.37	2.21	2.15	0.07
Lysine muscle[c]	0.40[f]	1.10[g]	1.65[f]	0.11
Lysine liver[c]	3.75[f]	5.90[g]	5.55[f,g]	0.44

[a]Means having different superscripts are significantly different ($P < .05$).
[b]mg/g wet sample.
[c]n moles lysine incorporated/mg RNA/min.
[d]3 rats/group

These results are in agreement with those of Naismith and Wikromanayaki (1953), Munro (1968), and Enwonwu and Munro (1970) for the relationship of dietary amino acid intake and RNA concentration in skeletal muscle and liver. Although the diet was not deficient in protein, the result of restricting intake limited the energy intake of the rats and undoubtedly a greater proportion of the protein intake was consequently used for gluconeogenesis or other energy metabolism. Table 10 shows that no significant differences were obtained for liver and muscle RNA concentrations of rats fed the high or low fat diet when compared at constant ages. Thus in contrast to the relationship observed between RNA concentration and dietary intake in Experiment I, RNA concentration was not significantly affected by high or low fat diets in Experiment II. However, the tissues of rats on the low fat diet tended to have slightly higher RNA values than those fed high fat. Hormonal effects such as those which resulted from the variation in diets used in this study might provide an explanation for the differences observed in RNA. The relationship of insulin and GH to tissue RNA will be discussed later.

DNA concentrations are measured in various tissues to estimate the "cellularity" or cell number of the tissue sampled. This is a relatively accurate method if the tissue is mononucleate. Since liver and muscle tissue are polynucleate; cell number as assessed by DNA is not accurately measured. Nevertheless, DNA concentrations of liver and muscle remain one of the best measures of cellularity available to date. During the early weeks of postnatal life, skeletal muscles of the rat undergo rapid growth, with the greatest increase in muscle mass occurring between 8 and 13 weeks of age (Gordon, Kowalski and Fritts, 1966). This postnatal increase in

muscle mass is largely due to muscle fiber hypertrophy, since it is generally accepted that muscle fiber number does not increase after birth (Goldspink, 1970; Rowe and Goldspink, 1969). During this period of muscle fiber hypertrophy the nuclei proliferate and DNA concentration increases proportionately. Pearson and Widdowson (1970) stated that the increase in nuclei and DNA is due to increased number of muscle fiber nuclei or "satellite cell" nuclei associated with lean tissue, and not to increased DNA per nucleus. Data from Experiment I (table 9) show that rats fed 100% and 75% ad libitum had significantly more DNA per gram of muscle than the 50% ad libitum group. Since the data show that increased feed consumption resulted in muscle nuclei proliferation (increased DNA) in this study it appears that muscle DNA can be nutritionally manipulated.

Although the liver DNA concentrations of the corresponding dietary treatments in Experiment I (table 9) were not significantly different, a similar trend was apparent in that feed intake and liver DNA concentration were positively related. The full fed group tended to have greater concentrations of liver DNA than the restricted fed rats.

Table 10 shows no significant differences in DNA concentration in muscle or liver of rats, within age groups, fed high fat or low fat diets. There was a slight trend for rats on the high fat diet to show higher DNA values in both tissues. Since the rats on the high fat diet gained significantly faster than those on the low fat diet, they had heavier muscle and liver weights which undoubtedly were associated with more nuclei. It is interesting to note from table 11 that muscle DNA concentrations are significantly lower at 10 weeks than at 3 weeks. These data

TABLE 10. MEANS AND STANDARD ERRORS OF TISSUE NUCLEIC ACIDS AND ^{14}C-LYSINE INCORPORATION RATES FOR EXPERIMENT II.

	3 weeks[c]		10 weeks[c]	
Item	Low fat	High fat	Low fat	High fat
Muscle RNA[a]	2.50 ± 0.19	2.43± 0.14	1.64 ± 0.10	1.90 ± 0.12
Muscle DNA[a]	0.79 ± 0.06	0.88 ± 0.05	0.60 ± 0.04	0.66 ± 0.04
Liver RNA[a]	13.61 ± 0.48	12.49 ± 0.45	13.00 ± 1.19	10.58 ± 0.68
Liver DNA	3.15 ± 0.22	3.54 ± 0.14	3.25 ± 0.28	3.24 ± 0.08
Lysine muscle[b]	1.15 ± 0.09	0.93 ± 0.17	2.26 ± 0.27	1.96 ± 0.39
Lysine liver[b]	2.90 ± 0.29	2.73 ± 0.50	2.44 ± 0.35	3.70 ± 0.70

[a]mg/g wet sample.
[b]n moles lysine incorporated/mg RNA/min.
[c]6 rats/group.
Note: There were no significant differences getween diet treatments at a constant age.

TABLE 11. MEANS AND STANDARD ERRORS OF TISSUE NUCLEIC ACIDS AND ^{14}C-LYSINE INCORPORATION RATES FOR EXPERIMENT II.

	Age[a]		Diet[b]	
Item	3 weeks[e]	10 weeks[e]	Low fat[e]	High fat[e]
Muscle RNA[c]	2.46 ± 0.11[f]	1.77 ± 0.08[g]	2.07 ± 0.16	2.17 ± 0.12
Muscle DNA[c]	0.84 ± 0.04[f]	0.63 ± 0.03[g]	0.70 ± 0.04	0.77 ± 0.05
Liver RNA[c]	13.05 ± 0.75	11.79 ± 0.36	13.31 ± 0.62[x]	11.53 ± 0.49[y]
Liver DNA[c]	3.34 ± 0.14	3.24 ± 0.14	3.20 ± 0.17	3.39 ± 0.09
Lysine muscle[d]	1.04 ± 0.11[f]	2.13 ± 0.23[g]	1.70 ± 0.22	1.46 ± 0.26
Lysine liver[d]	2.81 ± 0.30	3.10 ± 0.44	2.67 ± 0.23	3.24 ± 0.45

[a]Means for age having different superscripts are significantly different ($P < .05$).
[b]Means for diet having different superscripts are significantly different ($P < .05$)
[c]mg/g wet sample
[d]n moles lysine incorporated/mg RNA/min.
[e]12 rats/group

are in conflict with those of Pearson and Widdowson (1970) who reported that muscle DNA concentration increased during growth. The most plausible explanation for the observation in this study is that since the percent carcass fat increased during the feeding period, the muscle samples at 10 weeks contained more intramuscular and intermuscular fat than at 3 weeks, thus lowering DNA concentration. Liver DNA concentrations showed no significant change between age groups (table 11); however, DNA tended to decrease with increasing age. The livers of the older rats probably contained greater lipid concentrations than the younger rats which probably accounted for the decrease in DNA.

The study of protein synthesis rates in these experiments were modeled after the procedure of Henshaw et al. (1971). They indicated that several conditions must be met or assumed, if measurements of the rate of incorporation of radioactive lysine into protein are to yield valid assessments of the rate of protein synthesis under different metabolic conditions. These assumptions include; 1) the lysine content of protein synthesized in a given tissue must be the same in all animals; 2) the lysine injection must not alter the rate of protein synthesis during the experimental period; 3) all incorporated radioactivity must remain in the tissue during the period of measurement; 4) all radioactivity incorporated into protein must represent lysine; 5) the average specific activity of the intracellular lysine pool during the time course of the experiment must be known; 6) the specific activity of the precursor pool for protein synthesis must be the same as the total tissue pool which is measured. Henshaw et al. (1971) showed that these assumptions were sufficiently met under the fed

and starved states of nutrition included in their studies. One additional important assumption which applies to the present study is that the treatments did not significantly affect the lysine concentration of the tissue free amino acid pools. Data from Waterlow and Stephen (1966) show that variation in dietary protein intake did not significantly affect tissue free lysine content; however, lower protein intake tended to decrease lysine concentration in liver.

Although not reported herein, determinations of free tissue lysine content were extremely variable within treatments for muscle samples while liver free lysine concentrations were similar and decreased slightly with decreased dietary intake. Consequently, free tissue lysine values used for the calculations of liver lysine incorporation were the actual means for each group. A single value for muscle free lysine concentration was determined and used for all muscle lysine incorporation calculations in Experiment I, II and III.

Results of lysine incorporation rates in Experiment I (table 9) for muscle shows that restricting dietary intake significantly increased incorporation. In the case of liver lysine incorporation rates, the full fed group was significantly lower than restricted groups and the 75% group had the highest incorporation rates with the 50% group being intermediate. These results were somewhat surprising since Henshaw et al. (1971) reported that in all cases except one, muscle and liver tissues from faster growing animals incorporated larger amounts of lysine per unit of RNA than those from slower growing animals. A possible explanation for this observation may be related to the treatments' effect on the polysome action in these

tissues. Fishman, Wurtman and Munro (1969) reported that there are definite dirunal variations in rat liver and muscle polysomal profiles apparently due to eating habits. The polysomes are heaviest about 2 to 3 hr. after the eating period of greatest feed intake of the rat. Experiment I in this study markedly affected the eating habits of the rats. The ad libitum fed rats ate primarily at night, and the rats on restricted intake ate immediately when fed each day at about 7:30 am. Consequently, at the time of ^{14}C-lysine injection, the tissue ribosomes of the restricted rats would have been most actively synthesizing protein, while those of the ad libitum fed group would have been less active. Such variation in ribosomal activity could account for the differences in lysine incorporation rates observed in Experiment I.

Lysine incorporation rates of muscle and liver tissues from the rats fed the high and low fat diets showed no significant ($P > .05$) differences when compared at a constant age (table 10) or when compared by diet (table 11). However, the data in table 11 show that lysine was incorporated at significantly faster rates in muscle at 10 weeks compared to that at 3 weeks. The corresponding differences in liver incorporation rates were not significantly different, but they also tended to have greater rates at 10 weeks. Since the rats were fed the high and low fat diets ad libitum in Experiment II, the polysomal profiles between treatment groups were probably more nearly alike than those in Experiment I; however, these observations do not explain why the 10 week group had higher incorporation rates than the 3 week group.

Insulin And Growth Hormone

The serum RIA values of rats from Experiment I are shown in table 12. In general, the data do not show any consistent treatment effects. Since the rats were bled without fasting for a constant time before bleeding, the data tended to show large variation within treatments and between sampling periods. It is interesting to note that insulin was significantly lower for the ad libitum group than the restricted fed groups except at 6 weeks where the 50% group also had low values. However, these results generally agree with reports from Anderson and Long (1947), Coore and Randle (1964), Gagleardino and Martin (1966) and Pallotta and Kennedy (1968) who found that plasma insulin levels depended primarily on the quantity of glucose intake and the relationship of sampling to ingestion of glucose. The group effects on the insulin data shown in table 12 may possibly be explained by the fact that the ad libitum group ate primarily during the night, the restricted fed groups ate immediately after they were given feed (approximately 7:30 a m.), and the blood samples were taken between 9:30 to 11:00 a m. Consequently, insulin levels of the ad libitum fed rats were lower than those of the restricted fed groups which may reflect the more recent glucose ingestion among restricted fed rats. Serum insulin of the 75% group were probably higher because they ingested a greater amount of glucose than the 50% group. The GH values for the rats of Experiment I are not significantly different at constant ages except at the 2 week bleeding when the 50% group was significantly lower than the 75% group. It is generally believed that in humans, GH levels rise following increases in insulin (Glick and Goldsmith, 1968). This rise in GH has been demonstrated

TABLE 12. MEANS AND STANDARD ERRORS OF SERUM INSULIN AND GROWTH HORMONE FOR EXPERIMENT I[a].

		Percent ad libitum intake			
Hormone	Age	100	75	50	S.E.M.
Insulin[b]	2 weeks	67.7[d]	112.5[e]	108.4[e]	9.24
	4 weeks	112.3[d]	173.3[e]	154.7[e]	14.93
	6 weeks	98.3[d]	145.7[e]	87.7[d,e]	11.87
GH[c]	2 weeks	47.6[d,e]	70.7[d]	27.5[e]	12.62
	4 weeks	71.3	44.6	71.5	14.78
	6 weeks	55.4	39.9	50.0	12.27

[a]Means having different superscripts are significantly different ($P < .05$).
[b]μ Units bovine insulin equivalent/ml; 10 rats/group at each age.
[c]ng/ml; 10 rats/group at each age.

not to be due to the insulin *per se*, but insulin's effects of lowering blood glucose (Roth *et al.*, 1963; Luft *et al.*, 1966). These results have not been confirmed in the rat. Daughaday *et al.* (1968), Garcia and Geshwind (1968) and Schalch and Reichlin (1968) all failed to observe this rise in plasma GH following insulin administration to rats. The relationship between insulin and GH in Experiment I does not show any definite trends other than that the 100% and 50% *ad libitum* groups displayed increased serum insulin between 2 and 4 weeks, and decreased insulin between 4 and 6 weeks. The 75% group simply showed decreased serum GH with increasing age. Dickerman (1971) reported significant increases in plasma GH of rats from 23 to 64 days of age, from 64 to 120 days there was no significant changes, and at 240 days GH levels were significantly reduced to levels similar to those of 33 to 43 day old rats. The ages of the rats

in this study at the bleeding dates were approximately 35, 49 and 63 days, respectively. Thus, according to the observations of Dickerman (1971) serum GH would have been expected to increase throughout this study. Dickerman et al. (1969) and Trenkle (1970) have reported that starvation resulted in decrease plasma GH in rats. It appears that the restriction of feed intake in Experiment I did not cause differences in serum GH levels.

Results of the high and low fat diet study show consistent and significant differences for insulin and GH levels at both 3 and 10 weeks (table 13). In all cases, insulin levels were significantly ($P < .05$) higher among the rats fed the low fat diet than those on the high fat diet. In addition, GH levels were consistently higher for rats fed high fat than those fed low fat. Since the low fat group ingested glucose whereas, the high fat group did not, the differences in insulin were not unexpected.

TABLE 13. MEANS AND STANDARD ERRORS OF SERUM INSULIN AND GROWTH HORMONE FOR EXPERIMENT II[a].

	3 weeks	
Hormone	Low fat	High fat
Insulin[b]	51.1 ± 13.7[d]	18.4 ± 3.8[e]
GH[c]	24.6 ± 4.5	45.9 ± 13.7
	10 weeks	
Insulin[b]	116.4 ± 10.6[d]	44.3 ± 2.0[e]
GH[c]	27.4 ± 9.0[d]	94.0 ± 15.5[e]

[a]Means having different superscripts are significantly different ($P < .05$).
[b]μ Units bovine insulin equivalent/ml; 10 rats/group at each age.
[c]ng/ml; 10 rats/group at each age.

Blazquez and Quijada (1968) reported that 150 g rats fed a high fat diet showed a marked depression in plasma insulin levels which agrees with the data presented in this study. It is interesting to note that the GH levels of the low fat fed rats at 3 and 10 weeks are not significantly different, however, the serum levels of the rats on the high fat diet showed a two-fold increase between 3 and 10 weeks. This increase in serum GH is consistent with the age effects reported by Dickerman (1971).

Even though the effects of blood glucose on serum GH have not been confirmed in rats as they have in humans, they could account for the large differences observed between the rats fed the high and low fat diets. Since the high fat fed rats had no source of dietary glucose, they would be expected to have depressed levels of blood glucose and hence higher serum GH. Increased levels of GH among these rats would be expected to stimulate lipolysis (Beaton, 1956; White and Engel, 1959), thus elevating plasma free fatty acids (Raben and Hollenberg, 1959), increasing the hepatic lipid content and favoring the production of ketone bodies (Engel, Engel and McPherson, 1957). The net result of these reactions on lipid metabolism by GH is the promotion of amino acid conservation, protein sparing effect, and tissue growth (Raben, Matsuzaki and Minton, 1964).

Experiment III

RIA showed that the hypophysectomized rats in this study had no measurable serum GH. The only significant effect GH treatment had on tissue nucleic acids was that the rats injected with 50 μg of purified GH/day had significantly more RNA per gram sample than the 25 μg and control

group (saline). There were no significant ($P > .05$) differences in muscle RNA between the 25 μg and control groups (table 14). Liver RNA concentrations showed no response to the GH treatments. Korner (1960, 1964) has shown conclusively that GH treatment stimulated protein synthesis in the liver, muscle and other body tissues studied when he treated hypophysectomized rats in a similar manner to that used in this study. Likewise, the DNA concentrations of corresponding tissues did not show any response to the GH injection. Cheek, Powell and Scott (1965) have clearly demonstrated that when hypophysectomy was performed at weaning, it prevented DNA from increasing in muscle which occurred during growth of normal rats. Beach and Kostyo (1968) pointed out that GH treatment of hypophysectomized rats increased muscle DNA concentration, content, and individual muscle weight. Table 14 also shows that the GH treatments did not significantly affect lysine incorporation rates per mg RNA in either muscle or liver. Korner (1960, 1964, 1967) has clearly demonstrated that GH treatments of hypophysectomized rats increased protein synthesis rates and incorporation of labeled amino acid precursors into muscle, liver and other tissues.

The 50 μg group tended to have higher serum insulin levels than the other groups (table 15). The insulin levels in this experiment were lower than those in Experiments I and II. This is in agreement with Randle and Young (1956) who reported hypophysectomy decreased plasma insulin; however, they also reported that GH administration restored insulin levels to normal. Since the data observed in this study are generally inconclusive as to the purified porcine GH effects on protein metabolism and insulin, a valid conclusion might be that the purified porcine GH was not highly effective

TABLE 14. MEANS AND STANDARD ERRORS OF TISSUE NUCLEIC ACIDS AND ^{14}C-LYSINE INCORPORATION RATES FOR EXPERIMENT III[a].

	Growth hormone treatment			
Item	0[d]	25 μg[d]	50 μg[d]	S.E.M.
Muscle RNA[b]	0.82[d]	0.95[d]	1.23[e]	0.07
Muscle DNA[b]	0.62	0.55	0.65	0.04
Liver RNA[b]	8.18	8.89	8.52	0.39
Liver DNA[b]	1.77	1.58	1.58	0.07
Lysine muscle[c]	0.82	0.82	0.74	0.10
Lysine liver[c]	1.79	2.22	2.02	0.18

[a]Means having different superscripts are significantly different ($P < .05$).
[b]mg/g wet wt.
[c]ng lysine incorporated/mg RNA/min.
[d]8 rats/group.

TABLE 15. MEANS AND STANDARD ERRORS OF SERUM INSULIN FOR EXPERIMENT III[a].

	Growth hormone treatment			
Hormone	0	25 μg	50 μg	S.E.M.
Insulin[b]	16.4[c]	16.3[c]	21.0[d]	1.53

[a]Means having different superscripts are significantly different ($P < .05$).
[b]μ Units bovine insulin equivalent/ml; 8 rats/group.

due some lack of biological potency resulting either from purification, or the treatment levels were inadequate relative to the specific activity, or the treatment period length was inadequate to demonstrate any significant effects.

SUMMARY

Ninety-four rats were included in three experiments designed to study the effects of 1) limiting dietary energy intake, 2) fat or carbohydrate sources as dietary energy, and 3) hypophysectomy and GH treatment on growth and carcass traits, protein metabolism and serum insulin and GH. In Experiment I, thirty weanling male rats were randomly divided into 3 groups and fed either ad libitum, 75% ad libitum, or 50% ad libitum. The rats were bled every 2 weeks for insulin and GH assay. After 6 weeks, 2 rats from each group were injected with ^{14}C-lysine, sacrificed and muscle and liver samples were taken. Three rats from each group were used to determine liver and muscle RNA and DNA concentrations, and 5 rats from each group were used for carcass analysis.

In Experiment II, thirty weanling male Osborne-Mendel rats were randomly divided into 2 groups and fed either a high fat or high carbohydrate diet. After 3 weeks, 6 rats from each group were injected with ^{14}C-lysine and sacrificed, and muscle and liver samples were taken for RNA, DNA analysis and lysine incorporation rate into proteins. Five additional rats from each group were sacrificed for carcass analysis. After 10 weeks, 6 additional rats from each group were injected with ^{14}C-lysine and tissue samples were taken as described in Experiment I. The remaining rats, 3 from each group were sacrificed for carcass analysis.

In Experiment III, twenty-four hypophysectomized female rats were plateaued for 12 days, randomly assigned into 3 groups, and injected with either 50 μg or 25 μg of purified porcine GH, or isotonic saline for 4 days. On the 5th day all rats were injected with ^{14}C-lysine and sacrificed.

Livers and muscle samples were taken for analysis of RNA, DNA and lysine incorporation into protein. When the rats were sacrificed, blood samples were collected for analysis of serum insulin and GH.

Restricting feed intake (Experiment I) depressed daily gains, final weight, carcass weight, and percent carcass fat and increased percent carcass protein and moisture. The treatments significantly decreased muscle RNA and DNA, liver RNA and also tended to decrease liver DNA. Lysine incorporation into muscle proteins on a per milligram RNA/minute basis was significantly stimulated, and liver incorporation rates also tended to be stimulated by restricting feed intake. There was no consistent trends in serum insulin and GH even though insulin tended to be lowest for the 100% group, highest for the 75% group and intermediate for the 50% group. One possible explanation for these results could be the feeding habits of the rats in this experiment. The ad libitum fed group ate generally at night, while the restricted fed groups ate immediately after receiving feed at about 7:30 a m. each day. Consequently, the serum levels of insulin (blood obtained at 9:30 to 11:00 a.m. at each bleeding period) in the restricted fed groups tended to be higher due to more recent glucose ingestion. Also, the muscle and liver polysomes of the restricted fed groups may have been more active due to these same eating habits, and could account for the increased incorporation rates of ^{14}C-lysine among the restricted fed rats.

The rats on the high fat diet (Experiment II) consistently gained faster, had heavier carcasses, higher percent carcass fat, lower percent carcass protein and moisture, lower serum insulin and higher GH levels.

There were no significant differences in either muscle or liver RNA, DNA, and lysine incorporation rates into proteins between treatments.

GH administration (Experiment III) significantly increased muscle RNA and tended to increase serum insulin. Muscle DNA, liver RNA and DNA and muscle and liver lysine incorporation rates were not significantly different between treatments. A plausible explanation for the general lack of response on protein and nucleic acid metabolism is that either the biological potency of the purified porcine GH was low, or dose levels were too low, or length of the treatment period was too short.

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APPENDIX

Appendix I. Reagents used in radioimmunoassay

A. Reagents for Insulin RIA

1. 0.01 M phosphate buffer - 1% bovine serum albumin (IA_1)

$NaH_2PO_4 \cdot 2\ H_2O$	12.4 g
or $NaH_2PO_4 \cdot H_2O$	11.0 g
Thimersol	0.5 g
BSA	29.2 ml
Deionized H_2O	2970.8 ml

Mix over magnetic stirrer, adjust pH to 7.4, stored at 4 C.

2. 0.05 M sodium phosphate buffer, pH 7.4

Solution A

$NaH_2PO_4 \cdot H_2O$	27.8 g
Thimersol	0.1 g

Dilute to 1000 ml with distilled water.

Solution B

$NaH\ PO_4 \cdot 7\ H_2O$	55.65 g
Thimersol	0.10 g

Dilute to 1000 ml with distilled water.

Mix 19.0 ml solution A and 81.0 ml solution B, dilute to 200 ml with distilled water.

Adjust pH to 7.4 with NaOH, if necessary.

Store at 4 C.

3. 0.01 M phosphate buffered saline, pH 7.0 (PBS)

NaCl	143 g
Dibasic phosphate	260 ml
Merthiolate	1.75 g

Dissolve in distilled water and transfer to a large container.

Dilute to 17.5 liters with distilled water.

Adjust pH to 7.0, if necessary, store at 4 C.

4. 0.05 M EDTA-PBS, pH 7.0

Disodium ethylenediamine-tetraacetate (EDTA) 18.612 g

Add approximately 950 ml PBS.

Adjust pH to 7.0 with 5 NaOH while stirring.

Dilute to 1 liter, store at 4 C.

5. PBS-1% bovine serum albumin (PBS-1% BSA)

Add 990 ml PBS to beaker.

Add 10 g BSA (Fraction V 35% sterile Solution, Nutritional Biochemicals Corp., Cleveland, Ohio).

Mix over magnetic mixer.

Store at 4 C.

6. 1:400 guinea pig control serum (GPCS)
   Obtain blood from guinea pig that has not been used to develop antibodies.
   Allow blood to clot, recover serum and store the serum in convenient quantities at -20 C.
   Add 2.5 ml of appropriate serum to a 1 liter volumetric flask, dilute to 1 liter with 0.05 M PBS-EDTA, pH 7.0.
   Divide into 100 ml portions and store at -20 C.

7. 0.05 M barbital buffer pH 8.6
   Solution A
      Sodium barbital (veronal) 10.30 g
      Dilute to 1000 ml with distilled water.
   Solution B
      HCl 1.0 ml
      Dilute to 720 ml with distilled water.
   Mix 50.0 ml solution A with 6.0 ml solution B and dilute to 200 ml with distilled water.
   Adjust pH to 8.6 while stirring.
   Store at 4 C.

Appendix I.

B. Reagents for GH RIA

1. 0.025 M EDTA-PBS pH 7.6
   Disodium ethylenediamine-tetraacetate (EDTA) 9.306 g
   Add approx. 950 ml PBS
   Adjust pH to 7.6 with NaOH while stirring.
   Dilute to 1 liter and store at 4 C.

2. 1% BSA - EDTA - BSA
   Dilute 28.6 ml 35% BSA in 1 liter 0.025 M EDTA-PBS.
   Adjust pH to 7.6.
   Store at 4 C.

3. Monkey control serum
   Obtain serum from monkey that has not been used to develop antibodies.
   Add 1 ml to 500 ml volumetric flask, dilute to 500 ml with 0.025 M EDTA-PBS, pH 7.6.
   Store at -20 C in small quantities.

4. Rat GH standards
   Rat GH diluted to 0.1, 0.2, 0.5, 1.0, 1.5, 3.0, 5.0 and 10.0 ng/100 ul with 1% BSA - PBS (Appendix I.A.5) adjusted to pH 7.6.

Appendix I.

C. Reagents for radioiodination of GH

1. Chloramine-T, 3.5 μg/μl
   Store chloramine-T in tightly sealed vials covered with foil at -20 C.
   Dilute 35 mg chloramine-T to 10 ml with 0.05 M phosphate buffer (Appendix I.A.2.).
   Use within 30 min. of preparation.
   Discard all unused chloramine-T.

2. Sodium metabisulfate, 2.5 μg/μl.
   Dilute 25 mg $Na_2S_2O_5$ to 10 ml with 0.05 M phosphate buffer.
   Use within 30 min. of preparation.

3. Transfer solution

Sucrose	1.6 g
KI	0.1 g

   Dilute to 10 ml with distilled water.

4. Rinse solution

Sucrose	0.8 g
KI	0.1 g
Bromphenol blue	0.001 g

   Dilute to 10 ml with distilled water.

Appendix II.

A. Experiment I - Column Identification

1. Rat identification.
2. Treatment; 1. ad libitum, 2. 75% ad libitum, 3. 50% ad libitum.
3. Beginning weight in grams.
4. Final weight in grams.
5. Feed consumed in grams.
6. Muscle lysine incorporation rate, nanomoles/mg RNA/min. (one decimal place).
7. Liver lysine incorporation rate, nanomoles/mg RNA/min. (one decimal place).
8. Insulin at 2 weeks, μ Units bovine insulin equivalent/ml. (one decimal place).
9. Insulin at 4 weeks, μ Units bovine insulin equivalent/ml. (one decimal place).
10. Insulin at 6 weeks, μ Units bovine insulin equivalent/ml. (one decimal place).
11. GH at 2 weeks, ng/ml (one decimal place).
12. GH at 4 weeks, ng/ml (one decimal place).
13. GH at 6 weeks, ng/ml (one decimal place).
14. Muscle RNA in mg/gram wet sample (one decimal place).
15. Liver RNA in mg/gram wet sample (one decimal place).
16. Muscle DNA in mg/gram wet sample (one decimal place).
17. Liver DNA in mg/gram wet sample (one decimal place).
18. Carcass weight in grams (one decimal place).
19. Percent carcass water (two decimal places).
20. Percent carcass protein (two decimal places).
21. Percent carcass fat (two decimal places).

Appendix II A. Data from Experiment I - Limited Feed Study.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1001N	1	49	30	78	10		979	1128	770	225	905	1260					1908	6790	1854	979
1001NN	1	44	35	08	10		756	984	718	1131	1270	130					2162	6839	1861	978
1002N	1	42	30	97	60		739	1749	1156	140	163	264					1937	6484	1752	1372
1002NN	1	47	32	27	60	05	31	415	1174	881	150	130	905							
1003N	1	53	36	67	60	03	44	583	1057	948	965	1260	1280							
1003NN	1	47	31	57	60			622	413	454	1270	1269	879				2273	6688	1856	1200
1004N	1	49	31	07	68			520	1456	1082	530	124	149				1903	6698	1792	1188
1004NN	1	45	31	47	68			845	1498	1171	124	611	172	142	812	217	218			
1005N	1	47	31	27	88			717	1075	1565	109	149	320	167	1568	270	232			
1005NN	1	49	30	35	78	8		598	749	1087	1141	1250	181	138	1423	200	260			
751N	2	46	23	76	83			1709	1412	1459	159	137	1228					1506	7094	1831	686
751NN	2	52	28	26	83			696	994	893	267	194	163					1934	7009	1860	789
752N	2	51	23	96	83			980	2632	1498	288	1259	660					1576	6994	1814	868
752NN	2	41	26	03	83	13	67	671	1610	895	984	799	222							
753N	2	49	24	76	83			971	1595	946	1215	1114	330	065	894	207	205			
753NN	2	44	26	76	83			721	1979	1498	124	113	211					1805	6916	1842	952
754N	2	46	23	76	83			1426	1407	1373	1268	273	207					1670	7057	1852	759
754NN	2	52	25	05	83	09	51	1287	1651	2098	1269	131	527							
755N	2	50	26	05	83			1647	1667	2021	851	330	146	062	636	252	213			
755NN	2	45	26	76	83			1143	2384	1888	645	112	298	062	1210	170	245			
501N	3	47	18	73	87	20	69	1380	2639	1399	533	1193	1127							
501NN	3	42	18	58	87			918	1380	561	546	820	502	062	627	198	239			
502N	3	48	18	78	87	13	42	931	1353	386	270	1239	961							
502NN	3	52	20	23	87			641	962	764	113	435	164					1213	7212	1892	522
503N	3	46	18	53	87			1525	1430	878	120	172	484	054	965	182	202			
503NN	3	50	20	23	87			1067	1376	958	194	289	499					1211	7355	1874	403
504N	3	44	20	93	87			810	2422	1295	116	837	680					1321	7230	1942	456
504NN	3	44	16	02	87			945	1288	552	106	649	251					1006	7288	1954	336
505N	3	52	19	13	87			1364	1138	1319	206	1250	146	052	670	158	203			
505NN	3	52	17	73	87			1262	1482	660	543	262	120					1155	7484	1966	246

Appendix II.

B. Experiment II - Column Identification

1. Rat identification.
2. Age in weeks.
3. Diet treatment; 1. low fat, 2. high fat.
4. Muscle RNA in mg/gram wet sample (two decimal places).
5. Liver RNA in mg/gram wet sample (two decimal places).
6. Muscle DNA in mg/gram wet sample (two decimal places).
7. Liver DNA in mg/gram wet sample (two decimal places).
8. Insulin, μ Units bovine insulin equivalent/ml (one decimal place).
9. GH, ng/ml (one decimal place).
10. Muscle lysine incorporation rate, n moles/mg RNA/ml (two decimal places).
11. Liver lysine incorporation rate, n moles/mg RNA/ml (two decimal places).
12. Carcass wt. (two decimal places).
13. Percent carcass moisture (two decimal places).
14. Percent carcass protein (two decimal places).
15. Percent carcass fat (two decimal places).

Appendix II B. Data from Experiment II - High and Low Fat Diets.

1	2	3	4	5	6	7	8	9	10	11
1	3	1	254	1323	86	236	323	324	102	243
2	3	1	259	1237	88	329	194	155	157	281
3	3	1	276	1447	94	277	315	422	103	415
4	3	1	265	1218	72	316	373	132	98	223
5	3	1	158	1496	55	338	1016	240	107	329
6	3	1	286	1447	80	393	845	202	121	246
7	3	2	233	1367	75	384	316	248	58	190
8	3	2	260	1138	97	312	187	280	58	169
9	3	2	305	1410	106	408	273	652	87	176
10	3	2	205	1184	87	335	1111	060	173	313
11	3	2	235	1198	90	340	80	208	87	444
12	3	2	222	1197	73	366	138	308	93	245
13	10	1	135	1415	47	271	846	168	217	360
14	10	1	164	1206	58	448	1277	693	202	211
15	10	1	171	1092	62	297	1352	181	303	147
16	10	1	150	1833	70	265	1039	131	238	303
17	10	1	156	1036	52	308	952	122	283	291
18	10	1	210	1221	72	361	1521	346	116	154
19	10	2	183	1349	66	357	439	515	252	595
20	10	2	162	1024	52	313	398	905	91	231
21	10	2	213	1076	75	300	398	453	331	261
22	10	2	186	994	68	321	515	270	197	232
23	10	2	235	835	81	312	416	260	248	322
24	10	2	162	1067	56	340	494	240	74	313

1	2	3	12	13	14	15
25	3	1	160	710	519	35 726
26	3	1	130	682	017	281 042
27	3	1	130	675	317	751 094
28	3	1	150	660	016	991 314
29	3	1	135	067	318	231 011
30	3	2	139	067	459	331 329
31	3	2	132	065	981	536 1449
32	3	2	126	065	191	636 1434
33	3	2	129	062	301	593 1799
34	3	2	112	063	501	610 1652
35	10	1	207	067	961	743 1236
36	10	1	220	067	541	716 1314
37	10	1	214	068	681	696 1204
38	10	2	229	058	901	664 1944
39	10	2	335	056	461	478 2485
40	10	2	276	061	261	745 1805

Appendix II.

C. Experiment III - Column Identification

1. Rat identification.
2. GH treatment, μg GH/day.
3. Time from ^{14}C injection to death in minutes.
4. Muscle RNA in mg/gram wet sample (two decimal places).
5. Liver RNA in mg/gram wet sample (two decimal places).
6. Muscle DNA in mg/gram wet sample (two decimal places).
7. Liver DNA in mg/gram wet sample (two decimal places).
8. Muscle lysine incorporation rate, n moles/mg RNA/min. (one decimal place).
9. Liver lysine incorporation rate, n moles/mg RNA/min. (one decimal place).
10. Insulin, μ Units bovine insulin equivalent/ml (one decimal place).

Appendix II C. Data from Experiment III - Hypophysectomized Rat Study.

1	2	3	4	5	6	7	8	9	10
1	50	240	60	665	47	34	8	6	79
2	25	240	90	773	52	89	9	13	152
3		240	68	753	24	89	8	9	69
5	50	240	75	777	63	48	7	16	167
6	25	240	97	681	44	81	6	14	170
7		240	85	710	61	69	5	8	154
9	50	240	111	810	48	32	7	13	146
10	25	240	119	949	72	64	7	12	118
11		240	88	970	61	69	7	11	175
13	50	240	86	836	49	80	10	13	113
14	25	240	106	1006	57	51	7	10	192
15		240	83	880	64	80	8	10	116
17	50	20	104	1090	70	60	12	13	277
18	25	20	71	993	51	25	11	20	140
19		20	84	723	78	99	6	19	146
21	50	20	162	907	66	73	4	29	295
22	25	20	94	1130	53	76	7	27	134
23		20	102	792	73	89	4	24	198
25	50	20	144	860	98	43	7	21	255
26	25	20	74	808	42	120	3	42	154
27		20	50	813	61	130	4	29	186
29	50	20	144	860	77	88	8	30	152
30	25	20	119	767	67	56	6	40	140
31		20	81	906	67	129	14	33	169

Appendix III A. Correlation Coefficients from Experiment I - Limited Feed Study.

SIMPLE CORRELATIONS

	VAR NO.						
TRT	1	1.00000000					
B WT	2	0.06885806	1.00000000				
P WT	3	-0.96178032	-0.00198707	1.00000000			
FC	4	-0.99755067	-0.06242043	0.96022340	1.00000000		
C14MUS	5	0.90328672	-0.48970558	-0.89810547	-0.90105666	1.00000000	
C14LIV	6	0.54043252	-0.45388210	-0.49240441	-0.52745968	0.76153828	1.00000000
12	7	0.48432237	0.11003488	-0.55009494	-0.47132616	0.70401188	0.26036236
14	8	0.33549083	-0.21031239	-0.34770468	-0.33908847	0.38738328	0.18775902
16	9	-0.04926327	0.11125406	0.09140121	0.09936939	0.07630688	0.32064588
GM2	10	-0.19651923	0.05631980	0.21155072	0.19575253	-0.12038176	0.13865529
GM4	11	0.02132847	0.08892218	0.05302855	0.01205278	0.16348823	0.33034201
GM6	12	-0.05855066	0.10373399	0.05229155	0.05284432	0.13990258	0.34784140
RNA MUS	13	-0.89271694	-0.87969410	0.84179602	0.88593097	FREQ OF ONLY 0	FREQ OF ONLY 0
RNA LIV	14	-0.65864475	-0.02437745	0.65425699	0.65810497	FREQ OF ONLY 0	FREQ OF ONLY 0
DNA MUS	15	-0.59054494	0.00223377	0.52443037	0.59476802	FREQ OF ONLY 0	FREQ OF ONLY 0
DNA LIV	16	-0.45748106	-0.36570341	0.53114331	0.47071591	FREQ OF ONLY 0	FREQ OF ONLY 0
CARC WT	17	-0.92363244	-0.21854448	0.96593252	0.91864026	FREQ OF ONLY 0	FREQ OF ONLY 0
PCH20	18	0.93249868	0.37732205	-0.88906195	-0.91170939	FREQ OF ONLY 0	FREQ OF ONLY 0
PC PROT	19	0.74367825	0.17004388	-0.68908748	-0.71653860	FREQ OF ONLY 0	FREQ OF ONLY 0
PC FAT	20	-0.93421740	-0.31274251	0.89732575	0.91043940	FREQ OF ONLY 0	FREQ OF ONLY 0
ADG	21	-0.96356080	-0.06019394	0.99030434	0.98211764	-0.08468481	-0.27373313
FEED EFF	22	-0.05529432	0.02060582	-0.16466775	0.09599541	0.18641184	0.03704394
		1 TRT	2 B WT	3 P WT	4 FC	5 C14MUS	6 C14LIV

	VAR NO.						
12	7	1.00000000					
14	8	0.32141172	1.00000000				
16	9	0.43190669	0.53188228	1.00000000			
GM2	10	0.10602472	-0.08155241	0.09114122	1.00000000		
GM4	11	-0.16692706	-0.09574240	-0.37136158	0.22001264	1.00000000	
GM6	12	0.06214698	0.06404275	-0.13087650	-0.01771555	0.49586028	1.00000000
RNA MUS	13	-0.75587840	-0.50393999	0.06080690	-0.52267737	-0.05581493	-0.27241922
RNA LIV	14	-0.58759412	-0.22117437	0.19388523	-0.36308024	-0.23579903	0.01029156
DNA MUS	15	-0.26951912	-0.20498370	0.31198606	-0.06534309	-0.35578766	-0.12527309
DNA LIV	16	-0.65707975	-0.10230093	0.05255061	-0.20707054	0.02134033	-0.00193837
CARC WT	17	-0.38935155	-0.31784199	-0.02269893	0.52400744	0.27278226	0.10164771
PCH20	18	0.45184767	0.13976332	-0.08243457	-0.25337556	-0.15628773	-0.10004301
PC PROT	19	0.16337961	-0.08160212	-0.14009591	-0.00874513	0.13958436	-0.08958744
PC FAT	20	-0.43120623	-0.11672549	0.13792880	0.27995835	0.13411818	0.08540961
ADG	21	-0.59550372	-0.33483249	0.08479950	0.20786937	0.04775430	0.04013843
FEED EFF	22	0.31477471	-0.04277858	-0.00980346	0.01237927	-0.08469283	0.01464348
		7 12	8 14	9 16	10 GM2	11 GM4	12 GM6

Appendix III A. (continued)

	VAR NO.						
RNA MUS	13	1.00000000					
RNA LIV	14	0.67160487	1.00000000				
DNA MUS	15	0.58828314	0.26617937	1.00000000			
DNA LIV	16	0.44707301	0.57860608	0.06010445	1.00000000		
CARC WT	17	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	1.00000000	
PCM20	18	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	-0.[illegible]5208	[illegible]

PC PROT	19	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	-0.[illegible]2782	0.[illegible]2287
PC FAT	20	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	0.[illegible]1306	-0.[illegible]7291
ADG	21	0.84889317	0.65744855	0.52585899	0.55199883	0.[illegible]0103	-0.[illegible]1075
FEED EFF	22	0.49501196	0.21946991	0.00068177	-0.40595377	-0.[illegible]0906	-0.[illegible]2459
		13	14	15	16	17	18
		RNA MUS	RNA LIV	DNA MUS	DNA LIV	CARC WT	PCM20

	VAR NO.				
PC PROT	19	0.99999997			
PC FAT	20	-0.85552376	1.00000000		
ADG	21	-0.68843036	0.90196622	1.00000000	
FEED EFF	22	-0.02073375	0.00237877	-0.16552903	1.00000000
		19	20	21	22
		PC PROT	PC FAT	ADG	FEED EFF

Appendix III B. Correlation Coefficients from Experiment II - High and Low Fat Diets.

SIMPLE CORRELATIONS

	VAR NO.						
AGE	1	1.00000000					
DIET	2	0.00000000	1.00000000				
RNA MUS	3	-0.72141532	0.10157111	1.00000000			
RNA LIV	4	-0.30782922	-0.43348210	0.84927209	1.00000000		
DNA MUS	5	-0.65947873	0.24325035	0.07535540	0.11380859	1.00000000	
DNA LIV	6	-0.10814617	0.20467332	0.16326419	-0.03301586	0.04070297	1.00000000
INSU	7	0.55315051	-0.63517356	-0.52183581	0.17495771	-0.26788544	0.12073735
GH	8	0.33573857	0.58097179	-0.13056986	-0.53824865	-0.03161744	0.19818414
C14 MUS	9	0.67079499	-0.15282638	-0.46369493	-0.17548994	-0.35544914	-0.43899242
C14 LIV	10	0.11772143	0.23408192	-0.25320466	0.05856686	-0.14118526	-0.18443986
REPS	11	0.00000000	0.00000000	0.00830739	-0.15036402	-0.04431743	0.19778644
GH6	12	VAR APPX CON	VAR APPX CON	VAR APPX CON	VAR APPX CON	VAR APPX CON	VAR APPX CON
R-D MUS	21	-0.25222654	-0.21004542	0.39543482	-0.16637439	-0.09491455	0.21023615
R-D LIV	22	-0.12772117	-0.48256861	-0.08487981	0.78314387	0.03950570	-0.03174728
		1 AGE	2 DIET	3 RNA MUS	4 RNA LIV	5 DNA MUS	6 DNA LIV

	VAR NO.						
INSU	7	1.00000000					
GH	8	-0.21465733	1.00000000				
C14 MUS	9	0.39017491	0.01856885	1.00000000			
C14 LIV	10	-0.27084209	0.28971327	0.04748725	1.00000000		
REPS	11	0.18613722	0.14426998	-0.08888539	0.10000915	1.00000000	
GH6	12	VAR APPX CON	VAR APPX CON	VAR APPX CON	VAR APPX CON	VAR APPX CON	VAR APPX CON
R-D MUS	21	-0.03977056	-0.18986318	-0.31057563	-0.24376329	0.10178523	VAR APPX CON
R-D LIV	22	0.09118182	-0.47761243	0.02366874	0.09152084	-0.24499908	VAR APPX CON
		7 INSU	8 GH	9 C14 MUS	10 C14 LIV	11 REPS	12 GH6

	VAR NO.		
R-D MUS	21	1.00000000	
R-D LIV	22	-0.29232863	1.00000000
		21 R-D MUS	22 R-D LIV

Appendix III B. (continued)

AGE	1	1.00000										
DIET	2	0.00000	1.00000									
CARC AT	3	0.20833	0.25467	1.00000								
PCH20	4	-0.37105	-0.71749	-0.60764	1.00000							
PCHNO	5	-0.18326	-0.72065	-0.27133	0.64392	1.00000						
PCFAT	6	0.44241	0.74520	0.69973	-0.97190	-0.75806	1.00000					
CARC DAY	21	-0.36554	0.31102	-0.83088	0.94911	-0.16126	-0.11791	1.00000				
PROT DAY	22	-0.90762	0.09318	-0.79114	0.21745	0.13698	-0.33350	0.95482	1.00000			
TIMES	11	-1.00000	0.00000	-0.92833	0.57195	0.08326	-0.44241	0.88554	0.90762	1.00000		
TRT1	12	0.00000	-1.00000	-0.25867	0.71949	0.72065	-0.74520	-0.31102	-0.09318	0.00000	1.00000	
INTACT	13	0.00000	-0.25000	0.19656	-0.16554	0.41311	0.00075	0.08137	0.22627	0.00000	0.25000	1.00000
		1	2	3	4	5	6	21	22	11	12	13
		AGE	DIET	CARC AT	PCH20	PCHNO	PCFAT	CARC DAY	PROT DAY	TIMES	TRT1	INTACT

Appendix III C. Correlation Coefficients from Experiment III - Hypophysectomized Rats.

SIMPLE CORRELATIONS

	VAR NO.						
CNTRT	1	1.00000000					
TIMETRT	2	0.00000000	1.00000000				
RNAMUS	3	0.48462804	-0.07643753	1.00000000			
RNALIV	4	-0.14258169	-0.28855027	0.00703813	1.00000000		
DNAMUS	5	0.34894311	-0.45868273	0.38102256	0.17056890	1.00000000	
DNALIV	6	0.00290414	0.07138756	0.01235581	-0.08190967	0.15941207	1.00000000
C14MUS	7	-0.18822918	-0.28214019	-0.57145801	0.25994932	-0.17145203	-0.46009979
C14LIV	8	-0.10269923	-0.82968747	0.07727090	0.09078124	0.29996637	-0.44769211
INSULIN	9	0.33674115	-0.28317369	0.42874150	0.01972119	0.37255431	-0.08423253
GH	10	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0
RBPS	11	0.00000000	0.00000000	0.05916445	0.15157093	0.22958164	-0.02853064
RB MUS	21	0.23513407	0.31339091	0.64338627	-0.21155867	-0.42093669	-0.11094176
RB LIV	22	-0.15893412	-0.27793063	-0.06738307	0.70393096	-0.02098827	-0.79454963
		1 CNTRT	2 TIMETRT	3 RNAMUS	4 RNALIV	5 DNAMUS	6 DNALIV

	VAR NO.						
C14MUS	7	1.00000000					
C14LIV	8	0.39240252	1.00000000				
INSULIN	9	-0.16935685	0.17259538	1.00000000			
GH	10	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0	FREQ OF ONLY 0		
RBPS	11	0.20586762	0.26338696	-0.20007115	FREQ OF ONLY 0	1.00000000	
RB MUS	21	-0.43401313	-0.17807706	0.14063959	FREQ OF ONLY 0	-0.18148089	1.00000000
RB LIV	22	0.40598961	0.24368537	0.05334453	FREQ OF ONLY 0	0.08214346	-0.10159723
		7 C14MUS	8 C14LIV	9 INSULIN	10 GH	11 RBPS	21 RB MUS

	VAR NO.	
RB LIV	22	1.00000000
		22 RB LIV