THESIS

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PROTEIN SYNTHESIS, BREAKDOWN AND ACCRETION RATES IN SKELETAL MUSCLE AND LIVER OF YOUNG GROWING BOARS

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PROTEIN SYNTHESIS, BREAKDOWN AND ACCRETION RATES IN SKELETAL MUSCLE AND LIVER OF YOUNG GROWING BOARS

By

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ABSTRACT

PROTEIN SYNTHESIS, BREAKDOWN AND ACCRETION RATES IN SKELETAL MUSCLES AND LIVER OF YOUNG GROWING BOARS

By

Donald R. Mulvaney

Twenty four boar pigs were used to estimate fractional protein synthesis (FSR), breakdown (FBR), and accretion (FGR) rates of the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles and livers at 22 and 45 kg live body weight. A six hour continuous infusion of L-[U - ^{14}C]tyrosine was performed on 4 pigs at each weight to measure FSR. Four additional pigs were killed 1 week before and 1 week after each infusion to observe changes in composition, FGR and myofiber diameter. FBR was obtained by the difference of FGR and FSR. FSR and FBR of both muscle and liver were lower at 45 kg whereas FGR was lower at 45 kg for the liver, ST and BR musles. The LD at 22 kg and the BR at 45 kg exhibited similar high protein turnover values. Absolute rates of muscle protein, fat and nucleic acid accretion varied between periods.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

INTRODUCTION

The awareness of diminishing natural resources, increased costs of production and rapidly expanding human population has stimulated the need to seek out and research innovative means of achieving maximum utilization of resources to attain higher levels of animal agriculture productivity. In the past, attempts to increase the efficiency of the livestock animal's capacity to convert feed into meat, comprised of high proportions of muscle relative to fat, has involved sound nutritional and health practices as well as selective breeding programs. Proper managerial and breeding programs will continue to be of value in identifying and improving domestic animals that are efficient in the conversion of feed to the desired amounts of muscle and fat but with current knowledge, the improvements will not be of sufficient magnitude to meet the increase in meat protein production efficiency demanded of animal agriculture. Potential progress lies with a more in depth understanding of the animal's molecular biology and capabilities to synthesize protein. The knowledge of the mechanisms of protein synthesis and potential control points is gaining in magnitude (Bergen, 1975; Caskey, 1980; Clark,

1980; Hunt, 1980). The possibility of identifying animals or muscles within animals that differ in one or the other processes of protein synthesis or degradation or even altering the extent of turnover offers an intriquing avenue of enhancing the efficiency of the animal.

The primary thrust of the research endeavor was to make observations of the fractional protein turnover rates, growth rates and nucleic acid changes in liver and three anatomically different muscles of growing boar pigs during two different growth periods. Some turnover studies (Turner and Garlick, 1974; Millward et al., 1975; Garlick et al., 1976) indicate high rates of protein synthesis and breakdown (high turnover) during rapid muscle growth. When synthesis rate exceeds breakdown of protein, net protein accretion results. On the other hand, studies by Ogata et al. (1978), Maruyama et al. (1978) and Trostler et al. (1979) imply variable rates of protein breakdown during growth to account for net protein accumulation.

In this study, the longissimus, semitendinosis and brachialis muscles were selected to represent the middle, posterior and anterior sections of the animal as well as late, intermediate and early maturing muscles, respectively. Work by Berg and Butterfield (1976) on cattle indicates an anterior to posterior growth progression and muscle maturity pattern. This also has been shown in rabbits where individual muscles mature at different rates (Vezinhet et al., 1972).

LITERATURE REVIEW

Protein Turnover - Definition and Significance

The concept of protein turnover can be traced to work by Schoenheimer et al. (1939) in which they studied the relative distribution of ^{15}N in tissues of rats. This work along with earlier work by Borsook and Jeffreys (1935) showed that there was a constant but rapid metabolic turnover of proteins. Much of the current understanding of protein metabolism results from studies of liver and plasma proteins (Waterlow et al., 1978). More recently, there has been renewed interest in understanding whole body protein turnover, fractional turnover rates of tissues and the contribution skeletal muscle makes to whole body protein metabolism. Garlick et al. (1976) stated that muscle contributes 42% of whole body protein synthesis in the pig.

Waterlow et al. (1978) defined turnover as a process of renewal or replacement of a particular substance. Schimke (1977), Millward et al. (1976a), Garlick and Millward (1972) and Maruyama et al. (1978) described protein turnover in tissues in terms of the rate of one or the other of the two processes: protein synthesis and protein breakdown. Millward et al. (1976) indicated that the two processes are equal in rate in a steady state situation. In the nonsteady

state, the rate of one process can be calculated by subtracting the net change of protein mass or accretion from the other process. Waterlow (1970) stated that the overall protein turnover of the body is the sum of the turnovers of all the individual proteins and also compares this to basal metabolic rate. Garlick et al. (1976) estimated that protein turnover accounts for approximately 17% of the overall basal metabolic rate. Reeds and Lobley (1980) suggested that protein synthesis contributes a rather constant proportion (about 15%) of the total body energy expenditure to heat production.

Schimke (1973, 1975, 1977) discussed the properties of protein turnover in a general sense and stated that it is quite extensive with 70% of rat liver protein being replaced every 4 to 5 days. Furthermore, liver turnover is largely intracellular, has considerable variation in rate, is a random process and is correlated to the molecular weight of the protein molecule.

When the turnover of myofibrillar proteins is considered, there is much disagreement concerning the kinetics and properties of the process. Dreyfus et al. (1960) stated that the myofibrillar proteins have a finite lifespan which is in contrast to a more random and heterogeneous turnover indicated by Goldberg (1969a), Millward (1970a, 1970b), Low and Goldberg (1973) and Millward et al. (1976b).

The significance of protein turnover during the growth period is addressed by Millward et al. (1975) and Laurent and Millward (1980). They view the process as being wasteful. Young and Pluskal (1977) emphasized the wasteful aspect of protein metabolism when they showed that the net protein deposition during rapid growth represents only a small portion of the total amount of muscle protein synthesized. Laurent and Millward (1980) partitioned total protein synthesis, during stretch induced hypertrophy of adult fowl anterior latissimus dorsi muscles, into 68% for normal replacement, 9% for growth and 23% for wastage. However, Goldberg and Dice (1974), Goldberg et al. (1974), Morgan (1974), Goldberg and Chang (1978), Goldberg (1980) and Morgan and Wildenthal (1980) addressed the significance of protein turnover in terms of a more positive biological role or necessity. In this light, amino acids are released from protein breakdown and contribute to the pool for obtaining amino acids for gluconeogenesis. Goldberg and Dice (1974) and Schimke (1977) explained that the breakdown of protein is necessary to remove abnormal proteins, obtain amino acids for synthesis of enzymes and to help the animal adapt to adverse environmental conditions. While there are no definite answers to explain why animals have this continual energy drain, Schimke (1977) hypothesized that rather than have elaborate and specific degradative mechanisms for specific proteins, cells evolved with mechanisms to continually degrade all proteins.

Factors Affecting Protein Synthesis and Breakdown

The balance between protein synthesis and breakdown (turnover) regulates the accumulation of protein and growth of muscle (Millward, 1970a; Waterlow and Stephen, 1968; Morgan, 1974; Millward et al., 1975). In their reviews on protein degradation, Schimke (1970) and Goldberg and Dice (1974) indicated that proteolytic enzymes are important in protein degradation. As Bird et al. (1980) pointed out, the only proteinases found in muscle cells are calcium activated proteinase, cathepsin B and cathepsin D but there may be additional proteinases capable of degrading myofibrillar proteins. Mechanisms of degradation in normal physiological conditions have not been characterized. Bradley (1977) has shown that intracellular protein degradation is under physiological regulation and varies with physiological demand.

Hormonal Effects

Morgan (1974) and Morgan and Wildenthal (1980) listed various factors important in regulating protein synthesis. The availability of hormones like insulin, growth hormone, adrenal steroids and thyroid hormones are of major importance. For the control of degradation, insulin and glucagon were cited as viable hormones. The possible mechanisms given for control of protein synthesis or degradation are via enzyme induction and suppression at the

transcriptional or post-transcriptional level. Manchester (1976) illustrated possible hormonal control points in the protein synthesis scheme. These controls include transcription of DNA to RNA, increases or decreases of RNA concentrations, changes in the amounts of the relative species of RNA and changes in initiation or elongation factors. In his review of muscle protein synthesis, Bergen (1975) identified hormones as being important at various control points. Similarly, Jefferson et al. (1974) pointed out that protein synthesis could be regulated by amino acid availability, ATP and GTP, availability of RNA and ribosomes, initiation and elongation factors, all of which may be influenced by hormones. With the exception of studies like O'Malley and Schrader (1976), in which transcription was shown to be modulated by steroid hormones, there is little concrete evidence to prove or disprove hormonal effects at specific points along the protein synthesis scheme. However, some general observed effects of hormones on the processes of protein synthesis and degradation are available. Goldberg et al. (1980) showed lower rates of both protein synthesis and breakdown in skeletal muscle isolated from hypophysectomized rats and stated that lack of growth hormone was primarily responsible for reduced protein synthesis. The lack of thyroid hormones (triiodothyronine or thyroxine) is credited for lower protein breakdown. Increased breakdown was observed in fasted

adrenalectomized animals when treated with gluccocorticoids but this effect was not observed in well fed animals. Goldberg (1969a, 1969b) and Goldberg and St. John (1976) have shown skeletal muscle protein degradation to increase by denervation and addition of thyroid hormones or cortisone but is decreased by work induced hypertrophy, hypophysectomy, thyroidectomy, administration of insulin and high levels of branched chain amino acids.

Studies on the effects of insulin upon skeletal muscle have involved considerable variation in technique as well as results (Wool and Krahl, 1959; Wool and Cavicchi, 1966; Goldstein and Reddy, 1967; Short, 1969; Jefferson et al., 1974; Rothig et al., 1978; Frayn and Maycock, 1979; Albertse et al., 1979). In vitro incorporation experiments with muscle, by Goldstein and Reddy (1970) have shown a stimulatory effect on protein synthesis by insulin with controlled experimental conditions involving nonphysiological media, ionic strength and amino acid concentration. Similarly, Wool and Krahl (1959) used isolated rat diaphragms and showed an increase of radiolabeled amino acids into protein. Apparently, insulin stimulated entry of glucose into the cells and thereby had a nitrogen sparing effect. Goldstein and Reddy (1967) indicated that insulin enhanced the entry of amino acids into cells but did not necessarily directly affect protein synthesis. Wool and Cavicchi (1966) recognized the entry effect but also showed an effect of insulin on existing RNA templates

and believed the increase of protein synthesis from both in vivo and in vitro studies occurred more rapidly than a transcriptional effect would allow. Short (1969) investigated the effects of insulin on protein synthesis by red and white muscle in vitro and drew similar conclusions along with showing greater synthesis rate in red muscle than white. Jefferson et al. (1974) not only showed an insulin stimulating effect on protein synthesis in perfused skeletal muscle, but a suppression of protein breakdown. They indicated this lower breakdown was due to an insulin effect on the permeability of lysosomal membranes. The data of Rothig et al. (1978) provide some substantiation of this effect as they showed insulin reversibility of alkaline proteinases activity, which increases during starvation and in diabetic states. The effects of physiological concentrations of insulin on muscle protein synthesis and degradation, as measured by tyrosine release in the presence of inhibitors, was studied by Frayn and Maycock (1979). A stimulatory effect on protein synthesis was observed in both soleus and extensor digitorum longus muscles but depressed breakdown was observed only in the latter. In vivo experiment with diabetic and insulin treated rats by Albertse et al. (1979) showed a dramatic decrease in protein synthesis rate as insulin levels decreased. However, the fractional breakdown rate (obtained indirectly: FGR=FSR-FBR) exhibited the exact opposite relationship.

Young and Pluskal (1977) and Young (1980) summarized the role of various hormones in protein synthesis or breakdown. Growth hormone is considered to be anabolic due to stimulatory effects upon amino acid transport, DNA, RNA and protein synthesis. Similarly, the sex steroids increase RNA and protein synthesis but their role in breakdown is undefined. From the catabolic viewpoint, glucagon and gluccocorticoids are noted to decrease protein synthesis with the latter group of hormones depressing DNA and RNA synthesis and increasing protein breakdown. Goldberg (1969a) injected cortisone into rats intraperitoneally and observed large amounts of atrophy in the plantaris muscle and decreased protein synthesis along with increased degradation. Similar observations on protein synthesis were made by Shoji and Pennington (1977) but the rates of protein breakdown in rat extensor digitorum longus muscle were not significantly reduced.

Woodside et al. (1974) suggested that turnover of liver proteins is regulated by glucagon via both reduction of protein biosynthesis as well as stimulation of proteolysis. Bruer and Florini (1965; 1966) obtained results of enhanced muscle protein synthesis and increased template activity of DNA presumably due to testosterone. However, Florini (1970) was unable to show any stimulatory synthesis effects of specific proteins by androgens.

With little evidence, Young and Pluskal (1977) hypothesized the mechanism of hormone action to be a hormone receptor complex which acts directly on DNA to increase DNA replication and protein synthesis. Dube et al. (1976) and Mainwaring and Mangan (1973) indicated that muscle has high concentrations of binding sites but the responsiveness is variable for different muscles. Within male accessory organs, testosterone has been shown to exhibit some specificity of proteins synthesized (Parker et al., 1978). Grigsby et al. (1976) showed an increase of myofibrillar protein synthesis in testosterone implanted rabbits but serum insulin was elevated by the testosterone treatment. Trenkle (1974) postulated that insulin plays a role in protecting amino acids from catabolism. Vernon and Buttery (1978) tested the effects of injecting trienbolone acetate or testosterone into female rats and showed the rate of myofibrillar protein degradation was reduced within 3 days.

Growth hormone is also a candidate for promoting protein synthesis (Bergen, 1975) and may act by stimulating RNA synthesis (Korner, 1967; Manchester, 1970). In in vitro studies by Clemens and Korner (1970), enhanced protein synthesis and RNA labeling were observed when amino acids and growth hormone were added to the media, but these effects were abolished when cycloheximide was added.

Age and Species Effects

The body size of an animal affects the total protein metabolism of the body (Munro, 1969). Garlick et al. (1976) showed that the fractional protein synthesis rate in rat tissues is 2 to 3 times faster than in the pig. Dunlop et al. (1978) estimated protein breakdown in rat brain in vivo during development to be 2 to 2.5 times greater in young rats than in older rats and was 65% of the synthesis rate during rapid growth. Millward et al. (1975) showed that during development of rat skeletal muscle there was a fall in the rate of protein synthesis, and during rapid growth a corresponding high rate of protein breakdown. However, degradation was lower in fast growing strains compared to slow growing strains (Millward and Waterlow, 1978). The increased rates of protein breakdown was postulated to be a necessary part of muscle growth and results from the mechanisms that myofibrils use to proliferate. In contrast to Millward et al. (1975) and Laurent and Millward (1980), who stated growth is accompanied by high rates of both synthesis and degradation, Ogata et al. (1978) reported that growth of young rats is achieved by high rates of synthesis and low rates of degradation. In another study, the rates of protein synthesis of leg and breast muscles and whole body of growing chicks were measured by Maruyama et al. (1978) in which they showed a more rapid fractional synthesis rate (protein synthesized

per unit time divided by amount of protein in tissue) in breast than in leg muscles at 1 week of age but this difference was very small by 2 weeks. To account for the protein accretion in rapidly growing chicks a marked decrease in fractional rate of breakdown (FBR) was postulated.

Waterlow (1967) summarized the total protein synthesis rate in young and adult humans to show that the grams of protein synthesized per kilogram body weight per day were lower for adult than for children or young men. Waterlow et al. (1977) demonstrated a decline in overall protein synthesis rate with age. Using 3-methylhistidine to creatine excretion ratios as a measure of myofibrillar protein breakdown in humans, Tomas et al. (1979) showed a twofold decline of excretion from preterm neonates up to maturity. Furthermore, in studies with rats, Waterlow and Stephen (1967) demonstrated sex differences for plasma protein turnover with males having higher rates than females. Waterlow and Stephens (1968) showed that protein degradation rates in rat skeletal muscle decreased with increasing body weight. On the other hand, muscles of older rats have greater proteolytic activity than young rats (Waterlow et al., 1978). This age effect partially explains the slower protein accumulation rates and decrease in muscle mass during advancing age as noted by Young (1970).

When consideration is given to species, the rat has been the animal model most extensively used in protein turnover studies (Reeds and Lobley, 1980). For most species in general, the fractional protein synthesis rate is lower in skeletal muscle than visceral tissue. As Zak et al. (1979) and Reeds and Lobley (1980) pointed out, the problems with making assessments and comparisons of protein turnover across species lie with the variety of conditions which exist among various studies such as obesity, nutrition or disease and the technique used to measure turnover. One valid point made is that there is a similarity between species in terms of energy expenditure for protein synthesis and this is in the form of heat production. The process of protein turnover accounts for about 15% of the total body energy expenditure.

Nutrition Effects

As reviewed by Young (1970), protein metabolism in skeletal muscle plays a very important role in N economy of the whole body. The effects of protein malnutrition on turnover have been confounded by reutilization of the labeled amino acid during experiments. However, the results of protein deficient diets showed increased incorporation of label into liver protein and decreased incorporation into skeletal muscle protein (Millward et al., 1978). The importance of skeletal muscle protein synthesis and breakdown in the regulation of muscle mass and whole

body N balance has been investigated by Cahill (1970) and Millward et al. (1976b). Rats receiving inadequate diets had decreased protein synthesis. Increased breakdown rate is not always observed, as Millward et al. (1976b) and Young et al. (1973) showed reduced breakdown in rats fed protein free diets. Young and Alexis (1968) showed a decrease in RNA content of skeletal muscle for rats fed a protein deficient diet. The half-life of mixed muscle proteins of dietary protein depleted rats was reduced from 13.1 days to 10.2 day for controls, but upon refeeding increased synthesis and decreased breakdown brought about rapid accumulation of muscle protein (Young et al., 1971). In similar studies, Goto and Kametaka (1974) showed muscle protein synthesis to decrease, but liver protein synthesis to increase in early periods of protein depleted diets.

Other Effects

Earl et al. (1978) suggested that turnover rate of muscle protein is related to muscle function. Muscles that have tonic contractile activity have faster turnover rates than muscles of the intermittent twitch type. These workers also found RNA concentrations to be highly correlated with turnover rate. Using a pulse label of ^{14}C-aspartate in rats Swick and Song (1974) estimated the half-life of heart myosin to be 4 to 6 days while myosin from white skeletal muscle had half-lives of 25 to 45 days.

Hypertrophy of rat soleus muscles occurred after sectioning the tendon of the gastrocnemius muscles in studies by Goldberg et al. (1969a). They showed a greater decrease in sarcoplasmic protein breakdown than myofibrillar protein and concluded that during hypertrophy there is also decreased protein synthesis. In contrast, Laurent and Millward et al. (1980) studied fowl wing muscle hypertrophy and showed increases in both protein synthesis and breakdown. However, in similar studies by Laurent and Sparrow (1977) a 50% increase in protein synthesis rate was observed but only a negligible change in degradation rate was found. These changes in synthesis and degradation rates are corrobrated by Goldspink (1978a, 1978b) in denervated extensor digitorum longus muscles placed under passive stretch. Terjung et al. (1973) demonstrated a decrease in the half-life of skeletal muscle cytochrome C with exercise, and attributed the resulting overall increase of protein to a decreased degradation rate. Vandenburgh and Kaufman (1980) illustrated how muscle cell protein synthesis is increased and degradation decreased in an in vitro tissue culture study involving passive stretch. Goldberg (1972) discussed the biochemical changes that occur during skeletal muscle hypertrophy. Along with increased protein synthesis and decreased breakdown, there are increases in amino acid transport, RNA synthesis and DNA content.

The effects of muscle atrophy have been investigated by Goldberg (1972), Turner and Manchester (1973). After denervation, decreases in synthesis of new protein as well as greater breakdown of existing protein complement each other to decrease muscle mass. In dystrophic muscle, myosin is synthesized faster than in nondystrophic muscle but myosin heavy chains are destoyed at a greater rate than light chains (Rourke, 1975). Dice et al. (1973) indicated that in normal eukaryotic cells large molecular weight proteins are degraded more rapidly than lower molecular weight proteins, but the increased breakdown of protein observed during diabetic conditions is due to activation of different proteolytic mechanisms than exist in normal tissue.

In children suffering from burn injuries, Bilmazes et al. (1978) reported a greater amount of muscle protein breakdown per unit time than in healthy subjects. Waterlow et al. (1977) also showed a reduction of protein synthesis but unchanged breakdown in cases of surgical trauma of human adults.

Developmental Changes in Muscle Nucleic Acids and Protein During Growth

Changes in structure, composition and metabolism of tissues occur continuously from early embryogenesis to death. Complete differentiation results from changes in the pattern of gene expression (Waterlow et al., 1978; Young and Allen, 1979). For the mammal, the amount of muscle fiber hyperplasia seems to be complete shortly after birth (Goldspink, 1972; Swatland, 1976) at which time there is also the full complement of proteins characteristic of maximally differentiated cells (Waterlow et al., 1978). Subsequent cellular growth is estimated by measuring protein mass (Mirsky and Ris, 1949; Waterlow et al., 1978) and DNA and RNA content (Mirsky and Ris, 1949).

Since the DNA content of the diploid nucleus is constant, DNA content can be used to estimate the number of nuclei (Mirsky and Ris, 1949; Leblond, 1972). Enesco and Leblond (1962) estimated the amount of DNA per diploid nucleus to be around 6.2 picograms. However, Waterlow et al. (1978) pointed out that because of polyploidy in liver, estimations of cell number may be overestimated.

As indicated by Palsson (1955), Goldspink (1972) and Swatland (1976), postnatal development depends upon the degree of fetal development prior to birth and is species dependent. Palsson (1955) considered the pig to be less

mature physiologically than other domesticated animals. However, this depends upon the tissue as considered by Schain (1969) because pigs have more brain and organ development at birth than rats. Hakkarainen (1975) showed a similar developmental pattern for DNA and RNA in skeletal muscle for both pigs and rats, but the immaturity in the pig is compensated for as they have higher degrees of development in the proportions of the protein fractions in muscles used for locomotion and posture.

While Young (1970) pointed out that the terms hyperplasia and hypertrophy may not really be applicable to muscle growth, Leblond (1972) discussed hyperplasia as increases in total DNA and hypertrophy as the protein to DNA ratio. Enesco and Leblond (1962) and Winick and Noble (1966) indicated that protein to DNA ratio or tissue weight to DNA ratio can be used to determine the physiological cell size. Also, Moss (1969), Cheek et al. (1971) and Robinson (1971) discussed how there seems to be a maximal cytoplamic volume controlled by a particular nucleus and that a physiological cell size concept is a good measure of postnatal growth.

Leblond (1972) proposed three different postnatal cellular growth patterns and a fourth intermediate one to describe muscle cells. Enesco and Puddy (1964) and Leblond (1972) pointed out the skeletal muscle is made up of multinucleated cells and an increase in DNA content

represents an increase in nuclear number only and not an increase in cell number. Since work with rats (Winick and Noble, 1966) and pigs (Robinson, 1969) showed increases in total DNA during growth, hyperplasia (increased myonuclei number) is important during postnatal muscle growth. In reviewing work by Winick and Noble (1966), Allen et al. (1979) showed that 80% or more of the DNA content in rat muscle was accumulated after birth. Other studies indicate similar trends of increases of total DNA and RNA and decreased concentrations during growth. This was shown in rats (Devi et al., 1963; Enesco and Puddy, 1964; Winick and Noble, 1966; Howarth and Baldwin, 1971), pigs (Gordon et al., 1966; Robinson, 1969; Gilbreath and Trout, 1973; Tsai et al., 1973; Hakkarainen, 1975; Harbison et al., 1976), chickens (Moss et al., 1964; Moss, 1968a, 1968b) and ruminants (Laflamme et al., 1973; Johns and Bergen, 1976). Robinson (1969) reported increases in total DNA and RNA content in porcine skeletal muscle triceps brachii and semitendinosis as late as 100 days of age. On the other hand, concentrations of the two nucleic acids decreased from birth to 80 days of age. This observation is in agreement with work by Powell and Aberle (1975). Similarily, Harbison et al. (1976) reported increases in total RNA and DNA in pigs from 23 to 118 kilograms. Gilbreath and Trout (1973) found the DNA and RNA concentrations were highest at 1 day of age but decreased dramatically by 2 weeks and continued

to decline up to 12 weeks of age in porcine longissimus muscle. However, the RNA concentrations showed more variability. Tsai et al. (1973) and Hakkarainen (1975) pointed out that the concentrations decrease because of protein accumulation and therefore it is a dilution effect.

To explain the source of the increased muscle DNA during growth, the electron microscopy work by Mauro (1961) and the thymidine incorporation studies by Moss and Leblond (1970b, 1971) clearly show that a population of cells which lie between the plasma membrane and the basement membrane of myofibers called satellite cells are the source of the new nuclei. Cardasis and Cooper (1975) complemented this theory with results showing decreases in the total satellite cell population with age. Kelly (1978) found fewer satellite cells in the extensor digitorum longus muscle than the soleus muscle of both developing and mature rats and these differences in satellite cell number were correlated wtih myofiber nuclei density. The soleus had a greater rate of increase in myofiber nuclei per myofiber than the extensor digitorum longus as shown by an autoradiographic assessment of $[^{3}H]$ thymidine incorporation.

The work of Gordon et al. (1966) associated growth in rat skeletal muscle during the first 90 days with nuclei proliferation and fiber hypertrophy, but with hypertrophy alone during subsequent rat skeletal muscle growth. Moss (1968a, 1968b) and Swatland (1977) indicated a direct

relationship exists between muscle fiber diameter and the number of nuclei. As pointed out by Hakkarainen (1975) and Allen et al. (1979) there seems to be a preprogrammed increase of DNA preceding increases in RNA and protein. As summarized by Allen et al. (1979) the most rapid increase of DNA occurs during rapid growth periods. Also, the number of nuclei is directly related to fiber size and this may limit the quantity of protein in the myofiber.

Increases in total RNA is a precondition for increasing accumulation of protein (Hakkarainen, 1975) and is a good measure of protein synthesizing machinery (Wannamacher, 1972) as well as fractional protein synthesis rates (Millward et al., 1973; Garlick et al., 1976). Diet has also been shown to influence RNA levels (Howarth, 1971; Giovannetti and Stothers, 1975). Waterlow et al. (1978) indicated that the changes in fractional synthesis rate during development of brain, heart or skeletal muscle reflect changes in RNA content. Winick and Noble (1966), Powell and Aberle (1975) and Millward et al. (1975) showed that the ratio of RNA to DNA is indicative of the capacity to synthesize protein.

Henshaw et al. (1971) measured the rate of protein synthesis per unit of ribosome (the amount of radioactive lysine incorporated into protein per milligram of RNA) in liver and skeletal muscle of rats. In animals fed ad

libitum but growing at different rates, protein synthesis per unit of ribosomes varied directly with growth rate and was higher in the liver than muscle. However, Millward et al. (1975) also used an RNA activity concept to compare several rat tissues for the grams of protein synthesized per day per gram of RNA and noted that this activity declines during growth as does the fractional protein synthesis rates. As pointed out by Bergen (1974, 1975) rats on a restricted diet had lower weight gain than well fed controls but no differences in muscle protein synthesis efficiency related to overall growth were observed. Total carcass protein accumulation was similar between the two groups but the ad libitum fed rats gained proportionately more fat.

Methodology of Measuring Protein Turnover

In most instances, attempts to measure protein turnover have involved isotopic methods. In general terms, these methods are classified into two groups: those methods dependent upon the uptake of isotope into protein and those dependent upon isotope loss (release) from protein. Rates of synthesis can be estimated from the first method and information on both synthesis and degradation rates are obtained with the second type (Millward et al., 1976a; Garlick, 1969).

Before the most appropriate method for studying turnover can be selected, models must be proposed. The use of such models requires various assumptions to be made in

describing the biological system. These assumptions include the following: that precursor amino acid pools for exchange are in a steady state, that there is complete and instantaneous mixing of pools and that a constant fraction of a substance is transferred in a unit of time. The simplest model contains two pools; the free amino acid pool and the protein bound pool. In the case of protein turnover, there is considered to be an exchange of the protein pool with the free amino acid pool. The movement of one amino acid molecule into and out of protein involves the synthesis and breakdown of one molecule of protein. This indicates that the incorporation rate of each amino acid within the protein is proportional to its concentration in that protein (Waterlow et al., 1978).

The isotopic methods used generally involve the use of a single injection, continuous infusion or continuous feeding of labeled amino acids. With a single injection method, the specific activity of the free amino acid in plasma is initially high and then falls very rapidly. A single measurement at the end of the experiment is insufficient in describing the time course of specific activity. To avoid the rapid variations in specific activity after a single injection, the use of continuous infusion methods or methods involving injections of large quantities of labeled amino acids are employed. Since the single injection method characteristically has some

reutilization of label, the injection of large amounts of nonlabeled amino acid can expand the intracellular pool. The label which comes out of protein is then mixed with the extra amino acids and the specific activity declines and the reincorporation of label is less likely to occur. The use of this flood method extends the time that the specific activity of the precursor pool is at highest levels but measurement of specific activity of the precursor pool and protein are needed. With the single injection, protein synthesis is estimated by the proportion of the precursor label that is incorporated into protein and breakdown is estimated by the loss of isotope from the tissue (Waterlow et al., 1978).

With the continuous infusion method a constant precursor pool specific activity is obtained. During infusion, the specific activity of the free amino acid in blood rises to a constant plateau and the amount of amino acid leaving the free pool for protein synthesis can be calculated. While the specific activity rise in the precursor pool is not instantaneous and therefore some error enters into the calculations of protein synthesis, this lag time to reach maximum specific activity (plateau) becomes negligible over a period of time long enough to allow incorporation into protein. However, the infusion time of the experiment cannot be too lengthy as this could allow time for some reutilization of isotope (Garlick and Millward, 1972).

The change in specific activity (ie., dpm/nmole) of the amino acid in protein (S_B) is described as: $d S_B/dt = k_S (S_A - S_B)$, where k_S is the fractional rate of protein $d S_B/dt = k_S (S_A - S_B)$, where k_S is the fractional rate of protein synthesis, and S_B is specific activity of protein bound amino acis. S_A is the specific activity of the free amino acid. Since S_A must be larger than S_B for incorporation to occur, the measurement of S_A is important. With the single injection of tracer, the specific activity of protein bound and free amino acid are plotted over time, and protein synthesis rates are measured, but this requires several animals to accomodate the various time points. With continuous infusion, the specific activity of the precursor pool achieves a constant value after a short lag time initially and only one time point is required so measure the protein synthesis rate (Millward et al., 1977; Waterlow et al., 1978).

As shown by Waterlow and Stephen (1967), rats infused continuously with L - [U-^{14}C] lysine for 6 hours, the specific activity of free lysine in plasma (Sp) reaches a constant value or plateau (Sp max) and this pathway can be explained by a single exponential expression: $Sp = Sp\ max\ (1-e^{-\lambda pt})$, where λp is a fitted rate constant for the time course of rise of specific activity. The specific activity

of an infused amino acid at plateau may be used to calculate flux values for the amino acid which can be subsequently used to estimate whole body protein synthesis rates (Waterlow, 1967; Waterlow and Stephen, 1967; Garlick et al., 1973; Millward et al., 1977).

If the rise of specific activity of specific tissues (S_i) is considered, it will be lower than that observed in plasma, but it will be important because the amount of intracellular amino acid derived from protein breakdown is 1 - (Si max/Sp max). Also, if it is assumed the precursor pool for protein synthesis is largely made up by the total intracellular free amino acid pool, the rate of protein synthesis may be calculated by starting with the equation: $d\ S_B/dt = k_S\ (S_i - S_B)$. When this equation is integrated, two different equations can result depending upon whether the protein turnover in a tissue is rapid or slow and what labeled precursor amino acid is used. For tissues such as liver, kidney and viscera, the following equation is used:

$$S_B/S_i = \frac{\lambda p}{(\lambda p - k_S)} \cdot \frac{(1-e^{-k_S t})}{(1-e^{-\lambda p t})} - \frac{k_S}{(\lambda p - k_S)} .$$

For muscle the equation below is used:

$$S_B/S_i = \frac{R}{(R-1)} \cdot \frac{(1-e^{-k_S t})}{(1-3^{-Rk_S t})} - \frac{1}{(R-1)} .$$

If plasma specific activity is measured at several time points on different animals or the same animal, λp can be obtained since this is the exponential rate constant describing the time course of free amino acid specific activity rise in plasma. In the second equation, R is the ratio of the protein bound to free amino acid of the tissue. Garlick et al. (1976) found values of 80 $days^{-1}$ for λp and 400 for R. Neither λp nor R needs to be known accurately since they have relatively little influence on calculated k_S values. To solve the equations for k_S, S_B/S_i and t are measured at the end of infusion and p or R values are determined. There is a problem in solving for k_S since no rearrangement of the equations allows for solution of k_S. However, calculations may be performed graphically or by computerized iterative procedures. This involves calculating and plotting several S_B/S_i values for estimated k_S values. The value of a k_S corresponding to the experimentally obtained SB/Si is then obtained (Garlick et al., 1976; Waterlow et al., 1978).

Since the breakdown rate is not always directly estimated by incorporation experiments, alternatives for estimating k_D are possible. If the experiment is designed so that steady state conditions exist, the fractional synthesis rate (FSR) should equal the fractional breakdown rate (FBR). If the experiment is performed in a growing situation, the fractional growth rate (FGR, accretion) is

measured by the amount of protein mass changed over a period of a few days and FBR is obtained by the relationship of the following equation: FGR = FSR - FBR. This method has been used by Turner and Garlick (1974), Garlick et al. (1976), Millward et al. (1975; 1976b) for muscle and in liver by Conde and Scornik (1977) and Scornik and Botbal (1976).

Ogata et al. (1978) criticized this method of calculating k_S and k_D because measurements of growth were made over a period of days and allowances for diurnal variations were not accounted for. Also, the k_S values were measured only for a 6 hour period of time which is only representative of part of the 24 hour period. Garlick et al. (1973) recognized the diurnal variations in synthesis but stated the duirnal variations are small.

When FBR is measured by disappearance of label after a single pulse injection, there is a problem of heterogeneity of proteins and reutilization of the isotope. It is important to note that breakdown causes no change of specific activity but synthesis of new protein will dilute the specific activity in tissues. The problems of reutilization can be minimized through proper selection of labeled precursor. This involves selection of amino acids that are not stable within the tissues measured (Waterlow et al., 1978).

By measuring the decay of isotope incorporated into protein as a means of estimating protein degradation, reutilization may yield underestimations of degradative rates (Waterlow et al. 1978). A more desirable indicator of protein breakdown would be an amino acid which is not reincorporated into protein (Young and Munro, 1978). Asatoor and Armstrong (1967), Young (1970) and Young et al. (1973) have discussed the potential of using a methylated amino acid found in muscle as a marker for protein breakdown. Young and Munro (1978) and Ward and Buttery (1978) reviewed the criteria for selection of an amino acid to be used for in vivo muscle protein breakdown studies. The reliability of using a methylated amino acid for measuring muscle protein breakdown lies largely with its lack of charging with amino-acyl $_{t}$RNA, quantitative excretion and constant distribution in skeletal muscle.

While Young et al. (1972), Haverburg et al. (1975), Long et al. (1975), Young and Munro (1978) and Ward and Buttery (1978) have shown the urinary excretion of 3-methylhistidine to be a practical and valid method of estimating the degradation of myofibrillar protein of muscle, Nishizawa et al. (1977a, 1977b) have shown that 10% of the total amount of 3-methylhistidine in the body is found in skin and gastrointestinal tissues and therefore these tissues must be accounted for when measuring the urinary excretion of 3-methylhistidine.

Recent studies by Millward et al. (1980) suggested that the assumption that 3-methylhistidine in urine originates almost entirely from skeletal muscle is invalid since direct measurements of the amino acid in skeletal muscle, skin and gastrointestinal muscle contribute only 24.9, 6.8 and 9.8% of the total urinary excretion. However, Harris (1981) refuted the values reported by Millward et al. (1980) and stated that skeletal muscle actin and myosin account for 90% or more of the 3-methlhistidine in the urine. Even with the assumption that 3-methylhistidine is derived entirely from myosin and actin of muscle protein, Harris et al. (1977) and work by Milne and Harris (1978) substantiated results of Rangley and Lawrie (1976) concerning the unreliability of using 3-methylhistidine as a measure of muscle protein degradation in sheep or pigs. In these species, they found the methylated amino acid occurs as a nonprotein bound component.

MATERIALS AND METHODS

Design of the Experiment

Twenty-four crossbred uncastrated male pigs selected from eight different litters were used in the study (see Appendix D for breeding details). Table 1 summarizes the experimental design.

Table 1. Experimental Design

Treatment	Live body weight	
	22 kg	45 kg
	Group[a]	
Slaughter 1 week before Infusion	I	IV
Infusion of ^{14}C-tyrosine	II	V
Slaughter 1 week after Infusion	III	VI

[a]Four pigs per group

Twelve pigs were randomly allocated to each of two groups: one group was scheduled for radioisotopic infusion at approximately 22 kg and the other at 45 kg live body weight. All pigs received the same 16% crude protein corn-soybean diet throughout the experiment. One week before each infusion period, the 12 pigs within each weight group were allocated to one of three subgroups comprised of four pigs each. One subgroup of four pigs was designated to receive the infusion and the other two subgroups of four pigs each were scheduled for slaughter chronologically 1 week before and 1 week after the infusion at either 22 or 45 kg live body weight. Table 1 shows that group II and V were the infusion groups at 22 and 45 kg live body weight, respectively. Also groups I and IV were the pigs slaughtered 1 week before infusion at 22 and 45 kg live body weight, respectively, and groups III and VI the noninfused pigs slaughtered 1 week after infusion at 22 and 45 kg live body weight, respectively. Four noninfused pigs were slaughtered 1 week before and 1 week after the infusion groups at 22 and 45 kg (groups II and V, respectively) to observe changes in physical and chemical composition, particularly the accretion of skeletal muscle and liver protein over the 2 week growing period. The infused pigs (groups II and V) were analyzed for the amount of isotope incorporation into protein in the longissimus, semitendinosis and brachialis muscles of both right and left sides of the animal and liver at the end of a 6 hour infusion.

Proximate analysis was carried out on both the right and left side muscles and data expressed on combined muscles (right plus left) basis. This pooling of data was performed by taking the percentage moisture, fat or protein times the corresponding right or left side muscle weight, adding right and left side data and then dividing by the combined muscle mass. Nucleic acids, protein fractionation and myofiber diameter analysis was performed upon the right side muscles only.

Slaughter Procedure, Tissue Collection and Preparation

The noninfused pigs were held off feed for approximately 6 hrs before weighing and slaughter. None of these animals received immobilization treatment prior to severing the carotid artery and juglar vein. Shortly before cessation of bleeding had occurred, the pigs were placed on a table for excision of tissues. The longissimus, semitendinosis and brachialis muscles from the right and left side of each pig as well as the livers were removed within 5 minutes. In all cases, the three right muscles were removed first, then the three left muscles and the liver was removed last. Muscles were trimmed of subcutaneous fat, weighed to the nearest tenth of a gram and frozen rapidly in either Dry-ice and isopentane or in liquid N and placed in polyethylene bags. The muscle and liver samples were stored in a -90 C chest freezer.

Powdering of the Frozen Muscle and Liver

The frozen muscle and liver samples of infused and noninfused pigs were removed from the -90 C storage freezer and powdered essentially as outlined by Borchert and Briskey (1965). Powdering was performed in a -30 C walk-in freezer room. The tissues were broken into small pieces by use of a hammer after the frozen sample was placed in a denim cloth bag. The broken pieces of tissue were placed into either a Waring Blendor jar or a high speed impact mill along with crushed Dry-ice and pulverized for 20 to 30 seconds. The pulverized samples were sifted through a screen and the remaining coarse material was re-pulverized. The powdered samples were mixed thoroughly and subsamples placed in Whirl-Pac bags. The bags were left open at -30 C for 6 to 12 hrs (depending upon quantity of sample) to allow the carbon dioxide to escape. Samples were then sealed and again stored in the -90 C freezer for further analysis.

For the radioactive muscle and liver samples, a small stainless steel cart served as a base upon which a clear plastic tent was constructed. The powdering (as described previously) was performed inside the tent to reduce contamination of air and surfaces of the -30 C freezer.

Standardized Procedures of Tissue Analysis: Determination of Nucleic Acids, Skeletal Muscle Protein Fractionation and Proximate Analysis

Since these procedures are routinely performed, a detailed description will not be given. The method used for RNA and DNA estimation was a modified Munro and Fleck (1969) procedure and was described in detail by Mostafavi (1978). The protein fractionation procedure used was a modified Helander (1957) method. Again this method was detailed by Borton (1969), Eversole (1978) and Mostafavi (1978). The only difference was the use of a .05 M phosphate buffer to extract the low ionic strength extractable (sarcoplasmic) proteins. N determinations on all samples and skeletal muscle protein fractions were performed by a micro-Kjeldahl method as prescribed by the American Instrument Company (1961). AOAC (1975) methods were used for moisture and ether extractable fat determinations.

Procedure for Estimating Myofiber Diameter

The three right side muscles from each noninfused pig were analyzed for myofiber diameter. The tip of a laboratory spatula was used to measure small quantities (approximately 200 mg) of frozen powdered muscle. Samples were immersed in approximately 2.0 ml of a 1.0% glutaraldehydeBSS buffer (Appendix A.1). The small 5 ml

atomic absorption beakers containing the above mixture were refrigerated at approximately 4 C for 1 hour. At the end of 1 hr, the liquid portion was pipetted off and discarded. Two milliliters of BSS buffer (Appendix A.2) were added and pipetted off to wash the samples. Two milliliters of .02 M guanidine - HCl buffer (Appendix A.3) were added and the mixture allowed to stand at room temperature for ½ hour. The guanidine-HCl buffer was pipetted off and 2.0 ml of BSS buffer used to wash the sample. To all samples, 2.0 ml of BSS buffer plus two drops of methylene blue indicator were added and shaken gently at approximately 4 C for 2 days. At the end of 2 days, the beakers were swirled and one drop of the mixture placed on a microscope slide, and cover slip applied and lightly tapped to remove air bubbles. The slides were viewed through a light microscope with a X40 objective and a total magnification of 400. A minimum of 50 fibers was measured with the micrometer scale of the eye piece which was calibrated with a stage micrometer. Mean fiber diameters were expressed in micrometers.

Protocol for the Infusion of Isotope

Infusion Technique

All pigs designated for infusion were handled similarly with the exception of the first pig. Since the use of a sling apparatus with this pig was unsuccessful in keeping the pig from struggling during infusion, another method was tried which proved to be successful and was used in subsequent infusions. This procedure involved a V-shaped structure in which the pig was laid on his back and restrained with rubber belts around the thoracic and abdominal regions. The fore and hind legs were secured to prevent excessive leg movement. The head was immobilized by tying the snout to a U-shaped extendible rod.

An intravenous catheter from Becton Dickerson was inserted into an ear vein. This catheter consisted of a 20 gauge by 6.4 cm teflon tube with a 24 gauge inner needle and syringe connection. The right vena cava was punctured with a 12 gauge steel needle and 18 or 20 gauge tubing inserted into the vein through the needle for a distance of approximately 25 centimeters. Once the catheters were secured with tape, they were flushed with a heparinized saline solution. The ear catheter was connected to 20 gauge tubing which was connected to a 20 cc syringe containing the radioisotope. This syringe plunger was driven by a Harvard Infusion pump at the calibrated rate of 2.5 ml/hr. The vena

cava catheter tubing was used for withdrawing 5 ml blood samples. After the zero-time blood sample was taken and the infusion initiated, blood samples were withdrawn every 10 min for the first 2 hrs, every 15 min for the third hour and every 20 min for the remainder of the 6 hr infusion period. The blood samples were poured out of the syringe into heparinized Corex tubes and placed on ice. At the end of the 6 hr infusion, the pigs were given a 5 to 10 cc dose of phenobarbitol, removed from the infusion apparatus and the juglar vein and carotid arteries severed. Middle portions of the longissimus, semitendinosis and brachialis muscles from the right and left sides and the lateral lobe of the liver were removed, placed in polyethylene Whirl-Pac bags and frozen in liquid N. Twelve to 15 hrs after collection, the blood samples were centrifuged at 3,000 x g for 15 min and the plasma portion placed in small vials which were stored at -90 C.

Dilution of Isotope

A total of 750 μCi of uniformly labeled ^{14}C-tyrosine was purchased from Amersham Corporation (Arlington Heights, Illinois). For the first infusion period, 250 μCi of the radioisotope were diluted into 70 ml of sterile .9% saline. For the second infusion period, 500 μCi of the isotope were diluted with 60 ml of sterile .9% saline. The level of radioisotope infused at both periods was calculated to be approximately 11 μCi/kg body weight. The diluted isotope was then stored at subfreezing temperatures until used.

Specific Activity Assay

For the assessment of the specific activity of L-tyrosine in the muscle and liver tissues and blood samples from the pigs infused with L-[U-^{14}C] tyrosine, there were two major phases: the liquid scintillation counting and the relative quantification or estimation of L-tyrosine concentration. The specific activity of tyrosine in the supernates of TCA precipitated blood plasma and muscle or liver samples as well as, the tissue derived protein hydrosylates were determined by the method described by Garlick and Marshall (1972) and Garlick et al. (1976). The method involves the enzymatic conversion of L-tyrosine to tyramine via decarboxylation by bacterial L-tyrosine decarboxylase. This enzyme conversion is necessary to give greater assurance that the assay is specific for protein bound tyrosine and not other metabolic pathways (the tyramine estimation assay will react with phenylalanine, tyrosine or tyramine) or the D-isomers of tyrosine which may have been included in the infusate. After the enzymatic conversion step is completed, the tyramine is counted for radioactivity or estimated for relative quantities. The quantitation assay involves the reaction of tyramine with 1-nitroso-2-napthol and nitric acid solutions (Appendix A. 4) to yield a yellow solution which at concentration higher than 25 n moles can be read on a spectrophotometer, or read on a fluorometer at lower concentrations (Undenfriend and Cooper, 1952; Waalkes and Undenfriend, 1957).

Plasma Specific Activity

Five milliliters of blood were withdrawn from the infused pigs at specific times to eventually measure plasma specific activity. This provides a monitor for the progress of the infusion experiment as the specific activities of plasma at each sampling point are plotted and the rate of rise to maximum specific activity (λ) is estimated. Each blood sample was precipitated with 10% (w/w) TCA and centrifuged at 3000 x g for 15 minutes. The free or nonprotein bound tyrosine fraction was extracted three times with three volumes of ether to remove the TCA. The TCA precipitated, ether extracted samples were then placed in 50 ml culture tubes and the aqueous portion driven off by use of a sand trough heating mantle at 60 C and under a steady stream of N gas. The dried samples were resuspended in 50 ml culture tubes with 2 ml of .5 M citrate buffer, pH 5.5 and the pH of the resuspended sample adjusted with .2 N NaOH. These resuspended samples were then taken through the enzyme decarboxylation procedure (see Enzyme Decarboxylation section for discussion).

Tissue Specific Activity

The ensuing discussion involves only the steps involved in preparing the muscle or liver tissue for enzyme conversion. Both the free and bound tyrosine specific activities were estimated. To obtain the nonprotein bound or free amino acid pool in the powdered radioactive muscle

and liver samples, 5 g of each sample were precipitated in 50 ml Nalgene centrifuge tubes with 20% (w/w) cold TCA, allowed to stand on ice for 15 min and then centrifuged at 12,000 x g for 15 minutes. The supernate containing the free tyrosine was decanted and saved. The pellet was washed two additional times with TCA after breaking up the pellet and then recentrifuged. The supernates from each centrifugation were pooled and extracted three times with three volumes of ether. The pooled, ether extracted supernates were then dried (Appendix B), resuspended in 2 ml of citrate buffer, pipetted into 50 ml culture tubes and checked for pH adjustment to 5.5. The dried, buffer resuspended supernate samples were then taken through the enzyme conversion procedure (see Enzyme Decarboxylation Procedure).

In preparing the powdered radioactive muscle and liver samples for measurement of the protein bound pool, 1.0 g of each sample was precipitated in 15 ml corex tubes with 5.0 ml of cold 20% TCA, allowed to stand on ice for 15 min and then centrifuged at 12,000 x g for 15 minutes. The pellet was disrupted, reprecipitated and recentrifuged two additional times. The pellet was then taken through a sequential series of drying steps. Five milliliters each of 1% potassium-acetate in ethanol, ethanol-chloroform (3:1), ethanol-ether(3:1), ether and hot 2 N $HClO_4$ were used in the drying sequence. After the perchloric acid step, the

pellets were placed on Whatman paper to air dry and then stored in small vials until needed. The dried protein pellet derived from 1.0 g of tissue was then placed in hydrolysis tubes with 20 ml of 6 N HCl. A stream of N gas was added to each tube for approximately 30 sec and the tube capped tightly. The hydrolysis tubes containing the dried protein and HCl were hydrolyzed in an autoclave for 20 hr, at 107 C and 7 mm Hg. At the end of 20 hr, the hydrolyzed samples were dried down (Appendix B), resuspended in 2 ml citrate buffer, pipetted into 50 ml culture tubes, pH adjusted to 5.5 and then taken through the enzyme conversion procedure (see Enzyme Decarboxylation Procedure).

While tyramine estimation of the tissue free pool was performed on an aliquot of the supernates derived from the TCA precipitation of 1.0 g tissue samples, the estimation of tyramine in the protein bound pool required preparation of smaller quantities of tissue. Two tenths g of powdered radioactive muscle or liver was precipitated in 15 ml corex tubes with 2.0 ml of cold 20% TCA. The tubes were allowed to stand on ice for 15 min, centrifuged at 12,000 x g for 15 minutes (repeated twice) and the pellet taken through the drying sequence. The dried protein derived from .2 g of tissue was then hydrolyzed in 20 ml of 6 N HCl for 20 hr at 107 C and 7 mm Hg. The hydrosylates were dried (Appendix B), resuspended in 2.0 ml citrate buffer, pipetted into 50 ml

culture tubes, pH adjusted to 5.5 and taken through the enzyme conversion procedure (see Enzyme Decarboxylation Procedure).

Enzyme Decarboxylation

The enzyme L-tyrosine decarboxylase (L-tyrosine-carboxy-lyase) isolated from streptococcus faecalis and having V max activity such that one unit of enzyme liberates 1.0 mole of CO_2 from tyrosine per minute at optimum pH of 5.5 and temperature of 37 C was purchased from Sigma Chemical Co. The enzyme was kept frozen until weighing and suspension in buffer. For the free (supernate) fractions enough enzyme was weighed and suspended to make a concentration of 2 Units/ml or 3 Units/ml for the bound (hydrolyzed) fractions. The enzyme was prepared by washing twice with pH 5.5 citrate buffer and then suspending in .5 M pH 5.5 citrate buffer. Two hundred microliters of the enzyme suspension were added to the 2 ml resuspended supernates (derived from 1.0 or 5.0 g tissue) and .4 ml added to the 2 ml resuspended pH optimized hydrosylated (derived from either 1.0 or .2 g of tissue). The 50 ml culture tubes containing either the resuspended supernates or resuspended hydrosylates plus added enzyme were capped and then incubated at 37 C for 90 minutes. At the end of incubation, 1 g each of NaCl and Na_2CO_3 (anhydrous) were added to the tubes and the tubes shaken vigorously. Ten

milliliters of ethyl acetate were then added and the tube plus contents vortexed vigorously. The tubes were capped and then centrifuged at 240 x g for 10 minutes. The organic phase (top) only was transferred to another clean 50 ml culture tube and the salt phase discarded. To each tube with the organic phase, approximately 10 ml of chloroform were added. For the blood, 1.0 or 5 g tissue derived supernates and the .2 g hydrosylates, 4.5 to 5.0 ml of diluted sulfuric acid (1:500) were pipetted into the tubes with the organic phase and the tube shaken or vortexed vigorously. For the hydrolyzed samples derived from 1.0 g tissue, 2.5 ml of diluted sulfuric acid were used. The tubes that now contained the organic phase and an acidified aqueous layer were allowed to stand overnight at 4 C and were then centrifuged at 240 x g for 10 minutes. The aqueous layer for the plasma derived samples was divided into aliquots for counting (see Counting Procedure) and the tyramine assay. The aqueous layer for the supernate samples derived from 5 g of tissue and that for the 1.0 g hydrosylate was used for counting of the free and bound fractions, respectively. The aqueous layer for the .2 g sample hydrosylate was used for the tyramine assay to estimate bound tyrosine while the 1.0 g tissue derived supernate samples aqueous layer was used in the tyramine assay.

Tyramine Assay

Either 2 ml of the aqueous layer obtained from the decarboxylation procedure or known portions thereof were transferred to clean 50 ml culture tubes and volumes brought to 2 ml with diluted sulfuric acid. For the aqueous layer obtained from the decarboxylation procedure of the plasma samples and the 1.0 g tissue supernates, 2.0 ml were taken from the aqueous layer and used directly. For the .2 g tissue hydrosylates taken through the decarboxylation procedure, .1 ml of the aqueous layer was used and brought to 2 ml final volume with diluted sulfuric acid. To each of the 2 ml samples and prepared tyramine standards (appendix A 6) in the 50 ml culture tubes, 1 ml of a 1-nitroso-2 napthol solution and 1 ml of a nitric acid solution (Appendix A 5) were added. The tubes were shaken or vortexed vigorously and incubated at 55 C with shaking for 30 minutes. After cooling to room temperature, 10 ml of ethylene dichloride were added to extract excess nitrosonapthol. The mix was then vortexed and centrifuged at 240 x g for 5 minutes. The yellow colored aqueous phase of this mix is the top layer and this layer was aspirated and read on a fluorometer for concentrations less than 25 nmoles or on a spectrophotometer for concentrations above 25 nanamoles. The plasma derived samples were read on a Varian Spectrofluorometer (Model SF-330, Varian Associates, Inc., Palo Alto, California) with settings of 570 nm for excitation and 460 nm for the fluorescence. The estimation of tyramine in the bound pool

of muscle and liver was performed on the .2 g derived hydrosylates and in the free fraction supernates from 1.0 g tissue that had been taken through the decarboxylation procedure. The yellow colored aqueous phase for these samples were read on a Beckman Spectrophotometer (Model 24, Beckman Instruments, Irvine, California) at 460 nanometers.

Counting

After pipetting off the aqueous layer from the enzyme decarboxylation procedure, 1.0 to 2.0 ml of this aqueous layer was counted in 10 ml of a commercially prepared scintillation cocktail (Aquasol-2 Universal Liquid Scintillation Cocktail, New England Nuclear, Boston, Massachusetts) designed for counting aqueous samples. Plastic scintillation vials were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3310, Packard Instrument Company, Downers Grove, Illinois). All samples were counted for 50 min and counting efficiencies determined from a channels ratio method.

Preliminary Studies on Procedures

The most satisfactory results were obtained when the free pool obtained from 5 g of tissue was dried, resuspended, decarboxylated and used for counting while the free pool obtained from 1.0 g of tissue was used for tyramine estimation. However, aliquots from the aqueous layer of the last step of the enzyme decarboxylation procedure derived from the 5 g tissue supernate may also be

used for tyramine estimation and gives greater color development for spectrophotometer readings. Higher counting efficiencies were obtained for the bound pool when 1.0 g tissue samples were used. Any sample size larger than this yields problems in hydrolyzing as there seems to be more breakdown of carbon materials when the ratio of HCl to sample deviates below 20:1. Use of tissue sample size less than 1.0 g resulted in fewer counts. Use of sample sizes larger than 1.0 g resulted in lower (variable) counts but this may be a problem in having excessive amino acids which require considerable NaOH to bring pH of resuspended sample to 5.5 and this NaOH affected the enzyme.

For the tyramine estimation assay, .2 g of fresh liver or muscle sample was used for the bound tyramine but only .1 ml was taken from the 2.0 to 2.5 ml aqueous layer of these samples after the decarboxylation procedure. If much more than this is used in the tyramine assay, excess color development occurs.

In testing the overall procedure in preiminary studies, several approaches were used. Varying amounts (as measured by Cpm) of L-[U-^{14}C] tyrosine was taken through the entire procedure and the counting and tyramine assay performed. Inconsistencies occurred but a range of 70 to 110% of the counts were recovered. Quantities of tyrosine were taken through the decarboxylation procedure under optimum conditions and measured by a tyramine and tyrosine standard curves in the tyramine estimation assay. Recoveries of 80

to 95% were observed with this approach indicating some losses of radioactive tyrosine may occur in the assay. However, when tissue free and bound fractions were spiked with isotope and assayed, the ratios of bound to free specific activity showed negligible differences from nonspiked samples. It is concluded that while losses of tyrosine or tyramine occurs in the assay the relative specific activity ratios were less affected.

The amount of enzyme added was also tested upon known quantities of tyrosine and on aliquots of tissue free supernate and bound hydrosylates and the enzyme quantities given in the procedure were selected. Length of incubation had less effect on action of the enzyme and 60 min was sufficient time but 90 min incubations were used as a safety factor. The amount of enzyme added seemed to be less critical than having an optimum pH of 5.5 in the test tube.

RESULTS AND DISCUSSION

Live Body Weight and Muscle Weights

Table 2 shows the live body weight (LBW) means of each group within the 22 and 45 kg growth periods. LBW increased ($P < .02$) by 30.5 and 31.9% over the 2 week period at 22 and 45 kg, respectively.

TABLE 2. Live Body Weight Means and Net Change in Live Body Weight Over Two Week Growth Periods at 22 and 45 kilograms

	22 kg		Level of	45 kg		Level of
	I	III	significance[a]	IV	VI	significance[b]
LBW, kg	18.7	24.4	.01	42.6	56.2	.02
Change in LBW, kg	5.7			13.6		.08[c]

[a]Significance probability of F statistic between groups I and III

[b]Significance probability of F statistic between groups IV and VI

[c]Significance probability of F statistic of net LBW change over the 2 week period at 22 and 45 kg LBW

Figure 1. Live body weight change (increase) of pigs over 2 week growth periods at 22 and 45 kilograms.

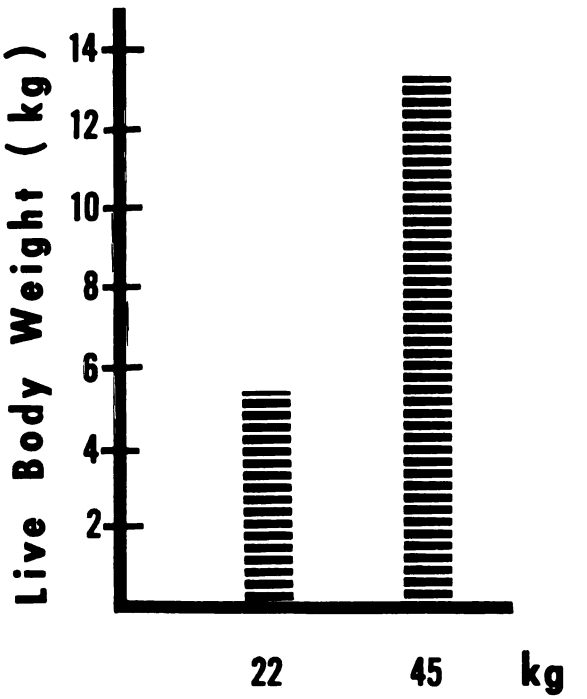

Figure 1.

The change in LBW over each 2 week growth period shown in Table 2. The absolute change of 13.6 kg at 45 kg was higher ($P < .08$) than the 5.7 kg increase over the 2 week at 22 kilograms. These observations are also plotted in Figure 1 to show a slightly steeper slope for the pigs at 45 kilograms. Since both increases were incurred over a 14 day interval, the 45 kg pigs had a more rapid growth rate than those at 22 kilograms. However, if the respective percentage increases of 30.5 and 31.9% are divided by 14 days and we assume growth rate was constant within each of the respective periods, then the percentage increase in LBW is 2.2%/day at 22 kg and 2.3%/day at 45 kilograms. The growth of these boars was considerably greater than the 1.0%/day found by Garlick et al. (1976) for 75 kg pigs. No mention of sex was made in that study but if the pigs used were castrates, the difference of that study to this study is understandable.

The observation of faster LBW gain for the pigs at 45 kg is not surprising since Becker et al. (1966) showed increased daily gains in pigs as they increased in weight from 1 kg to 100 kg LBW. This is also compatible with growth studies by Doorenbal (1971) in which the entire interval from weaning to 130 kg LBW is a period of intensive growth.

The combined weights of the right and left longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles are given in Table 3 for each group.

Muscle weights increased ($P < .05$) over each 2 week growth period except for the LD at 22 kg and the BR at 45 kilograms. Muscle weight increased by 12.2, 43.0 and 31.2% for the LD, ST and BR, respectively at 22 kilograms. At 45 kg, the LD, ST and BR muscles increased in weight by 28.4, 28.8 and 14.8%, respectively. This indicates that changes in growth rate are occurring for the muscles. Table 3 also shows the net increase in muscle weights that occured during the 2 week period at 22 and 45 kilograms. The 28.4% or 453 g increase of the LD at 45 kg was greater ($P < .02$) than the 12.2% or 84.5 g increase of the LD at 22 kilograms. Likewise, the 93.1 g increase of the ST at 45 kg was greater ($P < .04$) than the 56.8 g increase at 22 kilograms. Even though the changes over the 2 weeks at 22 and 45 kg are significantly different in absolute values for the ST, the percentage increase was less over the 45 kg period. The BR showed nonsignificant differences in the magnitude of muscle weight changes during the two periods. The data indicate the LD increased in weight at a faster rate at 45 kg LBW than at 22 kg while the ST and BR muscles showed a lower percentage increase in weight per day when the 22 kg LBW is compared to that observed at 45 kilograms. This is even clearer when the absolute weight changes are expressed on a per day basis. When the increase per day over the 22 kg period is compared to the increase per day at 45 kg, the LD changed from 6 to 32 g/day, the ST from 4 to 6.6 g/day and the BR from .8 to 1.0 g/day. At this point, without

TABLE 3. Effects of Live Body Weight on Muscle Weight, Muscle Weight as a Percentage of Live Weight and Net Increase in Muscle Weight Over 2 Week Growth Periods at 22 and 45 Kilograms

Muscle and Trait	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
Longissimus:						
Muscle weight, g	694.1	778.6	.32	1595.8	2048.8	.02
Muscle wt/LBW x 100	3.7	3.2	.09	3.5	3.7	.66
Change in muscle wt, g	84.5			453.0		.01[c]
Semitendinosis:						
Muscle weight, g	132.0	188.8	.01	323.0	416.1	.01
Muscle wt/LBW x 100	.7	.8	.11	.7	.7	.59
Change in muscle wt	56.8			93.1		.04[c]
Brachialis:						
Muscle weight, g	38.4	50.4	.02	94.7	108.7	.15
Muscle wt/LBW x 100	.2	.2	.95	.2	.2	.18
Change in muscle wt	12.0			14.0		.34[c]

[a]Significance probability of F statistic between group I and III.
[b]Significance probability of F statistic between group IV and VI.
[c]Significance probability of F statistic between the net change in muscle wt over each 2 week period at 22 and 45 kilograms.

mentioning composition of this gain, it appears the LD has a greater impetus for growth at 45 kg than at 22 kg while the ST and BR show a decreasing impetus at 45 kg compared to 22 kilograms.

The muscle weight expressed as a percentage of LBW were not different statistically over either period for any muscles as shown in Table 3. Muscles were maintained at about 3.5, .7 and .2% of LBW for the LD, ST and BR, respectively. This observation indicates proportionate development of the muscular tissues of the body as LBW increases. This has been substantiated previously by reports on the proportionate growth of individual muscles of cattle (Butterfield and Berg, 1966), sheep (Lohse et al., 1971) and in pigs (Richmond and Berg, 1971; Cole et al., 1976). Miller (1969) indicated how different tissues show variation in rate of weight increments so that the organ or tissue contribution to LBW varies during growth. Muscle mass increases more rapidly than LBW during growth and represents about 25% of the LBW at birth and over 40% at sexual maturity.

The muscle weight data of Table 3 and shown graphically in Figure 2 appear to show a disproportionate growth pattern for the 45 kg period compared to the 22 kg period. However, when the net increase in muscle weight over each growth period is expressed as a percentage of the net increase of LBW over each growth period, the 22 and 45 kg period value are 1.5 and 3.3%, 1.0 and .7% and .2 and .1% for the LD, ST

Figure 2. Net change in weight of the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles over 2 week growth periods of pigs at 22 and 45 kg live body weight.

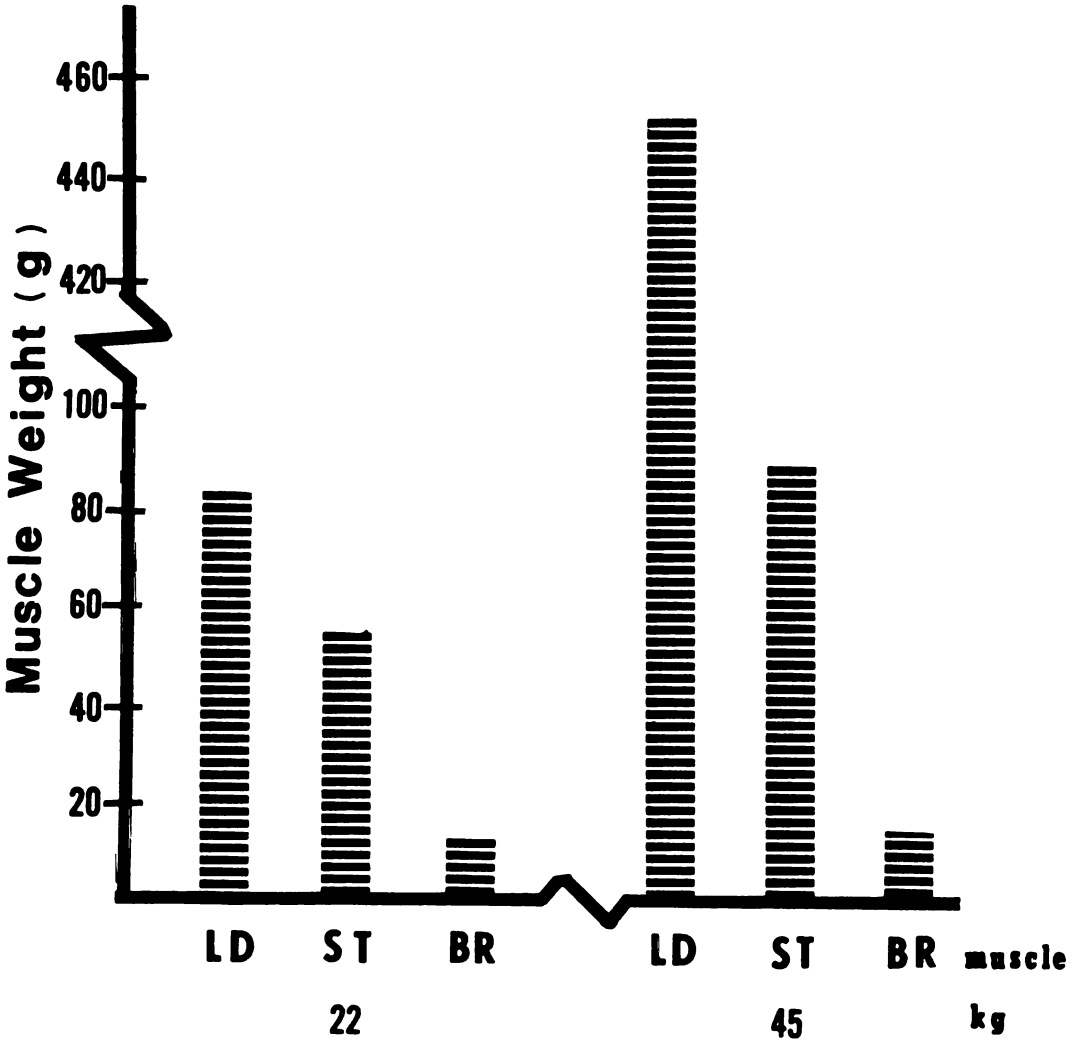
Muscle Weight (g)
460
440
420
100
80
60
40
20
LD
ST
BR
LD
ST
BR
muscle
22
45
kg

Figure 2.

and BR, respectively. Although these manipulations were not analyzed statistically, it appears muscles do change in their propensity for growth with increasing LBW. Additional growth periods need to be investigated to verify the observed rapid growth for the LD during the 2 week period at 45 kilograms.

Table 4 shows the fat free muscle weights and the change in muscle weight over each 2 week growth period. The fat free muscle weight data follow the same pattern as the gross muscle weight. These data indicate muscle weight changes in Table 3 or 4 were not significantly affected by the small differences in fat content.

Chemical Composition of Muscles

Table 5 lists the percentage moisture, fat and protein (Kjeldahl N x 6.25) of the three muscles for each group within the 22 and 45 kg growth periods. Other than significant increases in percentage fat at 45 kg for the LD and BR muscles and decreases in percentage moisture for the BR over both growth periods, the percentage moisture, fat and protein for the three muscles were not statistically different over the two growth periods. However, the BR had a decrease ($P < .08$) in percentage protein at 45 kg LBW. There was a definite trend for percentage moisture to decrease and percentage fat to increase over each 2 week growth period with no trend detected for changes in percentage protein for the three muscles.

TABLE 4. Fat Free Muscle Weight Means and Change in Fat Free Muscle Weight Over Each 2 Week Growth Period at 22 and 45 Kilograms

Muscle and trait	Weight					
	22 kg		Level of significance[a]	45 kg		Level of significance[b,c]
	I	III		IV	VI	
Longissimus:						
FFMW[d]	666.2	751.0	.31	1510.3	1923.7	.03
FFMW[d] change	84.8			413.4		.01
Semitendinosis:						
FFMW[d]	128.1	182.8	.01	310.0	396.6	.01
FFMW[d] change	54.7			86.6		.02
Brachialis:						
FFMW[d]	37.3	48.7	.03	90.9	103.1	.18
FFMW[d] change	11.4			12.2		.30

[a]Significance probability of F statistic between groups I and III.
[b]Significance probability of F statistic between group IV and VI.
[c]Significance probability of F statistic of the change in fat free muscle weight over each 2 week period at 22 and 45 kilograms.
[d]FFMW = fat free Muscle Weight in grams.

TABLE 5. Effects of Body Weight on Moisture, Fat and Protein of Longissimus, Semitendinosis and Brachialis Muscles

Muscle and trait	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
Longissimus						
% moisture	76.64	77.16	.80	74.56	73.75	.62
% fat	4.03	3.60	.55	5.34	6.14	.08
% total protein[c]	18.01	17.86	.80	18.18	18.18	.92
Semitendinosis						
% moisture	78.32	78.60	.72	76.88	76.20	.18
% fat	2.97	3.14	.56	4.01	4.64	.16
% total protein[c]	17.03	16.84	.48	17.44	17.60	.78
Brachialis						
% moisture	78.87	78.20	.01	76.86	76.23	.09
% fat	2.87	3.58	.57	4.14	5.50	.02
% total protein[c]	17.21	16.85	.63	17.81	17.16	.08

[a]Significance probability of F statistic between groups I and III.
[b]Significance probability of F statistic between groups IV and VI.
[c]Protein based on total Kjeldahl N x 6.25.

Miller (1969) provided an integrated approach to the events occurring during the development of protein synthetic activity of various tissues or organs of the growing animal. Protein synthetic activity increases as a result of increases in total DNA and RNA. Also, Forbes (1968) indicated that the percentage water decreased while percentage protein increased (expressed on a fat free body weight) during development with the rate of change slowing and eventually attaining a plateau. Hakkarainen (1975) substantiated this pattern in growing pigs. Work by Zucker and Zucker (1963), Bailey and Zobrisky (1968) and Searle et al. (1972) showed that protein and fat accretion occurs simultaneously in early growth periods but the rate of fat deposition superceded the rate of protein accretion at heavier body weights. Also, the increases of protein and fat and decreases in water content of muscular tissue have been found to be correlated with increasing animal age (Dickerson and Widdowson, 1960; Hakkarainen, 1975; Giovannetti and Stothers, 1975). The relationship of moisture, fat and protein on a percentage basis has been observed by others (Dickerson and Widdowson, 1960; Reid et al., 1968). Hakkarainen (1975) indicated that adult concentrations of protein are attained in a matter of a few weeks after birth in the pig. The decreases in moisture result from displacement of moisture by increases in fat and protein. Miller (1969) discussed development as a process

of continuous changes in body chemical composition. At birth, the protein content of most tissues is around 12% but this increases rapidly during the suckling period until a plateau of about 20% is attained. Nevertheless, not all tissues are synchronous in pattern and no one time point can be designated as the point of chemical maturity. Miller also indicated that skeletal muscle increases rapidly from immature to mature levels of protein concentration early in development.

The data over the 2 week period of 22 kg from Table 5 indicate that the three muscles were not changing dramatically in chemical composition. However, at 45 kg, all three muscles appeared to have increasing impetus to deposit fat but the BR showed more definite indications of reaching a point where rate of protein deposition was plateauing and being superceded by fat deposition and therefore significant ($P < .09$) decreases in water content was observed.

Although not shown in tabular form, if the proximate analysis data for the muscles from respective 22 and 45 kg periods are pooled and compared, similar trends of decreasing percentage moisture, increasing percentage fat and nonchanging percentage protein are observed.

It can also be mentioned that the values in Table 5 are pooled from analysis of the individual right and left muscles from each group. As can be seen in Appendix E, there

TABLE 6. Effect of Increasing Live Body Weight on Changes of Total Fat and Protein per Muscle[a,b]

Muscle and trait	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
Longissimus						
Total fat/muscle, g	27.6	27.9	.94	85.5	125.2	.01
Total protein/muscle, g	125.2	139.6	.42	288.6	371.9	.01
Semitendinosis						
Total fat/muscle, g	3.9	5.2	.16	13.0	19.4	.04
Total protein/muscle, g	22.5	31.8	.01	56.4	73.4	.01
Brachialis						
Total fat/muscle, g	1.1	1.7	.09	3.8	5.6	.02
Total protein/muscle, g	6.6	8.5	.04	16.9	18.6	.27

[a]Protein expressed as Kjeldahl N x 6.25.
[b]% fat and % protein times muscle weight; values from right and left side muscles were pooled.
[c]Significance probability of F statistic between groups I and III.
[d]Significance probability of F statistic between groups IV and VI.

is variation between right and left side muscles. Because of this observed variation, values were pooled as described earlier and expressed on the basis of the individual muscles mass.

Another way of looking at changes in chemical composition over time is by expressing data on a whole muscle basis. Table 6 summarizes the total grams of fat or protein (Kjeldahl N x 6.25) per muscle. The data in Table 6 illustrate two general trends: that muscles differ in their developmental pattern of changing chemical composition and that fat accretion is occurring at the same time as rapid muscle protein deposition. The LD and ST over the 2 week period at 22 kg had no changes in total fat deposition but at 45 kg had significant ($P < .04$) increases in total fat. The BR had small but significant increases in fat over both the 22 and 45 kg periods. These increases in fat were 1.1 and 46.4% for the LD, 33.3 and 49.2% for the ST and 54.5 and 47.4% for the BR for the 22 and 45 kg periods, respectively. Total protein per muscle increased ($P < .04$) for all muscles but nonsignificantly for the 22 kg LD and 45 kg BR. These increases represent a 14.4 and 28.9% increase for the LD, a 9.3 and 30.1% increase for the ST and a 28.8% and 10.0% increase for the BR for the 22 and 45 kg periods, respectively.

It appears that when there is no change in total fat per muscle but increases in total protein, there is a period of rapid protein synthesis and deposition. This would be

characteristic of the 22 kg LD and ST. The data in Table 6 seem to support data from Berg and Butterfield (1976) in that there seems to be an anterior to posterior progression of the animal with respect to fat deposition and chemical maturity (plateau of protein deposition, increased rate of fat deposition) with shoulder muscles having greater propensity to mature earlier than hindlimb muscles.

Table 6 shows that all three muscles over the 45 kg period and the BR muscle over the 22 kg period depositing significant amounts of fat while protein was increasing ($P < .04$) for the 45 kg LD, the 22 kg BR and the ST during from both periods. Although not shown in tabular form, if the data for each period are pooled and compared, the rate of fat deposition is more rapid during the 45 kg period than the 22 kg period. This is shown graphically in Figure 3.

Table 7 illustrates the changes in composition via the ratio of total protein (total Kjeldahl N minus nonprotein N) to total fat for each muscle. As shown in the table no statistical differences in protein to fat ratio were observed except for the 45 kg period BR. Also, these data show that as rate of fat deposition increases, the ratio of protein to fat will decrease unless the rate of protein deposition is also increased.

Figure 3. Net accretion of fat in the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles over 2 week growth periods of pigs at 22 and 45 kg live body weight.

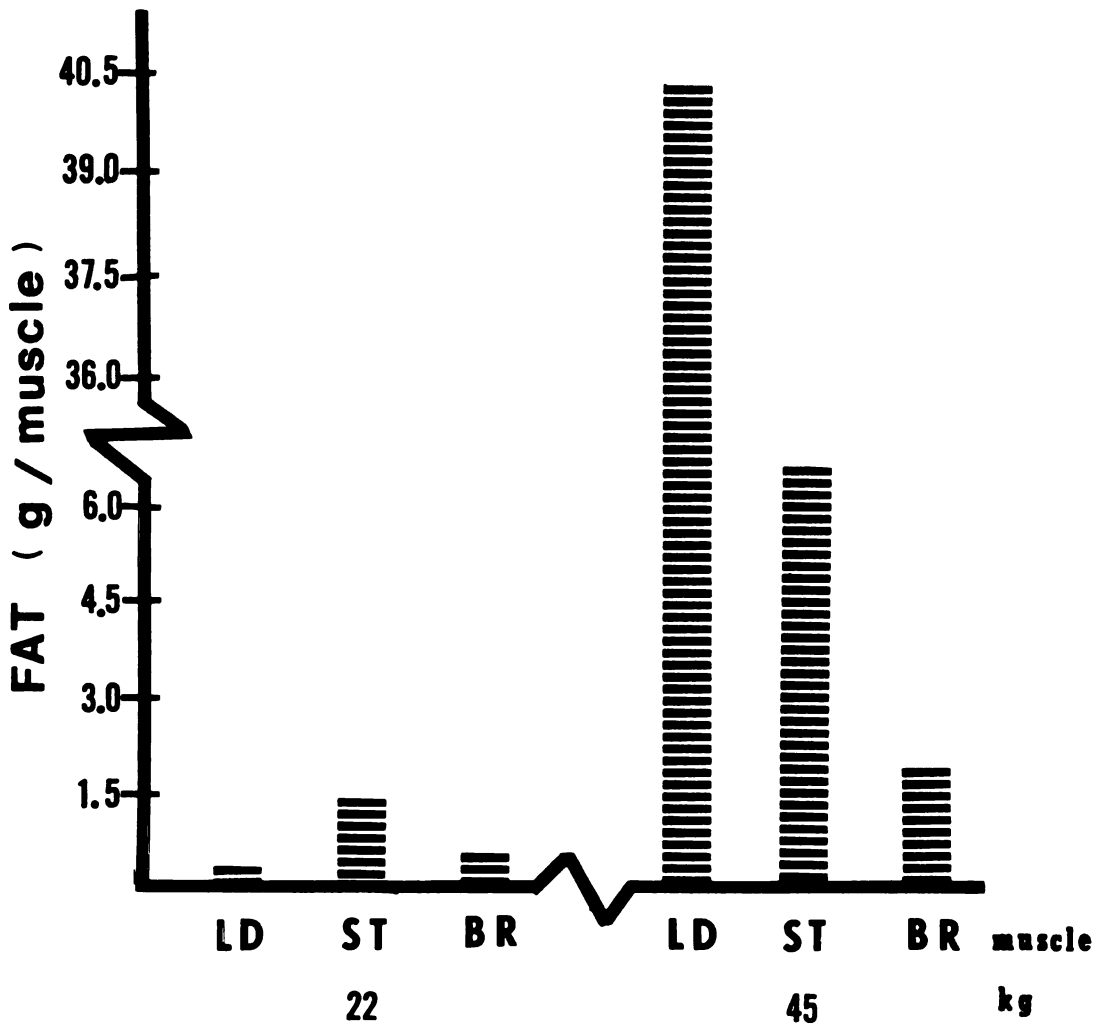
40.5
39.0
37.5
36.0
6.0
4.5
3.0
1.5
FAT (g/muscle)
LD
ST
BR
LD
ST
BR
muscle
kg
22
45

Figure 3.

TABLE 7. Effect of Time on Ratio of Protein to Fat in Longissimus, Semitendinosis and Brachialis Muscles[a]

Muscle	22 kg		Level of significance[b]	45 kg		Level of significance[c]
	I	III		IV	VI	
Longissimus	4.0	5.0	.32	3.4	3.0	.16
Semitendinosis	5.2	5.8	.54	4.4	3.8	.22
Brachialis	5.4	5.0	.69	4.5	3.4	.01

[a]Total protein is Kjeldahl N less nonprotein N times 6.25.
[b]Significance probability of F statistic between groups I and III.
[c]Significance probability of F statistic between groups IV and VI.

TABLE 8. Ratio of Protein to Fat Gain in Longissimus, Semitendinosis and Brachialis Muscles Over a 14 Day Period at 22 and 45 kg Live Body Weight[a]

Muscle	Maturity	22 kg	45 kg
Longissimus	Late	44.3	1.8
Semitendinosis	Intermediate	6.5	2.2
Brachialis	Early	3.0	.9

[a]The net change in protein (total Kjeldahl N minus NPN) per muscle divided by net change in fat.

When the total increase of muscle protein and fat over each 2 week period at 22 and 45 kg is analyzed as a ratio of protein to fat gain for the three muscles as shown in Table 8, the maturity patterns of development for the muscles is even more evident. The composition of gain changes dramatically with the increase in live body weight and has proportionately more fat, thus decreasing the ratio. The designations of late, intermediate and early for the LD, ST and BR, respectively are arbitrary classifications since the ST may also be a late maturing muscle. However, it appears that the BR is an early maturing muscle.

Muscle Protein Fractionation

Muscle protein extraction results are presented in Table 9 with the myofibrillar, sarcoplasmic and stroma protein fractions expressed as a percentage of total protein (total Kjeldahl N minus nonprotein N x 6.25). The percentage of sarcoplasmic protein changed for the LD ($P < .09$) and ST ($P < .01$) over the 2 week period at 22 kilograms. However, for the LD this change was a decrease in percentage but for the ST this was change an increase. The percentage myofibrillar protein increased ($P < .09$) for the 45 kg ST and 22 kg BR. These data show no detectable trends and as presented in Table 9 they offer little information about the protein synthetic capabilities of the animals. However, the data does show that when expressed as a percentage of the total protein, the myofibrillar proteins comprise over 50%, the sarcoplasmic proteins 25%

TABLE 9. Effects of Time on Protein Fractions of Longissimus, Semitendinosis and Brachialis Muscles From the Right Side Expressed as Percentage of Total Protein[a]

Muscle and Protein fraction	22 kg		Level of significance[b]	45 kg		Level of significance[c]
	I	III		IV	VI	
Longissimus						
% Myofibrillar	51.8	53.7	.34	51.1	51.9	.68
% Sarcoplasmic	24.0	25.9	.09	25.7	25.9	.89
% Stromal	24.3	20.8	.12	23.2	22.2	.77
Semitendinosis						
% Myofibrillar	51.9	51.7	.90	50.9	53.0	.09
% Sarcoplasmic	26.8	24.1	.01	25.3	26.3	.29
% Stromal	21.3	24.2	.15	23.9	20.8	.10
Brachialis						
% Myofibrillar	50.6	52.2	.05	52.5	51.2	.45
% Sarcoplasmic	25.3	23.4	.14	26.1	26.0	.85
% Stromal	24.1	24.4	.84	21.3	22.6	.52

[a]Total protein is calculated from total Kjeldahl N less nonprotein N.
[b]Significance probability of F statistic between group I and III.
[c]Significance probability of F statistic between group IV and VI.

and stroma proteins about 20 to 24%. The decreases in sarcoplasmic concentration over the 22 kg period corresponds to results by Young (1970) who showed that this fraction declined or remained constant during growth.

The percentage stroma fraction was not statistically different over either period at 22 or 45 kilograms. This is a valid criticism of the Helander (1957) procedure used since the stroma fraction is obtained by difference.

Since protein fractions are more traditionally expressed as a percentage of the total Kjeldahl N times 6.25, Table 10 was developed. Again, there are no detectable trends observed partly due to variation between animals. These data for the three muscles show values of approximately 46, 22, 20 and 10% for the myofibrillar, sarcoplasmic, stroma and nonprotein N fractions, respectively. With the exception of lower myofibrillar percentages, these values correspond with values of 53 to 56%, 20 to 25% and 11 to 13% for the myofibrillar, sarcoplasmic and nonprotein N fraction reported by Hill (1962). The myofibrillar percentages are within values obtained by Borchert and Briskey (1965).

Although not shown in tabular form, if the group means within the 22 and 45 kg periods are pooled, the percentages or concentrations of the protein fractions showed no detectable differences.

TABLE 10. Effects of Time on Protein Fractions and Nonprotein Nitrogen (NPN) of Longissimus, Semitendinosis and Brachialis Muscles Expressed as a Percentage of Total Nitrogen X 6.25

Muscle and Protein fraction	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
Longissimus						
% Myofibrillar	46.7	47.8	.46	45.8	46.4	.66
% Sarcoplasmic	21.6	23.2	.11	23.2	23.2	.91
% Stromal	21.9	18.7	.10	20.8	19.9	.81
% NPN	9.8	10.3	.06	10.1	10.5	.24
Semitendinosis						
% Myofibrillar	46.6	46.5	.91	45.4	47.6	.04
% Sarcoplasmic	24.1	21.7	.01	22.6	23.6	.25
% Stromal	19.1	21.7	.16	21.4	18.7	.13
% NPN	10.2	10.1	.61	10.6	10.1	.24
Brachialis						
% Myofibrillar	45.8	47.2	.07	47.1	46.4	.61
% Sarcoplasmic	22.9	21.1	.14	23.7	23.7	.94
% Stromal	21.8	22.0	.88	19.2	20.5	.47
% NPN	9.5	9.7	.40	10.0	9.4	.16

[a]Significance probability of F statistic between groups I and III.
[b]Significance probability of F statistic between groups IV and VI.

A more meaningful expression of the protein fractions is presented in Table 11 where the concentration (mg/g) of each fraction was taken times each muscle mass to obtain the total protein in each fraction per muscle. Total grams of protein increased by 11.8 and 28.0%, 41.6 and 26.7% and 30.5 and 10.6% for the 22 and 45 kg period LD, ST and BR muscles, respectively. However, only the increases for the 45 kg LD, 22 kg BR and both 22 and 45 kg ST were significant ($P < .05$). Since the myofibrillar fractions represent over 50% of the total protein, these data parallel the data for total protein. The net increases of total protein and myofibrillar fractions over each 2 week growth period are shown graphically in Figures 4 and 5. These figures represent the means of four pigs per group and show greater rates of protein deposition at 45 kg for the LD and ST but similar increases for the BR at both weight periods.

When the total protein and protein fractions per muscle data from each group at each weight are pooled, there was more ($P < .01$) protein or fraction at 45 kg than at 22 kilograms. Also, if the ratio of myofibrillar to sarcoplasmic protein is calculated from these pooled data, values of 1.06 and 1.98, 2.06 and 2.01 and 2.12 and 1.95 for the 22 and 45 kg LD, ST and BR, respectively are obtained. The increase in ratio for the LD and the decrease for the BR are both significant ($P < .05$) indicating possible changes in rates of synthesis of one or both fractions.

TABLE 11. Effects of Time on Protein Fractions of Longissimus, Semitendinosis and Brachialis Muscles[a,b]

Muscle and protein fraction	22 kg		Level of significance[c]	45 kg		Level of significance[d]
	I	III		IV	VI	
Longissimus						
Total, g	112.7	126.0	.39	261.2	334.4	.02
Myofibrillar, g	58.5	67.6	.37	133.9	174.0	.04
Sarcoplasmic, g	27.0	32.5	.18	67.4	87.6	.11
Stromal, g	27.1	25.9	.64	59.8	72.7	.09
Semitendinosis						
Total, g	20.2	28.6	.01	51.6	65.4	.03
Myofibrillar, g	10.5	14.8	.01	26.2	34.5	.01
Sarcoplasmic, g	5.4	6.9	.01	13.0	17.2	.02
Stromal, g	4.3	6.9	.02	12.3	13.7	.46
Brachialis						
Total, g	5.9	7.7	.05	15.1	16.7	.28
Myofibrillar, g	3.0	4.0	.04	7.9	8.5	.33
Sarcoplasmic, g	1.5	1.8	.06	4.0	4.4	.38
Stromal, g	1.4	1.9	.10	3.2	3.8	.28

[a]Total protein is calculated from total Kjeldahl N less nonprotein N.
[b]Total protein in fraction per muscle.
[c]Significance probability of F statistic between groups I and III.
[d]Significance probability of F statistic between groups IV and V.

Figure 4. Net accretion of protein (total Kjeldahl N minus NPN times 6.25) in the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles over 2 week growth periods of pigs at 22 and 45 kg live body weight.

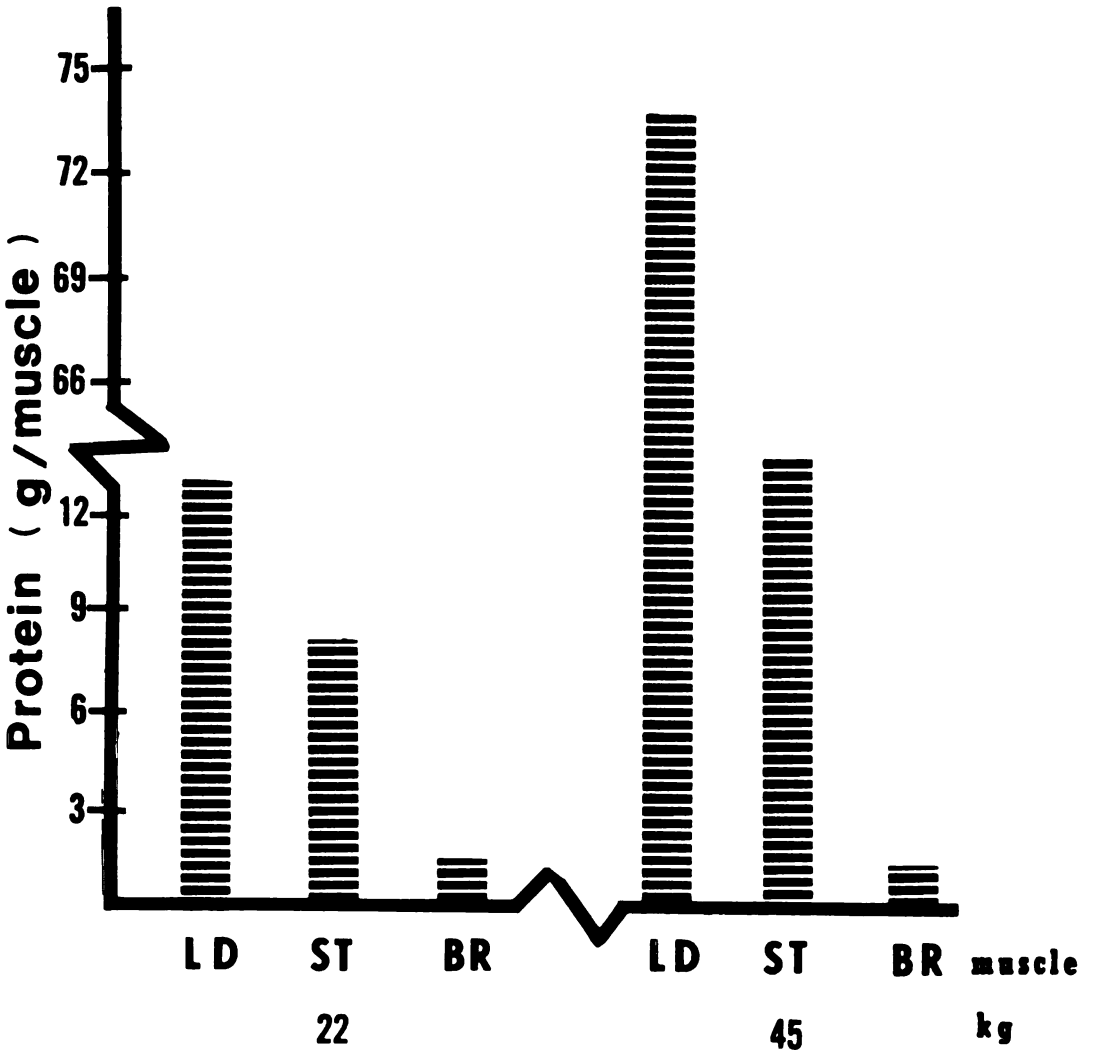
75
72
69
66
12
9
6
3
Protein (g/muscle)
LD
ST
BR
LD
ST
BR
muscle
kg
22
45

Figure 4.

Figure 5. Net accretion of myofibrillar protein in the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles over 2 week growth periods at pigs at 22 and 45 kg live body weight.

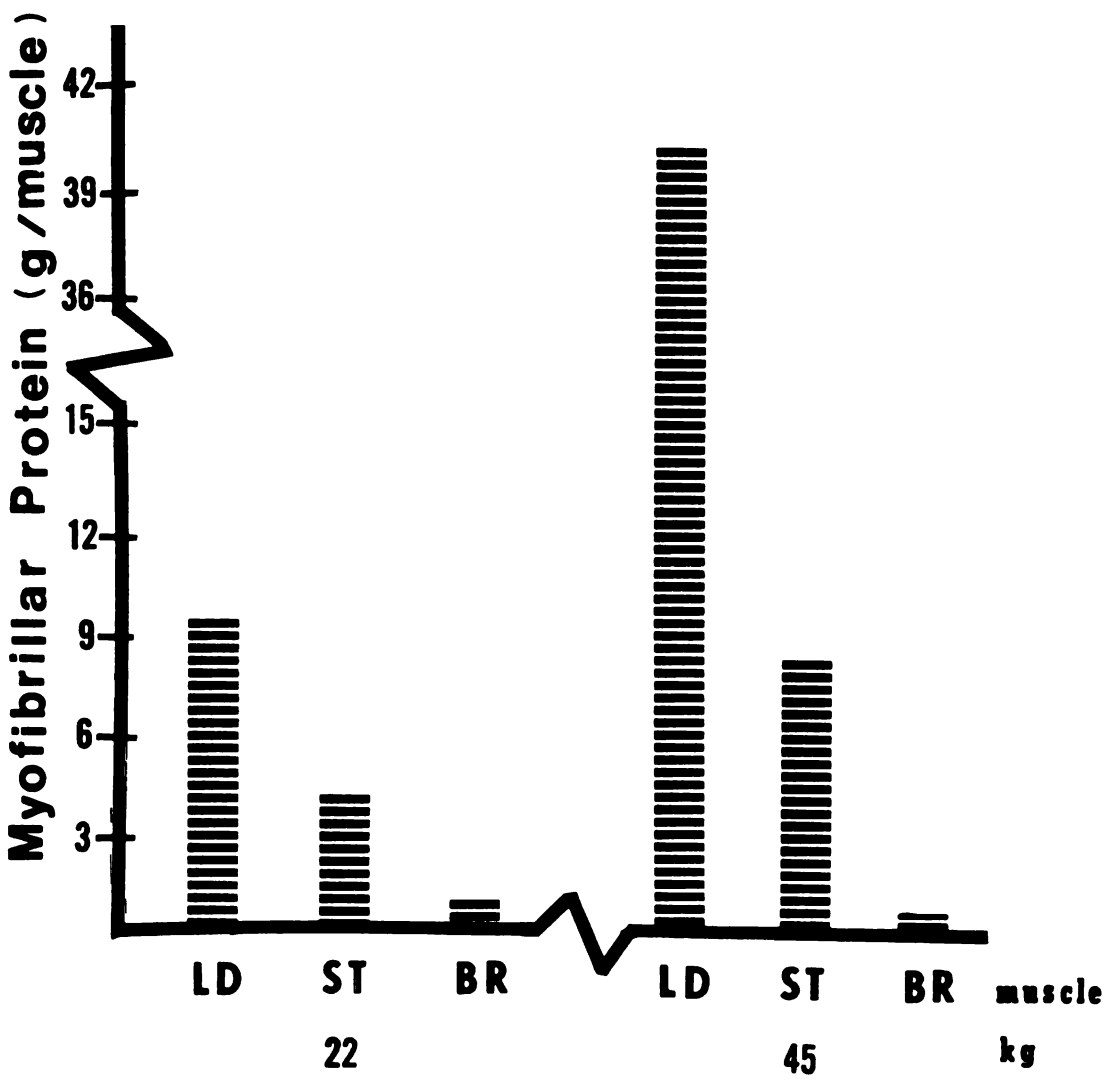
Myofibrillar Protein (g/muscle)
42
39
36
15
12
9
6
3
LD
ST
BR
LD
ST
BR
muscle
kg
22
45

Figure 5.

Table 12 presents the gross changes or accretion of protein, protein fractions and fat over each 2 week growth period. As already discussed, the LD and ST accumulated total protein and myofibrillar protein at a faster rate at 45 kg while the BR showed no statistical difference. Percentage increases for total protein at the 2 periods have already been mentioned, however, the increases for myofibrillar protein was 15.5 and 30.0%, 41.0 and 32.0% and 33.3 and 7.6% for the 22 and 45 kg LD, ST and BR, respectively. Thus, the LD accumulated total protein and myofibrillar protein at a faster rate at 45 kg ($P < .04$) compared to that at 22 kilograms. On an absolute gram basis, the ST was accumulating protein at a faster rate over the 45 kg growth period ($P < .02$) but the rate was less at 45 kg compared to 22 kg on a percentage basis. The evidence that the BR has slowed down in protein accretion rate is obvious both from the gravimetric data in Table 12 and when this net change is expressed on a percentage basis. Therefore, this further substantiates earlier statements about categorizing the BR as an early maturing muscle.

Muscle Nucleic Acids

The nucleic acid content for the LD, ST and BR muscles from each group at the 22 and 45 kg periods is presented in Table 13. RNA concentration (milligrams per gram muscle) did not change ($P < .25$) for any of the muscles over the 2

TABLE 12. Change of Fat, Protein and Protein Fractions of Longissimus, Semitendinosis and Brachialis Muscles Over 14 Day Periods of Two Weight Groups[a,b,c]

Muscle and Component	22 kg	45 kg	Level of significance
Longissimus			
Fat, g	.3	39.7	.01
Protein, g	13.3	73.2	.02
Myofibrillar protein, g	9.1	40.1	.04
Sarcoplasmic protein, g	5.5	20.2	.05
Stromal protein, g	-1.2	12.9	.02
Semitendinosis			
Fat, g	1.3	6.4	.05
Protein, g	8.4	13.8	.08
Myofibrillar protein, g	4.3	8.3	.02
Sarcoplasmic protein, g	1.5	4.2	.02
Stromal protein, g	2.6	1.4	.99
Brachialis			
Fat, g	.6	1.8	.02
Protein, g	1.8	1.6	.47
Myofibrillar protein, g	1.0	.6	.70
Sarcoplasmic protein, g	.3	.4	.32
Stromal protein, g	.5	.6	.33

[a]Change from initial to final for first weight period compared to change for second weight period.
[b]Total protein based on total Kjeldahl N less nonprotein N X 6.25.
[c]Totals per muscle.

TABLE 13. Effects of Time on Nucleic Acids of Longissimus, Semitendinosis and Brachialis Muscles[c]

Muscle and nucleic acid	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
Longissimus						
RNA, mg/g	3.94	3.88	.94	4.34	4.79	.61
DNA, mg/g	.85	.74	.02	.54	.55	.86
Total RNA, mg	2719.8	2919.1	.67	6382.5	9868.7	.08
Total DNA, mg	588.8	575.8	.81	772.6	1135.3	.01
Semitendinosis						
RNA, mg/g	3.14	3.38	.61	4.18	3.38	.25
DNA, mg/g	.82	.78	.00	.63	.59	.62
Total RNA, mg	414.7	641.7	.07	1259.6	1413.8	.56
Total DNA, mg	108.3	146.8	.01	184.1	246.4	.02
Brachialis						
RNA, mg/g	4.24	3.67	.72	2.06	1.96	.74
DNA, mg/g	.95	.94	.85	.71	.70	.95
Total RNA, mg	158.9	182.5	.72	184.9	215.8	.45
Total DNA, mg	36.2	47.2	.01	62.6	76.2	.09

[a]Significance probability of F statistic between groups I and III.

[b]Significance probability of F statistic between groups IV and VI.

[c]Concentrations and total RNA or DNA per muscle.

week period at either 22 or 45 kilograms. When the means for muscle RNA concentration at each period are pooled, only the BR showed a difference ($P < .03$) between the 2 periods with the 45 kg period having a 49% lower RNA concentration. Table 13 also shows a decrease ($P < .02$) in DNA concentration for the LD and ST at 22 kg only. However, when group means are pooled, DNA concentrations for all muscles was less ($P < .01$) at 45 kg by 31, 24 and 26% for the LD, ST and BR muscles, respectively.

Decreases in nucleic acid concentrations during growth postnatally have been shown to occur in other studies (Robinson, 1969; Howarth, 1971; Howarth, 1972; Tsai et al., 1973; Powell and Aberle, 1975; Hakkarainen, 1975; Harbinson et al., 1976). Even though Howarth and Baldwin (1971) suggest RNA concentration is a major determinant of protein synthesis rate and Enesco and Puddy (1964) and Moss (1968a) used DNA concentration as a measure of cellularity, nucleic acid data expressed on a concentration basis can be misleading. Hakkarainen (1975) has shown nucleic acid concentrations to increase slightly after birth but then decline dramatically and this decrease slows and concentrations eventually plateau in later stages of development in the pig. The decrease in nucleic acid concentration cannot be interpreted as a lowered capacity for protein synthesis but merely a dilution effect by accumulating cell protein and fat.

A more meaningful measure of protein synthesizing capabilites can be obtained from analyzing total RNA and DNA content. Total RNA and DNA per muscle is given in Table 13. There were increases of 7.3, 54.7 and 14.8% in total RNA over the 2 weeks at 22 kg while at 45 kg the increases were 54.6, 12.2 and 16.7% for the LD, ST and BR muscles, respectively. While there were these increases over each 2 week period for all muscles, only the increases at 22 kg for the ST and the 45 kg LD were significant ($P < .08$). At first glance, this may indicate more intense transcription of DNA to RNA or possible changes in RNA turnover during these periods. Total DNA per muscle over the 2 weeks at 22 kg increased ($P < .01$) by 35.5 and 30.4% for the ST and BR muscles, respectively, but decreased slightly (2.2%) for the LD. At 45 kg, the LD, ST and BR showed DNA increases ($P < .09$) of 46.9, 33.8 and 21.7%, respectively. These significant increases in total DNA and RNA coincide with previous reports by Robinson (1969), Tsai et al. (1973), Powell and Aberle (1975) and Harbison et al. (1976).

From the data presented in Table 13, it can be presumed from the DNA accretion, that there were increases in nuclei during each period of growth monitored and that transcription was actively occurring. However, it appears that at 45 kg the LD accumulated more protein synthetic capacity than at 22 kilograms. This is evident from the net

increase or change in total DNA over the respective periods as presented in Table 14. The group means (net change) for total DNA and RNA are shown graphically in Figures 6 and 7, respectively.

The percentage increases that the net changes in Table 14, or Figure 6 and 7, represent have already been discussed. On an absolute basis, the ST at 22 kg was accumulating more RNA ($P < .08$) than at 45 kilograms. However, the net change in DNA was greater ($P < .07$) at 45 kg for the ST. While there were net increases in nucleic acids over both periods for the BR, the net accretion rate between growth periods were not significantly different. If a relative constant turnover of RNA is assumed, the transcription rate during the 2 weeks at 22 kg could be said to be more rapid for the ST, more rapid at 45 for the LD and not much different for the BR over the 2 week periods at 22 and 45 kilograms.

In light of the review by Allen et al. (1979) who stated that at least 50 to 88% of the total DNA of muscle is accumulated postnatally, muscle DNA accretion of the pigs in this study is consistent with their observations. Although not shown in tabular form, if nucleic acid data are pooled for each 2 week period and values at 22 kg compared to those at 45 kg, the total DNA per muscle was higher ($P < .01$) at 45 kg and was 63.8, 68.8 and 65.2% greater than the levels found in the LD, ST and BR muscles at 22 kg, respectively.

TABLE 14. Net Change of Total RNA and DNA Per Muscle Over Each 2 Week Growth Period at 22 and 45 Kilograms

Muscle and nucleic acid	22 kg	45 kg	Level of significance[a]
Longissimus			
Total RNA, mg	199.2	3486.2	.02
Total DNA, mg	-13.0	362.7	.01
Semitendinosis			
Total RNA, mg	227.0	154.2	.08
Total DNA, mg	38.5	62.3	.07
Brachialis			
Total RNA, mg	23.6	30.9	.82
Total DNA, mg	11.0	13.6	.49

[a]Net change over the 2 week period at 22 kg compared to the net change at 45 kilograms.

Figure 6. Net accretion of DNA in the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles over 2 week growth periods of pigs at 22 and 45 kg live body weight.

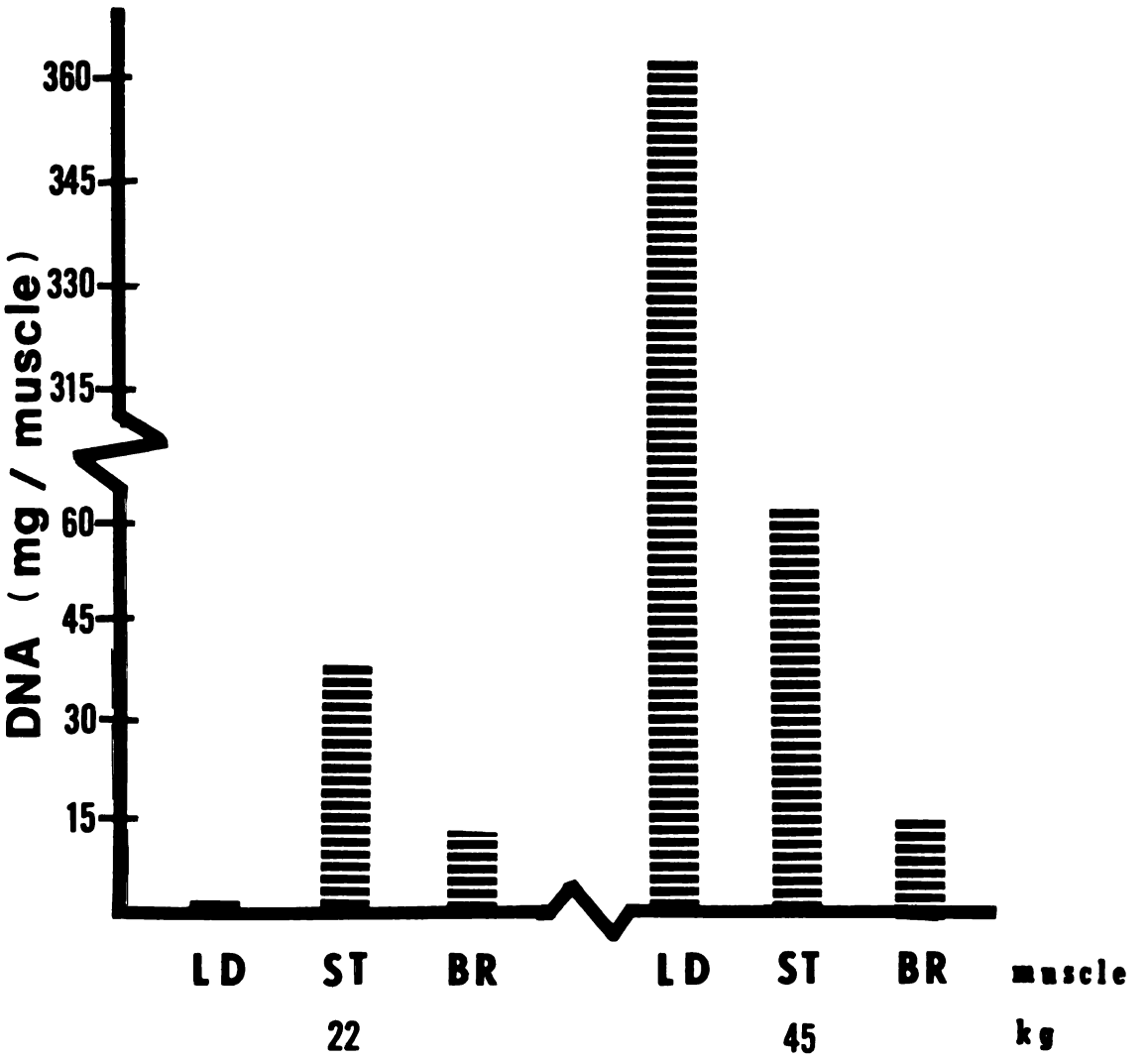
360
345
330
315
60
45
30
15
DNA (mg / muscle)
LD
ST
BR
LD
ST
BR
muscle
22
45
kg

Figure 6.

Figure 7. Net accretion of RNA in the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles over 2 week growth periods of pigs at 22 and 45 kg live body weight.

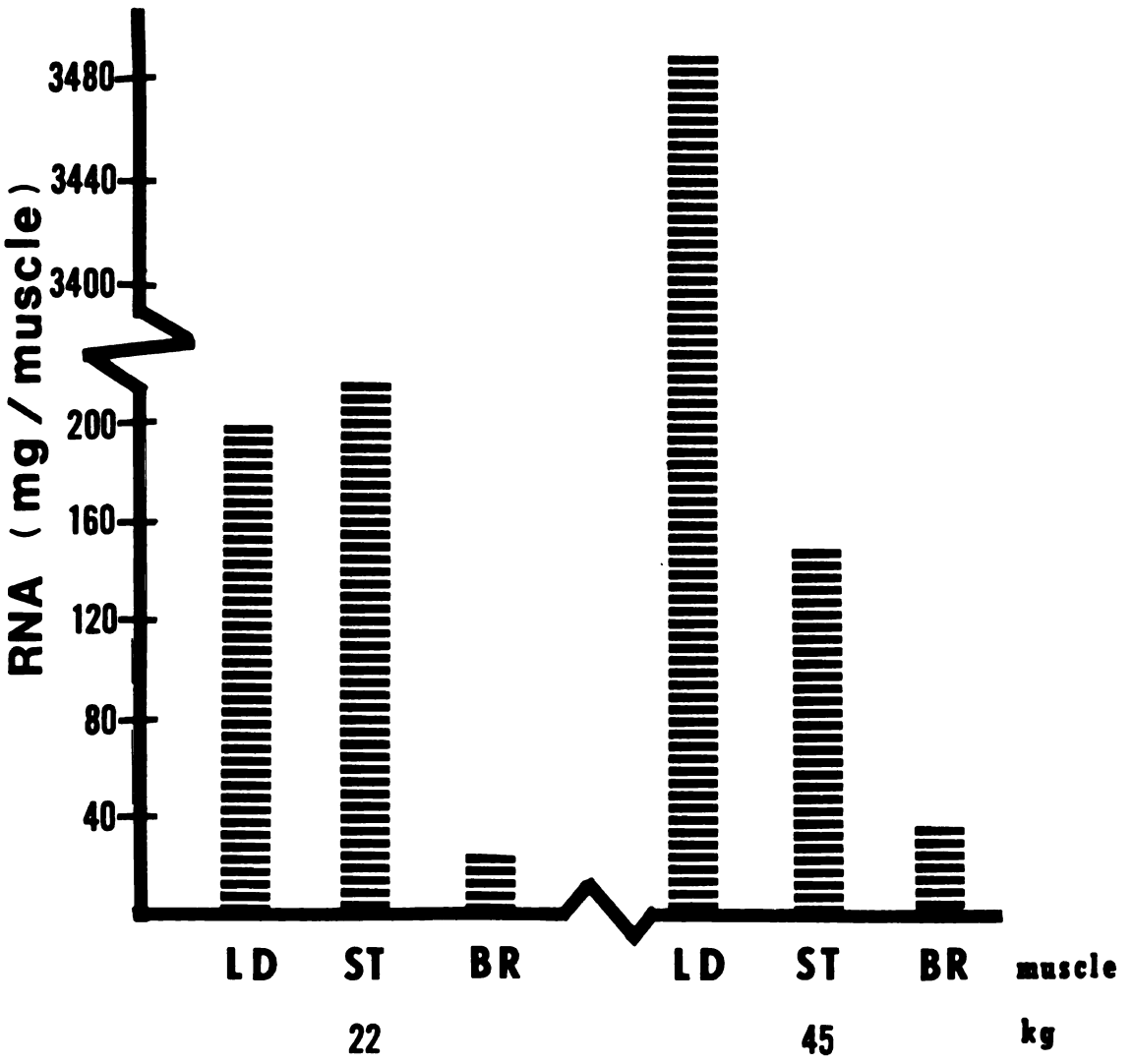
RNA (mg/muscle)
3480
3440
3400
200
160
120
80
40
LD
ST
BR
LD
ST
BR
muscle
kg
22
45

Figure 7.

Therefore, a substantial increase in nuclear DNA occurred between 22 and 45 kg in all three muscles and supports reports of satellite cell involvement in contributing nuclei to muscle postnatally (Moss and Leblond, 1970a; Moss and Leblond, 1971).

One measure of protein synthesis capability is the ratio of RNA to DNA (Winick and Noble, 1965; Powell and Aberle, 1975; Waterlow et al., 1978) and a low ratio is indicative of reduced protein synthetic capacity. Table 15 shows the group means for RNA to DNA ratios. Over each 2 week growth period at 22 or 45 kg, no significant changes in the RNA to DNA ratio occurred for any of the muscles.

However, when the ratios for the pooled means of each of the 2 week growth periods are compared, the LD and ST had higher ($P < .01$) ratios at 45 kg and the BR at 45 kg had lower ($P < .08$) ratios than at 22 kilograms. The LD, ST and BR muscles had ratios of 4.9, 4.1 and 4.1, respectively, at 22 kilograms. This represents only a 19% difference from lowest to highest. The respective muscles at 45 kg had ratios of 8.4, 6.2 and 2.8 or a 300% difference from the lowest to highest and a 35% difference between the LD and ST. This suggests that the LD and ST muscles had greater protein synthetic capacity at the 45 kg period and the BR had less when compared to that at 22 kilograms. RNA to DNA ratios correspond well with the protein accretion already presented in Tables 11 and 12. At 45 kg the LD and ST muscles had greater rates of protein accretion than the LD or ST at 22 kilograms.

TABLE 15. Changes in RNA to DNA Ratio in the Longissimus, Semitendinosis and Brachialis Muscles Over a 2 Week Growth Period at 22 and 45 kg Live Body Weight

Muscle	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
Longissimus	4.6	5.2	.57	8.3	8.6	.86
Semitendinosis	3.8	4.3	.39	7.0	5.7	.23
Brachialis	4.4	3.9	.72	2.9	2.8	.63

[a]Significance probability of F statistic between groups I and III.

[b]Significance probability of F statistic between groups IV and VI.

It should be mentioned that Enesco and Puddy (1964) pointed out that about 35% of the nuclei (estimated by DNA) may be derived from nonmuscle tissue. It is assumed that the proportion of muscle nuclei to other cellular nuclei remains constant during growth.

Millward et al. (1978) pointed out that the relationship of protein synthesis and RNA concentration is twofold: the capacity for protein synthesis expressed as RNA to protein ratio and the extent to which this capacity is utilized indicated by the rate of synthesis per unit RNA or RNA activity. For the latter measurement, the RNA content should be measured on the animals from which the fractional protein synthesis rate is measured which was not done in this study. Garlick et al. (1976) reported a high correlation between fractional synthesis rate per day and RNA concentration.

Another parameter measured in muscle growth studies as discussed by Leblond (1972) is that of cell size or the amount of cytoplasm under the domain of each nucleus which is estimated by weight to DNA or protein to DNA ratios. Table 16 summarizes the protein to RNA or DNA ratios and myofiber sizes for each group at 22 and 45 kilograms. As shown, only the LD exhibited significant changes in protein/DNA with a 14.4% increase ($P < .03$) over the 2 weeks at 22 kg and a 13.5% decrease ($P < .07$) over the 2 weeks at 45 kilograms. Since the 2 week period at 22 kg was the

TABLE 16. Longissimus, Semitendinosis and Brachialis Muscle Protein to DNA and RNA Ratios, Myofiber Size and Net Changes Over 2 Week Growth Periods at 22 and 45 kg Live Body Weight

Muscle and trait	22 kg		Level of significance[a]	45 kg		Level of significance[b,c]
	I	III		IV	VI	
Longissimus						
Protein/DNA	190.8	218.2	.03	340.9	294.8	.07
Change in Protein/DNA	27.4			-46.1		.01
Protein/RNA	41.6	46.8	.61	46.6	34.3	.25
Change in Protein/RNA	5.2			-12.3		.19
Fiber size, μm	39.3	41.4	.15	58.9	62.7	.16
Change in Fiber size, μm	2.1			3.8		.01
Semitendinosis						
Protein/DNA	186.1	194.7	.24	284.1	266.1	.51
Change in Protein/DNA	8.6			-18.0		.06
Protein/RNA	48.7	47.1	.82	41.3	51.4	.42
Change in Protein/RNA	-1.6			10.1		.32
Fiber size, μm	47.1	49.4	.53	63.5	72.1	.21
Change in Fiber size, μm	2.3			8.6		.02
Brachialis						
Protein/DNA	163.9	163.2	.94	241.3	220.2	.15
Change in Protein/DNA	-.7			-21.1		.08
Protein/RNA	41.5	55.8	.42	83.1	82.6	.97
Change in Protein/RNA	14.3			-.5		.59
Fiber size, μm	42.4	43.7	.49	59.2	62.0	.28
Change in Fiber Size, μm	1.3			2.8		.20

[a]Significance probability of F statistic between groups I and III.

[b]Significance probability of F statistic between groups IV and VI.

[c]Significance probability of F statistic of net change.

lowest for DNA accretion in the LD, the protein to DNA ratio increased in magnitude due to accumulation of protein. Neither the ST nor the BR muscles showed changes in protein to DNA ratio over either 2 week period. Although not given in tabular form, when the means at each period are pooled and compared, the LD, ST and BR muscles at 45 kg had higher ($P < .01$) ratios than at 22 kg and were 55.4, 44.5 and 41.1% greater, respectively. If the rate of change of protein/DNA is evaluated from Table 16, some assumptions may be made concerning the protein growth of the muscles. The LD and ST exhibited increases in protein/DNA at 22 kg while over the 2 week period at 45 kg these muscles decreased in protein/DNA. The BR decreased over each 2 week period at 22 and 45 kilograms. Therefore, it can be stated that the change in protein/DNA was decreasing in magnitude over the 2 weeks at 45 kg for all three muscles and these net changes in ratio differed ($P < .08$) from the period at 22 kilograms. Additional time points from a more extended study are needed to delineate the meaning of these decreases over the growth periods. Nevertheless, the overall larger protein/DNA values observed at 45 kg is in agreement with rat data reported by Bailey et al. (1973).

Table 16 also has the group means for protein/RNA and the net change in ratio over each 2 week period. These data support no general trends and no conclusions about

efficiency of RNA use can be drawn. It appears that the protein/RNA is of similar magnitude for the groups at both 22 and 45 kg for the LD and ST muscles and the 22 kg BR muscles. The pooled data for the BR at 45 kg had a larger ($P < .02$) protein/RNA ratio than at 22 kilograms. This difference is most likely due to the decrease in RNA content observed at 45 kg for the BR muscles.

Muscle fiber diameter was measured and group means are given in Table 16 along with the net increase in fiber size over each 2 week period at 22 and 45 kilograms. There were nonsignificant differences in fiber diameter over both 2 week periods for all muscles. However, when the pooled means of the 2 week period at 22 kg is compared to those at 45 kg, fiber diameters were 44.9, 39.5 and 37.7% larger ($P < .01$) at 45 kg for the LD, ST and BR muscles, respectively. These increases parallel the protein to DNA ratios for the three muscles. The net increase in fiber size shown in Table 16 are depicted graphically in Figure 8 and reveal that the rate of increase in fiber diameter was greater ($P < .01$) over the 2 week period at 45 kg than at the 22 kg for the LD and ST muscles. While this was also true of the BR, the difference was nonsignficant ($P < .2$). The differences in fiber diameters may be explained by referring to the total DNA and protein data. Not only did total DNA for the LD and ST at 45 kg and the ST at 22 kg increase but even larger increases in protein occurred and thereby

Figure 8. Net changes of myofiber diameter in the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles of pigs over 2 week growth periods at 22 and 45 kg live body weight.

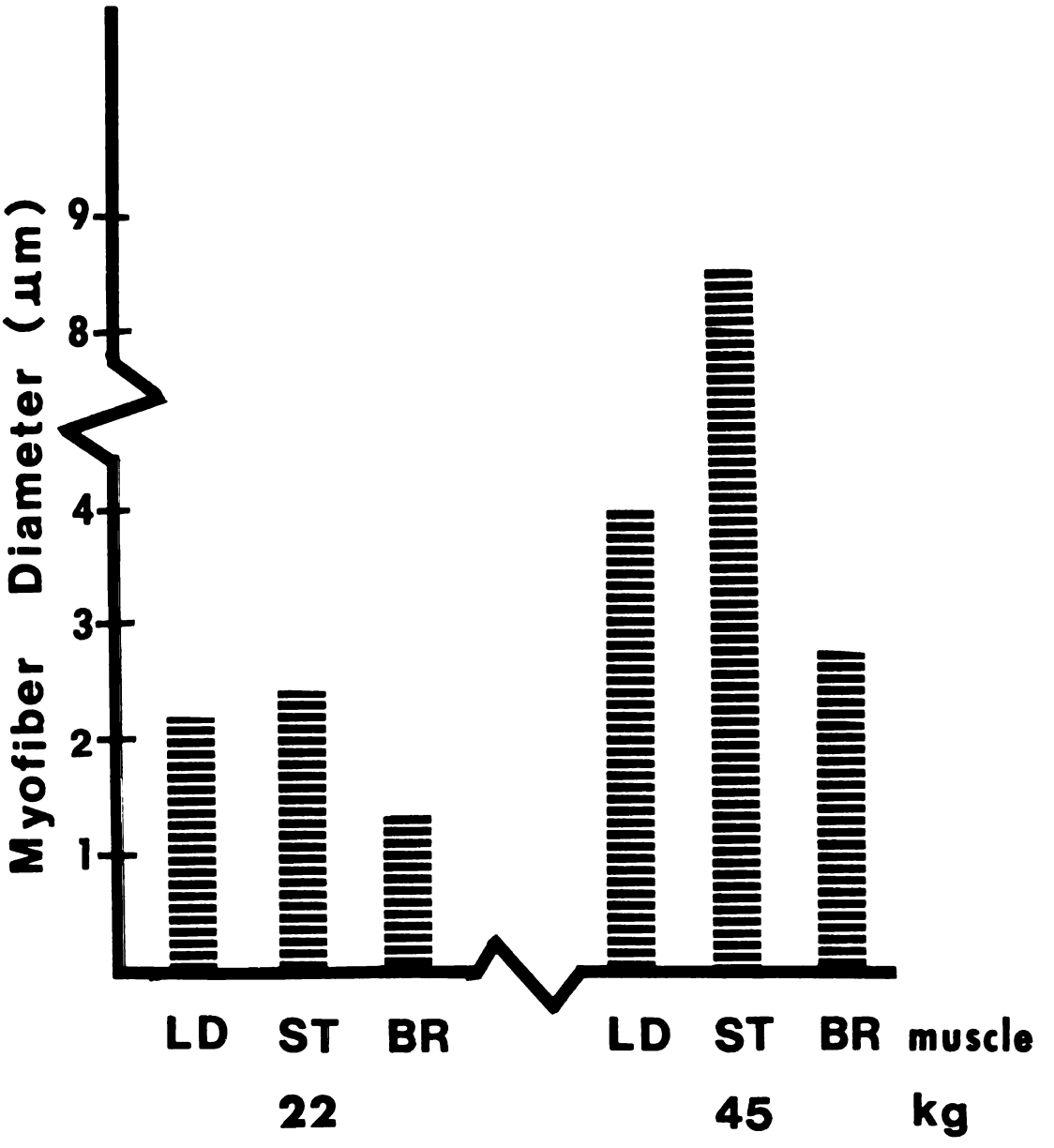

Figure 8.

reduced the protein to DNA ratios at 45 kilograms. However, protein had a much larger effect in accounting for the increased fiber size. The BR had less protein being accumulated and a lower protein to DNA ratio and the lower protein accretion resulted in nonsignificant differences of the magnitude of change in fiber diameter between the two growth periods.

It is important to note that the fiber diameter determinations made in this study were made after muscles had been subjected to cryogenic freezing and may not be comparable to previous studies. Nevertheless, Chrystall et al. (1969) found fiber diameters of the swine LD to increase with age and LBW but only by 10% from 100 to 125 days of age. Burleigh (1980) stated that fiber diameter is an inappropriate method compared to cross sectional areas or volume. For this study, it might be stated that the muscles at 45 kg possessed more nuclei, larger fibers and greater volume of muscle cytoplasm per nucleus than at 22 kg and this is in agreement with work summarized by Burleigh (1980).

Muscle Fractional Protein Synthesis, Breakdown and Accretion Rates

According to Young et al. (1975) and Millward et al. (1975, 1976b, 1978), the rate of skeletal muscle protein breakdown is greater during rapid muscle growth than in

adult or mature animals. Net protein accretion is a result of greater total synthesis rates relative to total degradation rates (Millward et al., 1975). To have high rates of protein turnover during development and growth seems inefficient and is somewhat disputed by studies by Ogata et al. (1978) in which growing rats had high rates of synthesis but low rates of breakdown. As pointed out by Reeds et al. (1980), there is a need to define the relationships that exist between protein accretion and protein synthesis because of the amount of energy required for forming peptide bonds and this contributes to the heat production of the animal and determines the amount of energy used in protein synthesis for each unit of protein deposited. Also, it is not known whether changes in rate of protein accretion during growth is due to alterations of protein synthesis, breakdown or both. When consideration is given to studies by Goldberg et al. (1980) where protein breakdown has been shown to be affected by hormones and when the highly ordered structure of muscle is considered, the breakdown of muscle proteins would seem to be obligatorily a highly regulated process much like synthesis.

The fractional protein accretion (FGR), synthesis rate (FSR) and breakdown rate (FBR) expressed as percent per day for the 22 and 45 kg growth periods are presented in Table 17. FGR was obtained by measuring the accretion of protein (Kjeldahl N minus NPN x 6.25) in each of the three muscles

TABLE 17. Fractional Protein Accretion, Synthesis and Breakdown Rates of Longissimus, Semitendinosis and Brachialis Muscles of 22 and 45 kg Pigs[a,b,c,d]

Muscle and trait	22 kg	45 kg	Level of significance
Longissimus			
FGR	.80	1.76	--
FSR	5.23 ± 0.45	4.21 ± 0.98	.02
FBR	4.43	2.45	.01
Semitendinosis			
FGR	2.46	1.68	--
FSR	6.41 ± 1.89	5.20 ± 1.88	.22
FBR	3.95	3.52	.26
Brachialis			
FGR	1.89	.72	--
FSR	6.45 ± 1.27	5.06 ± 0.86	.02
FBR	4.56	4.34	.40

[a]Expressed as % per day.

[b]Values from both right and left side muscles.

[c]Protein accretion based on total Kjeldahl N minus Nonprotein N.

[d]Values obtained indirectly: $k_A = k_S - k_D$.

over the 2 week periods at 22 and 45 kg LBW. FGR values increased from .8 to 1.76%/day for the LD, decreased from 2.46 to 1.68%/day for the ST and decreased from 1.89 to .72%/day for the BR at 22 kg compared to that at 45 kilograms. These FGR values represent differences of a 120% increase, a 31.7% decrease and a 61.9% decrease over the 2 growth periods at 22 and 45 kg for the LD, ST and BR, respectively.

The assumptions made here are that the pigs in the study were homogeneous in growth rate and that no dietary restrictions were experienced. However, it is acknowledged that since pigs were from 8 different litters and 4 sires (Appendix D), the pigs were not entirely homogeneous in type and composition.

Statistical analysis of FGR was not performed since it is based upon the change of total protein of the means of each group over the 14 day growth period at 22 and 45 kg, respectively, and therefore insufficient degrees of freedom for analysis of variance. Another assumption is that FGR here represents a valid average over the 2 week period.

When the data at 22 kg are compared to those at 45 kg for FSR, there was a decrease of 19.5, 18.9 and 21.6% (5.2 TO 4.2%, 6.4 to 5.2% and 6.4 to 5.1% per day) for the LD, ST and BR muscles, respectively. The decreases in FSR for the LD and BR were significant ($P < .02$). Since FBR was obtained by difference (FGR=FSR-FBR), the FBR also decreased when the

data at 45 kg is compared to that at 22 kg and the LD had a decrease ($P < .02$) of 44.7% (from 4.43 to 2.45%/day). The ST and BR muscles exhibited nonsignificant decreases of 10.9% (from 3.95 to 3.52%/day) and 4.8% (from 4.56 to 4.34%/day), respectively. While additional data points should be taken to substantiate the FGR obtained in this study and therefore allow a more accurate or substantiated FBR, the observations made concerning FBR, partially explain the rapid accretion of protein and muscle growth observed for the LD over the 2 weeks at 45 kg LBW. As presented previously, there were substantial increases in nucleic acids at 45 kg for the LD and even though the FSR was lower ($P < .02$) there was greater total protein synthesis and accretion at 45 kg because of reduced breakdown rates.

Table 18 illustrates the extent of protein turnover in the LD, ST and BR. While the FSR was lower for the pigs at 45 kg LBW, the absolute quantity (grams) of protein synthesized per day increased over the 2 week period at 45 kg as compared to that at 22 kilograms. Additionally, while decreases in FBR were observed at 45 kg compared to that at 22 kg LBW, the absolute quantity (grams) of protein broken down per day increased. Another manipulation of FSR and FBR is shown in Table 18 in which a turnover ratio (TR) is calculated two different ways. One turnover ratio (TR_1) can be calculated by expressing the grams of protein that are

TABLE 18. Use of FSR and FBR and Muscle Protein Content to Obtain Total Grams of Protein Synthesized or Broken Down Per Day*

LBW and Muscle	FSR %/d	FBR %/d	Protein g/muscle	Protein synthesized, g/day	Protein degraded, g/day	Net accretion, g/day	TR_1 [a]	TR_2 [b]	AR[c]
22 Kg									
LD	5.23	4.43	119.4	6.24	5.29	.95	84.7	15.2	.80
ST	6.41	3.95	24.4	1.56	.96	.60	61.5	38.5	2.46
BR	6.45	4.56	6.8	.44	.31	.13	70.4	29.5	1.91
45 Kg									
LD	4.21	2.45	297.8	12.54	7.30	5.24	58.2	41.8	1.76
ST	5.20	3.52	58.5	3.04	2.06	.98	67.8	32.2	1.67
BR	5.06	4.34	15.9	.80	.69	.11	86.2	13.8	.69

[a]Turnover ratio: percentage of protein syntheiszed per day that is broken down.

[b]Turnover ratio: percentage of protein synthesized that is deposited.

[c]Accretion ratio: percentage of protein deposited to that of total protein in muscle.

*Grams of protein synthesized/day = FSR x mass of protein.
Grams of protein broken down/day = FBR x mass of protein.

broken down per day as a percentage of the total grams of protein that is synthesized per day. This indicates the LD at 22 kg has turnover properties (high turnover) much like that of the BR at 45 kg LBW while the LD at 45 kg had less turnover and the ST not changing much from 22 to 45 kilograms. The significance of turnover is exemplified by the TR of 84.7% for the LD at 22 kg but this high value decreased to 58.2% (the lowest value) at 45 kilograms. Again this directly points to the characterization of late, intermediate and early maturing patterns for the LD, ST and BR, respectively.

Another turnover ratio (TR_2) is illustrated in Table 18 by expressing the grams of protein that is deposited per day (accretion) as a percentage of the total grams of protein synthesized per day. This TR_2 follows the same trend as TR_1. Also expressed in Table 18 is an accretion ratio (AR) which is the grams of protein that is deposited per day as a percentage of the total protein mass. Again this points out that accretion rates change during growth for different muscles.

Many previous studies on muscle protein turnover were conducted on rodent hindlimb muscles (Millward et al., 1978) or muscle exhibiting early maturity patterns (Goldberg et al., 1978) and generalizations about skeletal muscle protein turnover have been made. Furthermore, Perry (1975), Garlick et al. (1976), Edmunds et al. (1978) and Simon et al. (1978)

have measured the FSR in pig tissues but in all cases the studies were done at specific live weights and no measurement of protein accretion directly. Garlick et al. (1976) reported nonspecific leg muscle in pigs to have a FSR of 4.8%/day while Simon et al. (1978) using ^{14}C-leucine found pig muscle to have a FSR of 8.1%/day.

While this study was performed on only four animals at each of the two growth periods, there are indications that variations of protein turnover exist between muscles during growth and may be related to the maturation patterns of the muscle. It is also unwise to generalize about FSR differences between muscles even though other studies indicate higher synthesis rates in muscles that are comprised predominately of red fibers (Short, 1969).

Although no fiber typing was carried out on the LD, ST or BR, visual appraisal indicate the LD and ST are essentially white muscles and the BR more red than the other two muscles.

Millward et al. (1976a) discussed a steady state situation in adult muscle in which synthesis and breakdown are about equal. From Figure 9 the FGR (net accretion), FSR and FBR are compared with bar graphs of the data presented in Table 17. While the ST and BR within each period had similar FSR, the FBR was lower in the ST to allow for greater net protein accretion. Also, the highest FBR of the three muscles was observed for the BR in both periods.

Figure 9. Fractional protein synthesis (FSR), fractional protein breakdown (FBR) and fractional protein accretion (FGR) rates of the longissimus (LD), semitendinosis (ST) and brachialis (BR) muscles of male pigs at 22 and 45 kg live body weight. [FSR (%/day) corresponds to the height of the graph plus the standard deviation. FBR (%/day) corresponds to the hatched portion of the graph and FGR (%/day) is the difference: FGR = FSR - FBR.]

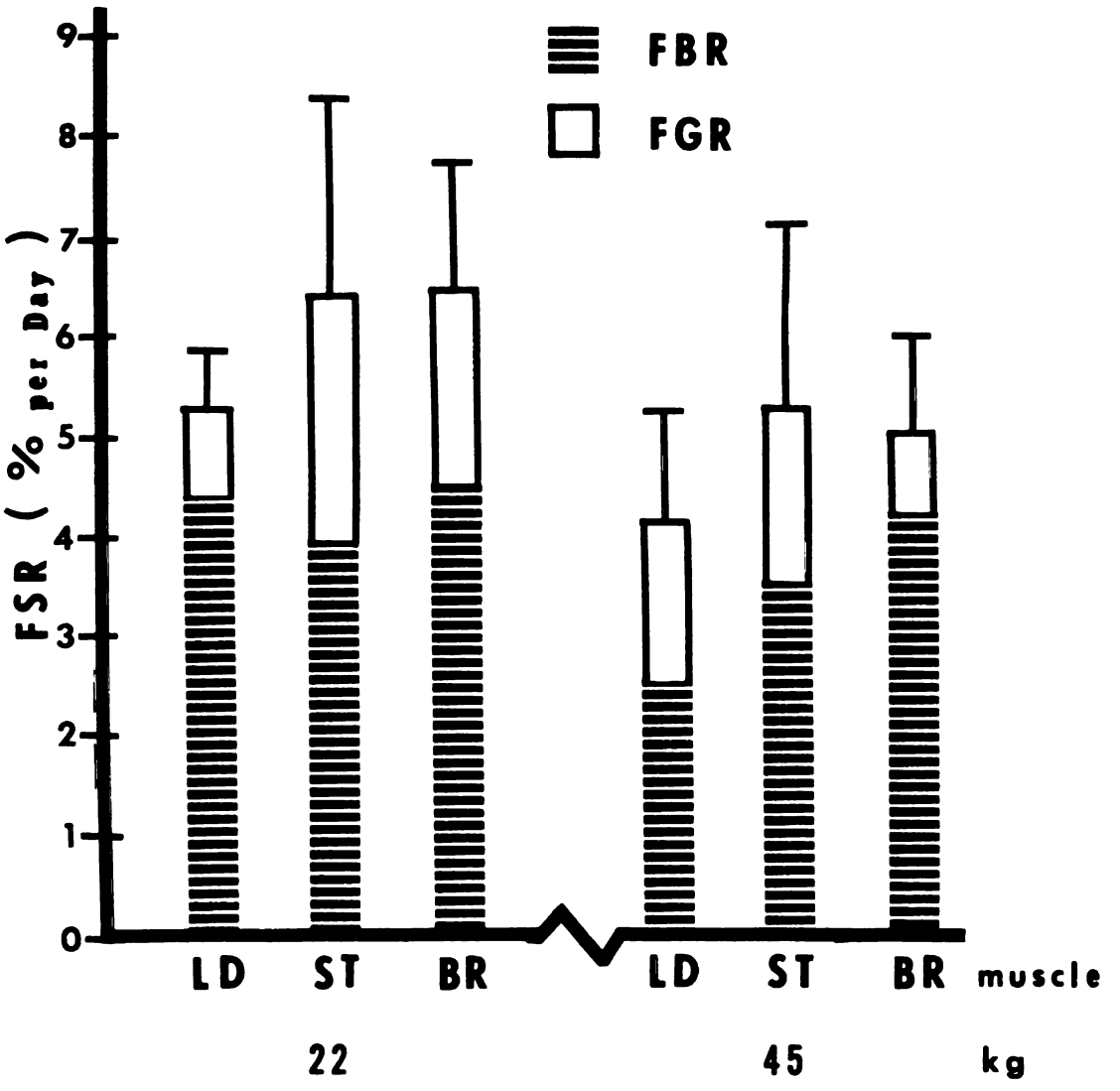

Figure 9.

The turnover data presented allude to the maturation pattern of the three muscles and that possibly protein breakdown is the process being modulated to affect net protein deposition. While the observed developmental decreases in FSR is in agreement with Millward et al. (1978), the dramatic decrease in FBR for the LD is not, but does coincide with theories by Ogata et al. (1978) and Maryuma et al. (1978) that breakdown rate is a possible regulated phenomenon. The FSR values found in this study are 6.0 and 4.8%/day when all three muscles are averaged for the 22 and 45 kg periods, respectively. These values fall within the 4.8%/day value found for 60 to 90 kg pigs in work by Garlick et al. (1976) and the 8.1%/day values found by Simon et al. (1978).

Liver Chemical Composition

The group mean liver weights, weight increase over each 2 week period and liver weights expressed as a percentage of live body weight are given in Table 19. Liver weight increases of 25.4% ($P < .03$) and 29.0% ($P < .02$) were observed over the 2 week periods at 22 and 45 kg, respectively. Furthermore, the increase over the period at 45 kg was greater ($P < .01$) than at 22 kilograms. Miller (1969) indicated that weights of livers relative to LBW are greatest around weaning but then plateau and eventually decline. While liver weight as a percentage of LBW did not change over either 2 week growth period, the liver weight

TABLE 19. Effect of Increasing Live Body Weight on Liver Weight Over 2 Week Growing Periods at 22 and 45 kilograms

Trait	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
Liver Weight, g	517.6	649.1	.03	988.2	1274.9	.02
Increase in Weight	131.5			286.7		.01[c]
Liver weight/LBW x 100	2.77	2.67	.72	2.34	2.28	.62

[a]Significance probability of F statistic between groups I and III.

[b]Significance probability of F statistic between groups IV and VI.

[c]Significance probability of F statistic between 2 week period at 22 and 45 kilograms LBW.

percentage was lower at 45 kg than at 22 kilograms. Although not shown in tabular form, livers were twofold heavier ($P < .005$) at 45 kg than at 22 kg LBW.

The percentage fat, protein or moisture did not differ significantly between groups within the respective growth periods as shown in Table 20 except for an increase ($P < .09$) in percentage protein over the 45 kg period. The total fat and protein increased as the liver increased in size. Total protein increased by 22.1% ($P < .04$) and 37.9% ($P < .02$) over the 2 week periods at 22 and 45 kg, respectively. While not significant, total fat increased by 17.1 and 28.3% over the 2 week periods at 22 and 45 kg, respectively. When the means of both groups at 22 kg are compared to those at 45 kg, total protein per liver was twofold higher ($P < .005$) and total fat slightly more than twofold greater ($P < .005$) at 45 kilograms.

The explanation for increased liver size is obvious when the DNA and protein to DNA ratios are examined in Table 21. Total DNA increased ($P < .01$) by 38.8% and 37.4% over the 2 weeks at 22 and 45 kg periods, respectively. When the data at each respective growth period are pooled and compared, the total DNA at 45 kg was 82% higher ($P < .005$) or almost twice that at 22 kg LBW. This increase in DNA indicates significant hyperplasia. Also, in Table 21 the protein to DNA ratio shows a reduction ($P < .08$) in value at 22 kg but nonsignificant differences at 45 kg LBW. When the

TABLE 20. Effect of Increasing Live Body Weight on Protein, Fat and Moisture Content of Livers of Growing Pigs at 22 and 45 kilograms[a]

Component	22 kg		Level of significance[b]	45 kg		Level of significance[c]
	I	III		IV	VI	
% Protein	18.09	17.68	.61	17.74	18.9	.09
% Fat	1.60	1.50	.53	1.63	1.64	.99
% Moisture	72.11	72.64	.20	72.09	71.69	.38
Total protein	93.61	114.29	.04	175.43	241.98	.02
Total fat	8.23	9.64	.15	16.38	21.02	.22

[a]Protein calculated from total Kjeldahl N x 6.25.

[b]Significance probability of F statistic between groups I and III.

[c]Significance probability of F statistic between groups IV and VI.

TABLE 21. Effect of Increasing Live Body Weight on Nucleic Acid of Livers of Growing Pigs at 22 and 45 kilograms

Nucleic acid and ration	22 kg		Level of significance[a]	45 kg		Level of significance[b]
	I	III		IV	VI	
RNA conc., mg/g	10.31	10.25	.88	9.72	9.99	.46
DNA conc., mg/g	4.14	4.60	.22	3.96	4.22	.42
Total RNA, g	5.32	6.65	.02	9.60	12.68	.01
Total DNA, g	2.14	2.97	.01	3.90	5.36	.01
Protein/RNA	17.58	17.32	.84	18.27	19.00	.55
Protein/DNA	43.74	38.57	.08	44.51	45.51	.90
RNA/DNA	2.49	2.26	.30	2.46	2.39	.70

[a]Significance probability of F statistic between groups I and III.

[b]Significance probability of F statistic between groups IV and VI.

pooled values of groups over each growth period are compared, the protein/DNA was higher ($P < .005$) at 45 kg revealing hypertrophy of liver cells.

Total RNA per liver increased by 25% ($P < .02$) and 32% ($P < .01$) over the 2 week periods at 22 and 45 kg LBW, respectively. The increased RNA would indicate more machinery for protein synthesis (Waterlow et al., (1978) but neither the efficiency of protein synthesis (protein/RNA) nor the intensity of protein synthesis (RNA/DNA) changed much over either 2 week growth period.

When the net change in total protein, fat, nucleic acids and the ratios of RNA to DNA and protein to DNA are evaluated in Table 22 some deductions about protein synthesis occuring over each 2 week growth period at 22 and 45 kg can be made. The net change in total protein was more than threefold greater ($P < .06$) over the 2 week period at 45 kg than at 22 kilograms. The net change in RNA was twofold greater ($P < .06$) and the change in DNA 1.8 times greater ($P < .07$) over the growth period at 45 kilograms. This indicates that protein was being accumulated at a faster rate over the second period primarily due to more cells and more protein synthetic capacity than at 22 kilograms.

As with muscle, liver protein, fat and nucleic acid data expressed on a concentration or percentage basis did not differ between growth periods. Also, summation of the percentage fat, protein and moisture total about 91%. Presumably the remaining 9% is made up of ash and glycogen.

TABLE 22. Change of Protein, Fat and Nucleic Acids of Liver Over 14 Day Periods of 22 and 45 Kg Pigs[a,b]

Trait	22 kg	45 kg	Level of significance
Total Protein, g	20.68	66.55	.06
Total Fat, g	1.41	4.65	.21
Total RNA, mg	1332.14	2688.24	.06
Total DNA, mg	832.78	1466.58	.01
RNA/DNA	-0.23	-0.07	.55
Protein/DNA	-5.26	0.16	.80

[a]Change from initial to final for first weight period compared to change for second weight period.

[b]Total protein calculated as Kjeldahl N X 6.25.

Liver Fractional Protein Synthesis and Breakdown Rates

After estimating λp values from plasma specific activity curves like that shown in Figure 10, protein FSR of the liver of infused pigs was derived interatively from the equations of Garlick et al. (1976, 1980) (computer program found in Appendix G.2). The means of the four infused pigs at 22 and 45 kg growth periods for liver FGR, FSR and FBR are listed in Table 23. These values indicate a 39.7% and 43.9% decrease in FSR and FBR, respectively when the 22 kg growth period is compared to that at 45 kg LBW. These data are comparable to values of 23.3%/day measured by Garlick et al. (1976) on 60 to 90 kg pigs. However, no mention of sex of those pigs was made. Garlick et al. (1976) also indicated that FSR in rat tissues tends to be 2 to 3 times faster than those for pigs. In another study, (Simon et al., 1978) values of over 110%/day were reported for FSR in pig livers.

Liver FSR data obtained from rats by Garlick et al. (1975) and Millward et al. (1978) show no real differences between young and old rats. However, Waterlow and Stephen (1968) using labeled lysine showed slight decreases in rat liver FSR with increasing age. Millward et al. (1978) stated that protein breakdown rate is a reflection of functional demand of a tissue and this results in variations in the changes of protein synthesis or breakdown during organ development. Furthermore, even though FSR declines with growth, the absolute protein synthesis increases.

Figure 10. Specific activity curve of plasma during a 6 hr infusion of [^{14}C-] tyrosine into pigs at 22 and 45 kg live body weight.

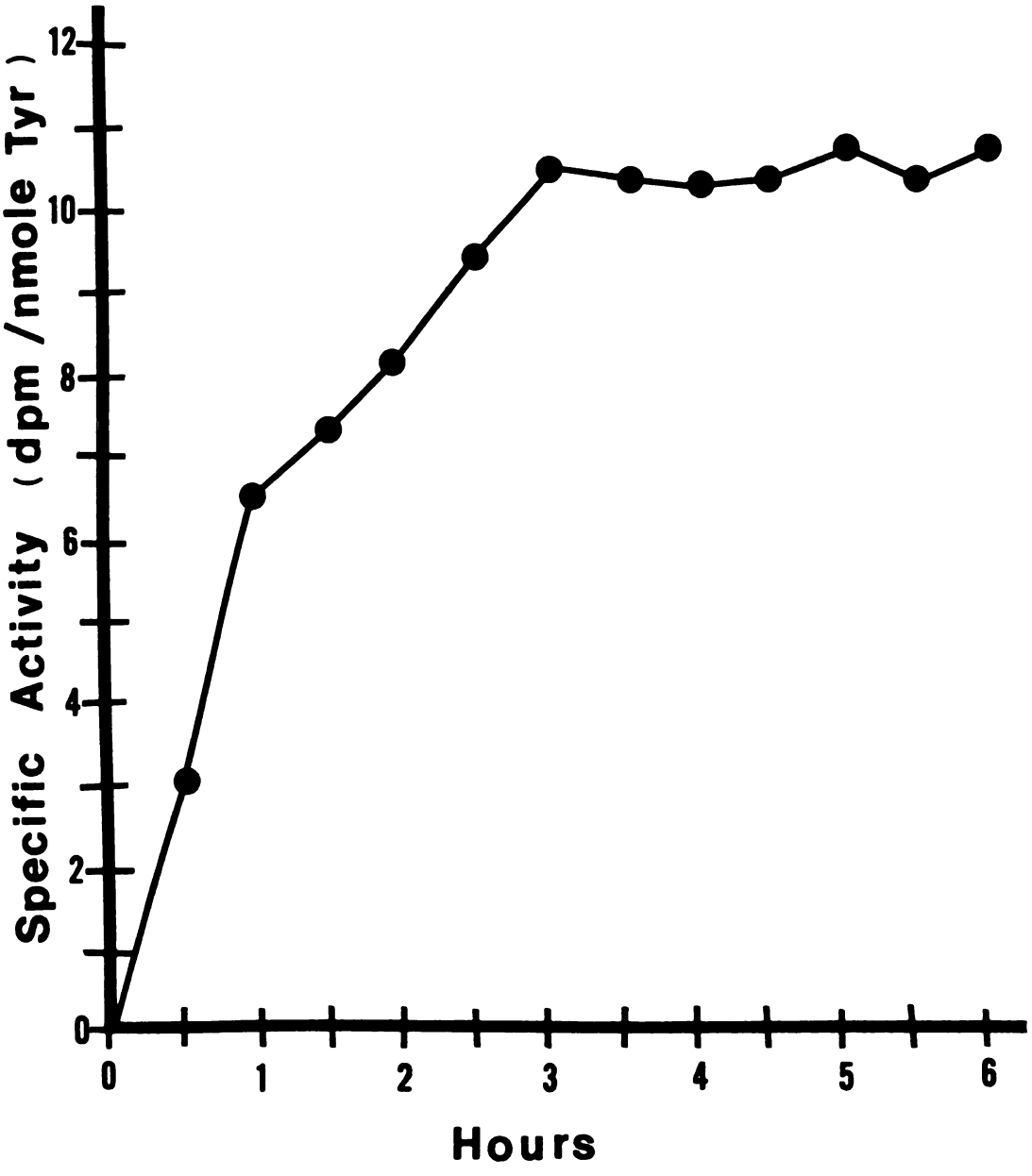

Figure 10.

TABLE 23. Liver FGR, FSR and FBR of Growing Boar Pigs at 22 and 45 Kg LBW[a,b]

Trait	22 kg	45 kg	Level of significance
FGR	1.42	2.28	
FSR	35.35±	21.30	.01
FBR	33.93	19.02	.01

[a]Values expressed as %/day.

[b]No correction for export proteins was made.

Since the grams of protein synthesized per day is equal to the FSR multiplied by the mass of protein of the tissue, the protein synthesized per day by liver at 22 kg was 36.7 g and 44.4 g at 45 kg or a 21% increase in the amount of protein synthesized per day.

SUMMARY

(1) Boar pigs at approximately 45 kg LBW were growing at a faster rate in terms of weight per day than pigs at 22 kilograms. However, percentage increases in LBW over each 2 week growth period at 22 and 45 kg were 30.5 and 31.9%, respectively.

(2) LD and ST muscles grew significantly faster in terms of weight increase per day at 45 kg LBW than at 22 kg while the BR showed no difference statistically between the 22 and 45 kg growth periods.

(3) Percentage increases in muscle weights were 12.2 and 28.4% for the the LD, 43.0 and 28.8% for the ST and 31.2 and 14.8% for the BR for the 2 week growth periods at 22 and 45 kg LBW, respectively. This indicates the LD was growing at a faster rate during the period at 45 kg LBW compared to that at 22 kilograms. While the rate of increase was lower for the ST and BR at 45 kg compared to that at 22 kg, the rate was markedly reduced for the BR muscle. This indicates that muscles change in their impetus for growth during LBW growth and that muscles used as indicators of growth should be selected according to the period of growth being studied and their maturity pattern.

(4) When muscle weight data is transformed into fat free values, the trends observed were not different from that prior to discounting for fat.

(5) Muscle weights as a percentage of LBW did not change during the two growth periods studied and were similar at 22 and 45 kg LBW for each muscle.

(6) Percentage moisture decreased with increasing LBW as muscles at 45 kg had lower percentage water than those at 22 kg LBW. They decreased were from 76.9 to 74.2% for the LD, 78.5 to 76.5% for the ST and 78.5 to 76.5% for the BR at 22 and 45 kg LBW, respectively. These decreases of 2.7, 2.0 and 2.0% in percentage moisture were offset by 1.9, 1.3 and 1.6% increases in percentage fat for the LD, ST and BR, respectively.

(7) There were no significant differences in percentage protein (Kjeldahl N x 6.25) between the two periods at 22 and 45 kg LBW.

(8) From a chemical maturity standpoint, the BR appeared to begin accumulating proportionately more fat during the 2 week period at 22 kg than the LD or ST as these two muscles showed nonsignificant changes in total fat over the growth period at 22 kilograms. All three muscles had greater fat deposition rates on a weight per day basis and a percentage increase over the 2 week period at 45 kg than at 22 kg LBW.

(9) Total protein (Kjelahl N x 6.25) per muscle increased more rapidly at 45 kg for the LD and ST and less rapidly for the BR as compared to the percentage increase in total protein per muscle at 22 kg LBW.

(10) Ratios of protein to fat in the muscles were lower at 45 kg compared to those at 22 kilograms. These comparisons indicate designations of late, intermediate and early for the LD, ST and BR, respectively, to describe their relative maturity patterns.

(11) No detectable trends were detected in analyzing the muscle protein fractions for changes over either 2 week growth period. All indications are that adult concentrations had been attained.

(12) Increases in total myofibrillar or sarcoplasmic protein per muscle parallels the increases in total protein (Kjeldahl N less NPN x 6.25) with the myofibrillar fraction representing approximately 50% of the total protein and sarcoplasmic representing 25%.

(13) Muscle DNA concentration was lower at 45 kg than at 22 kg LBW. Total DNA per muscle increased to indicate an increase in nuclei density and this was greater at 45 kg than at 22 kilograms.

(14) In general, RNA concentrations were higher at 45 kg for the LD and ST but lower for the BR than at 22 kilograms. Total RNA per muscle was higher at 45 kg than at 22 kg for all 3 muscles.

(15) The rate of both DNA and RNA accretion was greater at 45 kg for the LD while the rate was highest at 22 kg for the ST. However, for the BR over the 2 week period at 22 kg, RNA and DNA accretion was greater than at 45 kilograms.

(16) In general, nonsignficant changes in RNA to DNA ratios occurred over either 2 week period at 22 or 45 kg but the ratios at 45 kg were higher than those at 22 kg for the LD, ST and BR. This indicates different protein synthesizing intensities between the two periods at 22 and 45 kilograms.

(17) Fiber diameters and protein to DNA ratios were greater at 45 kg than at 22 kg for all muscles indicating hypertrophy was occurring. The rate of increase in fiber size was also greater over the 2 week period at 45 kg than at 22 kg for the LD and ST.

(18) The FSR and FBR (%/d) was lower for all three muscles of pigs at 45 kg LBW than at 22 kilograms. While each of the muscles at 45 kg LBW had roughly a 20% lower FSR, the LD, ST and BR had 44.7, 10.9 and 4.8% lower FBR, respectively. FSR values of 5.2, 6.4 and 6.4%/day at 22 kg and 4.2, 5.2 and 5.1%/day at 45 kg were observed for the LD, ST and BR, respectively. FBR values of 4.4, 4.0 and 4.6%/day at 22 kg and 2.4, 3.5 and 4.3%/day were observed at 45 kilograms.

19) Even though FBR was obtained from FSR and FGR, it may be stated that the relationship between FSR and FBR resulted in lower FGR for the ST and BR and increased FGR for the LD when values at 45 kg are compared to those at 22 kilograms.

(20) When the FSR and FBR are multiplied times the protein content of the muscles, the percentage of protein synthesized that was broken down per day was 85% for the LD at 22 kg and 86.2% for the BR at 45 kilograms. The ST was intermediate at both periods with 61.5 and 67.8% at 22 and 45 kg, respectively. At 45 kg, the LD shows only a 58.2% value or the lowest turnover rate. It appears that muscles change in protein turnover rate which accomodates changes in growth and protein deposition.

(21) Analysis of data pertaining to protein, fat and nucleic acid accretion in the LD, ST and BR over 14 day growth periods at 22 and 45 kg LBW, indicate that there are differences in maturity and growth patterns of muscles with the shoulder muscle (BR) being early maturing, the back muscle (LD) late maturing and the ham muscle (ST) being intermediate. These data indicate that increases in DNA is a necessary prerequisite for protein accretion and that even when protein accretion rates are high, some fat deposition is occurring and protein turnover is also quite extensive.

Furthermore, due to the dramatic changes in breakdown rate observed here, it indicates that muscle protein breakdown may indeed be as actively regulated during growth as synthesis. Although modulation of one or the other of these processes to achieve enhanced protein deposition may

be appealing the question of the desirability of how much of muscle protein synthesis and breakdown should be altered remains unanswered because of the extensive contribution of protein synthesis to heat production.

(22) Protein, fat or moisture expressed on a percentage basis of livers of pigs did not change over either growth period at 22 or 45 kilograms. However, at 45 kg livers had more total protein and fat primarily because the organ was larger. Livers remained a relatively constant 2.5% of LBW.

(23) While concentrations did not change, total RNA and DNA increased over each 2 week period at 22 and 45 kg and the livers at 45 kg had more RNA and DNA than livers at 22 kg LBW. The increase in DNA shows that hyperplasia was active and protein to DNA ratios indicate a significant increase in cell size when the 45 kg period is compared to the 22 kg period. However, a significant ($P < .08$) decrease in protein to DNA ratio was observed over the period at 22 kilograms. This decrease in ratio during the period at 22 kg may be due to a higher protein FBR resulting in a lower FGR while DNA accretion rate is high.

(24) Liver FSR and FBR were determined to be lower at 45 kg but the fractional accretion rate was higher. The percentage of the grams of protein synthesized per day that was broken down was 96 and 89% at 22 and 45 kg periods, respectively.

APPENDICES

Table A. 1 Glutaraldehyde-BSS Buffer

Make up a 1% glutaraldehyde solution in BSS buffer

Table A.2 BSS Buffer

Dissolve and mix the following in deionized water and bring final volume to 1 liter:

8.0076	g	NaCl
.2013	g	KCl
.1110	g	$CaCl_2$
.2033	g	$MgCl_2$
.0207	g	NaH_2PO_4
.1931	g	Na_2HCO_3
.5041	g	$NaHCO_3$
.9909	g	glucose

Table A.3 Guanidine-HCl Buffer

Make a .02 M guanidine-HCl solution in .05 M boric acid - KOH buffer, pH 9.5

Table A.4 1 - Nitroso - 2 - Napthol Solution for Tyramine Assay

Add weighed quantity of 1-nitroso-2-naphtol to 95% alcohol to make a .1% solution

Table A.5 Nitric Acid Solution for Tyramine Assay

Prepare nitric acid solution by adding .5 ml of 2.5% $NaNO_2$ solution to 24.5 ml of a diluted HNO_3 (1:5) solution

Table A.6 Tyramine Standards

Prepare standard solutions containing concentrations of tyramine ranging from 1 to 150 nmoles/ml in diluted sulfuric acid (1:500,v/v)

Table A.7 Citrate Buffer

Prepare .5 M citric acid and .5 sodium citrate solutions. Add citric acid to sodium citrate at the ratio of 1:5 and adjust to pH 5.5 with one or the other solution

Table B.1 Drying Procedure of Tissue Derived Supernates and Hydrosylates

1. After TCA has been removed from supernates by ether extraction and precipitates hydrolyzed and filtered through NO. 45 Whatman paper, transfer samples to 50 ml round bottom boiling flasks.

2. Attach flask to rotary evaporator with the water bath set at 50 C.

3. Just prior to total dryness, add 10 to 15 ml of deionized water and re-dry. Repeat two more times.

4. After the last water wash has been evaporated, and 2.0 ml of citrate buffer to the flask, swirl and dissolve material in the flask and transfer to a 50 ml culture tube.

Table C.1 Preparation of Muscle Samples For Amino Acid Analysis

1. Determine % protein of each sample.
2. Weigh out sample to equal 10 mg protein. Place in hydrolysis tubes with teflon lined screw caps.
3. Add 10 ml 6 N HCl to each tube plus 2 ml 12 N HCl.
4. Add 2 ml norleucine standard 1 mM.
5. Run stream of N gently into each tube for 30 seconds, cap quickly and screw tightly.
6. Autoclave for 18 hours. Cool.
7. Filter through No. 2 Whatman, wash filter well.
8. Rotary evaporate under vacuum just to dryness in 55°C water bath.
9. Add 20 ml H_2O; dissolve and evaporate again. Repeat.
10. Use ultra pure deionized water throughout.
11. Add 4 ml .01 N HCl to residue, dissolve and transfer to small tube for freezer storage.

TABLE D.1 Breeding Information on Pigs Used in Study[a]

PN	GN	MSU Litter No.	Dam	Sire
1	I	111-11	146	Dee
2	I	106-10	147	M.J.
3	I	104-13	140	Dee
4	I	104-14	140	Dee
5	III	102-11	121	Dee
6	III	106-12	147	M.J.
7	III	103-11	152	Denny
8	III	106-11	147	M.J.
9	IV	111-10	146	Dee
10	IV	H1-10	13	Denny
11	IV	102-10	121	Dee
12	IV	103-10	152	Denny
13	VI	109-12	141	Denny
14	VI	102-12	121	Dee
15	VI	H1-12	13	Denny
16	VI	111-14	146	Dee
17	II	108-10	174	Ron
18	II	109-10	141	Denny
19	II	108-11	174	Ron
20	II	104-12	140	Dee
21	V	109-13	141	Denny
22	V	106-13	147	M.J.
23	V	H1-11	13	Denny
24	V	111-13	146	Dee

[a]A total of 8 litters and 4 sires were used.

Table E.0 Definitions of Code Letters in Appendix E.

PN	-	pig number
GN	-	group number of pig
LBW	-	live body weight, kg
LDM	-	longissimus mass, g
STM	-	semitendinosis mass, g
BRM	-	brachialis mass, g
MO	-	% moisture
KP	-	% Kjeldahl protein
F	-	% ether extracted fat
DNA	-	deoxyribonucleic acid, mg/g
RNA	-	ribonucleic acid, mg/g
MFD	-	myofiber diameter, um
MY	-	myofibrillar protein (% of KP)
SC	-	sarcoplasmic protein (% of KP)
NPN	-	nonprotein nitrogen (% of KP)
STR	-	stromal protein (% of KP)
TP	-	KP less NPN
LW	-	liver weight

Table E.1 Individual Live Body Weights of Pigs and Proximate Analysis, Nucleic Acid, Myofiber Diameter and Protein Fractionation Data for the Longissimus Muscle of Pigs from Groups I and III.

				Proximate Analysis			Nucleic Acids			Protein Fractionation				
				% wet			mg/g wet			% wet				
PN	GN	LBW	LDM	MO	KP[a]	F	DNA	RNA	MFD	MY	SC	NPN	STR	TP[b]
1	I	17.5	617.6	77.08	17.28	4.30	.93	3.52	37.7	44.8	19.9	9.9	25.3	15.50
2	I	20.2	818.1	76.95	18.45	3.69	.79	3.74	40.6	48.5	21.9	9.5	19.8	16.74
3	I	18.6	605.2	76.73	18.09	3.65	.89	4.63	40.3	46.7	22.5	10.0	20.8	16.32
4	I	18.6	735.6	75.78	18.18	4.46	.80	3.87	38.7	46.9	22.1	9.5	21.4	16.44
5	III	24.3	712.4	75.42	16.83	5.47	.71	5.33	39.4	47.6	24.9	10.4	17.1	15.04
6	III	24.3	818.3	77.70	18.07	3.57	.72	2.33	43.7	48.6	22.2	9.7	19.4	16.34
7	III	23.1	660.9	78.30	17.78	2.49	.77	4.82	39.8	44.7	23.0	10.6	21.6	15.86
8	III	25.9	922.7	77.22	18.78	2.88	.75	3.01	42.9	50.3	22.7	10.6	16.4	16.78
ANOVA														
EMS[c]		369.920	14263.05	.171	.002	.409	.026	.008	137.697	2.403	4.890	.664	20.943	.012
EMS[d]		41.427	11905.056	2.504	.282	.994	.003	1.141	52.058	3.940	1.359	.121	5.603	.226
F statistic		8.930	1.198	.068	.007	.412	9.74	.007	2.645	.610	3.597	5.493	3.738	.053

[a] Kjeldahl N x 6.25
[b] (Kjeldahl N minus NPN) x 6.25
[c] Error mean square values between groups with 1 degree of freedom
[d] Error mean square values within groups with 6 degrees of freedom

Table E.2 Individual Live Body Weights of Pigs and Proximate Analysis, Nucleic Acid, Myofiber Diameter and Protein Fractionation Data for the Longissimus Muscle of Pigs from Groups IV and VI.

				Proximate Analysis			Nucleic Acids			Protein Fractionation				
				% , wet			mg/g, wet			% , wet				
PN	GN	LBW	LDM	MO	KP[a]	F	DNA	RNA	MFD	MY	SC	NPN	STR	TP[b]
9	IV	45.2	1595.8	74.56	18.19	5.34	.48	3.95	58.9	45.2	22.5	11.1	21.1	16.36
10	IV	44.5	1798.0	75.01	17.04	5.39	.46	5.60	57.0	47.7	24.8	9.7	17.9	15.38
11	IV	44.5	1390.3	74.22	19.39	5.07	.46	4.05	54.9	44.1	21.7	9.5	24.7	17.52
12	IV	46.7	1599.2	74.45	18.12	5.57	.53	2.20	64.9	46.5	23.1	10.9	19.5	16.14
13	VI	45.4	1967.8	73.99	17.63	6.13	.57	4.28	62.3	46.3	21.1	10.6	22.0	15.79
14	VI	59.9	1852.6	73.14	18.08	7.11	.57	4.75	63.6	43.2	20.6	10.3	25.9	16.26
15	VI	61.2	2406.0	74.28	17.68	5.86	.54	5.32	64.2	47.0	25.4	10.8	16.9	15.75
16	VI	58.1	1969.0	73.59	19.32	5.44	.54	4.81	60.5	49.3	25.8	10.2	14.7	17.30
ANOVA														
EMS[c]		369.920	4.1×10^5	.128	.038	1.246	.010	1.399	137.697	.873	.061	.431	1.071	.023
EMS[d]		41.43	4.4×10^4	.460	1.018	.275	7×10^{-4}	1.060	52.058	3.942	4.333	.259	17.314	.969
F statistic		8.930	9.395	.277	.853	4.526	13.783	1.320	2.645	.221	.014	1.662	.062	.024

[a] Kjeldahl N x 6.25
[b] (Kjeldahl N minus NPN) x 6.25
[c] Error mean square values between groups with 1 degree of freedom
[d] Error mean square values within groups with 6 degrees of freedom

Table E.3 Individual Proximate Analysis, Nucleic Acid, Myofiber Diameter and Protein Fractionation Data for the Semitendinosis Muscle of Pigs from Groups I and III.

			Proximate Analysis			Nucleic Acids			Protein Fractionation				
			%, wet			mg/g, wet			%, wet				
PN	GN	STM	MO	KP[a]	F	DNA	RNA	MFD	MY	SC	NPN	STR	TP[b]
1	I	127.0	78.24	16.82	2.97	.82	2.99	47.5	46.3	23.6	10.5	19.6	15.09
2	I	153.3	78.23	17.36	3.08	.80	3.15	43.3	45.8	22.7	9.5	21.9	15.69
3	I	119.8	77.62	17.24	3.39	.84	3.45	47.5	48.2	25.2	10.4	16.2	15.44
4	I	128.0	79.18	16.70	2.43	.83	2.99	50.1	46.2	24.8	10.2	18.8	14.98
5	III	206.7	76.60	16.14	5.06	.78	4.53	45.2	45.7	20.6	10.0	23.7	14.51
6	III	175.6	78.65	17.08	2.98	.76	3.34	44.8	49.4	22.0	10.0	18.6	15.38
7	III	171.8	79.29	16.44	2.67	.79	3.20	58.2	44.5	22.4	10.2	22.8	14.77
8	III	201.0	79.85	17.68	1.88	.78	2.46	49.2	46.6	21.6	10.0	21.9	15.92
ANOVA													
EMS[c]		6441.125	.405	.073	.110	.004	.112	187.114	.015	11.582	.032	13.714	.046
EMS[d]		262.669	2.762	.338	.295	2.7×10^{-4}	.389	19.268	2.687	.925	.111	5.205	.310
F statistic		24.52	.147	.217	.372	14.708	.288	9.711	.006	12.526	.285	2.634	.148

[a] Kjeldahl N x 6.25
[b] (Kjeldahl N minus NPN) x 6.25
[c] Error mean square values between groups with 1 degree of freedom
[d] Error mean square values within groups with 6 degrees of freedom

Table E.4 Individual Proximate Analysis, Nucleic Acid, Myofiber Diameter and Protein Fractionation Data for the Semitendinosis Muscle of Pigs from Groups IV and VI.

		Proximate Analysis				Nucleic Acids			Protein Fractionation				
		% wet				mg/g wet			% wet				
PN	GN	STM	MO	KP[a]	F	DNA	RNA	MFD	MY	SC	NPN	STR	TP[b]
9	IV	323.0	76.51	17.44	4.00	.57	3.93	63.5	45.5	22.3	10.9	21.3	15.56
10	IV	285.0	77.70	17.05	3.61	.56	4.68	60.4	45.2	22.2	10.8	21.8	15.20
11	IV	341.0	76.71	17.73	3.85	.49	3.41	66.1	45.3	22.3	9.7	22.6	15.88
12	IV	343.0	76.61	17.55	4.56	.66	3.71	64.0	45.7	23.5	11.2	19.6	15.60
13	VI	408.6	76.17	17.22	4.98	.60	3.27	70.1	47.5	21.7	10.4	20.4	15.44
14	VI	464.8	75.20	17.80	5.41	.62	4.37	69.8	46.8	23.8	10.0	19.4	16.04
15	VI	377.6	77.08	16.76	4.25	.58	3.87	80.1	49.9	25.0	10.4	14.6	15.03
16	VI	413.4	76.37	18.63	3.93	.56	2.00	68.1	46.2	24.0	9.4	20.4	16.88
ANOVA													
EMS[c]		1.7×10^4	2.142	.283	.811	9.6×10^{-4}	.616	187.114	.976	1.823	.515	14.112	.123
EMS[d]		1.0×10^3	.953	.712	.306	2.5×10^{-3}	.670	19.269	1.343	1.133	.305	4.519	.724
F statistics		17.092	2.249	.398	2.645	.380	.919	9.711	7.267	1.609	1.687	3.123	.170

[a] Kjeldahl N x 6.25
[b] (Kjeldahl N minus NPN) x 6.25
[c] Error mean square values between groups with 1 degree of freedom
[d] Error mean square values within groups with 6 degrees of freedom

Table E.5 Individual Proximate Analysis, Nucleic Acid, Myofiber Diameter and Protein Fractionation Data for the Brachialis Muscle of Pigs from Groups I and III.

			Proximate Analysis			Nucleic Acids			Protein Fractionation				
			%, wet			mg/g, wet			%, wet				
PN	GN	BRM	MO	KP[a]	F	DNA	RNA	MFD	MY	SC	NPN	STR	TP[b]
1	I	38.9	79.62	16.64	2.75	.97	5.03	45.4	45.3	22.8	9.9	22.1	15.02
2	I	44.6	79.16	17.11	2.67	.88	2.21	43.3	45.2	20.4	9.3	25.1	15.49
3	I	33.2	78.11	17.61	3.27	.99	4.44	38.1	45.5	23.7	9.3	21.5	15.98
4	I	36.9	78.59	17.49	2.78	.93	5.26	43.0	47.3	24.7	9.4	18.6	15.87
5	III	49.0	78.18	15.99	4.60	.96	7.67	42.5	47.0	20.2	10.4	22.3	14.33
6	III	52.8	78.11	16.69	3.89	.87	1.90	42.7	47.0	20.8	9.4	22.8	15.09
7	III	42.2	78.35	17.00	3.10	.97	2.60	43.6	46.5	22.6	9.4	21.5	15.38
8	III	57.4	78.17	17.71	2.72	.96	2.51	46.0	48.2	20.7	9.6	21.5	15.99
ANOVA													
EMS[c]		285.605	3.781	.039	.303	9.0×10^{-5}	.638	58.536	3.474	6.369	.121	.097	.055
EMS[d]		31.988	.312	.448	.851	2.4×10^{-3}	4.570	41.393	.717	2.190	.150	3.699	.434
F statistics		8.928	12.114	.086	.356	.038	.140	1.414	4.842	2.908	.807	.026	.128

[a]Kjeldahl N x 6.25
[b](Kjeldahl N minus NPN) x 6.25
[c]Error mean square values between groups with 1 degree of freedom
[d]Error mean square values within groups with 6 degrees of freedom

Table E.6 Individual Proximate Analysis, Nucleic Acid, Myofiber Diameter and Protein Fractionation Data for the Brachialis Muscle of Pigs from Groups IV and VI.

			Proximate Analysis			Nucleic Acids			Protein Fractionation				
			%, wet			mg/g, wet			%, wet				
PN	GN	BRM	MO	KP[a]	F	DNA	RNA	MFD	MY	SC	NPN	STR	TP[b]
9	IV	94.7	76.85	17.87	4.14	.66	1.95	59.2	47.6	22.9	10.1	19.4	16.10
10	IV	91.9	77.64	17.17	3.45	.64	1.87	58.4	49.2	23.1	10.5	17.2	15.38
11	IV	94.1	76.85	17.96	4.08	.63	1.61	53.4	48.1	25.7	9.6	16.6	16.25
12	IV	98.0	76.08	18.23	4.87	.71	2.36	65.8	44.0	22.4	9.9	23.7	16.43
13	VI	93.2	76.92	16.97	5.06	.74	2.18	68.9	47.7	22.6	10.3	19.4	15.25
14	VI	119.0	75.74	17.09	5.95	.77	2.36	56.3	44.6	25.0	9.1	21.3	15.56
15	VI	127.1	76.09	16.91	5.82	.64	1.99	63.4	47.2	23.8	9.3	19.8	15.34
16	VI	95.5	76.17	17.68	5.16	.66	1.32	59.4	46.1	23.3	9.1	21.5	16.10
ANOVA													
EMS[c]		393.401	1.758	1.072	2.711	.003	4.5×10^{-4}	58.536	1.009	.009	.591	3.336	.642
EMS[d]		146.371	.463	.174	.275	.002	.152	41.393	3.425	1.511	.236	5.721	.188
F statistics		2.688	3.800	6.153	9.848	1.396	.003	1.414	.295	.006	2.500	.583	3.41

[a] Kjeldahl N x 6.25
[b] (Kjeldahl N minus NPN) x 6.25
[c] Error mean square values between groups with 1 degree of freedom
[d] Error mean square values within groups with 6 degrees of freedom

Table E. 7 Individual Muscle Weight and Proximate Analysis Values for the Longissimus Muscle from the Right and Left Sides of Pigs from Groups I, III, IV and VI.

		LDM		KP		MO		F	
PN	GN	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt
1	I	307.9	309.7	17.92	16.65	76.37	77.78	4.71	3.90
2	I	405.1	413.0	18.22	18.68	77.05	76.85	3.73	3.66
3	I	295.6	309.6	17.64	18.52	78.99	74.57	2.11	5.12
4	I	359.3	376.3	18.27	18.09	76.05	75.52	4.65	4.28
5	III	360.4	352.0	16.54	17.13	74.18	76.69	7.02	3.88
6	III	418.3	400.0	17.82	18.33	77.65	77.76	3.80	3.33
7	III	338.3	322.6	18.01	17.53	78.23	78.38	2.61	2.36
8	III	450.0	472.7	19.09	18.49	77.23	77.21	2.82	2.94
EMS		4.85×10^3	2.40×10^3	.022	.022	.171	3.538	.138	2.703
EMS		2.59×10^3	3.46×10^3	2.504	.572	2.504	1.270	2.813	.427
F stat		1.914	.694	.068	.004	.068	2.786	.049	6.323
9	IV	813.0	782.8	18.27	18.10	74.28	74.85	5.73	4.94
10	IV	910.0	888.0	17.11	16.98	75.15	74.86	5.32	5.47
11	IV	712.0	678.3	19.57	19.21	73.28	75.20	6.18	3.90
12	IV	817.0	782.2	18.13	18.12	74.41	74.49	5.69	5.44
13	VI	992.6	975.2	17.34	17.92	74.08	73.90	6.36	5.90
14	VI	903.8	948.8	17.79	18.36	73.30	72.98	7.17	7.06
15	VI	1191.1	1214.9	17.96	17.40	74.70	73.86	5.43	6.29
16	VI	960.7	1008.3	19.85	18.81	74.03	73.18	4.82	6.03
EMS		7.92×10^4	1.37×10^5	.038	.009	.128	3.754	.092	3.823
EMS		1.11×10^4	1.11×10^4	1.018	.597	.460	.152	.596	.404
F stat		7.158	12.354	.853	.001	.277	24.669	.707	9.472

Table E.8 Individual Muscle Weight and Proximate Analysis Values for the Semitendinosis Muscle from the Right and Left Sides of Pigs from Groups I, III, IV and VI.

		STM		KP		MO		F	
PN	GN	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt
1	I	65.0	62.0	16.40	17.26	77.85	78.64	3.63	2.28
2	I	76.5	76.8	17.54	17.18	78.64	77.83	2.67	3.49
3	I	60.2	59.6	17.36	17.12	76.04	79.22	4.65	2.11
4	I	65.5	62.5	16.76	16.63	79.27	79.08	2.41	2.45
5	III	105.8	100.9	15.59	16.71	75.73	77.52	6.59	3.45
6	III	88.6	87.0	16.99	17.17	78.81	78.48	3.17	2.78
7	III	87.9	83.9	16.29	16.60	78.88	79.72	3.08	2.24
8	III	102.9	98.1	17.65	17.72	80.18	79.50	1.71	2.05
EMS		$1.74x10^3$	$1.48x10^3$	.073	$3.1x10^{-4}$	.405	.025	.177	.004
EMS		67.707	64.929	.338	.170	2.762	.709	2.679	.390
F statistic		25.706	22.873	.217	$2x10^{-5}$	.147	.036	.066	.012
9	IV	161.0	162.0	17.84	17.04	76.88	76.14	3.98	4.03
10	IV	142.0	143.0	17.08	17.03	77.97	77.43	3.32	3.89
11	IV	173.0	168.0	18.98	16.44	75.96	77.48	4.04	3.65
12	IV	168.0	175.0	17.45	17.65	76.71	76.51	4.58	4.54
13	VI	207.3	201.3	17.04	17.40	75.63	76.72	5.74	4.20
14	VI	237.9	226.9	17.50	18.12	74.73	75.70	5.87	4.92
15	VI	184.2	193.4	16.65	16.86	77.37	76.81	4.26	4.25
16	VI	211.7	201.7	18.65	18.61	75.65	77.12	4.61	3.22
EMS		$4.86X10^3$	$3.84x10^3$	.283	1.000	2.142	.183	2.599	.029
EMS		334.654	200.338	.712	.422	.953	.414	.458	.316
F statistic		14.511	19.170	.398	2.373	2.249	.442	5.680	.091

Table E.9 Individual Muscle Weight and Proximate Analysis Values for the Brachialis Muscle From the Right and Left Sides of Pigs From Groups I, III, IV and VI.

		BRM		KP		MO		F	
PN	GN	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt
1	I	19.0	19.9	16.37	16.90	80.19	79.07	3.00	2.51
2	I	22.2	22.4	17.38	16.84	79.53	78.79	2.91	2.44
3	I	17.0	16.2	17.69	17.52	78.55	77.64	3.57	2.95
4	I	18.9	18.0	17.40	17.59	79.12	78.04	2.95	2.60
5	III	24.5	24.5	15.9	16.07	77.89	78.48	5.80	3.41
6	III	27.0	25.8	16.96	16.40	77.58	78.67	4.56	3.18
7	III	20.6	21.6	17.14	16.88	78.50	78.21	2.99	3.20
8	III	29.4	28.0	17.84	17.57	77.92	78.43	2.74	2.70
EMS		74.420	68.445	.039	.657	3.781	.008	1.674	.495
EMS		9.392	7.099	.448	.348	.312	.236	1.074	.071
F statistic		7.923	9.641	.086	1.89	12.114	.033	1.558	7.00
9	IV	48.3	46.4	17.68	18.07	77.03	76.67	4.27	4.00
10	IV	47.0	44.9	17.10	17.75	77.74	77.54	3.58	3.32
11	IV	48.0	46.1	17.74	18.20	77.27	76.42	4.12	4.05
12	IV	50.0	48.0	18.20	18.26	76.09	76.06	5.10	4.64
13	VI	47.2	46.0	16.65	17.29	76.96	76.87	5.13	4.98
14	VI	59.0	60.0	16.75	17.43	75.75	75.73	6.36	5.54
15	VI	65.1	62.0	16.75	17.05	75.43	76.66	6.62	4.97
16	VI	47.5	48.0	17.50	17.86	76.24	76.11	5.10	5.21
EMS		81.281	164.711	1.072	.878	1.758	.218	4.712	2.750
EMS		39.901	38.114	.174	.083	.463	.333	.519	.181
F statistic		2.037	4.321	6.153	10.597	3.800	.653	9.085	15.149

Table E.10 Individual Liver Weights, Proximate Analysis and Nucleic Acid Data for Pigs of Groups I, III, IV and VI.

			Proximate Analysis, % wet			Nucleic Acids, mg/g	
PN	GN	LW	MO	KP	F	DNA	RNA
1	I	441.5	72.80	18.29	1.47	4.06	10.67
2	I	466.3	72.31	18.35	1.89	4.40	10.35
3	I	551.2	71.30	17.16	1.51	4.24	10.60
4	I	611.6	72.03	18.56	1.52	3.87	9.64
5	III	633.6	72.61	18.46	1.79	4.23	10.42
6	III	698.9	72.89	17.46	1.34	4.68	9.48
7	III	680.6	72.94	15.62	1.28	4.05	10.93
8	III	583.2	72.11	19.18	1.57	5.44	10.16
EMS		3.45×10^{4}	.556	.337	.021	.417	7.8×10^{-3}
EMS		4.41×10^{3}	.269	1.395	.046	.218	.294
F statistic		7.838	2.070	.241	.453	1.909	.027
9	IV	905.0	72.10	16.87	1.19	3.98	9.96
10	IV	920.0	72.48	18.11	1.57	4.31	9.88
11	IV	1009.0	71.64	18.20	1.71	3.79	9.26
12	IV	1119.0	72.14	17.76	2.06	3.75	9.76
13	VI	1070.3	71.86	17.45	1.36	4.80	10.71
14	VI	1382.0	72.67	19.47	1.77	4.17	9.18
15	VO	1378.6	70.84	19.42	1.66	4.45	10.12
16	VI	1268.6	71.40	19.26	1.75	3.48	9.96
EMS		1.64×10^{5}	.316	2.714	1.25×10^{-5}	.145	.150
EMS		1.55×10^{4}	.359	.656	.083	.190	.246
F statistic		10.573	.881	4.141	1.5×10^{-4}	.762	.609

Appendix Table F.1 Bound and Free Specific Activities of Right and Left Side Muscles[a]

Pig No.	Right Muscle: Bound Specific Activity (SB) dpm/ u mole	Right Muscle: Free Specific Activity (SI) dpm/ u mole	Right Muscle: SB/SI	Right Muscle: K_S[b]	Left Muscle: Bound Specific Activity (SB) dpm/ u mole	Left Muscle: Free Specific Activity (SI) dpm/ u mole	Left Muscle: SB/SI	Left Muscle: K_S[b]
				Longissimus				
17	24.3	2719.0	.0090	4.60	32.1	3056.5	.0105	5.20
18	26.8	2669.7	.0100	5.00	28.8	2530.9	.0114	5.57
19	27.3	2412.1	.0113	5.05	29.3	2313.6	.0127	6.10
20	45.7	4419.9	.0103	5.10	36.3	3488.4	.0105	5.20
21	39.3	3442.9	.0114	5.57	30.6	2566.2	.0119	5.77
22	18.5	3064.8	.0060	3.30	23.6	2792.5	.0084	4.26
23	25.8	4488.3	.0057	3.30	18.8	2860.3	.0066	3.54
24	19.2	2567.2	.0075	3.93	27.1	3379.4	.0080	4.16
				Semitendinosis				
17	46.9	3469.2	.0135	6.43	26.8	2318.0	.0116	5.65
18	23.9	2542.0	.0094	4.74	25.0	2740.3	.0091	4.62
19	45.0	3490.1	.0129	6.18	25.9	2458.4	.0105	5.19
20	47.1	2154.9	.0219	9.86	71.7	3794.3	.0189	8.63
21	21.8	2953.7	.0074	3.89	22.4	2973.8	.0075	3.93
22	41.2	2339.0	.0176	8.10	31.9	1836.1	.0174	8.02
23	21.3	3647.4	.0058	3.22	32.0	3528.7	.0090	4.61
24	34.6	4060.9	.0085	4.36	46.3	4167.8	.0111	5.45
				Brachialis				
17	25.9	2350.9	.0110	5.40	30.9	2614.6	.0118	5.73
18	23.8	2336.7	.0102	5.07	28.1	2525.6	.0111	5.45
19	42.8	2288.6	.0187	8.55	42.5	3082.6	.0138	6.55
20	37.8	2208.9	.0171	7.90	42.8	2885.7	.0148	6.96
21	21.0	2881.1	.0073	3.85	24.1	2613.9	.0092	4.66
22	27.8	2930.6	.0095	4.78	34.6	2966.1	.0116	5.65
23	31.0	3771.0	.0082	4.26	33.8	3394.5	.0099	4.95
24	39.0	3163.6	.0123	5.94	42.7	3176.2	.0134	6.39

[a] R values taken to be 400
[b] K_S values, %/day

Table F.2 Plasma Specific Activities of Infused Pigs from Groups II and V.[a]

Pig No.	Plasma Specific Activities (dpm/nmole) at Various Times (min)																									
	0	10	20	30	40	50	60	70	80	90	100	110	120	135	150	165	180	200	220	240	260	280	300	320	340	360
17	0	3.0	3.8	6.2	8.9	9.3	10.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5
18	.61	5.0	5.9	8.9	10.1	10.7	9.9	9.8	10.2	10.4	11.2	10.6	10.1	10.3	10.8	10.3	10.9	9.2	10.7	11.1	10.3	-	11.1	9.0	9.6	10.9
19	0	2.2	3.9	6.6	10.5	9.9	10.9	10.3	10.3	10.6	10.2	9.0	10.2	10.6	11.2	11.0	10.3	10.2	10.7	10.1	10.3	10.3	10.1	10.1	10.0	10.5
20	0	2.4	4.8	6.9	8.8	10.5	10.2	10.9	10.0	10.5	9.9	10.8	10.9	11.2	11.2	11.3	10.3	10.7	10.0	11.1	10.3	11.2	10.6	10.1	9.9	10.2
21	.25	1.5	2.6	3.6	4.8	-	-	-	-	-	-	9.8	10.5	11.3	-	11.4	10.2	12.6	11.1	10.6	10.3	11.0	11.0	12.3	10.8	10.0
22	.2	.8	1.2	1.0	1.4	1.6	1.5	-	-	2.0	-	2.6	2.3	2.6	-	6.2	5.6	4.3	4.4	5.6	5.7	5.7	5.8	5.2	5.1	5.2
23	0	1.7	-	2.5	3.9	6.2	6.5	7.0	-	7.4	8.2	-	8.1	9.1	-	10.5	10.6	10.9	10.6	9.5	10.5	10.8	10.8	9.5	10.2	10.8
24	.9	6.3	-	6.8	7.0	7.8	-	7.7	-	8.8	8.8	-	-	-	6.0	6.8	7.0	6.4	6.4	6.9	6.0	6.3	6.2	6.2	6.3	6.0

[a] missing samples designated by (-).

Table F. 3 Liver Free and Bound Specific Activities, λ Values and Fractional Synthesis Rate Constants of Infused Pigs of Groups II and V.

Pig No.	Bound Specific Activity (dpm/μ mole)	Free Specific Activity (dpm/μ mole)	S_B/S_I	λ (days $^{-1}$)	k_S
17	119.5	427.1	.280	.080	32.2
18	130.2	971.6	.134	.050	41.0
19	107.6	522.3	.206	.056	29.8
20	155.3	746.6	.208	.072	38.4
21	96.7	779.9	.124	.025	20.0
22	125.0	484.5	.258	.055	23.5
23	98.9	924.3	.107	.022	21.4
24	172.0	807.5	.213	.040	20.3

Table G.1 TI-59 Computer Program for Muscle FSR Values

To Feed In Program					
001	A	026	=	051	RCL
002	PRT	027	+/-	052	17
003	STO	028	INV	053	$\frac{\cdot}{\cdot}$
004	15	029	LNX	054	RCL
005	X	030	+/-	055	18
006	RCL	031	+	056	X
007	09	032	1	057	RCL
008	=	033	=	058	12
009	+/1	034	STO	059	=
010	INV	035	18	060	-
011	LNX	036	RCL	061	RCL
012	+/-	037	10	062	11
013	+	038	-	063	1/x
014	1	039	1	064	=
015	=	040	=	065	PRT
016	STO	041	STO	066	R/S
017	17	042	11		
018	RCL	043	RCL		
019	15	044	10		
020	X	045	$\frac{\cdot}{\cdot}$		
021	RCL	046	RCL		
022	09	047	11		
023	X	048	=		
024	RCL	049	STO		
025	10	050	12		

To Run Program

R value STO 10
t (.25) STO 09
Guess Ks (enter guess in keyboard)
A
Ratio of SB/SF will be printed
Make next guess
Press A
Match up SB/SF with observed.

TABLE G.2 TI-59 Computer Program for Liver FSR Values

To Feed In Program

001	PRT	026	1	051	RCL
002	STO	027	-	052	03
003	03	028	STO	053	−÷−
004	+	029	05	054	(
005	RCL	030	RCL	055	RCL
006	01	031	01	056	01
007	=	032	X	057	-
008	−÷−	033	RCL	058	RCL
009	RCL	034	02	059	03
010	01	035	=	060	)
011	=	036	+/-	061	=
012	1/X	037	INV	062	+/-
013	STO	038	LNX	063	+
014	04	039	+/-	064	RCL
015	RCL	040	1	065	05
016	03	041		066	=
017	X	042	0	067	X
018	RCL	043	=	068	RCL
019	02	044	−÷−	069	04
020	=	045	RCL	070	=
021	+/-	046	05	071	PRT
022	INV	047	=	072	R/S
023	LNX	048	1/X	073	RST
024	+/-	049	STO		
025	+	050	05		

To Run Program

λ_f STO 01
t_s STO 02
Guess K_s
RST (1st time)
R/S
Read Sb/Sf
Make successive guess
Press R/S
Match Sb/Sf with observed

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