QUANTITATIVE CHANGES IN TOTAL NITROGEN AND ITS COMPONENTS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT

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CHAPTER I

INTRODUCTION

In the past years animal scientists have searched for methods of establishing, as early as possible, the growth potential of the meat animal so as to be able to direct the animal's growth pattern during its later life phases. Such directional control would lead to the production of higher quality, meatier beef carcasses, which are becoming more essential in the market place today due to the competition from synthetic meat products. Gross indicies such as rate of gain, cutout value, conformation scores, yield grades and ribeye areas have been utilized and have been instrumental in improving the meatiness and quality of beef animals. Such gross measurements are directly or indirectly affected by the biochemical components which constitute muscle tissue. Thus a more basic understanding of growth in the bovine would seem necessary. To accomplish this a study of the components of the muscle tissue during growth and development of the bovine must be made.

The principal component in muscle tissue which affects muscling or meatiness is protein or more specifically, the protein of the contractile units of the muscle cell. Studies involving quantitative changes in these proteins have been made, yet this work has been done predominantly on rats, chickens, rabbits, pigs and humans. Some generalizations can be drawn from published works on laboratory animals with respect to protein changes during growth and development, however, specific inferences

between such results and the bovine could prove to be extremely hazardous.

In reviewing past literature, very little progress has been made in the analysis of the quantitative changes occurring in bovine muscle tissue during its development. Realizing the necessity of the information obtained from such a study and its implications on muscle growth in the bovine, a growth and development study of the total nitrogen and its components in the muscle cell from near birth to market age in the bovine was initiated.

CHAPTER II

LITERATURE REVIEW

The muscle fiber is obviously composed of many complex entities, however this review will be limited to a discussion of the proteins of the myofibril, sarcoplasm and connective tissue and the various factors affecting quantitive changes in these proteins during the development of the bovine. The review will be organized around two principal areas, muscle tissue analysis and muscle protein composition.

Muscle Tissue Analysis

Sample Procurement

Basically, there are two forms in which a muscle may be taken from the test animal. The first is in the ante-mortem form and the second, and probably most widely used, is in the post-mortem form. The selection of the proper form which a researcher must elicit is dependent upon the ultimate utilization of the tissue and the specific goals of the overall investigation. When it becomes necessary to obtain a sample in the pre-rigor state such that the tissue is in or very near its live physiological condition, a tissue aquisition in the ante-mortem state or immediate post-mortem state is necessary.

Favorable points for tissue aquisition in the pre-rigor, post-mortem form are the ease of muscle excision, elimination of involved sampling techniques, and the procurement of large samples. However, when the

tissue is acquired in such a way, the animal must be sacrificed. When monetary investments in test animals are high, as they are when using bovine, and when consistency in the utilization of the same animals throughout the sampling period is a requirement, then one must employ the use of live animal techniques for muscle procurement. There are definite disadvantages in the use of such live animal methods such as handling of the live animal, use of involved biopsy techniques, increased herd management practices and, as Bray (1963) pointed out, the presence of small sample size. Nonetheless, consistency of sampling, and economy of investments predispose the use of live animal methods.

Several authors have implemented live animal biopsy techniques on livestock and one of the earliest successful investigators was Bray (1953). He studied a method of tissue removal from the live bovine in order that further research could be performed on the same animals. Everett and Carter (1961), Spurlock (1962), Link (1967, 1970a and 1970b), Dikeman (1972) and Schmidt (1972) reported work on biopsy sampling and in all cases the tissue removal resulted in no ill effects upon the animal's health. Minor bleeding of the wound and stiffness of the animal were the only verified traumas which the animal experienced. However, Link (1967) demonstrated that connective tissue and fat would fill the sampling site in the place of the excised muscle, causing no adverse effect on the animal.

As stated earlier, the type of sample and the goals of the experimental investigations govern the form of sample used. In this study of the growth and development of the bovine, live animal techniques were enforced due to the points discussed above.

Freezing and Thawing of Muscle Tissue

Upon the removal of muscle tissue from the live animal, care must be taken to preserve the sample in its "live" condition or as close to it as possible.

Thoennes (1940) as cited by Perry (1950) studied the effect of freezing and thawing on fresh tissue. In his work he used liquid air to cool muscle to subzero temperatures rapidly after slaughter. Such rapid freezing (at scores of degrees per second) insured that the water was solidified immediately with no formation of ice crystals which could damage the internal structure of the cell as is done with slow freezing. Callow (1952) has shown that very quick freezing and holding at very low temperatures would cause very little, if any, structural damage in the protein or fat. This conclusion was reinforced with the work of Meryman (1956) who ascertained that protein damage was a function of time and temperature of freezing. Muscle tissue frozen quickly to a temperature of approximately $-20^{\circ}C$ in a blast freezer was found to result in some structural damage and a decrease in solubility with the number of days in storage according to Luijpen (1957). Similar conclusions were reported by De Fremery and Pool (1960) who also presented data on "thaw rigor". Thaw rigor is a very rapid, hard contracture of muscle when thawed after being stored in the pre-rigor state. Muscle tissue solubility is very low once "thaw rigor" begins. Connell (1960) publishing similar work illustrated that the protein molecule is capable of denaturing in several different ways, all of which tend to induce a state of insolubility.

Deatherage *et al.* (1960) made studies on differing freezing techniques one of which was, as described above, quick freezing in a blast

cooler and another was dry ice and acetone for quick freezing and storage at temperatures as low as -55°C. They found an increase in water holding capacity and no significant denaturation of proteins in the quick freezing with dry ice and acetone. Menz and Lujet (1961), as cited by Lawrie (1966), explained possible reasons for denaturation at higher freezing temperatures as related to lower freezing temperatures by proving that the rate of freezing and the point frozen and held are very important contributory items. They used isopentane (-150°C) for their freezing analysis and said at such low temperatures with such rapid freezing rates, water freezes between the actual filaments and crystalizes to such a small extent that no distortion of structure is observed. Theories of this nature were especially demonstrated by Partmann (1962) showing that with freezing temperatures below -20°C, there was a decrease in the percent insoluble structural proteins. Luyet (1966) outlined several changes caused by freezing and thawing at or around zero °C and these points included a dehydration and shrinkage, an impairment of the physiological functions, a disturbance of the structural framework, chemical alterations, all resulting in a dynamic breakdown of muscle tissue labeled as "thaw rigor" upon thawing.

Borchert and Briskey (1965) realizing the above changes, studied solubility and associated protein properties of fresh muscle tissue frozen instantly in liquid nitrogen. They found no loss of extractibility of the structural protein and large improvement of the emulsification properties of the muscle tissue.

Presently there is a lack of agreement among researchers on the relative amounts of damage to cells by differing rates of freezing. It is of general agreement that in freezing "living" tissue, intercellular

crystal formation is less damaging than intracellular ice development.

Cassens (1970) suggested, that slow freezing has the capacity to localize salts of the cell into highly concentrated areas and could have a denaturing effect on the proteins present. Cassens (1970) also illustrated that storage of fast frozen tissue should be maintained at similarly low temperatures for ice movement and large crystal formation could occur and cause cellular damages while in storage.

Protein Isolation

Sober (1965) has stated that a naturally occurring protein may be defined as a high molecular weight polymer of optically active alpha amino acids connected by peptide linkages. Distinguishing characteristics of proteins are a result of their large size, unique composition, sequences of the amino acids which make up the primary structure and their three dimensional form into which they are folded. Proteins have been recognized and named on the basis of the above stated properties. However, they are also classified according to such characteristics as electrophoretic mobility, ultracentrifugation and sedimentation properties, and solubility. It will be the object in this review to classify muscle proteins according to their solubility properties.

Theory and Mechanism. The proteins of muscle tissue have been classified according to one of the three major types of tissues to which they belong, these subdivisions being of the muscle cytoplasm or sarcoplasm, of the contractile units or myofibrils, and of the cell walls or connective tissues. They are given the name sarcoplasmic, myofibrillar or stroma proteins, respectively. Hamoir (1955) also identified them according to their solubility in salt solutions, the sarcoplasmic protein

being soluble in very dilute salts ($\Gamma/2 = 0.15$) but not completely in water, the myofibrillar proteins soluble in strong salt solutions ($\Gamma/2 = 0.50$) but not in highly concentrated salt solutions, and the stroma protein being insoluble in a salt solution. One can surmise from Hamoir's conclusions that it is well known that the solvent action of the salt solution on muscle protein is proportional to the ionic strength, μ, where $\mu = 1/2\ \Sigma cv^2$, where c is concentration or molarity and v is the valence. However, this is true only up to the point where "salting out" occurs.

Explanations of such protein solubility in salt solutions have shown that electrolyte ions do affect protein solubility. In small amounts they act to shield the protein molecules from each other by coming between opposing charges and interacting with them thus causing the protein to become soluble in the solution due to the bonds made with the salts of the solution. Young (1963) demonstrated this increase in solubility due to weak salt solutions is a phenomenon called "salting in".

Proceeding farther, we see that if high concentrations of salt is added to a protein solution, the solvent becomes organized about the salt ions to such an extent that the normal organization around the protein is decreased. This appears to result in a type of dehydration leaving the protein molecules to associate in a "solid phase". In such a case, precipitation of the protein occurs. This phenomenon is referred to by Green and Hughes (1955), as "salting out".

Solubility or the degree of solubility is a function of many variables. Sober (1965) listed these variables as concentration of ion used, type of ion used, hydrogen ion concentration (pH), and temperature. The concentration of the ions used has previously been discussed, thus, no

further time will be devoted at this point.

The type of ion present has a definite effect on the solubility of the protein. Dubuisson (1950) stated that one should avoid the use of electrolytes which are known to impair the polymerization or solubility of proteins. Dubuisson also illustrated that calcium or any of the heavy metals would impair the solubility of proteins. He concluded that the use of naturally occurring ions such as K^+, Cl^-, HCO_3^-, HPO_4^{--}, or $H_2PO_4^{--}$ would be preferred for extraction purposes. Dixon and Webb (1961) presented similar findings and advocated the use of phosphates in the extracting medium and as buffers. They reported phosphate buffers provide good separations, they do not interfere with nitrogen determinations, and are quite soluble providing the correct cations are chosen. Moreover, phosphates are most effective in the useful pH range of fractionation procedures. Here, again, the cation should be chosen carefully, for example, sodium phosphate, when at high ionic strength, is much more acidic than potassium phosphate buffers of the same composition (Dixon and Webb, 1961).

The pH is a very important factor in fractionation of muscle proteins. Proteins have a large number of titratable groups present (approximately 50 to 60 per 100,000 molecular weight). These titratable groups have the ability to react with the solvent and form hydrogen bonds. Since these groups are of different charges (COO^- and NH_3^+), they have the ability to form a salt bond. At any given pH, they will show a net charge, however, there will be one pH at which the negative and positive charged groups will exhibit a net charge of zero. At this point, (the isoelectric point), the protein exhibits the least solubility. Young (1963) indicated that it is at this point that solubility is

lowest thus indicating the use of isolation techniques at pH's above or below the isoelectric point. Also, since certain proteins are very susceptible to denaturation at very low or very high pH's, most fractionation techniques utilize a mammalian tissue physiological pH of 7.0 to 7.5.

The temperature of the extracting medium and environment influences protein solubility. Within a range of approximately $0^{\circ}C$ to $40^{\circ}C$, most proteins will increase in solubility with increasing temperature. This is a result of the increasing activity of the functional groups. Not withstanding, most muscle proteins are heat labile at temperatures greater than $5^{\circ}C$. Scheraga (1963) has shown that above $40^{\circ}C$ most proteins become unstable and tend to denature. As this happens, solubility is lowered. Sober et al. (1965) stated that in order for fractionation procedures to be reproducible, the temperature must be stated and must remain constant.

Hamoir (1955) showed that not only the previously mentioned factors but also factors such as physiological state of the muscle type of solvent, time of extraction and rate of extraction, all have a definite affect on the solubility of a specific protein. Sayre and Briskey (1963), in their work with protein solubility and the physiological conditions of the muscle, studied sarcoplasmic and myofibrillar protein solubilities immediately after slaughter, at the onset of rigor, at the completion of rigor, and at 24 hours post-mortem in muscles exhibiting a wide range of physiological conditions. They found that the solubility of the muscle proteins was highly affected by variations in pH and temperature at the onset of rigor. Myofibrillar protein solubility ranged from no reduction in the first 24 hours when pH remained high at the onset to 75 percent

reduction in solubility in the muscle having a low pH and high initial temperature. Also, the sarcoplasmic protein solubility at 24 hours was decreased to 55 percent of that observed at zero hour in muscles exhibiting high temperatues and low pH at rigor onset. They also stated that the pH at 24 hours had little effect on protein solubility.

Dubuisson (1950), in his discussion of length of extraction time, displayed data showing its positive affect on protein recovery. He pointed out that the extraction was used to disintegrate the structure of the proteins in such a way to expose them to a solubilizing salt medium. Proteins showed an increased extractibility with increased extraction time up to a point at which time the proteins were transformed to less soluble configurations which resulted in decreased protein extractability. Similar results were reported by Hamoir (1955).

Dubuisson (1950) pointed out that the degree of agitation, or rate of extraction, is a very important factor when fractionating proteins. He explained that one must be very careful when agitating salt solutions, especially high ionic strength solutions, for if the homogenizer is set too fast, frothing may occur which tends to denature some proteins due to their surface active properties. Thus, the technique should be utilized so that this type of denaturation does not occur.

The effect of the solvent on protein solubility is of primary importance in protein separation. An example of such an effect was stated by Young (1963). Water-miscible neutral organic solvents, such as ethanol or acetone, decreased the solubility of most proteins in water. This solubility decrease will cause a precipitation of the proteins from the solution. Protein solubility at some pH and ionic strength is a function of the dielectric constant of the aqueous medium and the addi-

tion of the solvent has the tendency to decrease the hydration of the ionic groups. Since these solvents have a lower dielectric constant than water, the decrease in hydration due to their addition decreases the repulsive forms between two like charges. The solvent's addition decreases the protein molecule's mutual repulsion and promotes protein coalescense. In so doing, the proteins precipitate from solution. This denaturation, coagulation and precipitation is partially the mechanism behind trichloroacetic acid (TCA) precipitation of proteins.

Fractionation Procedures. Isolation of protein fractions have, for quite sometime, been the basis for muscle tissue analysis. Furth (1895), as reported by Robinson (1952a), made systematic studies on muscle tissue and was one of the first to initiate such investigations. Also very important ground work for tissue isolation was established by Bate-Smith (1934) when he published a process for separation of the intracellular proteins (soluble in water and/or salt solutions) and extracellular proteins (insoluble in water and salt solutions). Meyer and Weber (1933), as reported by Bate-Smith (1934), also reported similar procedures but found a globulin which had been unreported. This globulin was soluble in very dilute salt solutions and was labeled as one of the proteins of the myogen (sarcoplasmic) fraction. Later Bate-Smith (1937) published a slight revision of his earlier procedure and confirmed Meyer's globulin "x".

The above cited references are more or less the basis upon which much of the protein partitioning has been done in the last several decades. These procedures or modifications of these procedures have been used to extract certain fractions, certain protein complexes, individual proteins, or to quantitate total protein. The procedures which quanti-

tate total protein are of primary importance in this review and will be discussed from the very early techniques to some of the most modern.

Meyer and Weber (1933) in their initial work quantitated nitrogen from the various fractions (stroma, myosin, globulin x, and myogen) from both red and white muscle and obtained fairly good results. Later Smith (1937) and Reay and Kuchel (1936), as cited in Bailey (1944), reported similar data on rabbit and fish, respectively. Bailey (1944) summarized earlier researchers' procedures and presented a modified procedure with the better techniques obtained from each of these earlier authors. Herrmann and Nicholas (1948) used a technique very similar to Bailey's method in which the muscle proteins were separated into fractions insoluble in strong salt solutions, soluble in strong salt solutions, and soluble in weak salt solutions. In this procedure, potassium chloride was used as the extracting salt. A similar extraction technique was reported by Dubuisson (1950) but sodium was used in the place of potassium. Csapo and Herrmann (1951) worked with chick muscle tissue and quantitated a total protein value from the subdividions which were made by a glass homogenizer. Their work with the glass homogenizer was quite different for most of the earlier researchers had used fine, sterile sand to pulverize or grind the muscle with the buffer using a mortar and pestal.

Robinson (1952a) presented a fractionation procedure which helped to standardize protein isolation (Figures 1 and 2). Fraction one consists of the sarcoplasmic proteins which includes the enzymes of the glycolytic pathway. Fraction two contains the myofibrillar proteins but may contain deoxyribonucleo proteins of the cell. Fraction three consists of denatured or native insoluble proteins such as actomyosin.

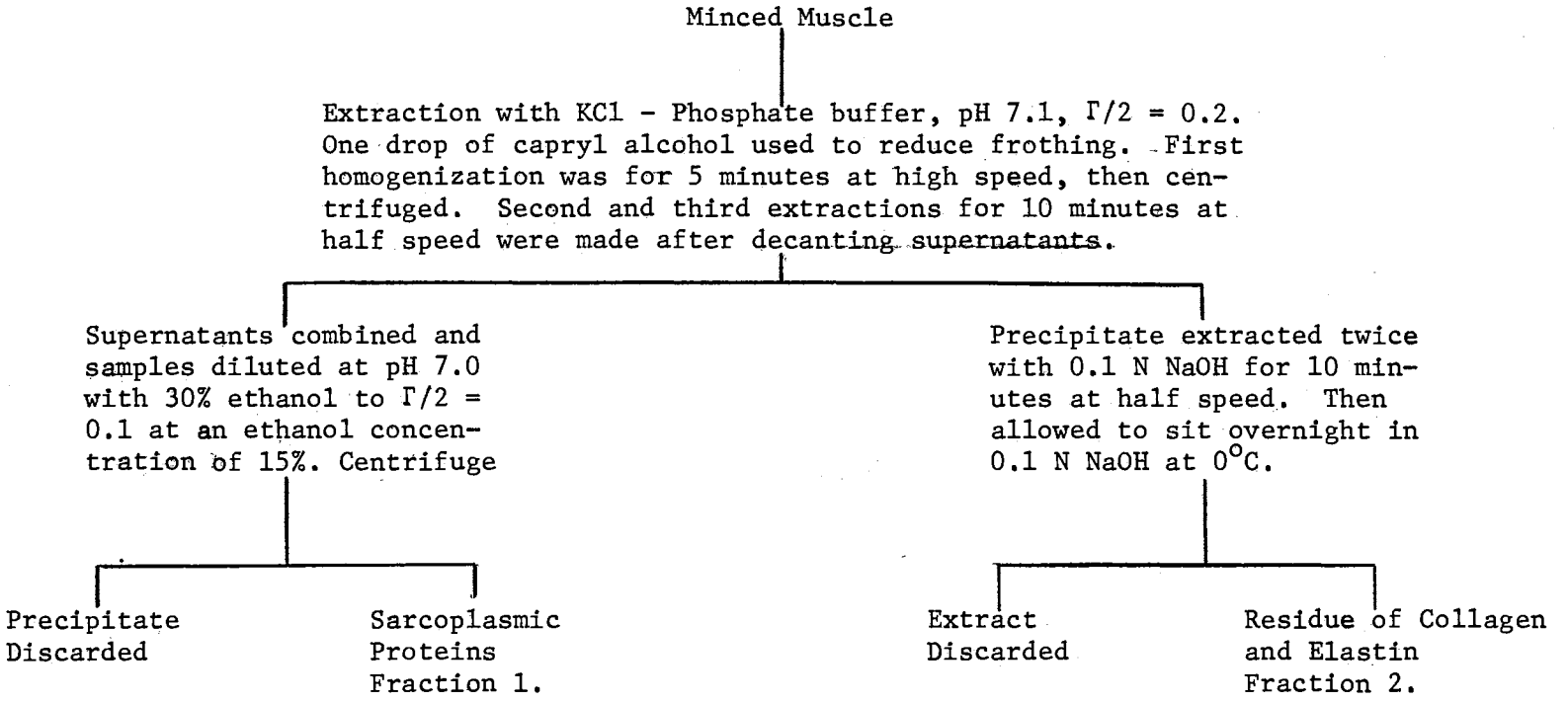


Figure 1. Scheme Showing Fractionation Achieved With Dilute KCl - Phosphate and 0.1 N NaOH Solutions, A, From Robinson (1952a)

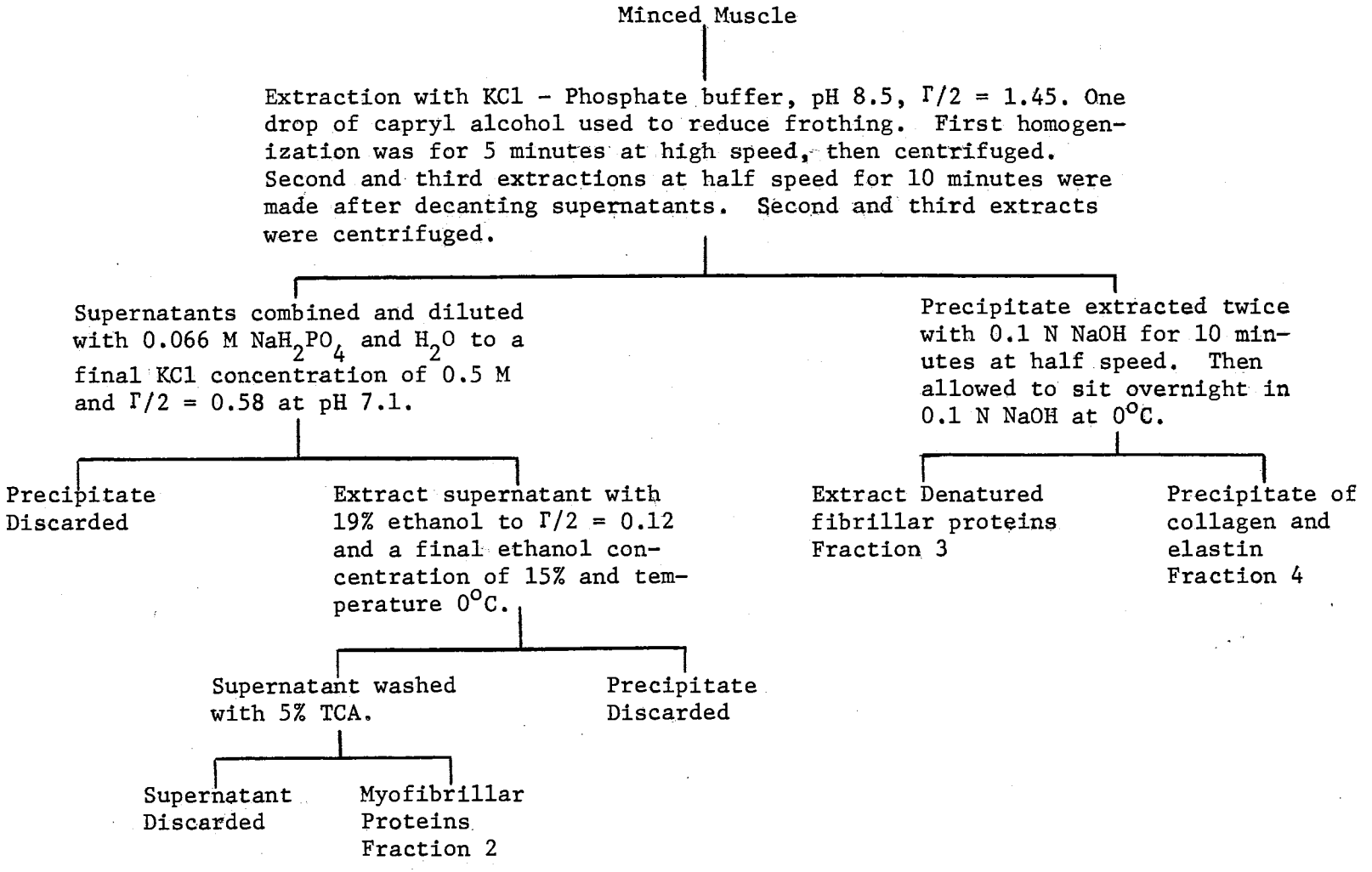


Figure 2. Scheme Showing Fractionation Achieved With Dilute KCl-Phosphate and 0.1 N NaOH Solutions, B, From Robinson (1952a)

Also according to Dische and Osnos (1950), as stated by Robinson (1952a), the mucopolysaccharides would probably be extracted here. Fraction four consists of extracellular proteins, reticulin, elastin and collagen. As can be noted in the flow diagrams, there are two positions in which there is part of the extract discarded. With this type of situation, quantitation is difficult to accomplish, nonetheless, the overall procedure was instrumental in presenting a routine fractionation procedure. Robinson (1952a) also made some analysis on the type of blender used in isolation procedures. Differences in results were tested on the Latapie Mincer followed by the glass homogenizer, a treatment of sand grinding after mincing with scissors, using a stainless-steel homogenizer, and using a Waring Blender. He stated that best results were obtained from the stainless steel homogenizer.

One of the most reliable quantitative protein isolation procedures was presented by Helander (1957). In his procedure, muscle was partitioned into sarcoplasmic protein, myofibrillar protein, stroma protein, and non-protein nitrogen. By this method, the proteins were exhaustively extracted from muscle tissue by continuous extractions with a dilute salt solution and then a strong salt solution which upon centrifugation at the proper steps divided the tissue into its 3 basic components. Trichloroacetic acid was used to precipitate the protein nitrogen from the non-protein nitrogen (NPN) in each fraction to give a quantitative total NPN fraction. Helander's procedure can be seen in Figure 3. Weinberg and Rose (1960), Dickerson and Widdowson (1960), Helander (1961), and Dickerson (1960), all presented in summary a method of muscle isolation but were either modifications of earlier procedures or used, as did Helander (1961), the exact procedure of such previous articles. Khan

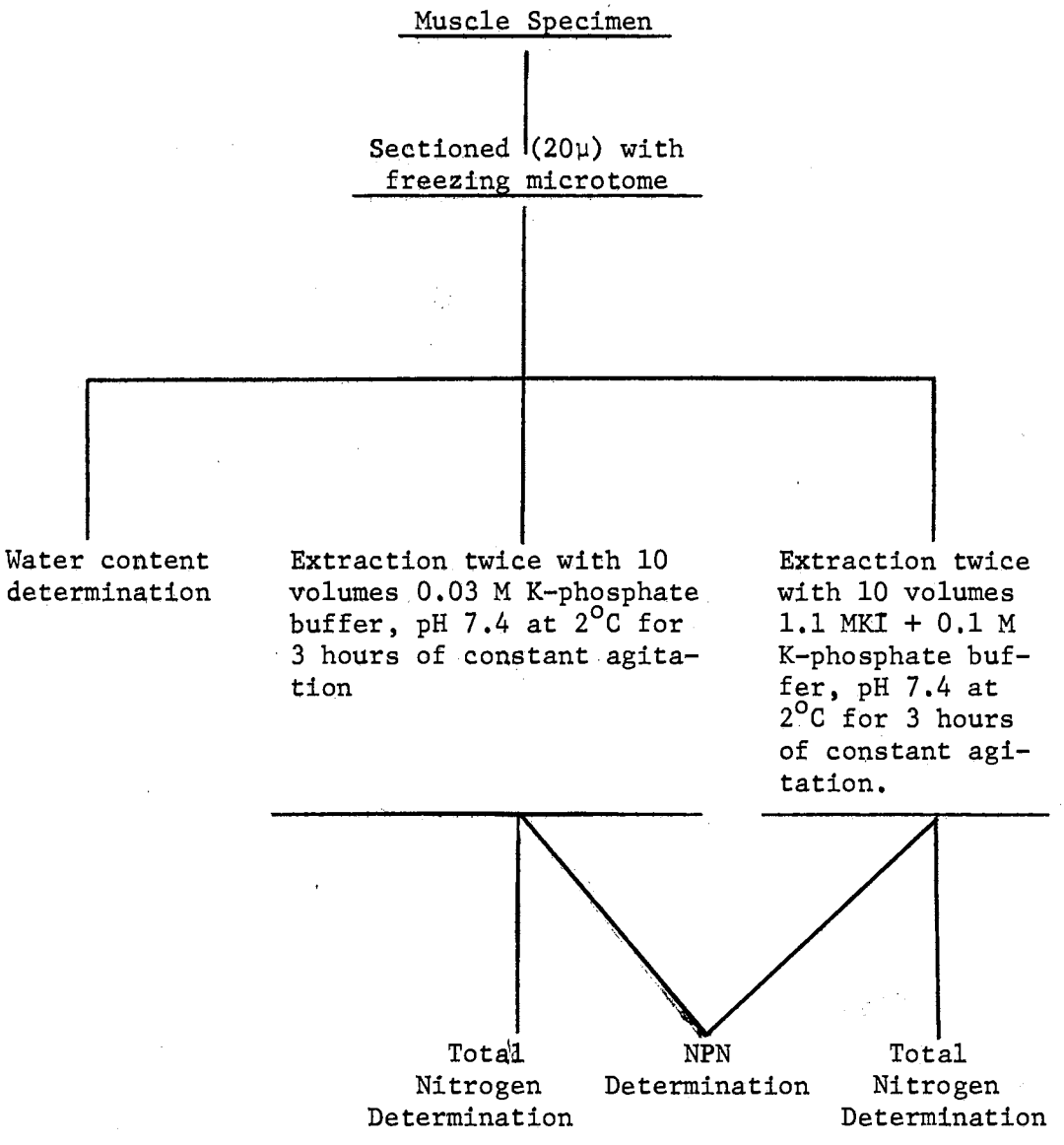


Figure 3. Procedure for Quantitative Nitrogen Determination. From Helander (1957)

(1962) reported a partitioning procedure in which he took modifications of several of the earlier procedures and incorporated them into a very functional quantative outline. Khan and Vanden Berg (1964) published data utilizing the procedure of Khan (1962), Borchert and Briskey (1965) and Sayre and Briskey (1963) presented information utilizing Helander's (1957) procedure. Hegarty, Bratzler, and Pearson (1963) utilized a procedure developed by Seagram (1958) and Turner and Olson (1959), however, it is clear upon its appraisal that due to several discarded fractions, quantitation would be rather difficult. Yamatani and Kandatsu (1967) used a modification of Robinsin's (1952a) technique utilizing an exhaustive extraction method. Rickansrud (1969) developed, in this laboratory, a method for isolation of myofibrillar proteins and sarcoplasmic proteins and with the work of Helander (1957), Robinson (1952a) and Rickansrud (1969) in mind this author was able to develop a satisfactory quantitative method of protein isolation which will be discussed below.

Nitrogen Determination

Protein nitrogen can be determined in a number of ways including the turbidimetric, biuret, and Lowery methods according to Layne (1957), micro and macro Kjeldahl methods and absorbance at 280 nanometers. The spectrophotometric methods of protein determination are very fast and easy to use, but a major disadvantage of these methods is that, in order to obtain a reading or value for estimation of protein concentration, the protein must be in solution. In quantitative protein fractionations the generation of such insoluble constituents such as the stroma proteins makes it very difficult to use such colorimetric determinations.

The micro and macro Kjeldahl methods will give a precise account of

the protein regardless of its physical state. It should be noted, however, that the Kjeldahl analysis is only as accurate as the protein isolation technique allows. Bradstreet (1965) and Young (1963) pointed out that investigators making quantitative fractionations use the Kjeldahl method due to its completeness and accuracy. Disadvantages of utilizing the Kjeldahl methods result from the necessity of preparation of standard acids and basis and the volume of reagents used if many determinations are made. Also length of analysis time is a disadvantage unless facilities are efficiently located in the laboratory. It is of importance to note that all nitrogen in the sample will be quantitated, thus necessitating the use of separation of the tissue initially into protein and non-protein nitrogen components.

Muscle Composition

Composition and Structure

Due to the nature of the material in this thesis, a brief review of muscle composition and structure is necessary before a discussion of muscle constituents is initiated. In view of the method in which the following discussions are presented, the review of muscle components is divided into the major fractions of muscle tissue, that is, proteins of the sarcoplasm, myofibrils, and connective tissues with a brief discussion on protein-lipid complexes.

Sarcoplasmic Proteins. As illustrated earlier, the sarcoplasmic proteins are those which are extractable in weak (low ionic) salt solutions. These proteins make up approximately six percent of the typical adult mammalian muscle after rigor but prior to degradative changes as

shown by Helander (1957). Walls (1960) has shown that the sarcoplasm is usually referred to as undifferentiated protoplasm of a semifluid consistency in which are embedded the structural units of the cell. The major protein constituents of this fraction amount to about 50 components, according to Lawrie (1966), with twenty or so being the glycolytic enzymes which range in molecular weight from 30,000 to 100,000 with isoelectric points of most around pH 6.8, but ranging from pH 6 to pH 7 as shown by Bendall (1964). Also within the weak salt extract are mitochondrial fragments and particles of the sarcoplasmic reticulum. Cassens et al. (1970) demonstrated that the mitochondria has as its function in the muscle cell to recover energy contained in foodstuff and to convert it by phosphorylation into the phosphate bond of ATP. The sarcoplasmic reticulum could be seen as having a three dimensional form with a complex arrangement of tubules and vesicles that run in a longitudinal direction with the fiber and completely surround each fibril. Transverse tubules interrupt the regular arrangement of the longitudinal system once every couple of sarcomeres as shown by Bodwell et al. (1970). Bendall (1970) stated that the transverse tubules had no direct connection with the longitudinal system and were usually not included as part of the sarcoplasmic reticulum. The longitudinal tubular system ("L" system) as stated earlier completely enwraps the fibril and in mammals runs from A-I band junction to A-I band junction at terminals called triads. Bendall (1970) verified that at these triads, the "L" system and the "T" system (transverse tubular system) intersect. The function of the sarcoplasmic reticulum is to conduct impulses from nerve endings to the "T" system which relays the message to the "L" system which, in turn, stimulates the muscle fiber to work. A complete explanation of

such functioning is not within the limits of this review. Of course, included in the sarcoplasmic fraction is the myoglobin and hemoglobin which are the respiratory pigments of the muscle. These compounds play a minor role in the percentage of the sarcoplasmic proteins (Lawrie, 1966) if the animal to be used is slaughtered and bled before taking the sample. If the animal has not been exsanguinated, then the hemoglobin content will be relatively higher.

Bodwell (1970) pointed out that the proteins of the soluble sarcoplasm are heat labile and care should be taken to eliminate denaturation processes during experimental analysis.

Myofibrillar Proteins. Myofibrillar proteins have been referred to as those proteins soluble in high ionic strength salt solutions. The myofibrillar proteins are separate in chemical make-up but are classified as a group because of their insolubility at cellular ionic strength according to Briskey et al. (1971) and degree of maximum solubility at ionic strength greater than 0.15 especially 0.35 and 0.50 as concluded by Dubuisson (1950). Helander (1957) has shown these proteins compose about 11 percent of the total composition of a typical adult mammalian muscle after rigor but prior to degradative post-mortem changes. Briskey and Fukayawa (1971) pointed out that the major proteins which have been isolated from the myofibril include myosin by Szent-Gyorgi, 1941-1943, actin by Straub in 1942, tropomyosin by Bailey in 1948, α-actinin by Ebashi and Ebashi in 1965, β-actinin Maruyama in 1965, and troponin by Ebashi and Kodama in 1968.

Myosin is the major component of thick filaments of striated muscle tissue. It has a molecular weight of about 500,000 (Bendall 1964) but the reported molecular weight varies with the reference which one cites.

Bate-Smith (1937) proved that myosin's isoelectric point is around 5.4 to 5.5. Myosin's importance to the muscle system lies in the fact that it has great adenosine triphosphatase (ATPase) enzymatic activity which is activated by calcium ions and inhibited by magnesium ions. The most important modifier of myosin ATPase activity is actin (Bendall, 1970). At low ionic strength, in the presence of actin, magnesium ions activate ATPase activity to almost the same extent as calcium ions (Bendall, 1964). Actin in its monomeric state is of a globular nature and is thus called globular actin (G-actin). Hayaphi et al. (1964), as stated by Briskey and Fukazawa (1971), demonstrated that it has a molecular weight of 50,000, an isoelectric point of pH 4.7 - 4.8 and is globular in nature. When neutral salt is added to G-actin, it has the ability to polymerize to its fibrous ("F") form. Briskey et al. (1971) has shown that F-actin consists of a double helix each of which consists of globular monomers of G-actin.

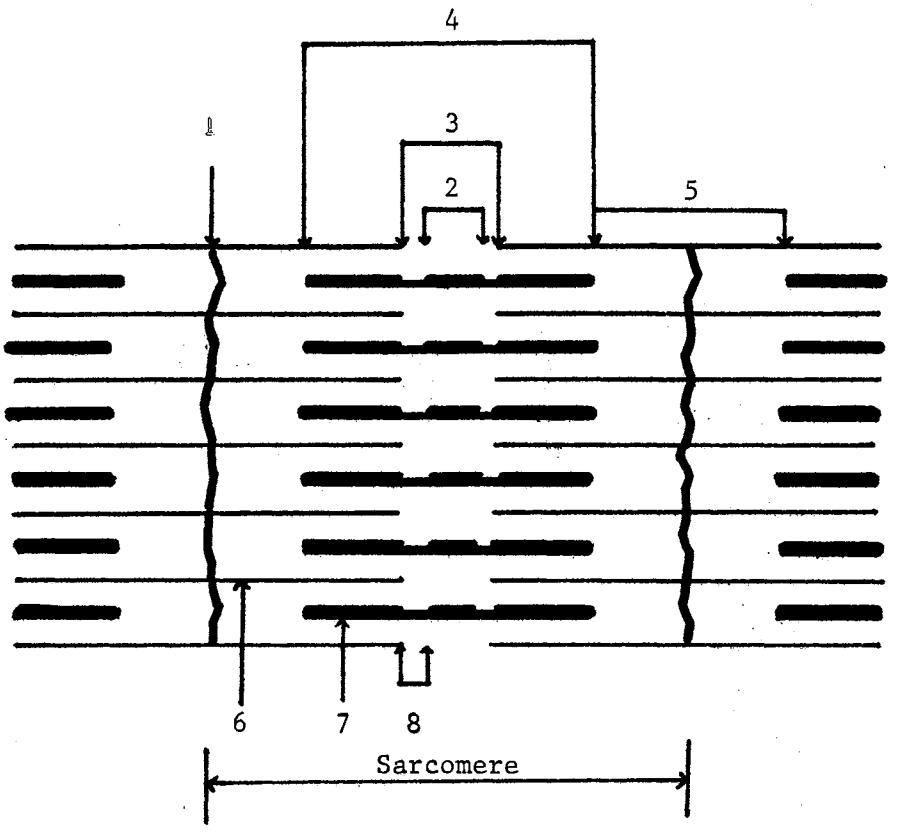
Briskey (1971) pointed out that actin and myosin make up the majority of the fibrillar proteins but other very important proteins exist there and are essential for muscular activity. Tropomyosin or tropomyosin-B (after Bailey, who first isolated it) is a very similar protein to myosin in its isoelectric point and solubility properties and amino acid composition. However, unlike myosin, it has no ATPase activities and, unlike actin, it decreases in viscosity with increasing salt concentrations. Bailey (1948), as cited by Briskey et al. (1971), said it could be considered a new fibrous protein since it differed so much from actin and myosin. Tropomyosin has a molecular weight of about 70,000, an isoelectric point of 5.1, and 94 percent helical as compared to 56 percent for myosin and 30 percent for actin. Briskey et al. (1971) has

shown that a very unique property of tropomyosin is its ability to form three dimensional crystals. Bodwell et al. (1970), verified that tropomyosin exerts its influence on the myofibril by adding stability to the fiber plus its necessity for muscle contraction and Bendall (1970), pointed out that tropomyosin supplied a code limiting the length of the actin chain with the aid of troponin.

Troponin has a molecular weight of 50,000, and lies in close association with tropomyosin on the actin filament. It has a unique calcium binding property which is not modified by ATP, actin or myosin. According to Briskey (1971), troponin's influence on the fibril can be exerted by the binding of calcium, by exerting a conformational change on the structure of actin through tropomyosin or by undergoing a conformational change itself.

The last important functional proteins of the myofibril which will be discussed are the actinins (alpha and beta). β-actin at present has no established specific function but several factors are summarized by Bodwell et al. (1970) for α-actinin. It appears to have a regulatory function in the muscle as demonstrated by Maruyoma (1966) and the amino acid properties of both α and β actinin are similar to actin. α-Actinin has the ability to stop F-actin gelation and to initiate a precipitating effect. It also has the ability to accelerate G-actin polymerization to F-actin. Briskey et al. (1967) and Goll et al. (1964) stated that the actin filaments (thin) are probably cross linked by α-actinin in or near the Z disc in the myofibril.

The organization of the myofibrillar proteins can be summarized into a structural picture for better understanding (Figure 4). The protein of the thick filament is myosin and the proteins of the thin fila-



1: Z-line (tropomyosin, troponin, α-actinin)
2: M-line
3: H or pseudo H zone
4: A band
5: I band
6: Thin filament (F-actin, α-actin, β-actinin, tropomyosin, troponin)
7: Thick filament (myosin)
8: L-line

Figure 4. The Structure and Components of the Contractile Proteins of the Sarcomere

ment are F-actin, tropomyosin, and troponin. α-actinin appears to be associated with the Z disc material. When a muscle is stimulated the thick and thin filaments slide past each other by a making and breaking of bonds on the active sites of the myosin and actin proteins causing contraction to occur. Upon a second stimulation the reversal of this process takes place for relaxation to occur. During rigor, a contracture of the muscle takes place but depletion of ATP will not allow relaxation to occur.

Connective Tissue. Connective tissue protein, generally referred to as stroma protein, is thought to be insoluble in salt solutions (Lawrie 1966). Connective tissue makes up about one percent of the muscle cell of a typical mammalian adult post rigor but prior to degradative changes according to Helander (1957). It consists of formed elements such as collagen, elastin, and reticulin and it includes a ground substance in which the formed elements are embedded. Seifter and Gallop (1966) have shown that collagen is probably the most abundant tissue in the body usually representing up to 20-25 percent of the body's total protein. Collagen is composed of fibers which are straight, non-stretching, non-branching and white in color. Elastin, which is yellow in color, has fibers which are elastic, or extensible, and form a lacelike branching network, makes up very small quantities of the total connective tissue in mammals except in walls of large arteries and in ligaments. Elastin cannot be broken down by heat where as collagen can. Collagen fibers show a regular pattern of structure but elastin fibers are very random in their orientation. Collagen can be associated with providing rigid form for a body but elastin is usually associated with providing a structural support such as the case with ligaments.

Recticular fibers are the least understood of the connective tissues. These fibers are small, delicate, wavy, and do possess some degree of branching. These fibers are closely associated with the muscle fiber. Bodwell et al. (1970) indicated that they appear to form a network between the muscle fiber membrane (sarcolemma) and the collagen and elastin which surround each fiber. The connective tissues of the body has as its function to maintain body form. The skeletal system, of course, holds the body erect but the connective tissues attach the muscle systems to the skeleton so as to retain form. Also, the connective tissue help direct the force of muscular contraction and relaxation toward a given force.

The last proteinaceous substance to be discussed will be the lipid-protein complex which is most often associated with mammalian blood plasma. Formation of such compounds occur when polar lipids are synthesized and introduced into the bloodstream. Hanahan (1962) indicates that here they associate with specific proteins and with non covalent linkages form the specific lipo-protein. Fisher and Gurin (1964) and Marsh (1963), as cited by Cornwell (1964), presented data showing positive evidence for covalent linkages between the protein and lipid in the complex. Young (1963) and Cornwall (1964) stated that the type of linkages between the lipid and protein moities were not known at this time. Hanahan (1962) concluded that however closely bonded the linkages may be, this complex acts as a transportation media of lipids from the small intestine via the blood albumin to the liver and from the liver to the fat deposits and other tissues.

The lipo-proteins are classified according to their densities from very light-lipo-proteins (chylomicrons) to very high density lipo-pro-

teins. Cornwall et al. (1964) has shown protein content of these lipo-proteins to range from two percent in the chylomicrons to 50 percent in the very high density lipo-proteins. The structure of the lipo-protein is not completely clear, however, Hanahan (1962) states that apparently the protein moiety is located on the outer surface where it forms a thin hydrophilic coat around part of the micellular lipid structure. He stated that it is presumed that the hydrophilic phospholipid molecules are oriented toward the water phase.

Bierman (1965), as cited by Emery (1969), reported that because of their low densities, lipo-proteins can be separated from protein extracts after centrifugation because of their flotation properties. Bierman also indicated that, by this method, contaminating serum proteins can be removed from an extract. Apparently the complex is a very stable one due to the total removal of the lipo-protein from an isolated fraction with extracting solutions (Cornwall et al., 1964).

Besides the previous protein substances which have been discussed that are found in the muscle cell, there is one subsubstance which is nitrogenous in nature but non-proteinaceous. This substance, or group of substances is called, appropriately, non-protein nitrogen (NPN). According to Dickerson and Widdowson (1960), it is the only nitrogen in the tissue which is present in true solution. Substances which are classified as NPN in nature and found in the muscle cell are urea, creatin, purines, amino acids, and some polypeptides. These substances are separated from the protein fraction by trichloracetic acid precipitation of the true protein components.

Developmental Protein Changes

This review of quantitative changes in proteins during development will be discussed according to the changes made in the major components, that is, sarcoplasmic, myofibrillar, stroma proteins, non-protein nitrogen, and the lipo-proteins.

Sarcoplasmic Proteins. Herrmann and Nicholas (1948) traced changes in the protein development of rat tissue from intrauterine life to 24 days postpartum. They found sarcoplasmic proteins to increase from about eight days prior to birth to about eight days after birth at which point they leveled off until the 24th day and at such time they started a decrease in their quantity. Robinson (1952a) concluded that the sarcoplasmic proteins increased from prior to hatching all the way through the growth period of chick muscle. This conclusion was based on the fraction being expressed as a percent of the fresh weight. However, when the fraction was expressed as a percentage of the total nitrogen, its values decreased throughout the embryonic period and after hatching, it made a slight increase and then leveled off rapidly. Sarcoplasmic protein changes in cattle with age was investigated by Helander (1957). His work demonstrated very little change in the sarcoplasmic protein from a late gestation period (seven months) to immediately after birth (seven days). However, a dramatic increase was noted up to about six weeks of age at which point a slight decrease and plateauing effect was realized. These values were based on protein nitrogen per gram of tissue. Dickerson and Widdowson (1960) reported data on the quantitative changes of human and porcine tissue with age. Their finding shows very little change in the concentration of sarcoplasmic proteins in man, but

in the pig there was a slight decrease prior to birth. In the growth period after birth the sarcoplasmic proteins increase both in human and porcine tissue. These conclusions hold true whether expressing the protein nitrogen either on the weight of the sample or on percentage of total nitrogen. Similar data reported on chick muscle by Dickerson (1960) indicate that sarcoplasmic proteins increased from birth until four weeks of age. At this time the values plateaued and an almost adult composition was reached. As in previous reports, this held true whether expressed on a tissue weight basis as on a percent of total nitrogen basis.

A summary of the sarcoplasmic protein changes of the various authors reviewed here are presented in Table I. Trends of sarcoplasmic changes can be compared between the species.

In the above discussions, changes associated with age were reviewed. At this time it is essential for a review of changes in sarcoplasmic protein composition with various states of activity. Helander (1961) published data illustrating extensive work with effect of exercise and restricted exercise on quantities of sarcoplasmic proteins in guinea pigs and rabbits. The results of his work with guinea pigs showed no significant difference in normal and exercised animals but there was a definite drop in the quantity of sarcoplasmic proteins expressed on a mg per gram of wet tissue basis. In his work with rabbits he presented data which illustrated significant increases in the amount of sarcoplasmic proteins with restricted activity. This increase was very pronounced when inactivity was induced for three years. He concluded that restricted exercise causes reduction in myofilimental density and such vacated space in the fiber is filled with the sarcoplasm. This is a re-

TABLE I

CHANGES IN MYOFIBRILLAR AND SARCOPLASMIC PROTEINS IN MAMMALIAN SKELETAL MUSCLE WITH AGE

Species	Reference	Immature		Mature	
		S.P.[2]	M.P.[3]	S.P.	M.P.
Human[4]	Dickerson and Widdowson (1960)	3.2	11.2	4.3	12.8
Pig[4]	Dickerson and Widdowson (1960)	3.5	10.6	5.2	12.8
Cattle[4]	Helander (1957)	5.0	8.4	5.3	13.0
Rat	Goldspink (1962)	4.1	10.0	5.2	11.2
Pig[5]	Sink and Judge (1971)	3.3	8.5	4.2	7.6
Chicken[4]	Khan (1962)	5.9	10.6	5.4	9.5
Chicken[4]	Dickerson (1960)	6.6	16.9	11.0	19.4
Cattle[6]	Lawrie (1961)	6.2	15.4	8.9	19.0

1. Values expressed as grams of protein per 100 grams of tissue.
2. Sarcoplasmic Protein
3. Myofibrillar Protein
4. Fresh tissue basis
5. Fat-free, moisture-free basis
6. Fat-free basis

versal of a normal growing and working fiber. Van Linge (1962) determined in his work that strenuous exercise caused an increase of sarcoplasm, not necessarily the sarcoplasmic proteins, whether it be from untrained muscle or trained muscle. Lawrie (1963) pointed out that this may be explained by a necessary increase in myoglobin in exercised muscle tissue and, in which case, cause an increase in the sarcoplasm function.

A point made by Dubuisson (1950) may be cited here while discussing exercise. He performed work on quantitative extraction of rabbit muscle in its normal state and in fatigued and contracted states. He concluded that no difference could be found in the sarcoplasmic proteins between the normal muscle state and fatigued muscle. Similar conclusions were made between the rested and contracted states.

Myofibrillar Proteins. According to the data submitted by Herrmann and Nicholas (1948), myofibrillar protein of rat muscle tissue increased starting 8 days prior to birth and throughout the complete experimental period. The increase in this fraction prior to birth was steady but not nearly as sharp the increase as after birth. This fraction made a very evident jump approximately at the 27th day of age at which time the sarcoplasmic fraction made concomitant decreases in quantity.

Csapo and Herrmann (1951) investigated contractile protein changes during chick development. Their findings are similar to the finding of Herrmann and Nicholas (1948) in that the myofibrillar proteins increase during intrauterine life and at birth make even sharper developmental increases. However, in the chick it seems that at approximately 50 days a plateau is reached and increasing increments of the protein are not very pronounced. Differences in this aspect may be due to the fact that

the rat was not kept until maturity had been attained. Csapo and Herrmann also stated in their results that the quantities of the various proteins in the fibrillar fraction developed at different rates with actin forming first and then myosin forming at least four days later. They determined this by the quantitative measurement of actomyosin present in the tissue.

Very similar work to Csapo and Herrmann' was reported by Robinson (1952a). He showed that myofibrillar proteins made marked increases from intauterine life up until about 50 days post partum. At this point increases did not stop but remained very low. Robinson expressed his values on a grams/100 grams of tissue basis and as a percentage of total nitrogen. In both cases, increases were very similar. Robinson pointed out, though, that the myofibrillar fraction prior to birth was very high in deoxyribonucleo-proteins since their solubility properties are very similar to those of the contractile proteins of the myofibril. Herrmann and Nicholas (1948) studied this nucleic acid myofibrillar protein complex. They stated that these nucleic acids did not affect contractile protein determination, however, Robinson did not agree with this conclusion. He developed (Robinson, 1952b) a second scheme for protein quantitation which would correct for the nucleic acid presence. This was accomplished by dilution of strong potassium chloride phosphate salt soluble proteins by ethanol and then extraction by 0.1 N sodium hydroxide. This correction brought all of their results on a comparable basis.

Changes in bovine myofibrillar proteins was investigated by Helander (1957). He found that apparently the myofibrillar proteins made considerable increases from a two month pre-natal period to about seven days of age. After the first week of life, these proteins decreased somewhat

until after the second month at which time considerable increases were made until one and one half years of age was attained. At this point "maturity" was attained, for very little changes were made in the following years.

Dickerson and Widdowson (1960) studied developmental protein changes in the human "thigh" muscle and in pig muscle. In human tissue the myofibrillar proteins made slight, steady increases from the 14th week of gestation to just after birth. As soon as the baby was born, the protein of this fraction increased at a tremendous rate until after seven months of age. After this age the contractile protein content (on a gram/kg fresh tissue basis) changed very little throughout the experimental period. The picture changes somewhat when the protein is based on percent total nitrogen. The protein content after 14 weeks of gestation rises until about the 22 week. However, at birth, the percentage falls again to levels near that of the 14 week embryo. After seven months the level has increased back to the level of a 22 week old foetus. From this point the percent protein rises gradually until the adult stage is acquired. Thus, one could say that although the fibrillar proteins do not make a marked increase along with the mass of muscle tissue, they do form an increasing amount of the protein substance within the muscle.

Dickerson and Widdowson (1960) in their study of pig muscle, showed a continual, steady increase in fibrillar proteins from the 45th day of gestation until about the third or fourth week of life. After this time, very slight increases exist until the adult stage is reached. These results are those based on a gram protein/gram of fresh tissue. When looking at the fibrillar proteins as a percent of total nitrogen, a marked increase can be observed from tissue of a 46 day foetus to that

of the 90 day foetus. Contractile proteins decrease in percent immediately after birth but then increases can be noticed until about the fourth week. At this time the protein composition is similar in quantity to that of the adult muscle. It can be noticed that changes in composition of the pig and human muscle tissue is very similar.

Dickerson (1960) examined changes in avian tissue during development. He found that myofibrillar proteins, when based on gram/kg of muscle, increased from time of hatching throughout the experimental period. However, greatest increments of change was realized from hatching to the second week of life. After this point only a gradual increase was noticed. When basing the conclusion on a percent of the total protein, differences can be seen. The fibrillar protein content increased from hatching to two weeks of age just as did when expressing in the other manner. But, at four weeks of age, a definite decrease in myofibrillar protein was realized and the tissue remained at this level throughout the analytical periods. This decrease was accompanied by a concomitant increase in sarcoplasmic proteins.

The above discussion has been centered upon age associated changes of the myofibrillar protein in muscle tissue. A brief outline of activity associated changes of these proteins in muscle tissue can be initiated at this time. Van Linge (1962) made histological studies on muscle changes during strenuous exercise. His findings reveal that after prolonged training, formation of new fibers can be observed. Also, he observed that increases in myofibrillar as well as sarcoplasmic proteins could be seen in strongly exercised muscles.

Helander (1960) made investigations on the effect of reduced activity on guinea pig and rabbit muscles. He proved that exercise in guinea

pigs caused an increase in the amount of contractile proteins. However, when these animals were restricted in their movements, no differences could be noticed between the normal and restricted muscle tissues.

When he worked with rabbits, he found that restricted activity caused significant decreases in fibrillar proteins. The decrease was even more marked when the rabbits were restricted for three years. Differences in results between the rabbits and guinea pigs were explained by Helander (1960) to be a result of not enough restriction on the guinea pigs to show any significant decrease in percent of contractile protein. Helander (1957) ascertained the affect of a plaster cast on the protein composition of rabbit leg muscles. The normal side was allowed to act as the control and both hind limbs were utilized in the analysis. He found that the presence of the case, which decreased muscular activity in the leg, cause a significant reduction in the fibrillar proteins when compared to the normal leg. The sarcoplasmic proteins made slight increases to make up the space difference in the muscle.

Stroma Proteins. Herrmann and Nicholas (1948) made investigations on rat protein development. They reported several protein fraction changes including those associated with stroma proteins. In these results, they showed that stroma proteins in the rat increased from 8 days prior to birth until about the 14th day of age. From this point, they concluded there was a steady decrease throughout the remainder of the rat's life.

Robinson (1952a), in his work with chick muscle, published similar data to Herrmann and Nicholas (1948) and their work with rat tissue. Robinson displayed data illustrating the stroma protein increasing with embryonic development but decreasing somewhat after birth, and forming

a constant percentage of muscle tissue. Robinson (1952a) also verified that stroma protein appeared to be the first component fully differentiated in muscle tissue.

Helander (1957) performed similar work but was on bovine tissue. He concluded that stroma proteins increased until birth but after that time formed decreasing amounts of the total protein in the muscle even up to a period of 14 years of age.

Dickerson and Widdowson (1960) showed somewhat different results using human tissue. Their work showed an increase of connective tissue proteins from early in gestation, up through birth and on to seven months of age. From this point until adult ages were reached, there was a steady decline in the quantity of the proteins in the human muscle. Similar conclusions can be drawn whether looking at the results on a gram per 100 gram tissue basis or on a percentage of total nitrogen. These two authors also studied pig muscle stroma protein changes. Conclusions here follow similar patterns as with human tissue. The stroma proteins increased in quantity from 46 days of gestation to about the third week of life. At this point they decreased on through the experimental period. Again, as in human tissue, the results show the same changes whether expressed as gram/100 grams or percent of nitrogen.

Fowl muscle was the subject of Dickerson's (1960) work, in which he studied stroma protein changes. His results show that such proteins form a decreasing amount of total tissue weight as age progresses. This is also evident when expressing as percent of total nitrogen. It can be seen that after the fourth week of age, the quantity of stroma proteins is very constant throughout the remainder of development. Goll et al. (1963) and his work with bovine tissue reported similar results as pre-

sented in the above discussion. They also presented methods for isolation of specific amino acids of the connective tissues.

Hunsley et al. (1971) and Chiskulas et al. (1965) worked with age associated changes in muscle tissues. The former worked with bovine tissues and the latter studied rat tissues. Both presented similar conclusions and were the same as those presented earlier in the discussion. That conclusion being that after birth (or hatching) stroma protein makes up a decreasing quantity of the total protein nitrogen as age progresses.

When looking at activity associated changes in stroma proteins as a faction of the wet tissue weight we see a decrease due to restricted activity and a very marked increase with exercise. In his work with rabbits, however, Helander (1961) found no changes in the stroma when muscles were restricted in their activities even for a period of three years. Helander (1957) immobilized the hind limb in a cast and checked for protein changes. He stated that, due to the inactivity, the stromal proteins increased in quantity. Considering Helander's work, conflicting statements seem evident. However, he pointed out that in the last study, there was an approximate 20 percent atrophy in total muscle tissue and very little atrophy in the connective tissues which would certainly make the stroma look as if it had increased. Actually, there was very little, if any, increase in stroma protein nitrogen in his immobilized rabbit studies.

Non-Protein Nitrogen. Leslie and Davidson (1951) in their work on the chemical composition of the chick embryonic cell, studied changes of protein nitrogen and nonprotein nitrogen (NPN) from prenatal to short periods postnatally. They found NPN to increase from eight days of

setting to two days after hatching. Of course, the protein nitrogen increased during the same period but to a much greater extent. Very similar results are reported by Kendrick - Jones and Perry (1967) in their work with enzymes during development of rabbit skeletal muscles.

Helander (1957) working with bovine tissue found similar results. His data showed an increase from the seven month foetus to a period of about 1.0 to 1.5 years of age. The increases during this time were noted to be very gradual and the changes after this time were very insignificant.

Dickerson and Widdowson (1960) noted NPN changes in pig and human muscle to be quite similar with steady increases from prior to birth until five months in the human and four weeks in the piglet after which time the quantity, as a fraction of fresh muscle, remained fairly consistant at a relatively low value. However, when they expressed their data on percentage of total nitrogen, they found very little changes in the human tissues until maturity was reached and at this point, the percent NPN dropped. In the pig, there was an increase in NPN percent from a 46 day foetus until after birth. Immediately after birth, the percent dropped and then rose again up to the fourth week. After the four to six weeks, the quantity decreased very slightly throughout the remainder of the experimental period.

Dickerson's (1960) report on chicken muscle showed an increase after hatching until about the second week. After this the NPN became relatively constant throughout the growing period.

Helander's (1957) work with restricted activity in rabbit muscle showed very little changes in percent NPNdue to such restrictions. Similar findings were also published on rabbits (1961) which had been re-

stricted up to three years. In such extensive restriction experiments the reports could not find any changes in NPN percentages in the cell.

Summary

To summarize the previous review on protein composition and its age associated changes in growth and development, it can be seen as pointed out by Goldspink (1962) and confirmed by almost all recent data that there is an increase in intracellular protein during growth and somewhat of a decrease in extracellular proteins. In general sarcoplasmic proteins increase with age to a point where they level off and continue as a constant value throughout development. Myofibrillar proteins increase throughout development, however, when maturity is reached, the increase becomes very slight. Of course, the way that the data looks may depend on the way in which the researcher presents it. Intracellular proteins can be expressed as a weight of the total muscle sample weight, as a percentage of the total protein content, or as a percentage of each other (sarcoplasmic: myofibrillar). Given the same data, one could draw three completely different graphs using three different ratios.

Moulton's (1923) work is very applicable to the review summary presented. He stated that a point at which the concentration of water, proteins and salts become comparatively constant in the fat free cell could be called the point of chemical maturity of the cell.

CHAPTER III

MATERIALS AND METHODS

General

Eight purebred Angus cows with calves were selected for this study from the Lake Carl Blackwell Range of the Oklahoma Experiment Station Herd. The cow - calf pairs were chosen with two criteria in mind; that is, the calves must be bulls and they must be as young as possible. After selection and grouping, they were brought into their first analysis period at an average age of 46 days and an average weight of 52.5 kg.

The calves were castrated at approximately three months of age and were weaned from their dams at an average age of 190 days and an average weight of 164.6 kg. At weaning, they were brought to a feedlot and fed ad libitum a standard growing and finishing ration. The steers remained in feedlot until attaining a weight of 383.1 kg at a mean age of 428 days. At this point the steers were brought to the Meat Laboratory, where they were held off feed for 24 hours but were supplied with adequate, fresh water. Following this holding period, the steers were slaughtered and processed according to methods described by Deans (1951) and Wellington (1953).

Procedures

Biopsy Procedures

The calves were delivered for surgery approximately 20-24 hours prior to anesthesia, for this length of time off feed induced most efficient responses and recoveries of the calves after the surgery.

Immediately before surgery, floors, squeeze chutes, tables, and any other equipment used during the biopsy was sanitized by scrubbing with soap and water and rinsing well with hot water. The steers were also scrubbed and curried free of manure and dirt that had accumulated on the hair coat.

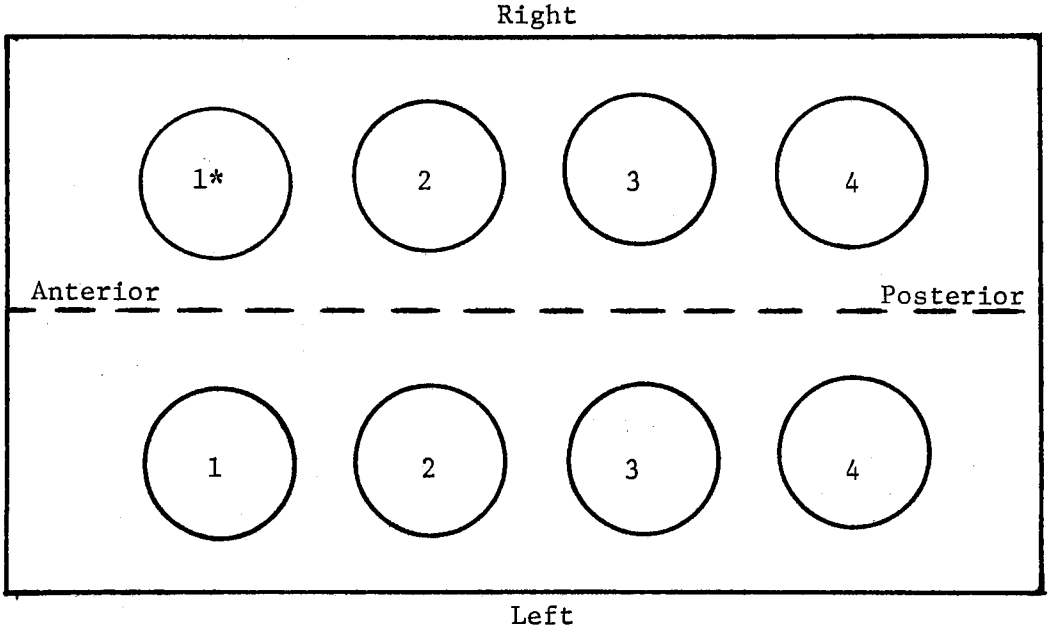
After preparations were finished, the steers were haltered and restrained either manually while young or via squeeze chute in the latter stages of the experimental trials. The anatomical area for biopsy, which had been previously designated, was located by palpation, the hair clipped very closely, the hide scrubbed with Phi-So-Hex and rinsed with water and then alcohol. Lastly, this area was disinfected with an iodine solution.

Once these preparations were accomplished, a general anesthetic was administered via the jugular vein at a dosage of five (5) milligrams of 10 percent Surital per pound of live weight. The anesthetic was administered rapidly and within one to one and a half minutes, the animal was ready for surgery. An incision was made laterally through the skin and transverse to the Longissimus dorsi. The incision was extended about 10 cm. and deep enough to penetrate the skin but not to disturb the epimysium. Very quickly the skin was retracted by allis tissue forceps so as to allow entry of the biopsy device. This device consisted of a

stainless steel corer (similar to corers used in shear test analysis) attached to a Black and Decker 1/4", type "D", electric hand drill. Two sizes were available for use, that is, one-half inch and one inch. Specifically, the size used depending upon the age of the steer for when the calves were young, the half inch corer was used to eliminate large holes being left in the muscle which would predispose infection and possibly loss of the animal. During the later analysis periods, the large corer was used with no ill effects on the animals.

Immediately after retracting the hide, the corer was admitted through the incision and into the muscle with a slow steady force. The depth of entry was regulated by the presence of a rib in positions 1R, 1L, 2R, and 2L and the transverse process of the lumbar vertebrae in positions 3R, 3L, 4R, and 4L (Figure 5). As soon as the deep border of the muscle was reached, the corer was retracted and long, curved surgical scissors were used to clip the attached end. Immediately after excising the core of muscle, the wound was covered with Furacin, a topical, antibacterial, soluble, veterinary powder. The incisions were closed with sufficient mattress sutures of Vetafil to give most efficient healing results.

Within four hours past surgery all steers were on their feet and within six hours all were alert and ready to be returned to the pasture or to the feed lot depending upon the experimental period. Seven days post surgery provided sufficient time for all swelling to subside and fourteen days after surgery proved to be ample time to find complete adhesion of the scar tissue, hence, the sutures were removed at this time. By the end of the fourth week post surgery, almost complete scar tissue recovery could be observed.



*Biopsy of the Longissimus muscle was made at predesignated positions of:

1. 11th Rib Area
2. 13th Rib Area
3. 2nd Lumbar Area
4. 4th Lumbar Area

Figure 5. Anatomical Location of Sampling Site in Bovine Longissimus Muscle

It should be noted that while on their dams, no problem was ever encountered by a refusal of the calf by the dam. Also problems of infections were never observed and minor stiffness and swelling lasted but a few days after surgery.

All instruments used in the biopsy had been scrubbed with soap, autoclaved, and then soaked in a 1-4000 solution of Nolvasan until used in surgery. The corer and all surgical equipment was scrubbed and soaked in Nolvasan between each calf during the procedure.

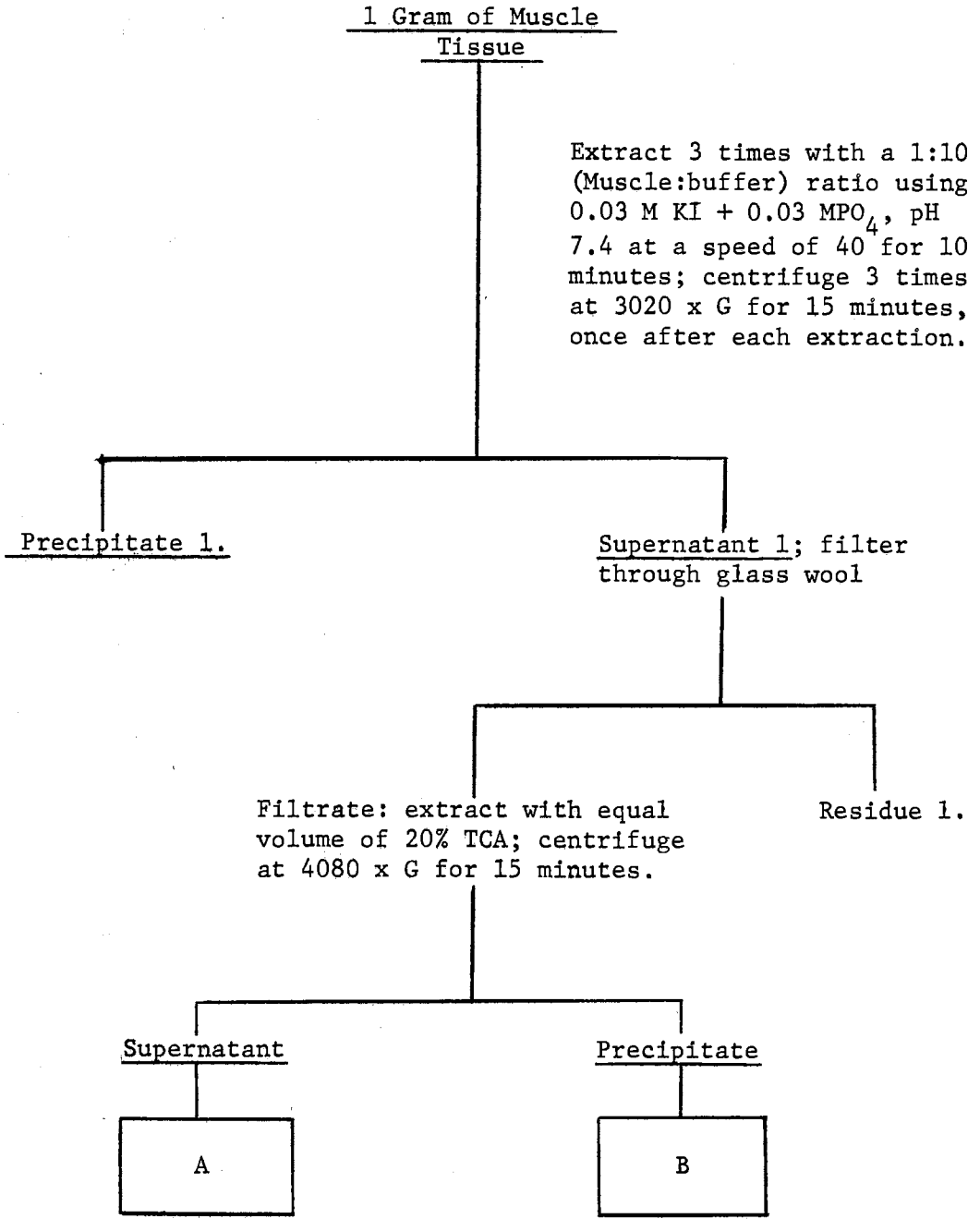
Freezing and Storage of Tissue

Immediately, upon the removal of the tissue from the animal, an approximate weight was determined so as to insure that enough muscle had been removed by the biopsy for all biochemical analysis. The muscle was wrapped in heavy aluminum foil in order to insure a tight seal. The small packets were then immersed into a flask of liquid nitrogen ($-170^{o}C$) and held there until all bubbling ceased. Once all activity had ended, the sample was then removed from the liquid nitrogen and placed in a precooled ice chest, then transported to the Meat Laboratory and stored below $-20^{o}C$.

Prior to storage, the samples were placed in Cry-O-Vac plastic bags to reduce moisture loss and eliminate misplacing of the samples.

Protein Isolation

Care was taken in all instances to keep the extracting environment at one to four degrees centigrade in order to maintain protein stability and prevent conformational changes which might decrease protein solubility.



A. Sarcoplasmic Non-Protein Nitrogen.
B. Sarcoplasmic Protein Nitrogen.

Figure 6. Extraction Scheme for the Sarcoplasmic Protein and Non-Protein Nitrogen

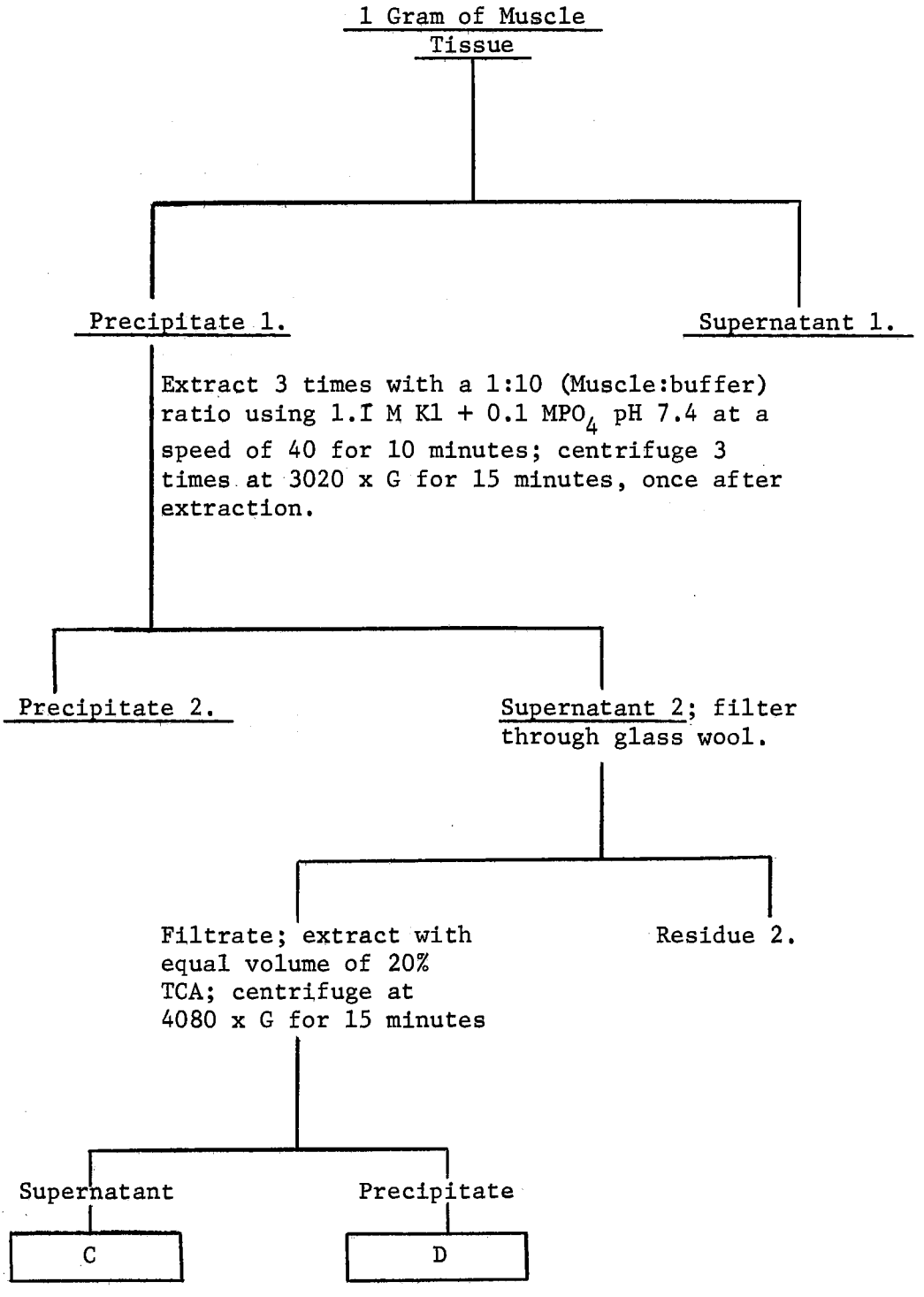
Small chips of tissue were removed from the biopsy samples and weighed on a Mettler H10T analytical balance. The approximate one gram aliquot thus obtained was accurately weighed and immersed immediately into 10 volumes of cold 0.03 MKI + 0.03 MPO_4 buffer, pH 7.4. The muscle tissue was then extracted three times with the above buffer. Extraction lasted for 10 minutes with a rheostat setting of 40. After each extraction, the homogenate was centrifuged for 15 minutes at 3020 x G in a Sorvall automatic refrigerated centrifuge, Model RC2-B. After each centrifugation, the supernatant was decanted and filtered through glass wool and the filtrate and residue retained in a one degree centigrade cold room. The supernatant obtained here was the weak salt soluble or sarcoplasmic proteins.

After the third centrifugation the precipitate which remained was resuspended and extracted three times in a 1:10, muscle buffer ratio using a 1.1 MKI + 0.1 MPO_4 buffer, pH 7.4.

Extraction was made as before for 10 minutes at a speed of 40. Centrifugation was performed as before at 3020 x G for 15 minutes and the supernatant was decanted and filtered through glass wool. The filtrate and residue was retained, as before in a one degree centigrade cold room. This supernatant obtained was the strong salt soluble or myofibrillar proteins.

After the third centrifugation the precipitate which remained was called the insoluble or stroma proteins.

After obtaining these isolated protein fractions, equal volumes of 20 percent trichloroacetic acid (TCA) was added to the supernatants and 20 ml of the 20 percent TCA was added to the stroma protein precipitate. The supernatants were shaken vigorously for 5 seconds and the stroma



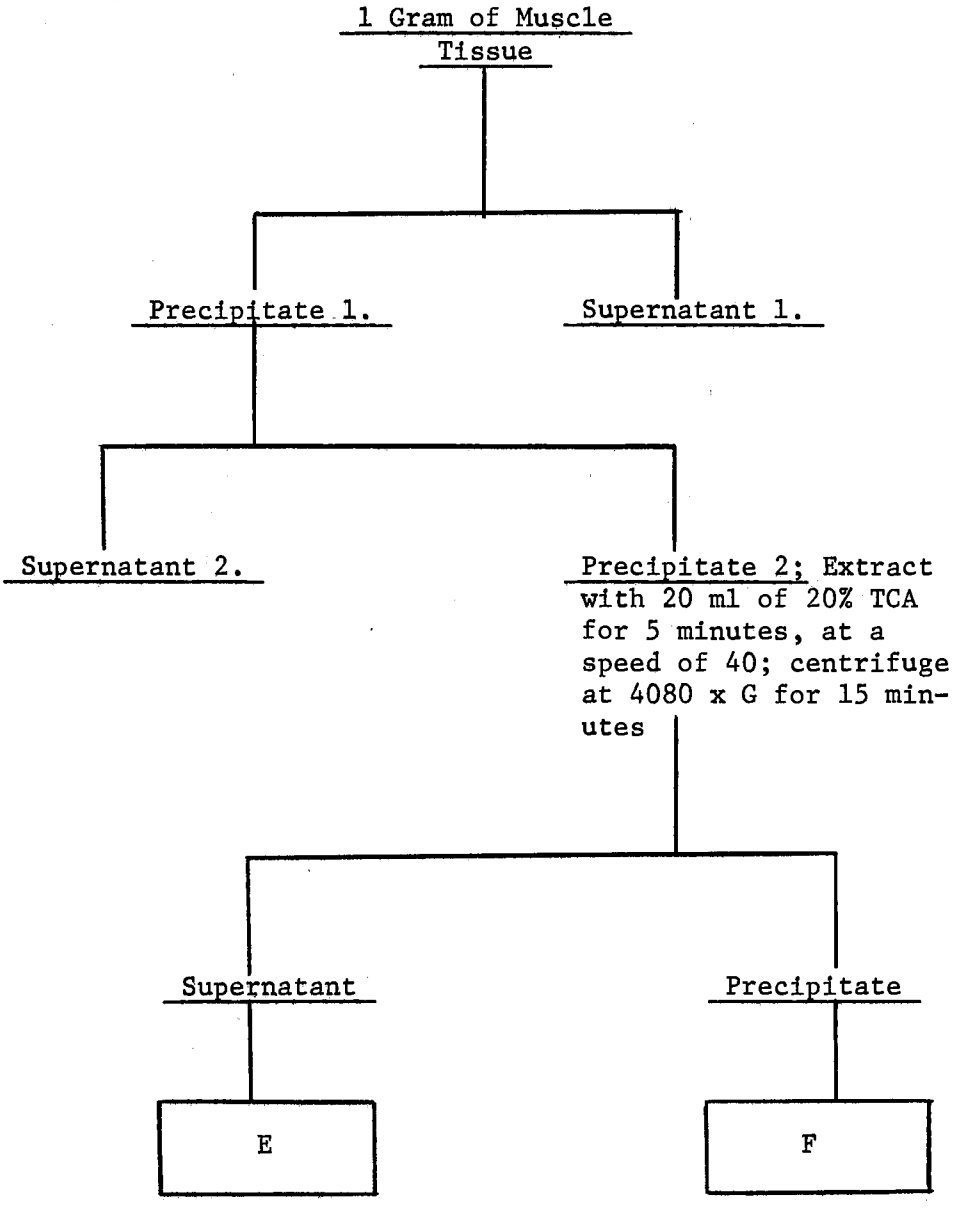
C. Myofibrillar Non-Protein Nitrogen.
D. Myofibrillar Protein Nitrogen.

Figure 7. Extraction Scheme for the Myofibrillar Protein and Non-Protein Nitrogen

proteins were homogenized for five minutes at a speed of 40.

The suspensions were then centrifuged at a speed of 4080 x G for 15 minutes and the resulting supernatants decanted and filtered through glass wool directly into separate 800 ml Kjeldahl flasks. These supernatants were called the non-protein nitrogen (NPN) of their respective fraction. The precipitate was removed and deposited into separate 800 ml Kjeldahl flasks and with these precipitates was added the rinsed glass wool through which their respective NPN fractions had been filtered. Immediately 25 ml of concentrated H_2SO_4 was added and the flasks sealed with rubber stoppers to insure no loss of nitrogen and to start the digestion of the sample. The residue which was obtained in the glass wool after each extraction and centrifugation still remained to be separated.

The filtrate from the weak salt soluble proteins was extracted with the 0.03 MKI + 0.03 MPO_4 pH 7.4 buffer at a speed of 20 for five minutes to insure the removal of all sarcoplasmic proteins. The suspension then was centrifuged at 4080 x G for 15 minutes and the supernatant decanted directly into a Kjeldahl flask. This fraction was referred to as the sarcoplasmic lipo-nitrogen extract using the low ionic strength buffer. The precipitate which remained was extracted with the 1.1 MKI + 0.1 MPO_4, pH 7.4 buffer to insure that no myofibrillar proteins may have been trapped in the lipo-nitrogen complex. The extraction lasted for five minutes with a rheostat setting of 20. The mixture was centrifuged for 15 minutes at 4080 x G. The supernatant was decanted directly into a Kjeldahl flask and was labeled the sarcoplasmic lipo-nitrogen extract using the high ionic strength buffer. The precipitate which remained was called the sarcoplasmic lipo-proteins and was extracted with 20 ml



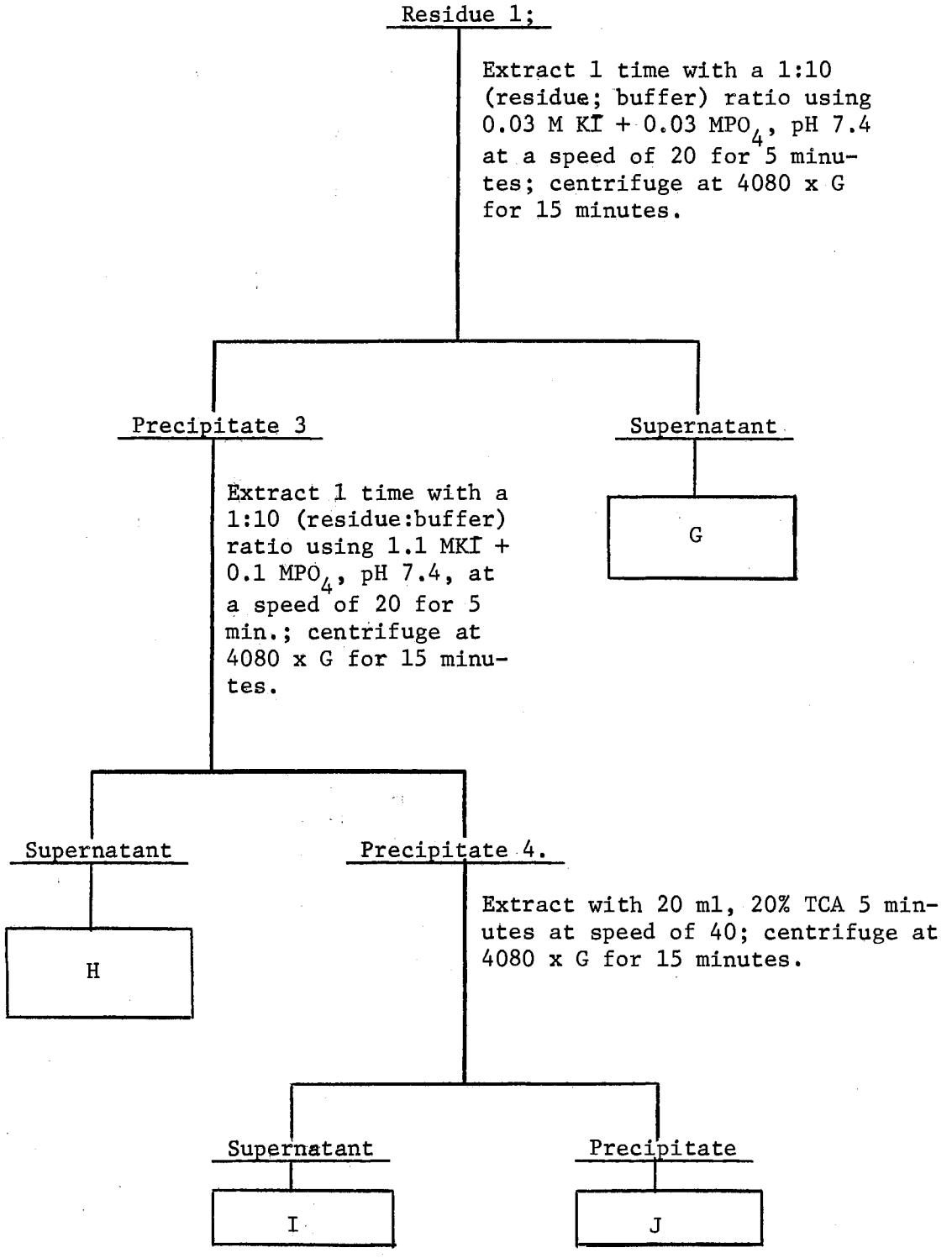
E. Stroma Non-protein Nitrogen.
F. Stroma Protein Nitrogen

Figure 8. Extraction Scheme for the Stroma Protein and Non-Protein Nitrogen

of 20 percent TCA at a speed of 40 for 5 minutes. The suspension was then centrifuged at 4080 x G for 15 minutes and the resulting supernatant decanted into a Kjeldahl flask and called sarcoplasmic lipo-nitrogen NPN and the precipitate deposited into a separate Kjeldahl flask and labeled sarcoplasmic lipo-nitrogen. Immediately, 25 ml of concentrated H_2SO_4 was added and the flasks sealed with rubber stoppers.

The residue remaining from the strong salt soluble extracts was extracted with 1.1 MKI + 0.1 MPO_4, pH 7.4, buffers for five minutes at a speed of 20. This homogenate was then centrifuged for 15 minutes at 4080 x G and the resulting supernatant decanted directly into a Kjeldahl flask and labeled myofibrillar lipo-protein extract. The remaining precipitate was resuspended in 20 ml of 20% TCA and extracted for five minutes at a speed of 40. The suspension was then centrifuged at 4080 x G for 15 minutes and the resulting supernatant decanted directly into the Kjeldahl flask and labeled myofibrillar lipo-nitrogen NPN. The precipitate was then deposited into a Kjeldahl flask and labeled myofibrillar lipo-proteins. Immediately 25 ml concentrated H_2SO_4 was added and the flasks sealed with rubber stoppers. In all cases where the omnimixer, centrifuge tubes or glass wool was rinsed and where buffer was not required, de-ionized, glass distilled water was used. Blanks were prepared for the respective fractions and H_2SO_4 added just as previously performed on the actual samples.

It should be noted here that, in all cases, all glassware and utensils utilized in the isolation procedure was washed in soapy water, tap water rinsed, washed in a sulfuric acid - dichromate cleaning solution, tap water rinsed, and finally deionized, glass distilled water rinsed. The utensils were oven dried and stored in drawers separate from other



G. Sarcoplasmic low ionic strength lipo-nitrogen extract.
H. Sarcoplasmic high ionic strength lipo-nitrogen extract.
I. Sarcoplasmic lipo non-protein nitrogen.
J. Sarcoplasmic lipo-protein nitrogen.

Figure 9. Extraction Scheme for Sarcoplasmic Lipo-Nitrogen

laboratory materials and equipment.

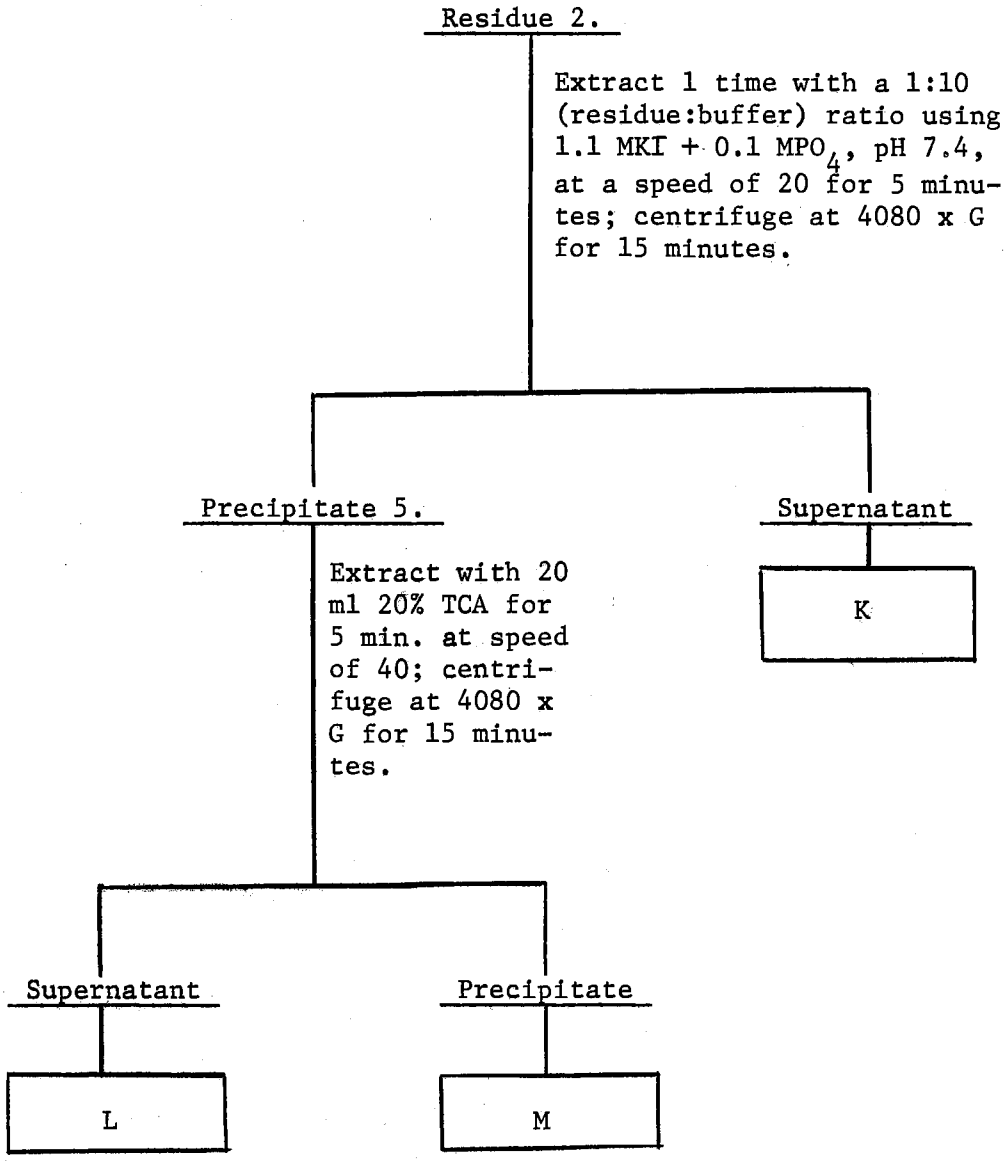
Protein Determination

Protein in the muscle isolates was quantitatively determined by the Kjeldahl method for organic nitrogen.

Each fraction was emptied into a large, 800 ml, Kjeldahl flask and 25 ml of concentrated sulfuric acid added. The flasks were sealed and held overnight to allow for disintegration and partial digestion of the sample. This standard holding time was performed on all samples throughout the experimental period.

After this holding period, the samples were opened and 10 grams of sodium sulfate, and four Hengar Selinized Granules were added. The flasks were then placed on a digestion rack over high temperature and allowed to digest for twice the length of time which the samples required for clearning.

After the allowed digestion time, the flasks were removed from heat, stoppered, and were allowed to stand for 10 minutes in order to cool. After cooling, 400 ml of distilled water was added to dilute the digest followed by the addition of 75 ml of concentrated NaOH for neutralization and three pieces of mossy zink as boiling chips. Immediately the flask was placed on the distillation rack with receiving flasks in place to eliminate the possibility of ammonia loss. The samples were distilled into a receiving flask containing 50 ml of five percent boric acid until 350-375 ml of distillate was obtained. The heat was then removed and the distillate titrated for nitrogen determination. The condensate was titrated to neutrality immediately so that no ammonia losses would be realized. A standard 0.1253N sulfuric acid with a methyl red, methylene



K. Myofibrillar Lipo-nitrogen Extract.
L. Myofibrillar Lipo-nitrogen Non-protein Nitrogen.
M. Myofibrillar Lipo-protein Nitrogen.

Figure 10. Extraction Scheme for Myofibrillar Lipo-Nitrogen

blue mixed indicator was used for this titration. The normality was standardized at 0.1253 N in order that one ml of titrating acid would be equal to one percent protein in the sample assuming muscle protein to be 16.0 percent nitrogen.

It should be noted that deionized, distilled water was used to rinse all glassware and that standards were utilized in order to present consistant titrations from day to day.

Statistical Analysis

The statistical analysis of this experiment was accomplished by a completely randomized block design. The animals served as the experimental units and the age periods served as experimental treatments. Since the location of the biopsy was the same with each animal at any given time, the location effects were completely confounded with age. All analysis were accomplished according to Snedecor and Cochran (1967).

CHAPTER IV

RESULTS

The data obtained from the Kjeldahl analysis were corrected for a blank reading and for sample weight. Such adjustments enabled the elimination of all extraneous sources of nitrogen and permitted the expression of the results on a unit tissue basis, as "percent protein" on a wet tissue basis. Since it was not feasible to obtain the volume or weight of each of the thirteen nitrogenous components resulting from the complete fractionation of the muscle samples all results had to be expressed as a percent of the original sample weight rather than on an individual fraction basis.

Calculation of the data was performed using the formula (uncorrected reading - blank reading) divided by sample weight equals percent protein nitrogen in a gram of sample. However, facilities utilized in the Kjeldahl determinations were located in the Agronomy Research Laboratory and the standard titrating acid (0.1253N) was prepared such that one milliliter of acid equaled one percent of wheat protein. Since differing quantities of nitrogen make up muscle and wheat protein (16.000 percent and 17.543 percent respectively), a constant was derived and used to correct for muscle protein. Derivation of this constant was made by the following mathematical calculations:

$$100 \div 17.543 = 5.7003$$
$$100 \div 16.000 = 6.2500$$

so,

$$\frac{\underline{X} \text{ ml titrating acid}}{5.7003} \quad (6.25) \quad = \quad \% \text{ muscle protein}$$

or,

$$(X) \quad \frac{6.25}{5.7003} \quad = \quad \% \text{ muscle proteins}$$

Hence,

$$(X) \quad (1.0964) \quad = \quad \% \text{ muscle protein}$$

Thus, the final equation for obtaining a corrected protein value was:

$$\frac{\text{Uncorrected reading - blank reading}}{\text{sample weight}} \quad (1.0964) = \% \text{ muscle protein on a wet tissue basis}$$

All values thus obtained could readily be converted to percent nitrogen on a wet tissue basis by dividing the above corrected protein by 6.2500.

As alluded to earlier, the nitrogen content of bovine longissimus muscle was partitioned into 13 different "fractions". Changes in the nitrogenous content of these fractions were followed during the pre- and post-weaning phases of the beef calves' life via procedures developed in our laboratory and described in Chapter III of this thesis. To facilitate the discussion of the quantitative changes in the nitrogenous components of muscle during growth and development, various combinations of the 13 fractions analyzed were grouped into what was considered the principal nitrogen subunits of muscle, such as; total nitrogen, total

protein nitrogen, total NPN, and total lipid fraction nitrogen. Each of these principle nitrogen subunits were then partitioned into their respective major subunits and discussed accordingly.

It is pointed out that the "lipid nitrogen fraction" mentioned above was not considered in previous studies of this nature, even though, as will be shown in the results to follow, this fraction may have a significant influence on the sarcoplasmic and myofibrillar nitrogen isolates of developing bovine muscle.

Total Nitrogen

The mean values plotted in Figure 11 reflect the overall changes in total nitrogen, total protein nitrogen, total NPN, and total lipid fraction nitrogen throughout all experimental periods. The analysis of variance (Table V, Appendix) indicated no significant change in total nitrogen from the initial to the final study periods. Although non-significance has been ascertained, definite trends in pre- and post-weaning phases are suggested by the data plotted in Figure 11. Total nitrogen can be seen to show a steady decrease of 0.21 percent from the first to the fourth test periods. It was during this phase of the calves' life that nutrients were obtained from the pasture and from their dam's milk. When the calves were put on feed (periods five through eight), there was a sharp initial increase in total nitrogen (period five). Reasons for this increase cannot be precisely identified, but may be due in part to the sharp increases in the NPN content (Figure 11). The average values plotted for periods six, seven, and eight showed a steady increase in total nitrogen during this part of the feedlot period. There was a net increase of 0.41 percent in total nitrogen during these periods. The

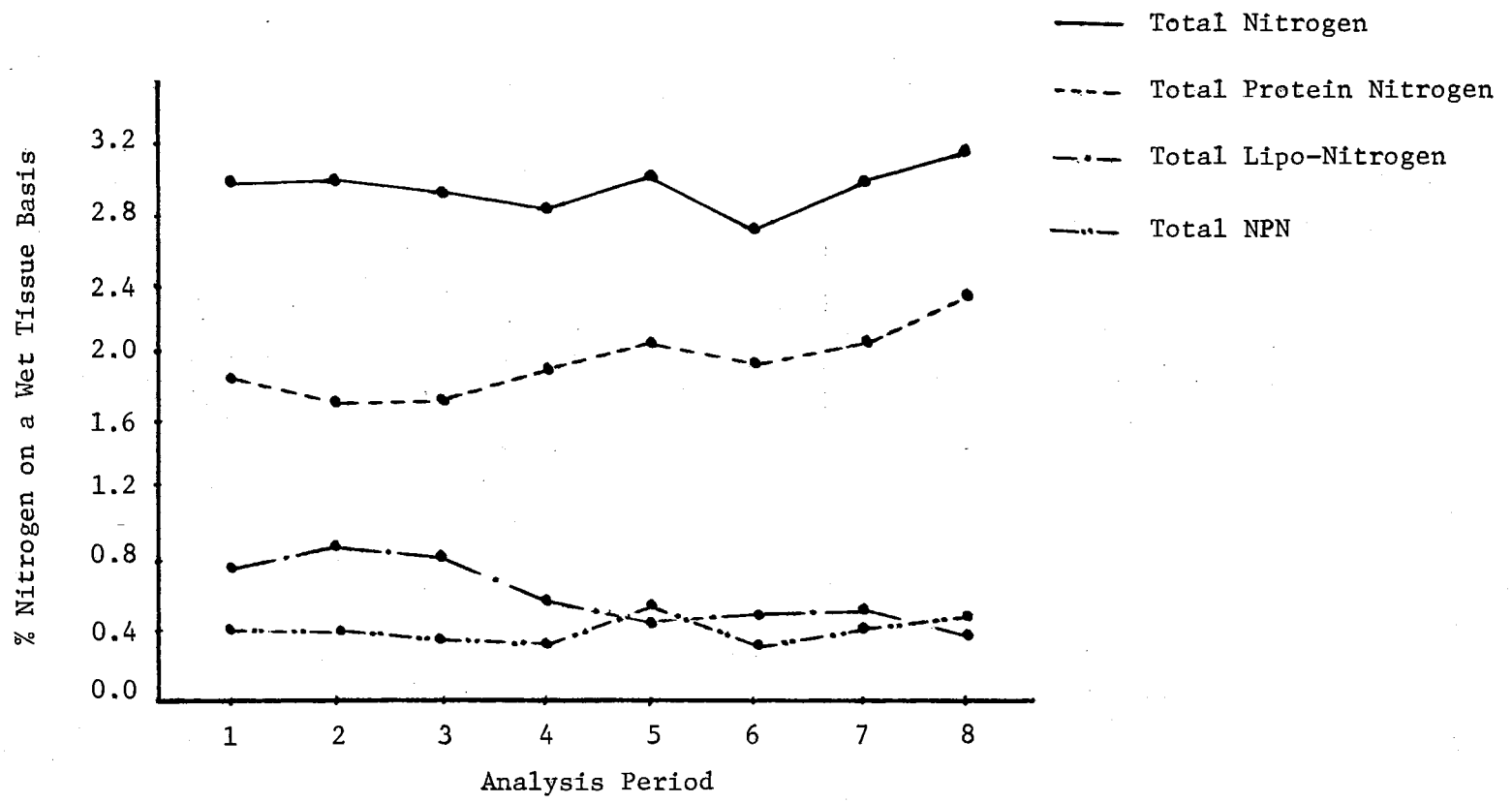


Figure 11. Quantitative Changes in Total Nitrogen and Its Major Subfractions in Bovine Longissimus Muscle During Growth and Development

overall net increase in the total nitrogen (periods one through eight) amounted to only 0.8 percent. A more detailed discussion of the trends indicated by the data in this and in subsequent figures will appear in Chapter V.

Of the subfractions of the total nitrogen, possibly the one of particular concern was the total protein nitrogen. This subfraction comprised approximately 61 percent of the total nitrogen at the first test period and 74.3 percent during the last period. Although definite trends can be noted and an increase in the quantity of this fraction was calculated (Table XII, Appendix), no significance can be declared (Table V, Appendix). The fluxuations which the protein nitrogen made in that phase of the calves' life prior to weaning resulted in an initial abatement of 0.14 percent in protein nitrogen from period one to period two. Here a static condition was retained until the final analysis period prior to weaning (period four), where an enhancement of the protein nitrogen resulted in a 0.19 percent increase. At this point, a more or less stationary position was attained and extended through period six. However, in the last two analysis periods, noticeable increases occurred which resulted in an overall net increment of 0.45 percent.

The NPN fraction exhibited no statistically significant change (Table V, Appendix) in quantity during the course of this study. In the first analysis period post-weaning (period five), an accretion was noticed, for this period only, which resulted in an overall increase of 0.21 percent nitrogen. As stated above, very minor changes were observed prior to and after this point of inflection although slight trends can be seen showing declension pre-weaning and enhancement post-weaning.

The quantitative changes which occurred in the lipid nitrogen sub-

fraction were highly significant ($P < .01$) as shown in Table V, Appendix. Changes which occurred from the first study period to the first period post-weaning show a net decrease of 0.24 percent nitrogen. A definite trend can be observed in the steady decrease from period two to period five and relative stability from this point to the end of the experimental study. As can be observed from Figure 11 and Table XII, Appendix, it is this lipid nitrogen fraction that appears to be responsible for the relatively high values obtained in the total nitrogen in the first periods of analysis. A discussion of the changes which occurred in this fraction and its influence on the sarcoplasmic and myofibrillar subfraction of the protein nitrogen will appear later.

Total Protein Nitrogen

The total protein nitrogen has been subdivided into its major components, i.e., sarcoplasmic protein nitrogen, myofibrillar protein nitrogen, and stroma protein nitrogen as depicted in Figure 12. For a meaningful understanding of the changes which were displayed by the total protein nitrogen, a description of the changes which occurred in its subfractions must be elicited.

Sarcoplasmic protein nitrogen displayed no significant change (Table VI, Appendix) throughout the duration of this investigation. Overall, there was a small net decrease (0.11 percent) in sarcoplasmic protein nitrogen from period one to period eight. Average values for each of the eight research periods for the sarcoplasmic protein nitrogen, as well as the other subfractions of the total protein nitrogen, can be obtained from Table XIII, Appendix.

Myofibrillar protein nitrogen variations were very evident as re-

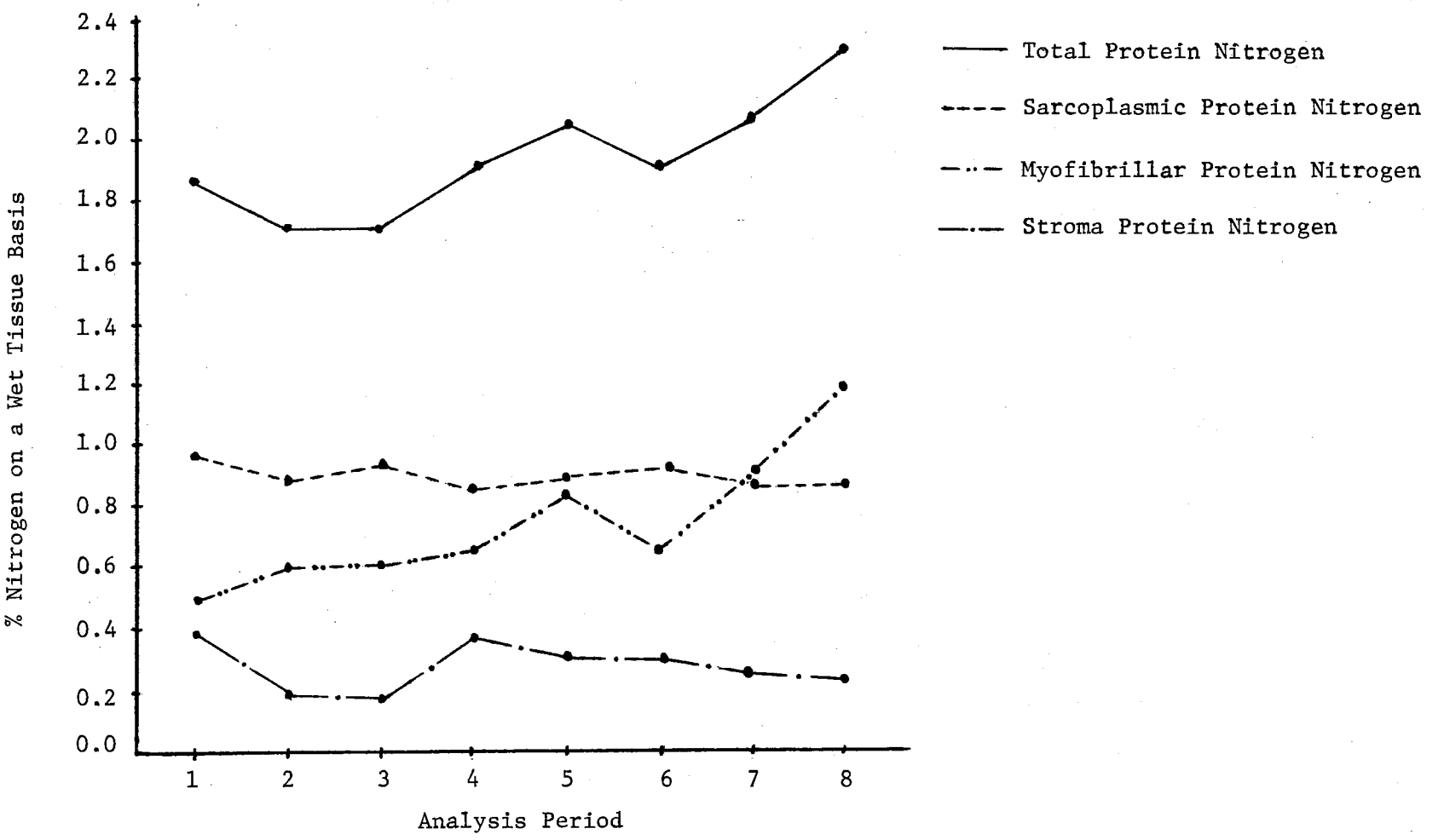


Figure 12. Quantitative Changes in Total Protein Nitrogen and Its Subfractions in Bovine Longissimus Muscle During Growth and Development

flected by the data in Figure 12. The analysis of variance (Table VI, Appendix) indicated a highly significant ($P < .01$) difference in the period means in this fraction. There was a net increase of 0.69 percent nitrogen from period one to period eight, with the greatest inflection (0.51 percent) occurring from period six to period eight. An initial strong increase was observed in this fraction from period one to period two, showing a positive difference of 0.12 percent nitrogen. At periods two, three and four, very little changes were evident, however, period five, (the 1st period post-weaning) reflected a prominent peak which subsided to a position of equal intensity at period six as found in period four. The sharp increases noted in period seven and eight have been described above, however, explanations for inflections which occurred at periods two, five, seven and eight will be discussed, together with the changes in the other fractions in Chapter V of this thesis.

Results for the stroma fraction indicated no statistically significant differences among the eight period means (Table VI, Appendix). A decrease of 0.21 percent stroma protein nitrogen was observed from period one to period three followed by a marked increase of 0.20 percent nitrogen at period four. From periods four to eight there was a steady decline in stroma protein nitrogen. Overall, there was a net decrease of 0.15 percent in stroma protein nitrogen from periods one through eight.

Total Non-Protein Nitrogen

The non-protein nitrogen has been divided, as the protein nitrogen into its components, i.e., of sarcoplasmic NPN, myofibrillar NPN, and stroma NPN. The quantitative changes of these fractions are depicted graphically in Figure 13 and recorded arithmetically in Appendix Table

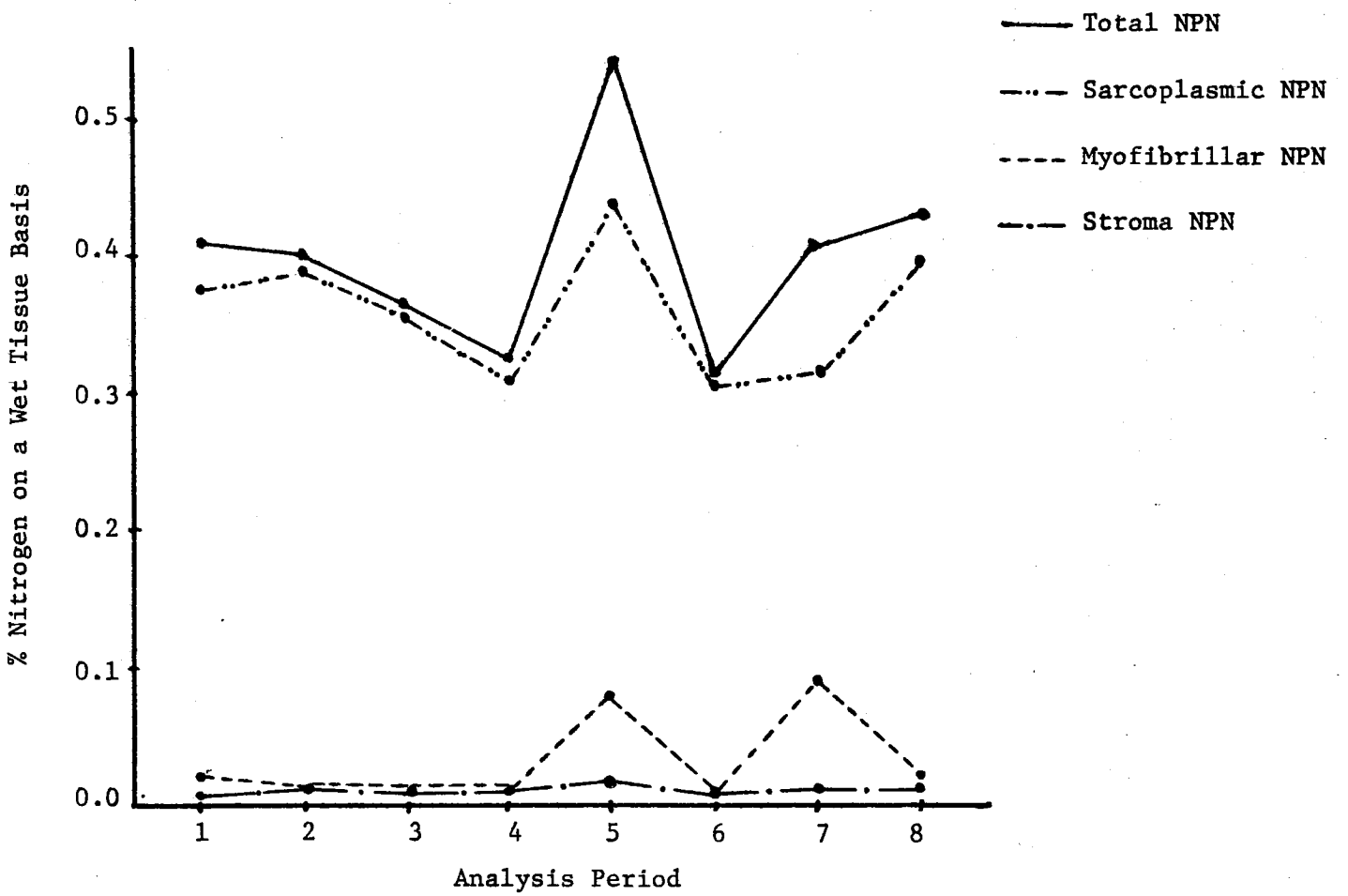


Figure 13. Quantitative Changes in Total NPN and Its Subfractions in Bovine Longissimus Muscle During Growth and Development

XIV.

As reflected in Figure 13, the sarcoplasmic NPN showed several very strong fluctuations which, according to the analysis of variance (Table VII, Appendix), proved to be significant ($P < .05$). Very little change was noted between the mean values in periods one and two, however, a very steady decline was observed from period two to period four. The loss in NPN between these two period extremes amounted to 0.08 percent. At period five, a strong inflection in this fraction was detected which resulted in a 0.13 percent nitrogen change which subsided by a like amount at period six. Another increase, (0.08 percent) in NPN occurred between periods seven and eight. From the first to the last study periods, a net positive change of 0.02 percent was obtained indicating that the significance declared in the analysis of variance was due to fluctuations within the growth and developmental periods and not to an overall age effect.

Myofibrillar NPN showed no significant changes (Table VII, Appendix). In the pre-weaning period (periods one through four), no changes were observed in this fraction but very noticeable peaks were seen in periods five and seven with similar low points occurring at periods four, six and eight.

The stroma NPN fraction exhibited no significant change (Table VII, Appendix). As may be observed in Figure 13, this fraction was relatively static throughout the growth and development study.

Total Lipid Fraction Nitrogen

The lipid fraction nitrogen, alluded to earlier as being one of the major subfractions of the total nitrogen, was partitioned somewhat dif-

ferently than those fractions described previously. This fraction was divided into the total lipid protein nitrogen, which was a composite of that lipid nitrogen from the sarcoplasmic and myofibrillar fractions, the total lipid NPN, composed of the sarcoplasmic and myofibrillar lipid NPN, and the total lipid extractable nitrogen which was obtained from the sarcoplasmic and myofibrillar fractions as described in Chapter III. The total lipid protein nitrogen data as shown graphically in Figure 14 and arithmetically in Table XV, Appendix, indicate no general trends which were maintained for more than two periods during the experimental study. Moreover, no statistically significant differences can be shown (Table VIII, Appendix) between the period means.

Increases in the lipid protein nitrogen occurred from period one to period three with a difference in the end points being 0.18 period. Almost opposite decreases were then observed from period three to period five. After period five, very small increments occurred through period seven. At this time, a sharp decline to the period eight mean value was noted and a 0.10 percent decrease in protein nitrogen was recorded. Collectively, the pre-weaning lipid protein nitrogen values (periods one through four) averaged 0.24 percent higher than that obtained during the feedlot phase of their study.

The fraction which apparently causes most of the fluctuations in the lipid nitrogen was the lipid extractable nitrogen as can be seen in Figure 14. The differences in the period means of this fraction was highly significant ($P < .01$), according to Table VIII, Appendix. The general trend of this fraction follows a very marked decrease from period one to period eight.

The values in periods one and two were very similar however a sharp

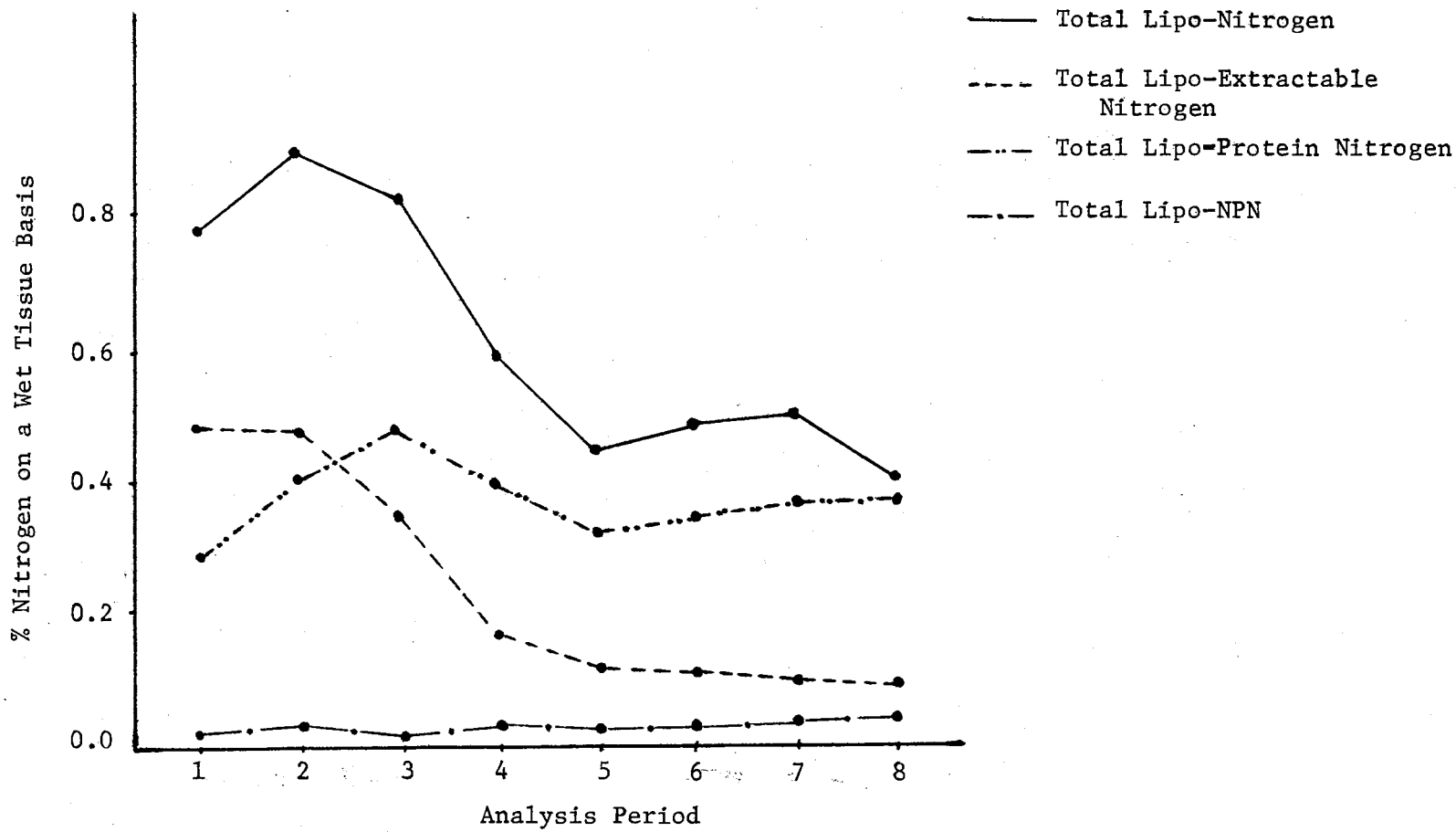


Figure 14. Quantitative Changes in Total Lipo-Nitrogen in Bovine Longissimus Muscle During Growth and Development

declension was noted from period two to period five. At this point a fairly static condition was maintained throughout the latter study periods. The overall decrease from period one to period eight was 0.40 percent but as stated earlier, the major diminution occurred between periods two and five, showing a 0.36 percent decrease.

The lipid NPN fraction followed a very static level from period one to period eight with a slight enhancement throughout the study resulting in a 0.03 percent increase. As can be seen in Table VIII, Appendix, no significance was obtained between the period means.

As stated earlier, the literature on previous studies involving the partitioning of muscle nitrogen makes no reference to a "lipo-nitrogen fraction". A careful study of the literature suggests that in these previous studies, the lipo-nitrogen components, as shown in Figure 14, were included in the sarcoplasmic and myofibrillar nitrogen fractions.

Thus, it was believed to be in order to determine what influence the lipo-nitrogen fraction might have on the course of the sarcoplasmic and myofibrillar protein and non-protein nitrogen results presented in Figures 12 and 13 if the lipo-nitrogen data were included in these values. To do this the total lipo-nitrogen fraction was partitioned and added to the respective sarcoplasmic and myofibrillar protein and non-protein nitrogen fractions from which it was initially extracted. The resulting "combined" data are presented below.

Sarcoplasmic Fraction Nitrogen

Figure 15 reflects the changes which occurred in the sarcoplasmic protein nitrogen and NPN fractions after the addition of the appropriate lipo-nitrogen components. The original sarcoplasmic protein nitrogens

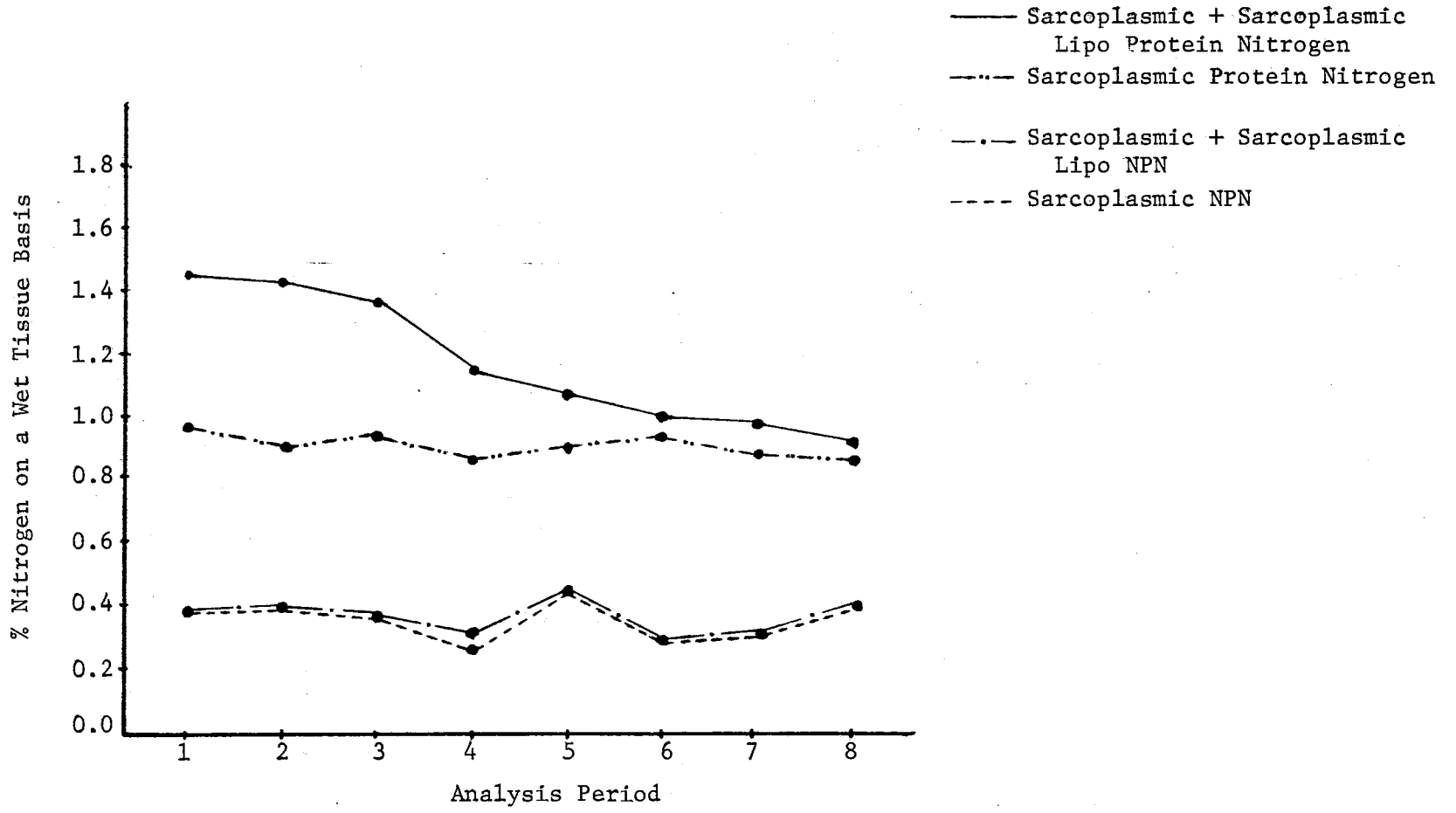


Figure 15. The Influence of the Lipo-Nitrogen Fraction on Quantitative Changes in Sarcoplasmic Protein Nitrogen and Sarcoplasmic NPN of Bovine Longissimus Muscle During Growth and Development

and NPN groups, shown in Figure 12, were included in Figure 15 for use as reference points only. As shown in Figure 15, the addition of the lipo-protein nitrogen fraction resulted in a significant alteration in the course of the original sarcoplasmic protein nitrogen curve. Whereas the mean values for sarcoplasmic protein nitrogen without the lipo-protein nitrogen displayed only a slight and non-significant decrease over the test periods one through eight (with much interim variation), the "combined" values showed a highly significant decrease ($P < .01$) over these test periods (Table IX, Appendix).

Results indicated that throughout all study periods there was virtually no difference in the curves for sarcoplasmic NPN with or without the lipo NPN included (Figure 15).

Myofibrillar Fraction Nitrogen

In Figure 16, the graph for the myofibrillar plus lipo-protein along with the graph for the original myofibrillar protein nitrogen are presented for comparisons. The analysis of variance (Tables VI and X, Appendix) showed both fractions to exhibit highly significant ($P < .01$) increases from period one to period eight with a net increase of 0.70 and 0.69 percent, respectively for the aforementioned fractions. As the curves indicate, very similar trends were followed in both fractions throughout the study. This similarity suggests that a constant quantity of lipo-protein nitrogen was obtained in each of the test periods of the experiment. The comparisons here and the comparisons between the total and original sarcoplasmic protein nitrogen fractions (Figure 15) present very unique and interesting differences.

The changes which occurred by the addition of the appropriate lipo-

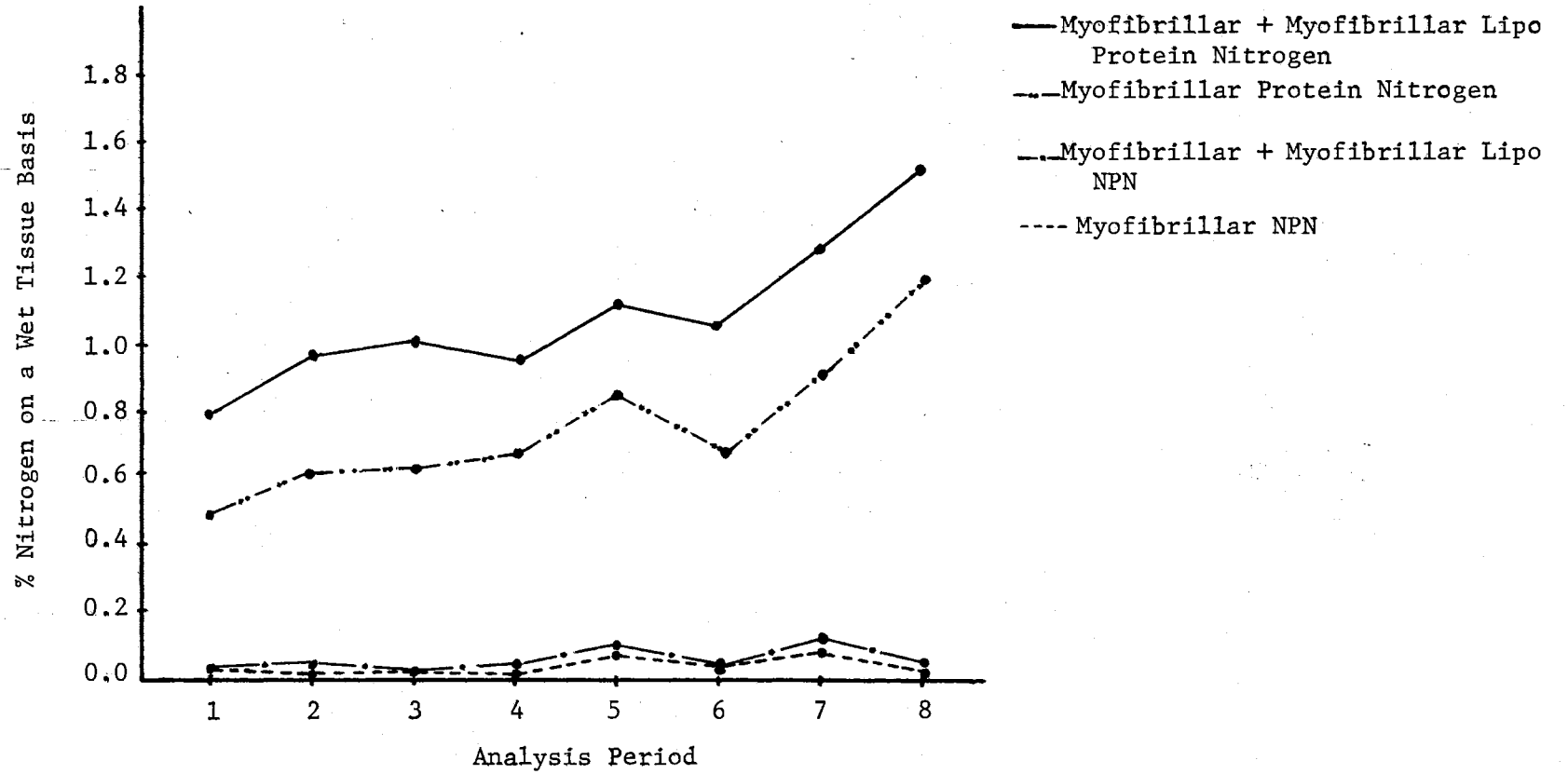


Figure 16. The Influence of the Lipo-Nitrogen Fraction on Quantitative Changes in Myofibrillar Protein Nitrogen and Myofibrillar NPN of Bovine Longissimus Muscle During Growth and Development

nonprotein nitrogen isolate to the original myofibrillar NPN can be seen in Figure 16. Statistical analysis (Tables VII and X, Appendix) has shown neither fraction to be significant in the changes which they displayed throughout the experiment. It can be seen from their graphs that very little changes were made in the inflection of the original myofibrillar NPN due to the addition of the appropriate lipo-nitrogen values.

CHAPTER V

DISCUSSION

Total Nitrogen

The discussion to follow is designed to put into perspective the various quantitative changes which occurred in the total nitrogen of the bovine, longissimus muscle and its subfractions, during growth and development. To do this it is necessary to reconstruct overall changes in the principal components of this fraction and discuss the relative alterations throughout the pre-weaning and feedlot phases of this experiment.

The principal component of total nitrogen is, of course, the protein nitrogen fraction, which, as is shown in Figure 11, displayed irregular changes with an initial decline in the muscle tissue nitrogen after the first analysis period. Such losses, which indicate a reduction in the proteinaceous elements of muscle tissue, have not been reported in previous research (Robinson 1952, Helander 1957, Dickerson and Widdowson 1960, or Lawrie 1961). However, it should be noted that not only were animals of different species used but also the results from the above mentioned reports were expressed on different basis than those of the present study. Care must be taken in comparing the present and previous results for contrasts of results between different species could lead to erroneous conclusions. Moreover, comparisons of works within the same specie could also lead to improper statements if the data are presented

on different bases.

Most previous works, especially that of Lawrie (1961), show protein nitrogen to exhibit significant increases in quantity post-natally, with near adult levels being reached at approximately eight months of age. In the present study a decrease in protein nitrogen was observed during the early weaning test periods.

Table II shows that weight gains in the present test animals exhibited normal increases from the first to the last experimental periods. Also, as seen in Figures 17 and 18, it has been shown by Guenther et al. (1965) and Berg (1967) that the sequence of tissue development in the "normally" growing bovine is bone tissue first, muscle tissue second, and fat tissue last due to their respective demands for nutrients. Considering the above facts, it is entirely feasible that bone development of the test calves, at the time of the first experimental period, could have been demonstrating maximal growth and attained a peak at, or just prior to, period two. Muscular growth would be in its early stages of development at this point but growing nonetheless in order to maintain body composition and support because of the rapidly growing skeleton. Also, during this time, the digestive system of the young calves would be developing and enlarging resulting in an increase in body weight. All of the above factors point towards a conclusion that the animal's weight increased due to the organ, system and skeletal growth primarily and muscle growth secondarily.

In periods four and five, analysis have shown a marked increment in protein nitrogen from period three. This increase could possibly have resulted from the onset of the major development of the muscle tissue.

TABLE II

AVERAGE AGE, WEIGHT, AND GAINS OF EXPERIMENTAL CALVES AT SPECIFIED EXPERIMENTAL PERIODS DURING GROWTH AND DEVELOPMENT

Period	Age in Days	Weight (kg)	Total Gain (kg)	Gain in kg/day by Period
1	46[a]	52.5	52.5	1.14
2	90	99.0	46.5	1.06
3	148	141.0	42.0	0.72
4	190	164.6	23.6	0.56
5	246	224.2	59.6	1.06
6	301	285.9	61.7	1.11
7	364	344.1	58.2	0.94
8	428	383.1	39.0	0.61

[a]Values indicated are period means for the experimental steers utilized in this study.

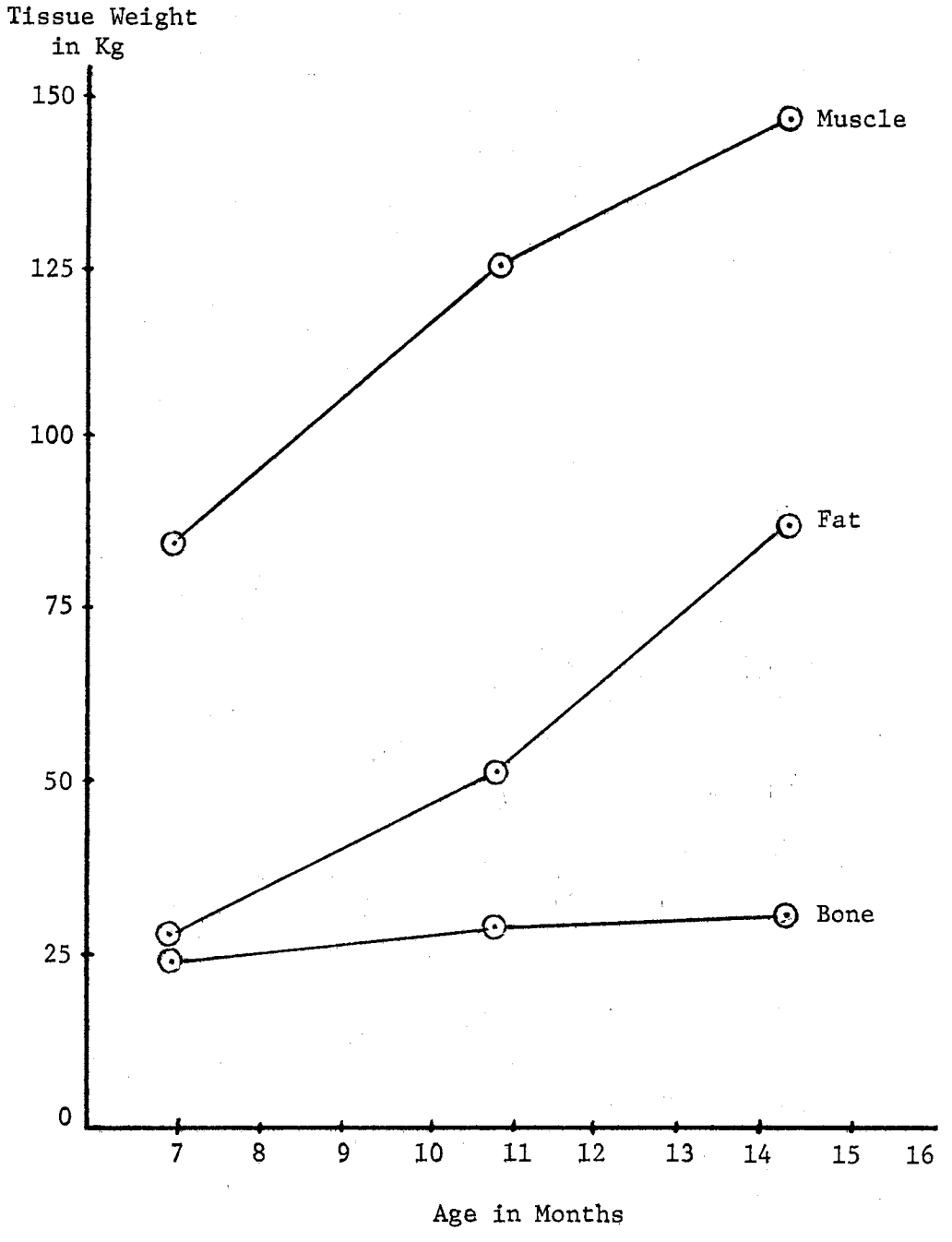


Figure 17. Growth Curves of Bovine on a High Plane of Nutrition Based on Total Carcass Values From Guenther, et. al. (1965)

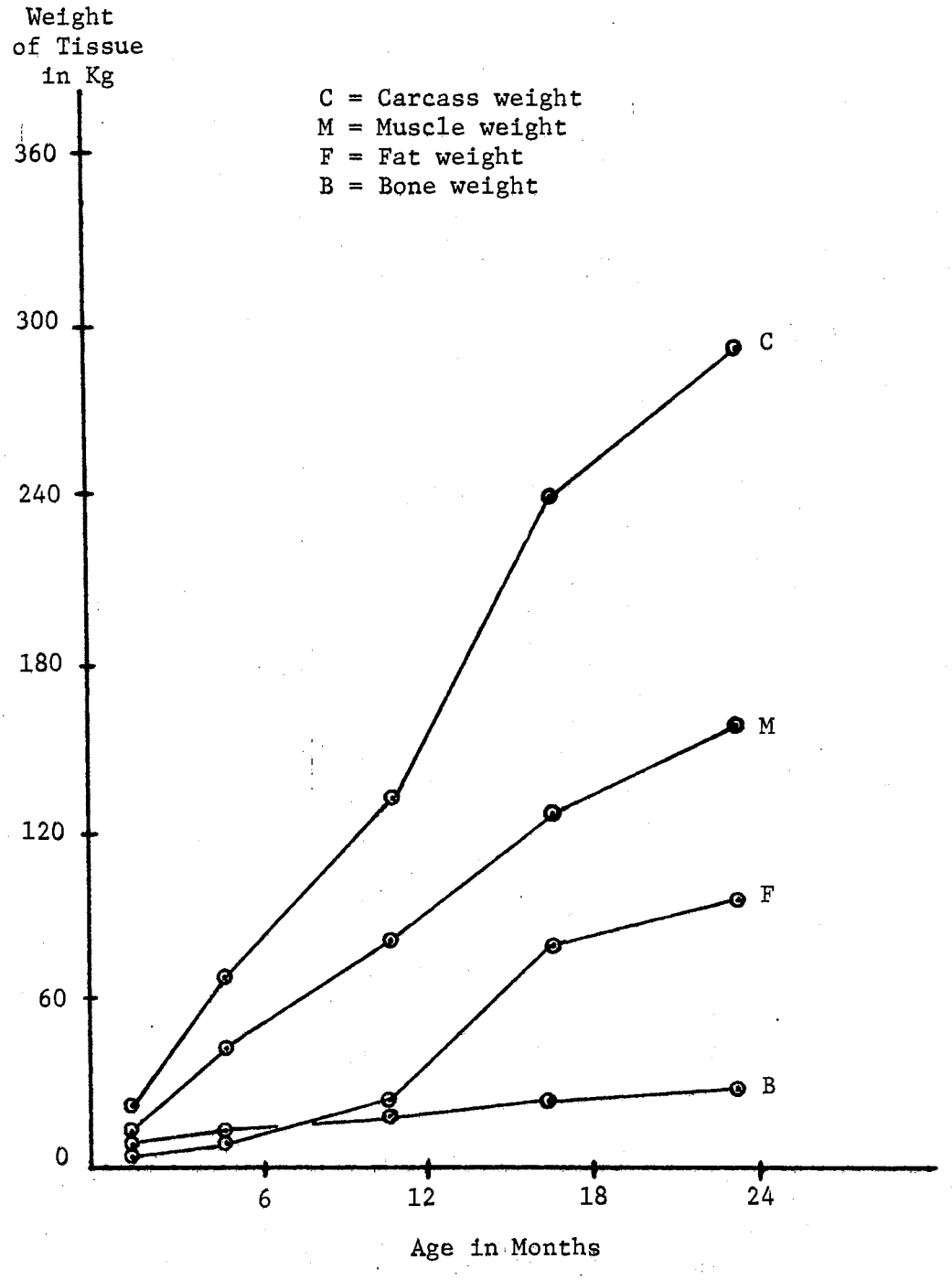


Figure 18. Carcass and Tissue Weights From Hereford Steers Slaughtered at Six-Monthly Intervals From Berg, R. T. (1967)

Since muscle systems and individual muscles grow by increasing the quantity of proteins within the fiber causing it to expand, not by increasing the number of fibers, a quantitative increase in the total true protein nitrogen suggests development of the muscular system in the animals during these periods. This continued enhancement in the percent protein nitrogen in the tissue continued throughout the length of the study except for a small decline which occurred in period six. This increase during the entire study period has indicated a continued enhancement in the quantity of protein in the muscle cell resulting in overall muscle tissue growth. Guenther et al. (1965) and Berg (1967) have shown that the major impetus for growth of muscle tissue peaks at approximately 11 months of age whereupon fat tissue begins its primary surge of growth. The author realizes that data presented by the above references were collected on a different breed of cattle, yet that breed has been shown to be slower maturing than the one used in this study. Whem comparisons are made with the data obtained in this study and the normal developmental patterns of muscle tissue, it can be seen that inconsistencies occur here, for a very sharp inflection in the total protein nitrogen graph (Figure 11) occurred in the last experimental periods. By observing the graph no possible indication has been displayed concerning the stabilizing of muscle tissue growth or fat tissue enhancement.

Since a lengthy period of muscle growth extending from the fifth month of age to the 14th month of age was not expected, an assumption could be made that the animals had an extended growth period for some reason, possibly nutritional in nature. The pastures on which the animals were grazed could have possibly been of sufficiently low quality that the cows were restricted and the calves resultingly became nutri-

tionally stressed. The continued muscle growth later in life could, in view of the above discussion, have been derived from "compensated" growth or growth ensuing at a later time in life compensating for a restricted growth earlier in life. From the data collected post slaughter, it was realized that fat growth had occurred and to a sufficiently large extent to result in intramuscular fat deposition. Yet this adipose tissue development apparently did not restrict the increases of the total protein nitrogen during the last stages of the experimental trial. Another possibility which could have caused the fluctuations in the protein nitrogen to occur was between side variation. Previous research, as well as research in this laboratory have shown definite side differences in biochemical attributes of the longissimus dorsi muscle. The data in Figure 11 and Table III show that protein nitrogen in the left longissimus dorsi exhibited steady increases from the initial to final analysis periods whereas that from the right longissimus dorsi tended to fluctuate in this regard.

Two final areas of possible discontinuities in the results which may be discussed here are concerned with experimental procedures and techniques. As explained earlier, samples were taken by live animal biopsy. After the final analysis period, the animals were slaughtered, and the carcasses were chilled for final carcass analysis. After chilling, sections from the rib and short loin (the biopsy areas) were observed. These observations revealed that fat had infiltrated the areas which had been biopsied. Thus, during the course of the experiment, muscle denervation had probably occurred at each sampling site, causing fat to infiltrate and occupy these areas. Due to this adipose tissue growth, possible errors in tissue nitrogen could have been made if the

TABLE III

BIOPSY LOCATION ACCORDING TO ANALYSIS PERIOD

Period	Area	Side	Position
1	2nd Lumbar	Right	3
2	2nd Lumbar	Left	3
3	11th Rib	Right	1
4	11th Rib	Left	1
5	13th Rib	Right	2
6	13th Rib	Left	2
7	4th Lumbar	Right	4
8	4th Lumbar	Left	4

infiltration was sufficiently widespread in the muscle tissue. As can be seen in Figure 5, some of the sampling areas would have been more susceptible to fatty tissue deposition than others, causing possible isolated problem areas. Accordingly, the most trouble would have evolved from position two in periods five and six. Nevertheless, protein nitrogen continued to increase throughout the study, suggesting that error due to fat infiltration was at least limited.

The final problem alluded to earlier, was the possibility of poor isolation technique which would result in unexplainable flucuations in the analysis throughout the study. As shown in Table IV, the recoveries of the nitrogen in the muscle tissue were very highly consistent throughout the test periods, averaging approximately 96.3 percent. With data such as this which suggests very consistent technique and in light of the above discussion, a conclusion must be drawn indicating that satisfactory total protein nitrogen results were obtained in this study.

Since the total protein nitrogen exhibited a loss in the early stages of the experiment and then showed increases throughout the remainder of the study and since this initial loss was expressed as a percent of a gram of muscle tissue, obviously another type of tissue necessarily had to make up the difference in the percent of the total nitrogen.

The total NPN of the muscle tissue displayed no statistically significant changes during the course of this study. A slight NPN decline in the periods pre-weaning has suggested a loss of the nonproteinaceous nitrogenous substances in the tissue. Possibly the most important of these substances during the postnatal stages of development was the amino acids, peptides, short chain polypeptides, and the purines and

TABLE IV

RECOVERY OF THE FRACTIONATED MUSCLE PROTEIN AS A PERCENT OF THE WHOLE MUSCLE PROTEIN IN BOVINE LONGISSIMUS MUSCLE[1,2]

Period	% Protein in Muscle Whole Sample			% Protein From Combined Fractionates			% Recovery
	A.	B.	Avg.	A.	B.	Avg.	
1	21.04	21.07	21.06	20.71	20.52	20.62	97.91
2	21.35	21.16	21.26	20.81	20.66	20.74	97.55
3	20.10	19.36	19.73	18.87	18.90	18.94	96.00
4	20.97	21.18	21.08	19.97	19.70	19.84	94.12
5	20.32	19.23	19.78	20.02	19.01	19.52	98.69
6	19.18	18.46	18.82	18.24	18.39	18.32	97.34
7	20.23	20.81	20.52	18.69	19.18	18.94	92.30
8	20.35	20.78	20.57	20.72	18.81	19.77	96.11

[1]One steer per period was chosen at random to use for a recovery check on the isolation procedure.

[2]All samples were analyzed in duplicate. Percent recovery was calculated from the average values.

pyrimidines. Neonatally, the calves' body was rapidly growing by increasing the bone tissue, organs, major systems and also muscle tissue. However, muscle tissue in this study appeared to start its major increase at approximately four months of age due to the influx of protein nitrogen at that point. As can be seen from Figure 11, no increase was noted in the NPN substances until after weaning where a single period enhancement was noted at period five. The decrease in NPN postnatally presents a first impression of possible error, however, on closer analysis it also may be stated that the tissue is growing rapidly enough so that on a percent per gram of tissue basis the NPN substances utilized less and less of the tissue "area". It should be noted here that if the percent NPN, as well as the percent protein nitrogen, were expressed on a muscle weight or total lean weight basis, an increase would definitely be seen.

The sharp increase in NPN noted at period five can be related to a possible nitrogen retention due to the elevated nitrogenous feeding regime. If one simply looked at the apparent increase at period five, it could be assumed that intense protein synthesis was occurring (due to compensatory growth) which would be paralleled by a high percentage of amino acids, peptides, polypeptides and nucleotides. But prior to period six a concomitant decrease in NPN was noted which would indicate one of two possibilities, that is, one protein synthesis occurred at such a rapid rate that the amino acids and peptides could not be incorporated into the protein chains efficiently prior to period five and, two, a situation of "catching up" occurred after period five. In the periods following period six, an increase was noted which stabilized during the last two trials. The increase at this point could possibly have been affected by the strong trend of protein nitrogen and total nitrogen de-

position which occurred in the same periods, caused by intense protein synthesis and followed by slight increases in NPN. Since no large changes occurred in the protein nitrogen, some other fraction necessarily must have fluctuated because all nitrogen values were based as a percentage in a gram of sample.

In this experiment the lipo-nitrogen, referred to in Chapter IV, was responsible for the major nitrogen changes which occurred pre-weaning. It can be seen from Figure 11 that this fraction exhibited a noticeable increase after the 1st period, peaking at period two and abating during periods three to five. After period five very little change was observed. This fraction, alluded to in some of the literature as the "floating material" has never been quantitatively separated from the various protein complexes by previous researchers. In the present study, the lipo-nitrogen fraction was noted to comprise a sizable portion of the total nitrogen and thus was separated, not only for interest but also for total quantitation purposes. The initial increment and relatively high quantities of this lipo-nitrogen recorded during the pre-weaning stages comprised approximately 25.7 percent of the total nitrogen in the initial period and near 30.0 percent in the second period, thus its importance was rather obvious from a recovery standpoint. Specific conclusions concerning this fraction should await results from further study, however, a few general points may be enumerated at this time.

It would appear that the lipo-nitrogen fraction may fluctuate in content with changes in the diet. For instance, when the test animals were young and depended largely on their dams for nutrients, this fraction was noted to be very high. However, when the calves became less

dependent on their dams and more dependent on pasture for nutrient supply, the lipo-nitrogen fraction started to decline. After weaning, this fraction remained at a low, constant level.

Purportedly, nutrient source has a significant effect on the growth and development of the various stomach compartments of the ruminant. During early post-natal life, when the calf is dependent upon milk and milk fats for subsistance, the abomasium growth and development is stimulated. Subsequently, when the calf becomes more and more dependent upon forage for subsistance, an increase in ruminal size, papillae, and activity occur. At periods one through three, the lipo-nitrogen was highest, coinciding with maximal abomasum development. Thus, at this time, the calf could utilize large quantities of dietary milk fat.

In periods four through five, lipo-nitrogen showed a significant decrease, becoming relatively static after the fifth test period. The above test periods (four through five) would coincide with that life phase during which the calves' rumen developed and became active. At this time, the calves could no longer metabolize large quantities of dietary fat, per se.

Thus it would seem that the changes in lipo-nitrogen content of the muscle cell was directly related to the state of development of either the abomasum or the rumen, which, in turn, is influenced by the animal's diet, both factors affecting the type of energy utilized by the muscle cell.

In close accord with the above discussion would be the differentiation of red to white muscle fibers. The percentage of red muscle cells is reported to be highest in the young bovine while the large percentage of white muscle cells prevail in the mature beef animal. In addition,

the red cell is associated with elevated lipid metabolism, whereas the white cell is primarily glycolytic in nature. Thus, during early post-natal life significant quantities of lipid might be found in muscle tissue probably bound within the fiber as a lipo-nitrogen complex as suggested by the data in this thesis. It would follow that as the bovine matures, intrafibrillar lipid content would be expected to decline as indicated by the above data, due to the prevalence of glycolytic type muscle fibers.

Total Protein Nitrogen

The gross changes which occurred in the total protein nitrogen were presented in Chapter IV. Specific changes and reasons for these changes in total protein will follow by a discussion of the components of this fraction, i.e., sarcoplasmic, myofibrillar, and stroma protein nitrogen as has been presented in Chapter IV.

The sarcoplasmic protein nitrogen, that protein nitrogen originating from the sarcoplasm or cellular fluids, expressed no statistically significant changes during the course of this experiment, although a slight decrease was noted in comparing the values from periods one and eight. This small decline in sarcoplasmic protein nitrogen is not in complete agreement with earlier results published by Helander (1957) Dickerson and Widdowson (1960), Dickerson (1960) and Lawrie (1961). However, as was previously stated, animals of different species were utilized in some of the above studies and different methods of expressing the data were followed. Although extra specie comparisons may not be entirely valid, it is of interest to note that in one occasion (Robinson, 1952a) the sarcoplasmic proteins were shown to decrease slightly from the

first to the last study periods. In this study, Robinson expressed his data as absolute concentrations and as a percent of the total protein nitrogen. In the former method, the sarcoplasmic protein nitrogen was seen to show rapid initial increases and a leveling off during the course of the experiment, whereas the latter method of expressing the data resulted in a definite sarcoplasmic protein nitrogen decrease. Even though Robinson's work cannot be directly compared with the data presented in this thesis, parallelisms between the method of expression of the data can be visualized. For instance, if, in the present study, the sarcoplasmic protein nitrogen data were expressed on a total lean or individual muscle weight basis, definite increases would be observed during the study, but when expressed as a percent of a gram of muscle, which in this case is the same as a percent of total nitrogen, the sarcoplasmic protein nitrogen showed a slight but non-significant decrease. Thus, the percentage method of expressing the sarcoplasmic protein nitrogen data utilized in this thesis accounts, in part, for the lack of complete agreement with these results and those found in previous literature.

What follows are possible reasons for the lack of a positive change in this fraction when expressed on a percent basis. It is a fairly well accepted fact that as a percent, the sarcoplasmic proteins comprise a high quantity of the muscle tissue (Lawrie, 1966). When the animal is young, there is a greater proportion of cellular fluids than there is of contractile protein units. As the calves developed, muscling occurred by increasing the size of the cells not by increasing the number of cells (Van Linge, 1962). This may be accomplished by the formation of additional myofibrillar protein within the cell, causing the fiber to increase

in diameter. During this expansion, the sarcoplasmic proteins also increase in quantity due to need for additional nutrient transporting media, yet the contractile protein units increased at a greater rate than did the sarcoplasmic proteins. This, in itself might account for initial superior sarcoplasmic protein nitrogen quantities which, with normal growth, inverted to superior myofibrillar protein nitrogen quantities.

It was also realized that a small percent of salt soluble collagen existed (Gross, 1957) and portions of it could be removed and retained in the sarcoplasmic and myofibrillar protein nitrogen fraction by the isolation techniques used in this study. Gross (1955) also stated that if the tissue had been frozen for more than a week, very little salt extractable collagen could be obtained. Basing these facts on the method of extraction used in this study, it can be realized that this soluble collagen would have caused very little differences to occur in the sarcoplasmic protein nitrogen fraction in the initial stages of the study for the tissue remained frozen for several weeks prior to extraction. Later in the study, an almost immediate extraction was made, imposing the possibilities of soluble collagen contamination in the sarcoplasmic and myofibrillar fractions. This added protein nitrogen could have enhanced the protein nitrogen values obtained in the sarcoplasmic fraction yet according to statements by Gross (1955) it can be assumed that more soluble collagen protein nitrogen would have been extracted with the myofibrillar fraction than with the sarcoplasmic fraction due to the extractable collagen's maximum solubility at $T/2 = 0.60$. With this in mind, it can be seen that the presence of soluble collagen probably aided a slight decrease in sarcoplasmic protein nitrogen and a

overall significant increase in the myofibrillar protein fraction.

Myofibril growth and soluble collagen presence have both attributed to an overall slight decrease in sarcoplasmic protein nitrogen quantity. It can be of interest to ascertain the effects of protein synthesis per se, on sarcoplasmic protein nitrogen quantities. Bloom and Fawcett (1970) have stated that protein synthesis occurs in the ribosomes, which according to Cambell (1966) is extracted with the sarcoplasmic proteins. As stated earlier, the myofibrillar proteins increased at a faster rate than did the sarcoplasmic proteins causing greater quantities of the former to be formed in the growing cell than the latter. Yet due to ribosomal extraction with the sarcoplasmic fraction and some newly synthesized myofibrillar proteins to be separated with this fraction, very slight decreases in sarcoplasmic proteins could be realized during growth and development of the calves.

The author is very much aware of the presence of marbling during growth of the animals and possible fat infiltration due to biopsy denervation. However, fat accumulations in the muscle tissue in these instances have no effect on intrafibrillar proteins and their relationships to each other, but on the other hand, as Figure 14 depicts, the fat tissue shows its affect on total nitrogen in the muscle tissue due simply to an increasing fat quantity in a gram of muscle tissue which results in the decrease in the percent of tissue nitrogen (Figure 11).

The changes which have occurred in the myofibrillar protein nitrogen fraction (Figure 12) has been shown to be highly significant ($P < .01$) in this study. This fraction has demonstrated a continued increase in the quantity from the first to the final analysis periods. In order to show such an increase, the contractile proteins must increase in

quantity in the muscle fiber tissue for, as stated earlier, no evidence has been reported confirming an increase in number of muscle fibers in the bovine longissimus dorsi. It is well known that the fibrillar proteins increase very rapidly in quantity at an early stage of life as confirmed by Dickerson (1960) and Dickerson and Widdowson (1960), but Lawrie (1961) has shown a point of leveling off in the growth to be approximately eight months. In this study, the myofibrillar protein nitrogen has shown a general increase from periods one through eight with a point of very little growth existing between periods two and five. One could say that in such circumstances something, possibly nutritional restrictions, has caused a lack of protein synthesis which would result in no fiber enlargement and no muscle growth. Possibly at this point, the nutritional needs of the essential organs and skeleton of the body demanded a greater share of the nutrients than the muscle, leaving an insufficient quantity for protein deposition. At period five a principal increase of protein nitrogen occurred followed by a sharp decline to period six. This fluctuation could possibly indicate a very unstable condition in the muscle with most of the nitrogen being the result of elevated nitrogen retention. After period six the myofibrillar protein nitrogen tremendously increased indicating very high rates of protein synthesis although at this time, it is usually found that the major increases in protein synthesis have already been made. Such latent growth would, as referred to earlier, suggest a possible nutritional restriction earlier in life.

In general the trend has been one of a continued myofibrillar protein increase throughout life even though several factors could have limited its progress. As cited earlier, the side from which the biopsy

was taken could have definitely affected its chemical composition at any period of analysis during the study. Considering this, it can be surmised that possible side variation could have been very instrumental in causing unexplainable fluctuations in the myofibrillar protein nitrogen in this study.

The denervation effects, alluded to earlier, demonstrated no visible adverse changes in the myofibrillar protein nitrogen in this study for a continued growth of this fraction was very evident during this study. Yet it can be concluded that if the sarcoplasmic fraction has shown a decrease due to the fat infiltration, as discussed earlier, the myofibrillar protein nitrogen would have made up the difference in percent of the total sample; however, since no loss of total protein nitrogen and total nitrogen was observed it is very improbable that losses could have occurred in either of these major fractions during development.

It should be explained at this point that soluble collagen, mostly soluble at the ionic strength of the myofibrillar protein nitrogen fraction, could have changed the inflection of the graph of this fraction. As explained earlier, and as confirmed in this lab by the author, soluble collagen was lost with storage but when muscle tissue was extracted very soon after sample acquisition, maximum quantities could be removed. As this study approached the last several stages of analysis, the muscle samples were extracted soon after procurement and could have resulted in significant amounts of collagen to be extracted with the myofibrillar protein nitrogen fraction. This would have caused an increase, although small, in the inflection of the overall graph, however, conclusions concerning the overall results of the myofibrillar protein nitrogen would

have still been the same.

The stroma protein nitrogen fraction, Figure 12, has shown an immediate decrease in content after period one. This initial decrease has been referred to in the literature by Robinson (1952a) and Dickerson and Widdowson (1960). They have stated that the stroma protein nitrogen is very high in the embryo at term and after birth but as the animal grows, the stroma protein nitrogen comprises less and less of the quantity of the total nitrogen in the muscle cell. However, Lawrie (1961) has shown that on a percent stroma protein nitrogen in a gram of fat free tissue, the stroma proteins increase until about the sixth month of age and then form a constant portion in the quantity of the muscle cell. In this study, the initial decrease follows literature responses. Thus, it can be stated, as Dickerson (1960) has stated, that the size of the fibers within the muscle tissue exhibited notable increases in diameter due to increases in the quantity of the myofibrillar protein nitrogen. This has been shown to be very evident as can be seen in comparisons with the graphs of the stroma and myofibrillar protein nitrogen in Figure 12. The increased size of the fiber with its greater quantity of protein nitrogen caused a decrease to be observed in the stroma protein nitrogen when expressed on a percent of a gram of muscle tissue.

Periods two and three presented no evidence of change, however, period four exhibited a very sharp increase (Figure 12). The static condition in the stroma nitrogen, in light of the above discussion, has resulted from the steady state of the myofibrillar elements. Yet in period four the myofibrillar nitrogen made slight increases paralleled by very noticeable increases in the stroma protein nitrogens. This type of increase follows an antagonistic trend that was developed in the first

three periods. It could be suspected that the denervation of the muscle tissue, alluded to earlier, had caused increased stroma protein nitrogen due to the possibility of the fat and connective tissues spanning the area between biopsy sites and infiltrating the tissue, causing the analysis in the 4th and subsequent periods to be high in that fraction. Yet Link (1967) has shown that no significant increases in stroma protein nitrogen was evident as a result of the denervation which occurred in their animals after live biopsies were made. However, when the remainder of the analysis periods were viewed, a steady increase was noted which followed the original statement that had been made explaining that the stroma proteins make up a decreasing amount of the muscle time during development.

It should also be noted here that the salt soluble collagen, discussed earlier, could have inversely changed the inflection of the graph of this fraction when comparisons were made with the myofibrillar fraction. Due to reasons explained earlier, very little change could have developed in the stroma fraction during the early stages. But when the extractions were made shortly after the biopsy, maximum amounts of the soluble collagen could have been removed from the "insoluble" stroma protein nitrogen resulting in less stroma present as the analysis progressed. In general, it can be seen that the sarcoplasmic protein nitrogen followed a very steady, almost static course throughout development while the stroma protein nitrogen, except for fluctuations during the first three periods, showed a constant, diminishing trend during growth of the animals. Also it can be seen that the myofibrillar protein nitrogen increased during the span of the experimental analysis. In view of the above changes it can be concluded that the major changes which

occurred in the total protein nitrogen were due to the changes displayed by the myofibrillar protein nitrogen.

Total NPN

In viewing the total NPN data from Figure 13, it can be seen that notable variation was observed during the experimental trials even though, as was stated earlier, no statistical significance could be declared. In order to ascertain the reasons for such changes, an analysis of the individual fractions which collectively made up the total NPN must be made.

The sarcoplasmic NPN fraction demonstrated significant changes ($P < .05$) during the study. A small drop in this fraction was observed during the pre-weaning periods which paralleled a similar decline in total nitrogen and a small resemblance to the total protein nitrogen changes. It has been discussed earlier that protein synthesis occurs in the ribosomes which are extracted in the sarcoplasmic fraction. When TCA is used to extract the sarcoplasmic nitrogen complex, two fractions evolve, sarcoplasmic protein and non-protein nitrogen. The non-protein nitrogen, as covered earlier, consists of amino acids, peptides, and other nonproteinaceous substances which are not large enough to be precipitated from solution with TCA. During protein synthesis, the amino acids and peptides are formed and used to build the long protein chains. If the muscle tissue is analyzed during a period of intense protein synthesis, it is entirely feasible that these newly formed nonproteinaceous materials could be present in the NPN fraction. Thus one might assert that when the sarcoplasmic NPN level follows a decending pattern, protein synthesis might be slowing. Since the total nitrogen and total

protein nitrogen fractions show a decrease during the initial periods of this study and this same trend was followed by the sarcoplasmic NPN fractions, it would be a logical assumption that protein synthesis had decreased in the later experimental periods, pre-weaning.

Due to its function as a nutrient transporting medium, the sarcoplasmic NPN fraction might be expected to reflect dietary changes in the experimental calves. Hence, when the calves were placed in the feedlot and thus on a higher proteinaceous diet, a marked increase in the sarcoplasmic NPN fraction (and subsequently an elevated rate of protein synthesis) might be expected. The data plotted in Figure 13 (periods five, seven, and eight) appear to substantiate this assumption.

The myofibrillar NPN fraction, Figure 13, showed very little changes during the pre-weaning stages of the study, indicating a very slow protein turnover in the myofibrillar fraction. In the feedlot periods, post-weaning fluctuations from period to period were very evident. A very high level of myofibrillar NPN was found at periods five, which might indicate that more amino acids and peptides were being formed and transported to the contractile units than could be incorporated into the growing myofibrillar proteins. A low point was attained at period six as was true for by the sarcoplasmic NPN fraction. Periods seven and eight exhibited fluctuations similar to those in periods five and six.

The stroma NPN displayed no significant changes during this growth and development study. Very consistently low quantities were evident during the entire study period indicating very low protein turnover occurred in this fraction.

Lipo Nitrogen

During the extraction of the muscle tissue for the portitioning of the various protein and non-protein fractions, a floating material was noticed in the extracts of the sarcoplasmic and myofibrillar fractions. The literature in some cases described such floating material as being a lipo-protein complex as in Emery (1969) but indicated no specific protein composition or changes made during growth in the bovine. Realizing that not much data of this type was available, these floating materials were separated and analyzed by procedures described in Chapter III. It was found that this lipo-nitrogen fraction contained approximately 25.7 percent of the total nitrogen in the muscle tissue at the first analysis period and, as stated earlier, became very important from a recovery standpoint.

Figure 14 reflects the changes in the total lipo-protein nitrogen. This fraction exhibits an increase during the first two analysis periods and an almost equal decrease during the next two analysis periods. These fluctuations in the total lipo-protein may be related to the development of the "true stomach" (abomasum) of the bovine. It is common knowledge that the ruminant's true stomach develops very early in life and then, as the animal's roughage intake increases, the rumen, reticulum and omasum begin to develop and function in order to utilize roughages, grains and other low fat feeds.

While the calves were young, their stomachs performed as a non-ruminant's (simple) stomach and in so doing, utilized the dam's milk as the primary source of nutrients. Milk, high in fat could be digested by this enzymatically active stomach, primarily the abomasum, and be transported to the liver and muscle for further degradation or to the liver

for fat biosynthesis.

It should be noted here that at a very young age, the muscle tissue in the bovine has a predominance of red fibers. These red fibers are rich in mitochondria and obtain their energy by oxidative metabolism. Long chain fatty acids can be utilized by these tissues as a source of energy and such long chain fatty acids can be obtained from the digestion of milk. The fatty acids are transported to the muscle tissue and are broken down there for energy. Thus it is reasonable to say that due to two factors, that is, the primary development of the true stomach and the presence of oxidative (red) fibers, a lipo-protein nitrogen fraction could be found in the young calf.

The decrease which was observed in the latter pre-weaning periods could be due to one or both of the above factors also. The development of the ruminant stomach results in less efficient utilization of fat as a source of energy due to the fact that most of the energy in the fully developed ruminant comes from the volatile fatty acids evolved from the digestion of feedstuffs by ruminal microorganisms. Fat, or rations high in fat, disrupts the sequence of digestion and very poor utilization of the fat results. Thus, due to changes in the stomach, fats are inefficiently utilized as energy sources in the more mature ruminant.

Also, as the animal develops, the red fibers which are abundant in the young bovine muscle differentiate into white fibers as the calf ages (Buller et al., 1960). The white fibers are primarily glycolytic in nature. Thus, as the animal ages, there is less lipid present in the muscle due not only to a smaller requirement but also to a limit as to how much lipid could be metabolized by the muscle. Thus, a decrease in the quantity of lipo-nitrogen observed during the development of the

bovine might be expected.

The lipo-NPN fraction, as shown in Figure 14 occupies a very small percent of the muscle tissue and exhibits a steady, almost unchanging state throughout the study.

The last portion of the lipo-nitrogen fraction to be duscussed is the lipid extractable nitrogen, or that nitrogen which was extracted from the lipid complex by the low and high ionic strength buffers. In the initial period, this fraction contained approximately 62 percent of the total lipo-nitrogen and from that point a statistically significant decrease ($P < .01$) was noticed which continued throughout the course of the study and, in the final analysis period, comprised approximately 30 percent of the lipo-nitrogen. The reasons for the extractions of the lipo-nitrogen complex were discussed earlier but it may be said that this fraction probably resulted from entrappment of the actual sarcoplasmic and myofibrillar protein nitrogen which, due to physical extraction, caused the release of said fractions, or the possible disruption of the strong forces which held the lipid and protein together in a complex resulting in a less concentrated lipo-nitrogen complex. Due to the very mild extraction performed at this step in the procedure, the possibility of a disruption of the interbonding becomes very slight. The graph of the lipo-protein nitrogen, Figure 14, depicts no similarities or parallels with the lipo-extractable nitrogen fraction thus indicating that the nitrogen which as extracted from the total lipid nitrogen fraction was very likely to have been entrapped in the lipo-nitrogen network and was later removed by re-extraction.

The reasons for its changes would be purely speculation at this point although a relationship seems to exist between the conformation of

the lipid complex and the amount of nitrogen entrapped. Further studies on this fraction would seem in order.

Since past literature concerning data of this type made no mention of a lipo-nitrogen fraction, and since the only way in which the total nitrogen values obtained in this study would coincide with published total nitrogen values, it was assumed that the lipid nitrogen was retained with its respective protein fraction and, accordingly, should be presented as such. Thus, it was of interest in this study to combine the lipid nitrogen fraction with its parent nitrogen fraction and determine this collective fraction's quantitative changes during development.

Sarcoplasmic Nitrogen + Sarcoplasmic Lipid Nitrogen

The changes which occurred in this sarcoplasmic protein nitrogen complex are reflected in Figure 15, in which the total sarcoplasmic protein nitrogen complex is compared to the sarcoplasmic protein nitrogen fraction. It can be seen that at the 1st analysis period the sarcoplasmic protein nitrogen complex is about 50 percent greater than the sarcoplasmic protein nitrogen alone. Very little change is made in this "complex" during the first three periods but then a sharp decrease is observed until period six and very little change occurs after this period. Thus, quite a bit of the lipo-nitrogen existed in the sarcoplasmic protein fraction during the first several stages of the study, with very little being evident during the last periods. This "complex" presents a very similar curve to that obtained by Robinson (1952a) when he expressed his data as a percent of the total nitrogen, although it is realized that extra species comparisons might be invalid.

As noted in Figure 15, very little changes were observed in the com-

bined NPN fractions of this complex.

Myofibrillar Nitrogen + Myofibrillar Lipid Nitrogen

Figure 16 depicts the changes occurring when the myofibrillar protein nitrogen and the myofibrillar lipid protein nitrogen were added to create a myofibrillar protein nitrogen "complex". It can be seen that the changes which occurred between the fibrillar protein nitrogen and the myofibrillar "complex" were almost parallel in all cases during development in the bovine.

It can also be observed that the changes which occurred in the myofibrillar "complex" NPN fractions, like those of the sarcoplasmic complex NPN fractions, were very small during the course of this study.

From the above discussions and from the comparisons of the graphs in Figures 15 and 16 with those graphs in Figure 14, it can be seen that the sarcoplasmic "complex" follows, very similarly, the changes which were displayed in the total lipid nitrogen graph, while those changes noticed in the myofibrillar "complex" exhibited no similar patterns. Accordingly, it might be concluded that most of the lipo-protein nitrogen originated from the sarcoplasmic protein nitrogen fraction, and a very constant, stable amount from the myofibrillar nitrogen fraction.

It should also be noted that the values at the final study period for the total myofibrillar "complex" were near published quantitative values for total myofibrillar protein nitrogen.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study was designed to assess the quantitative changes in total nitrogen and its subfractions in bovine longissimus dorsi muscle during the pre-weaning and feedlot life phases.

Experimental materials for this study were acquired from six grade Angus steer calves obtained from the Oklahoma Agricultural Experiment Station Lake Carl Blackwell herd. At the first analysis period the calves averaged 52.5 kg live weight and 46 days of age. The calves were allowed to remain with their dams until six months of age and then were placed in a feedlot where they were fed, ad libitum, a standard growing and finishing ration. The average daily gain during pre-weaning life was 0.87 kg per day. At the final analysis period in the study, the average weight was 331.1 kg and the average age was 428 days. The average overall daily gain was 0.90 kg.

At eight, six to eight week intervals, the calves were biopsied at previously designated sampling sites and this muscle tissue was used as the experimental materials for the study. The tissue was frozen immediately in liquid nitrogen and stored -20 degrees centigrade until analysis. One gram of muscle, freed of obvious fat and connective tissue was partitioned into thirteen nitrogen fractions. All fractionations were accomplished at zero to four degrees centigrade. These isolated components were analyzed via Macro - Kjeldahl procedures and the data were

converted to percent nitrogen on a wet tissue basis. All procedures utilized in muscle fractionation were developed in this laboratory and Macro-Kjeldahl procedures were modified to conform to fractionation analysis.

Results showed that there were no statistically significant changes in total nitrogen throughout the study. This was not unexpected as total nitrogen, as well as all results, was expressed as a percentage of the wet tissue sample. Quantitatively, the principal component of total nitrogen was total protein nitrogen which, on the average, comprised 65.5 percent of the total muscle nitrogen. Although the mean values of total protein nitrogen suggest a continued increase in this fraction as the calves matured, these changes were not of sufficient magnitude to be statistically significant. The total lipo-nitrogen fraction, however, which accounted for 20.7 percent of the total muscle nitrogen in this study, showed a highly significant ($P < .01$) decrease. These results showed a strong negative trend pre-weaning and a relatively stable trend post-weaning. The total NPN fraction, which averaged 13.5 percent of the total muscle nitrogen was the smallest component. This entity made very little changes overall except for a large inflection at period five, resulting in no statistical significance.

Statistically nonsignificant changes of the total protein nitrogen were a result of the opposing trends of its components, the sarcoplasmic, myofibrillar, and stroma protein nitrogen fractions. The sarcoplasmic entity, averaging 46.7 percent of the total protein and 30.6 percent of the total muscle nitrogen was quantitatively the most abundant of the aforementioned components. Its values remained very constant throughout the study and displayed no statistical significance. The myofibrillar

protein nitrogen, averaging 38.9 percent of the total protein nitrogen and 25.5 percent of the total muscle nitrogen displayed a very strong increase from the first to the last study periods, resulting in significance at the one percent level of probability. The stroma protein nitrogen displayed average values of 14.7 percent of the total protein nitrogen and 9.6 percent of the total muscle nitrogen placing it lowest in quantitative importance. This fraction showed a general negative trend throughout the study although rather inconsistent values were obtaind in the pre-weaning growth phase. Due to such variation, no statistical significance could be declared.

NPN from the sarcoplasmic, myofibrillar, and stroma components of the total muscle nitrogen was collectively referred to as total NPN. Based on past literature, it was known that most of the NPN would be isolated in the total cell soluble fraction. The work presented here reflects such conclusions, for the sarcoplasmic NPN fraction comprised 90.4 percent of the total NPN and 12.2 percent of the total muscle nitrogen. No statistical significance was declared for the total NPN yet statistical significance ($P < .05$) was found for the sarcoplasmic NPN. The myofibrillar NPN isolate made up a relatively steady portion, 7.6 percent of the total NPN and 1.0 percent of the total muscle nitrogen, throughout the study except for small increases and decreases in the post-weaning phase of growth. The stroma NPN subfraction remained at very low levels throughout the entire study comprising only 2.4 percent of the total NPN and 0.3 percent of the total NPN and 0.3 percent of the total muscle nitrogen. Both the myofibrillar and stroma NPN portions were found to show no statistically significant changes in their period mean values during the study.

In previously published works on muscle protein partitioning, a lipo-protein referred to as "floating material" has been reported. This "complex" was usually discarded or combined with the respective fraction from which it was isolated, such as the sarcoplasmic or myofibrillar nitrogen fractions. In this study the floating material obtained from the sarcoplasmic and myofibrillar nitrogen fractions was portioned into total lipo-extractable nitrogen, total lipo-protein nitrogen, and total lipo-NPN. Collectively, these three fractions were termed total lipo-nitrogen. The lipo-extractable nitrogen displayed statistical significance ($P < .01$) in its decrease from the first period of study to the first period post-weaning. At that point a slight negative trend was noted through the final study period. It averaged 37.6 percent of the total lipo-nitrogen fraction, indicating that it had a major role in the changes which the total lipo-nitrogen fraction displayed. Also, this fraction averaged 7.8 percent of the total muscle nitrogen. The total lipo-protein nitrogen displayed a relatively irregular increasing trend during this study, which in the analysis of variance displayed no statistical significance. This subfraction averaged 48.7 percent of the total lipo-nitrogen and 12.2 percent of the total muscle nitrogen. The lipo-NPN exhibited trace quantities at all times during this study averaging 3.4 percent of the total lipo-nitrogen and 0.7 percent of the total muscle nitrogen. Here, as in the lipo-protein nitrogen, no statistical significance could be declared.

When the lipo-nitrogen subfractions were combined with their respective parent subfractions, as appears to have been done in some past research works, very noticeable changes were observed. The addition of the sarcoplasmic lipo-protein nitrogen to the sarcoplasmic protein nitro-

gen resulted in a highly significant decrease ($P < .01$) while independently, the sarcoplasmic protein nitrogen suggested no significant decrease during growth. After combining these fractions, they averaged 35.6 percent of the total muscle nitrogen. When the myofibrillar protein nitrogen and myofibrillar lipo-protein nitrogen were collected, a fairly steady increase was observed displaying statistical significance at the one percent level of probability. The inflection, which the myofibrillar lipo-protein nitrogen added, resulted in a very consistent proportion indicating that a very steady quantity of myofibrillar extractable lipo-protein nitrogen was present at any one trial period throughout the study. Collectively, this fraction averaged 32.6 percent of the total muscle nitrogen. After combining the fractions as described above and comparing such data to previous literature findings on animals near slaughter age, it can be seen that in order to obtain values approaching those of past research, the respective lipo-nitrogen component must be added. When the lipo-NPN was added to its respective sarcoplasmic and myofibrillar subfractions, no changes could be observed in the statistical variation although slight increases in their quantities could be observed at all experimental periods.

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APPENDIX

TABLE V

ANALYSIS OF VARIANCE OF TOTAL NITROGEN AND ITS MAJOR COMPONENTS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT[a]

Source	DF	Total Nitrogen	Total Protein Nitrogen	Total NPN	Total Lipo Nitrogen
Corrected Total	95				
Animal	5	1.301	5.309	1.909	7.783
Period	7	7.872	17.599	2.133	18.130*
Animal X Period	33[1,2]	3.600	11.790	1.960	3.900
Duplicates (An Per)	44[1,2]	1.491	4.701	2.001	1.981

*Significant ($P < .01$).

[a]All values denote mean square.

[1]Error terms.

[2]The degrees of freedom do not sum to the Corrected Total degrees of freedom due to the loss of two animals in the last period which necessitated the use of missing data calculations.

TABLE VI

ANALYSIS OF VARIANCE OF THE MAJOR SUBFRACTIONS OF TOTAL PROTEIN NITROGEN IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT[a]

Source	DF	Sarcoplasmic Protein Nitrogen	Myofibrillar Protein Nitrogen	Stroma Protein Nitrogen
Corrected Total	95			
Animal	5	0.589	6.601	0.621
Period	7	0.541	22.470*	2.862
Animal X Period	33[1,2]	2.230	4.660	1.560
Duplicates (An Per)	44[1,2]	1.180	1.932	6.739

*Significant ($P < .01$).

[a]All values denote mean square.

[1]Error terms.

[2]The degrees of freedom do not sum to the Corrected Total degrees of freedom due to the loss of two animals during the last period which necessitated the use of missing data calculations.

TABLE VII

ANALYSIS OF VARIANCE OF THE SUBFRACTIONS OF THE TOTAL NPN IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT[a]

Source	D.F.	Sarcoplasmic NPN	Myofibrillar NPN	Stroma NPN
Corrected Total	95			
Animal	5	0.441	0.522	0.033
Period	7	1.120*	0.512	0.013
Animal X Period	33[1,2]	0.429	0.669	0.019
Duplicates (An Per)	44[1,2]	0.399	0.670	0.020

*Significant ($P < .05$).

[a]All values denote mean square.

[1]Error terms.

[2]The degrees of freedom do not sum to the Corrected Total degrees of freedom due to the loss of two animals in the last period which necessitated the use of missing data calculations.

TABLE VIII

ANALYSIS OF VARIANCE OF THE SUBFRACTIONS OF THE LIPO-FRACTION NITROGEN IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT[a]

Source	D.F.	Lipo-Protein Nitrogen	Lipo-Non-Protein Nitrogen	Lipo-Extractable Nitrogen
Corrected Total	95			
Animal	5	4.034	6.029	6.608
Period	7	1.989	6.050	14.161*
Animal X Period	33[1,2]	1.901	0.049	0.839
Duplicates (An Per)	44[1,2]	1.250	0.032	0.478

*Significant ($P < .01$).

[a]All values denote mean square.

[1]Error terms.

[2]The degrees of freedom do not sum to the Corrected Total degrees of freedom due to the loss of two animals in the last period which necessitated the use of missing data calculations.

TABLE IX

ANALYSIS OF VARIANCE OF THE SARCOPLASMIC + SARCOPLASMIC LIPO-NITROGEN FRACTIONS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT[a]

Source	D.F.	Sarcoplasmic + Sarcoplasmic Lipo-protein Nitrogen	Sarcoplasmic + Sarcoplasmic Lipo-NPN
Corrected Total	95		
Animal	5	2.878	0.410
Period	7	21.883**	1.092*
Animal X Period	33[1,2]	4.111	0.429
Duplicates (An Per)	44[1,2]	2.301	0.410

*Significant ($P < .05$).

**Significant ($P < .01$).

[a]All values denote mean square.

[1]Error terms.

[2]The degrees of freedom do not sum to the Corrected Total degrees of freedom due to loss of two animals in the last period which necessitated the use of missing data calculations.

TABLE X

ANALYSIS OF VARIANCE OF THE MYOFIBRILLAR + MYOFIBRILLAR LIPO-NITROGEN FRACTIONS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT[a]

Source	D.F.	Myofibrillar + Myofibrillar Lipo-Protein Nitrogen	Myofibrillar + Myofibrillar Lipo-NPN
Corrected Total	95		
Animal	5	1.87	0.67
Period	7	22.58*	0.60
Animal X Period	33[1,2]	2.76	0.69
Duplicates (An Per)	44[1,2]	1.54	0.74

*Significant ($P < .01$).

[a]All values denote mean square.

[1]Error terms.

[2]The degrees of freedom do not sum to the corrected total degrees of freedom due to the loss of two animals in the last period which necessitated the use of missing data calculations.

TABLE XI

ANALYSIS OF VARIANCE ON THE 13 ORIGINAL SUBFRACTIONS OBTAINED FROM THE COMPLETE FRACTIONATION OF BOVINE LONGISSIMUS MUSCLE TISSUE DURING GROWTH AND DEVELOPMENT[a]

		Sarcoplasmic Nitrogen						Myofibrillar Nitrogen					Stroma Nitrogen	
Source	D.F.	Protein	Non-Protein	Lipo-Protein	Lipo-Non-Protein	Lipo-low Buffer Extractable	Lipo-high Buffer Extractable	Protein	Non-Protein	Lipo-Protein	Lipo-Non-Protein	Lipo-high Buffer Extractable	Protein	Non-Protein
Corrected Total	95	1.391	0.440	1.210	0.002	0.865	0.303	4.515	0.609	0.780	0.028	0.065	1.126	0.018
Animal	5	0.588	0.439	1.969	0.002	0.249	0.293	6.604	0.518	1.343	0.019	0.051	0.617	0.027
Period	7	0.539	1.122*	4.745**	0.007*	8.177**	1.770**	22.472**	0.514	1.941	0.031	0.246	2.859	0.012
Animal X Period	33[1,2]	2.233	0.428	1.190	0.003	0.471	0.276	4.660	0.670	1.091	0.040	0.107	1.560	0.020
Duplicate (An Per)	44[1,2]	1.184	0.400	0.740	0.001	0.190	0.130	1.930	0.673	0.400	0.030	0.020	0.740	0.020

*Significant ($P < .05$).

**Significant ($P < .01$).

[a]All values denote mean square.

[1]Error terms.

[2]The degrees of freedom do not sum to the Corrected Total degrees of freedom due to the loss of two animals in the last period which necessitated the use of missing data calculations.

TABLE XII

PERIOD MEAN VALUES FOR TOTAL NITROGEN AND ITS MAJOR COMPONENTS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT*

Period	Total Nitrogen	Total Protein Nitrogen	Total NPN	Total Lipo-Nitrogen
1	3.05	1.86	0.41	0.78
2	3.02	1.72	0.40	0.90
3	2.93	1.73	0.37	0.83
4	2.84	1.92	0.33	0.59
5	3.03	2.05	0.54	0.45
6	2.72	1.92	0.32	0.48
7	2.97	2.07	0.41	0.49
8	3.13	2.31	0.43	0.39

*All values expressed as percent nitrogen on a wet tissue basis.

TABLE XIII

PERIOD MEAN VALUES FOR THE TOTAL PROTEIN NITROGEN SUBFRACTIONS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT*

Period	Sarcoplasmic Protein Nitrogen	Myofibrillar Protein Nitrogen	Stroma Protein Nitrogen
1	0.97	0.50	0.39
2	0.90	0.62	0.20
3	0.94	0.62	0.18
4	0.86	0.68	0.38
5	0.90	0.85	0.31
6	0.93	0.68	0.32
7	0.89	0.91	0.26
8	0.88	1.19	0.24

*All values expressed as percent nitrogen on a wet tissue basis.

TABLE XIV

PERIOD MEAN VALUES FOR THE TOTAL NPN SUBFRACTIONS OF BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT*

Period	Sarcoplasmic NPN	Myofibrillar NPN	Stroma NPN
1	0.38	0.02	0.01
2	0.39	0.01	0.01
3	0.36	0.01	0.01
4	0.31	0.01	0.01
5	0.44	0.08	0.02
6	0.31	0.01	0.01
7	0.32	0.09	0.01
8	0.40	0.02	0.01

*All values expressed as percent nitrogen on a wet tissue basis.

TABLE XV

PERIOD MEAN VALUES FOR THE TOTAL LIPO-NITROGEN SUBFRACTIONS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT*

Period	Total Lipo-Protein Nitrogen	Total Lipo-NPN	Total Lipo-Extractable Nitrogen
1	0.29	0.01	0.48
2	0.40	0.02	0.47
3	0.47	0.01	0.34
4	0.39	0.03	0.17
5	0.32	0.02	0.11
6	0.35	0.02	0.10
7	0.37	0.03	0.09
8	0.37	0.04	0.08

*All values expressed as percent nitrogen on a wet tissue basis.

TABLE XVI

PERIOD MEAN VALUES FOR THE TOTAL SARCOPLASMIC NITROGEN SUBFRACTIONS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT*

Period	Sarcoplasmic + Sarcoplasmic Lipo-Protein Nitrogen	Sarcoplasmic + Sarcoplasmic Lipo NPN
1	1.44	0.39
2	1.43	0.39
3	1.37	0.36
4	1.15	0.32
5	1.07	0.44
6	0.99	0.31
7	0.98	0.32
8	0.92	0.41

*All values expressed as percent nitrogen on a wet tissue basis.

TABLE XVII

PERIOD MEAN VALUES FOR THE TOTAL MYOFIBRILLAR NITROGEN SUBFRACTIONS IN BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT*

Period	Myofibrillar + Myofibrillar Lipo-Protein Nitrogen	Myofibrillar + Myofibrillar Lipo-NPN
1	0.79	0.03
2	0.96	0.03
3	1.00	0.02
4	0.95	0.03
5	1.11	0.09
6	1.07	0.03
7	0.28	0.12
8	1.50	0.05

*All values expressed as percent nitrogen on a wet tissue basis.

TABLE XVIII

PERIOD MEAN VALUES FOR THE 13 ORIGINAL SUBFRACTIONS OBTAINED FROM THE COMPLETE FRACTIONATION OF BOVINE LONGISSIMUS MUSCLE DURING GROWTH AND DEVELOPMENT*

	Sarcoplasmic Nitrogen						Myofibrillar Nitrogen					Stroma Nitrogen	
Period	A Protein	B Non-Protein	G Lipo-Protein	H Lipo-Non-Protein	K Lipo-low Buffer Extractable	L Lipo-high Buffer Extractable	C Protein	D Non-Protein	I Lipo-Protein	J Lipo-Non-Protein	M Lipo-Buffer Extractable	E Protein	F Non-Protein
1	0.97	0.38	0.14	0.00	0.34	0.06	0.50	0.02	0.15	0.01	0.09	0.39	0.01
2	0.90	0.39	0.23	0.01	0.30	0.15	0.62	0.01	0.17	0.02	0.03	0.20	0.01
3	0.94	0.36	0.31	0.01	0.12	0.19	0.62	0.01	0.17	0.01	0.02	0.18	0.01
4	0.86	0.31	0.23	0.01	0.06	0.09	0.68	0.01	0.16	0.02	0.02	0.38	0.01
5	0.90	0.44	0.14	0.00	0.03	0.05	0.85	0.08	0.19	0.01	0.02	0.31	0.02
6	0.93	0.31	0.04	0.00	0.01	0.03	0.68	0.01	0.31	0.02	0.06	0.32	0.01
7	0.89	0.32	0.06	0.01	0.02	0.03	0.91	0.09	0.30	0.03	0.04	0.26	0.01
8	0.88	0.40	0.03	0.01	0.01	0.03	1.19	0.02	0.24	0.03	0.04	0.24	0.01

*All values expressed as percent nitrogen on a wet tissue basis.

VITA

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