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Growth of the *longissimus* muscle in male cattle

by

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INTRODUCTION

Muscle tissue has a very high biological value as a food in the human diet. The demand for this quality product has greatly increased over the years, especially in the case of cattle muscle tissue. It thus becomes important to attempt to maximize the rate and efficiency at which this tissue is synthetized. Major improvements in growth of cattle in the future will require a better understanding of the changes occurring in muscle during growth.

Castration of meat producing animals is probably as old as the domestication of animals. The decrease in efficiency and the changes in conformation in the castrated animal may have also resulted in differences in growth. Thus, there is a need for further study of the effects of this management practice in cattle on the muscular growth.

Another factor important for a better understanding of growth are the differences among breeds. For example, some breeds were selected for their milk production while others for their meat producing abilities. Therefore, there is interest for the study of the breed characteristics as a tool to affect the general muscle growth process of cattle.

Since the longissimus muscle is the largest muscle of the body, and since it is thought to develop at a similar rate as the total body, this muscle would be a very good tool to work with to study the progressive changes during growth of different types of cattle.

The goal of this work was to study the general growth of the longissimus muscle of male cattle. To reach this goal, two breed types and two types of male cattle were used. Therefore, our objectives were to:

1) Determine the effect of progressive live weight changes on different muscle protein fractions, DNA concentration and fiber diameter in the longissimus muscle of 45, 145, 245, 345, 445 kg cattle.

2) Compare intact and castrated male cattle for the changes in the various parameters studied in the longissimus muscle during growth.

3) Compare beef cattle and dairy cattle males for the changes in the various parameters studied in the longissimus muscle during growth.

4) Reevaluate the general muscle growth gradients based on the parameters studied in the longissimus muscle and maybe better understand the general growth of muscle.

Abbreviations Used

The following abbreviations will be used throughout this thesis:

DNA deoxyribonucleic acid

RNA ribonucleic acid

TDN total digestible nutrient.

LITERATURE REVIEW

General Growth

Development is:

> "a unified process which includes the appearance of orderly, recognizable patterns as a consequence of (or at least accompanied by) the formation of new constituents, the fabrication of these constituents into larger units, and their arrangement in space" (Ebert, 1966).

Within development, differentiation would correspond to a complex of changes leading to orderly diversification of structure and function of cells while growth would simply be the permanent enlargement of existing structures. Excess fat and double-muscling are not regarded as essential for body life and thus are not considered as part of the normal development process.

Hammond and Appleton (1932) introduced a complete dissection technique with sheep. Based on data from detailed anatomical work using this technique, they postulated two gradients of growth: one going from the head toward the hindlimbs and another one going from the end of the extremities, moving toward the spine of the body. This theory was further studied by Huxley (1932) who concluded that the chronological changes which take place in the conformation and composition of the body during development follow the allometric equation $y = bx^{\alpha}$ where y is the weight of the part, b a constant, x the weight of the animal or other independent variable, and α the growth

coefficient of the part. The absolute size is thus the only factor determining the form of an animal which is not subjected to environmental or nutritional limitations. Thus, the various parts of an animal develop according to a very definite order and rate.

The idea of gradient of growth implies a differential growth rate of the muscles within each part of the body. While Hammond and Appleton (1932) were able to group these muscles on their early or late maturity, the first major classification was initiated only recently (Butterfield, 1963a,b; Butterfield and Berg, 1966). Their study of 95 muscles in several breeds of cattle varying in age showed four classes of muscles. They showed that the muscles which develop early in life were: 1) those which arose from the distal half of the vasti and articularis genu; 2) those of the thoracic limb which arose from the scapula or proximal half of the humerus; 3) those of the thoracic limb which arose from the distal half of the humerus, from the radius or from the ulna. They showed that certain muscles were late developing. They were: 1) those of the pelvic limb which arose from the ox coxae, together with the vasti and articularis genu muscles; 2) those of the abdominal region. Also, they showed that others are very late developing. Those included: 1) the muscles of the thorax which were attached to the thoracic limbs; 2) muscles of the neck which were attached to the thoracic limbs and 3) the muscles of the neck and thorax. In their classification, only one group of muscles developed at the same rate as the body. This was the group of muscles surrounding the spinal column in the thoracic and lumbar regions like

the longissimus muscle. This classification was adopted completely or partly for pigs (Cuthbertson and Pomeroy, 1962a,b; Richmond and Berg, 1971), sheep (Lohse, Moss and Butterfield, 1971) and cattle (Luitingh, 1962). For the most part, these workers used females and castrated males to illustrate their classification.

Bonsma (1967) stated that intact males would grow somewhat differently from castrated males because the sex hormones present in the intact males promoted the closure of the epiphyses of long bones and indirectly influenced related muscles. However, Berg (1968), Mukhoty and Berg (1971) did not observe any significant differences between intact and castrated males or females for the gradient of growth of cattle. Breeds did not have any effect on these gradients either (Berg and Butterfield, 1968; Mukhoty and Berg, 1971). So, of the ages studied, no factors affected the growth gradients of the body tissues. This was in complete agreement with Huxley (1932) who postulated the allometric growth pattern in animals.

In addition to the gradients of growth for the different parts of the body, it appeared that the first tissue to be synthesized is the nervous tissue, then bone, muscle and finally fat (Hammond and Appleton, 1932). The different locations of the fat deposits also followed a gradient. Interpretation of growth data has brought conflicting views, in particular due to the fat deposition during growth. The nutrition is a factor recognized by Huxley (1932) that might affect the allometric growth because of its relation to the deposition of excess fat. Hammond and Appleton (1932) initiated a series of

experiments to study the effect of nutrition on the carcasses of sheep of the same strain. Such studies were also done with pigs (McMeekan 1940a,b,c) and with fowl (Wilson 1954b). These experiments were quite similar in their objectives and procedures so they will be discussed as a whole. These workers reared animals on two different planes of nutrition and then, around weaning time, switched the diets for half of the animals. Thus some were reared on a high or low plane of nutrition for either part or the whole time the trial lasted. Based on a rather limited number of individuals of each species, it was concluded that a period of restriction during growth had differential effects on the various tissues and parts of the body, correlating with the stage of growth of the tissue at the time of restriction. The growth of a tissue was retarded the most in a tissue with the highest natural growth impetus at the time of restriction.

Pomeroy (1941) reared some castrated male pigs to 150 kg and then fed them only straw and water. At regular intervals, a few pigs were slaughtered until the last ones weighed only 90 kg. He confirmed the results of McMeekan (1940c) and proposed that the tissues of the body were influenced in reverse order to their maturity by this submaintenance feeding regime.

Palsson and Verges (1952) fed groups of ewes and their lambs a high or low level of nutrition starting at early gestation and lasting until the lambs were 41 weeks old. They reported that a nutritional restriction affected the growth of the body in inverse relation to the organ or the part with the maximum growth impetus at the time of

restriction. They confirmed the results seen earlier by Hammond and Appleton (1932).

The previous conclusions were all based on comparisons of animals of similar live weights. Wallace (1948a,b) fed several ewes with different energy levels. Some of the lambs, produced by these ewes, were sacrificed during foetal stages and some were fed a high or low nutritional regime postnatally. He reported some marked differences in live weight of the lambs as shown by Hammond and Appleton (1932) and Palsson and Verges (1952). Wallace (1948a,b) also showed extreme differences in shape or appearance in these lambs and thus proposed that good nutrition was essential for the optimum growth of a lamb.

Wallace (1948c) questioned the conclusions of McMeekan (1940a, b,c). Wallace thought that the growth of a tissue based on its weight should be expressed on an equal tissue or part weight basis like total muscle or total weight of a limb. Wallace (1948c) argued that the proportion of each tissue in a joint was constant when examined on the same weight of that tissue, but may have been different if confounded with other parts or tissues of the body. A reassessment of the data of McMeekan (1940a,b,c) using logarithmic graphs (Wallace, 1948c) or growth curves (Elsley, McDonald and Fowler, 1964) suggested that plane of nutrition affected uniformly the overall rate of growth, but not with respect to fat tissue. Fat deposition was probably the cause of the disagreement between the two schools of thought. The conclusions of Wallace (1948c) were also obtained in fowl (Wilson, 1954a,b) and in dwarf goats (Wilson, 1958a,b). Moulton (1923) studied the changes

in form and live weight of castrated male cattle on different planes of nutrition. He reported that the mature sizes and proportions were the same in all the animals. His results agreed indirectly with the allometric theory of Huxley (1932) which said that at a tissue weight, the tissue had a certain shape or composition. Wallace's conclusions (1948c) were also in complete agreement with these concepts.

Muscle Proteins and Structure

The chemical and structural analysis of muscle has been often centered around the determination of the various protein fractions. These components are responsible for the shape and activity of muscle fiber. The myofibrillar proteins, soluble in concentrated salt solution, constitute the basic structural proteins responsible for contraction (Helander, 1957). The sarcoplasmic proteins, soluble in water or dilute salt solution, are used as storage nutrients. They are also involved in the intermediary metabolism of the muscle cell and constitute the auxiliary proteins of the cell. Finally, the connective tissue fraction contains those proteins that are insoluble in salt solution and which give rigidity, strength and some intrinsic toughness to the muscle. They represent a large portion of the tendons of a muscle. The main protein of the connective tissue is collagen which corresponds roughly to 75 percent of this fraction in the longissimus muscle of cattle (Wilson, Bray and Phillips, 1954). This percent of collagen within the connective tissue will vary between

muscles and species of livestock.

The total protein content of muscle increases very rapidly in early postnatal life, and then levels off (Spray and Widdowson, 1950; Usborne, Kemp and Moody, 1968; Robinson and Bradford, 1969). This plateau occurs at different ages depending on the species, but it seems to correspond to a chemical maturity (Moulton, 1923). It has been shown that the leveling off is initiated during adolescence in pigs (Dickerson and Widdowson, 1960; Sink and Judge, 1971), in cattle (Lawrie and Kirton, 1961), in fowls (Robinson, 1952; Dickerson, 1960), in rats (Cheek, Powell and Scott, 1965b), and in mice (Robinson and Bradford, 1969).

Myofibrillar and sarcoplasmic proteins increase very rapidly at first and then level off as does the total protein content (Helander, 1957; Dickerson and Widdowson, 1960; Gordon, Kowalski and Fritts, 1966). A good alternative way to show that the protein content reaches a plateau is the lack of increase in these two muscle protein groups when the animals are older (Usborne et al., 1968; Link et al., 1970b; Sink and Judge, 1971). The concentration curves of the myofibrilliar and sarcoplasmic proteins seem to follow the same general concentration curve of the total protein. These parallel curves are also found during the prenatal life (Dickerson, 1960; Dickerson and Widdowson, 1960).

The variation in total protein content among the different muscles might be important and is probably related to the postnatal growth curve and maturity of each muscle. Lawrie and Kirton (1961) studied the protein concentration in the psoas major and longissimus muscle of

cattle. They reported larger values for myofibrillar and sarcoplasmic proteins in the longissimus muscle than in the psoas major muscle. Scow and Hagen (1955) also reported greater protein concentrations for the temporal over the biceps femoris muscle of guinea pigs. Lawrie (1961) reported differences within the longissimus muscle of mature cattle. While a variation within a muscle may have existed, it did not mean that the protein composition of this muscle varied at random. In fact, work-induced growth in leg muscles brought an increase in the incorporation of amino acids with no change in the qualitative synthesis of myofibrillar or sarcoplasmic proteins (Goldberg, 1968).

Gonadectomy of male guinea pig was without effect on the total, myofibrillar and sarcoplasmic proteins of the rectus femoris muscle, while it sharply decreased the total amount of these proteins in the temporal muscle of older guinea pigs (Scow and Hagen, 1955). The same kind of experiment on the thigh muscle of rats (Scow and Hagen, 1957) showed no effect of testosterone on protein concentration. Link et al. (1967) presented data supporting the idea that there was no difference in protein concentrations between the longissimus muscles of female and castrated male cattle. The results are not always as conclusive because of the differences between muscles. Generally, the myofibrillar and sarcoplasmic protein concentrations follow the total protein concentration of the whole muscle.

Because of the high contents of connective tissue in tendons (Lawrie, 1966) and the various needs for strength in the attachments

of muscles working hard, it is normal to observe differences between muscles for the collagen content. Lawrie (1966) showed that collagen was the most important protein of connective tissue. It was found that the amount of collagen per gram of protein varied between muscles and between species. The longissimus muscle contained more collagen per gram of protein than the psoas major muscle (Lawrie, 1966) but much less than the extensor carpi radialis (Lawrie, 1966), soleus and plantaris muscles (Chiakulas and Pauly, 1962), semitendinosus muscle (Bendall and Voyle, 1967) and approximately the same as the biceps femoris muscle (Nottingham, 1956; Lawrie, 1966). There was a close association between work and collagen concentration.

Bendall and Voyle (1967) determined the collagen content of the longissimus and semitendinosus muscles of castrated male cattle ranging from 1 to 24 months of age. They reported that the collagen concentration was high at early ages, but it decreased after six months to stay constant thereafter. Chiakulas and Pauly (1962) had previously reported the same findings in young littermate rats while working with the plantaris, soleus and extensor carpi radialis muscles. Goll, Bray and Hoekstra (1963) reported a lower concentration of collagen in 18 month old castrated male cattle than in one month old calves in the biceps femoris. Carpenter et al. (1963) observed no real difference in collagen concentration of pigs ranging from 4 1/2 to 42 months of age.

Total collagen content per muscle has been shown to increase with live weight in rats (Cheek et al., 1965a,b), in cattle (Mitchell, Hamilton and Haines, 1928; Mackintosh, Hall and Vail, 1936) and in

sheep (Bate-Smith, 1948). In a study of mature castrated male and intact female cattle, Yeates (1964) observed that the connective tissue is quantitatively a relatively stable component of the muscle compared to muscle fiber size and the fat fraction. So the collagen concentration was high in early postnatal life and then decreased to stay constant, while its total amount increased with age like the other protein fractions.

Mackintosh et al. (1936) observed no difference in collagen per gram of muscle nitrogen between yearling castrated male calves full-fed different nutritional regimes. This lack of response to feeding regimes was also reported by Batterman, Bray and Phillips, (1952) when these regimes were served to aged female cattle. Wilson et al. (1954) showed no effect of grade or fat percent in the carcass on the collagen content of the fat-free carcasses of intact and castrated male and female calves.

DNA and Muscle Fiber Development

The various histochemical studies done with mammals support the idea that the total number of muscle fibers is reached during prenatal life or soon afterwards (Cowdry, 1942; Joubert, 1956b; Goss, 1966; Hedrick, 1968; Widdowson, 1970). Hyperplasia has been shown to occur postnatally in most other body tissues except nervous tissue (Enesco and Leblond, 1962; Goss, 1966; Harbison, 1970). However, it is possible to conceive an elongation of the muscle fiber by a form of hyperplasia

or fusion of new myogenic cells to existing fiber (Holtzer, Marshall and Finck, 1957).

Most body cells are mononucleated and thus contain the same number of nuclei as the number of cells. Because of the muscle fiber is a multinucleated cell, the number of nuclei is not a good measure of fiber number (Cheek et al., 1971). It merely represents the physiological muscle cell numbers that may be quite different from the number of anatomical fiber cells.

Skeletal muscle represents a very large proportion of the body of mammals. The relation of mature muscle mass of a specie to its live weight has been shown to be constant, irrespective to the size of the animal (Munro, 1969). Munro and Gray (1969) analyzed the DNA content of the liver and thigh muscle of various animals. They showed that the DNA concentration per gram of total body weight or per gram of muscle decreased to 45 percent from adult mice to mature horses. However, the total DNA content of the muscle increased by several hundred folds due to the weight differences. They proposed DNA values of 137mg/kg of body weight and 30mg/100g of muscle for a 450 kg castrated male calf.

The effect of age or weight on the total content of DNA within a muscle has been extensively studied. Robinson and Bradford (1969) reported a steady increase in DNA total content of mice between birth and 12 weeks of age. The DNA content of the hind leg of mice was shown to increase with time up to 200 percent (Robinson and Lambourne, 1970).

Enesco (1961) reported several fold increases of the total DNA content of various muscles of rats 17 to 95 days old. This finding in rats was also shown by Enesco and Puddy (1964). Cheek et al., (1965a) observed a similar increase of the DNA content in the quadriceps muscles (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius) between one and eight week old rats.

As the total DNA content of a muscle increases, the weight of this muscle increases also, but at a greater rate. Srivastava and Chaudhary (1969) studied the changes in protein and ribonucleic acid metabolism in the hindleg of mice between one and 320 days of age. Using their RNA and RNA/DNA values, the DNA concentration can be calculated. It appeared from these data that the concentration of DNA per gram of muscle decreased with time to reach a plateau level when the synthesis of DNA is stopped. This general curve was reported also in other experiments using rats (Devi et al., 1963; Cheek et al., 1965a; Howarth and Baldwin, 1971a,b), chicks (Moss, Simmonds and McNary, 1964; Moss, 1968a), guinea pigs (Kochakian, Hill and Harrison, 1964), pigs (Harbison, 1970) and lambs (Norton and Walker, 1970). The time necessary to reach this plateau is very poorly defined in the literature. For example, Winick and Noble (1965) reported the cessation of DNA synthesis in muscle after 100 days or more while Gordon et al. (1966) did not find any DNA concentration changes as early as 78 days of age. Cheek et al. (1965a) and Devi et al. (1963) showed very little change after 150 g live weight or six to eight weeks of age. In sheep, the plateau was seen very early in life (Master, 1963; Norton and Walker, 1970) and any protein

to DNA ratio as a measure of cell size will thus follow closely the protein concentration curve. It is however definite that the DNA concentration curve reaches a minimum level and this plateau coincides with maturity of the specie.

Boivin, Vendrely and Vendrely, (1948) extracted DNA from isolated calf cells and they proposed that the DNA concentration was constant within cells. Vendrely and Vendrely (1948, 1949) determined the DNA content per cell of most tissues and organs of many species. They suggested that the DNA content between the various tissues was constant within a specie. Sex, diets and live weights did not affect the DNA content of the cell (Thomson et al., 1953). So Vendrely (1955) proposed values for different species for the DNA content per cell nucleus. He found 6.6 picograms of DNA per nucleus in cattle. This constant DNA concept has been used by several workers to do nuclei counts. The increased number of nuclei, based on DNA analysis, suggested an increase in total DNA content of muscle with age or live weight in rat (Winick and Noble, 1965), in fowl (Moss et al., 1964; Moss, 1968a,b) and in mice (Cheek et al., 1965a).

Different muscles have been compared for their concentration of DNA per gram of muscle. Cheek et al. (1971) described the DNA concentration in five muscles of a 2.5 year old monkey. The values were approximately 0.95mg/g of muscle for each of the muscles. While the relative concentrations were different between species, the similarities between muscles, at maturity, were reported in the frog (Iordache and

Vasu, 1969), rat (Enesco, 1961) and fowl (Moss, 1968a). The decrease in DNA concentration during growth was observed in many species for the gastrocnemius muscle (Enesco and Puddy, 1964; Moss, 1968a), biceps muscle (Enesco, 1961; Cheek et al., 1971), pectoralis muscle (Cheek et al., 1971; Moss, 1968a), longissimus muscle (Herold and Nelms, 1964; Harbison, 1970) and several other muscles of lesser importance. The muscles of all the species studied acted in the same way during growth. However, they probably were somewhat dependent on their specific growth gradient for the time at which they reached their maximum concentrations.

The practice of castration in the male does not seem to affect the final DNA content of muscle (Kochakian et al., 1964). These workers castrated two groups of 550 g live weight male guinea pigs. Subcutaneous implantation of 15 mg pellets of androgen for intervals up to 35 days were given at 21 or 220 days after castration. These implants did not change the weight or the total DNA content of the oblique and gastrocnemius muscles. The temporal and masseter muscles were retarded in the attainment of the adult level of DNA reached by the intact males.

If the DNA content is an indication of the number of physiological cells, the ratio of protein to DNA would be an estimation of physiological cell size. Winick and Noble (1965) studied the quantitative changes in DNA and protein concentrations during prenatal and postnatal growth of experimental rats. They observed that the protein content of a cell did not reach its maximal concentration during the stage of rapid cell

division or at the time of maximal DNA concentration. Thus, the ratio of protein to DNA would increase. The ratio becomes larger and larger in muscle since the protein concentration increased relatively faster than the DNA concentration. This increase in the ratio could also be seen in the data from pigs (Harbison, 1970), mice (Robinson and Bradford, 1969) and lambs (Norton and Walker, 1970). Cheek et al. (1971) reviewed some of the literature on this parameter and proposed a cubic equation for the growth of muscle cell of male rats. This could probably be reflected by the fact that the peaks of protein and DNA concentrations in muscle tissue were not attained at the same time resulting in a slowing down, followed by an acceleration in the slope of the curve. A linear equation did not really show the relation, during growth, between muscle mass and live weight of male eviscerated rats (Cheek et al., 1965b) while the ratio becomes quite constant at maturity (Munro, 1969).

Myofibrillar and sacoplasmic proteins are two main muscle proteins that reach their maximal concentration per cell later than DNA. Gordon et al. (1966) determined the concentration of muscle proteins and DNA in rat quadriceps muscle during growth from 78 days to 155 days of age. They reported a constant concentration of DNA during that short period of time while the myofibrillar and sarcoplasmic proteins increased by more than 70 percent. Moss et al. (1964) demonstrated with fowl, ranging in age from birth to 28 weeks, that the amount of cytoplasm per nucleus increased with time. With mice, Srivastava and Chaudhary (1969) found that the myofibrillar protein concentration increased up

to 320 days of age while DNA concentration reached its maximum level at 150 days of age. Since 85 percent of the total protein in muscle is composed of myofibrillar and sarcoplasmic proteins, these two proteins are probably responsible for most of the increase observed in total protein content per nucleus or cell.

Muscle Fiber Diameter

Animals can grow either by increasing cell number or by increasing cell size. It has been shown that the physiological cell number varied with age and the muscle itself. More discussion is needed on the changes or transformations of the already present muscle fiber with respect to diameter or cross-section.

The live weight or age of an animal has long been associated with changes in muscle fiber size as shown in the review paper by Cheek et al. (1971). They reported that the skeletal muscle fiber diameter may double or even triple in size postnatally. Mayeba (1890), Gauthier and Padykula (1966) observed that muscle fiber size was not always directly proportional to the mature size of the species studied. Bohman (as reported by Joubert, 1956b) showed that the fish had the largest fibers and the bird the smallest. Those of the mature rat, rabbit and human were all approximately 40 microns in diameter (Gauthier and Padykula, 1966). Within limits, the values cited here are representative of other data reported with those same species. Warringsholz (1903) stated that at maturity the muscle fiber diameter

was larger in pigs than in cattle, larger in cattle than in horses and larger in horses than in sheep. Mature cattle have muscle fiber diameters of approximately 70 microns (Joubert, 1956b; Swanson, Kline and Goll, 1965; Henrickson and Gillis, 1968).

Eliot, Wiggington and Corbin (1943) studied the changes in the fiber size of the soleus muscle of weaned littermate rats for 375 days. They reported that the cross-sections of the muscle fiber seemed related to the animal live weight while the number of fibers was unaffected by the postnatal live weights. A similar study was done by Chiakulas and Pauly (1962) with the extensor carpi radialis, plantaris and soleus muscles of rats. While the experiment lasted only from birth to 24 weeks of age, 10 fold increases in fiber diameter were observed in these three muscles.

Goldspink (1962a) and Goldspink and Rowe (1968) proposed the idea of phasic growth when one studies the distribution of the fibers within a muscle sample. Goldspink (1962 a,b) and Rowe and Goldspink (1969) fixed, immediately after death, muscles of mice and rats. They observed that the muscle diameter was consistently 20 microns for the young mice and that the diameter doubled to 40 microns when the mice reached a live weight of approximately 34 grams. In rats, the sternomastoideus muscle fibers could double or even triple in size with time when this procedure was used (Goldspink and Rowe, 1968). Rowe and Goldspink (1969) repeated this work on the sternomastoideus muscle of mice and found again a triple phase of growth in this muscle and double phase in the biceps brachii and tibialis anterior muscles. Strangely, they

only found one phase of growth for the soleus and extensor digitorum longus muscles. Based on these findings, it appeared that depending on the muscle, growth would be expressed in terms of one, two, or even three different phases. Comparable differences were reported by Shafiq, Gorycki and Milhorat (1969) with the extensor digitorum longus, soleus and gastrocnemius muscles of mice.

Hegarty and Hooper (1971) studied the growth of the sternomastoideus and other muscles in mice like Goldspink and Rowe (1968). They found no difference between these different post-rigor muscles. They proposed that the muscles with an abnormal standard deviation from the fiber diameter mean could be the result of a mixture of large pre-rigor fibers and smaller post-rigor fibers. They postulated that this was the result of the slow penetration rate of the fixative and the rapid rate at which muscles went into the rigor state. In small muscles, the fixative had time to penetrate the fiber completely before the rigor state could be observed. Thus, unless one used a chemical that prevented the development of contraction, the measurements of muscle fibers could be very misleading. Their observations were also repeated in the biceps brachii (Hegarty, 1970, 1972).

Staun (1963) reviewed the various factors affecting the diameter of muscle fiber of pigs. He found that the diameters varied with the age and weight of the animals. This was also reported by Chrystall, Zobrisky and Bailey (1969) in their investigation of the changes in diameter of the longissimus muscle of pigs. They showed a 100 percent

increase in fiber diameter during the first 25 days of age, but much less thereafter. An increase of only 10 percent was shown between 100 and 125 days of age, followed by very little change until 311 days. In this study, the pigs ranged from 1 to 175 kg in live weight. Elson et al. (1963) had previously shown a very similar marginal change for pigs sacrificed at regular intervals of 45 days. They had observed that rapid growth occurred between birth and 39 kg live weight, followed by a transition period up to 67 kg and little change until approximately 82 kg which was the end of the experiment. Livingstone, Blair and English (1966) reported similar findings with pigs ranging up to 57 kg live weight. Carpenter et al. (1963), in a study of the carcasses of pigs ranging from 4.5 to 42 months of age, showed very little change per day between those pigs. However, they reported that a 100 percent increase in fiber diameter was measured between the two extreme age groups. Staun (1963) suggested a 0.484 micron increase per kg of live weight pig from 61 kg until the end of the general growth period.

The increase in muscle fiber diameter follows a curvilinear curve in the semitendinosus, psoas major, semimembranosus, and longissimus muscles of pigs (Elson et al., 1963). A more accentuated slope was found in the late maturing muscles (Elson et al., 1961). This increase in fiber diameter was associated with a greater coefficient of variation in the fiber size (Staun, 1963), which was contrary to the findings of Chrystall et al. (1969). This might have been the result of the use of fixatives as previously discussed by Hegarty and Hooper (1971).

Janeba (1933) compared the effect of age on fiber diameter in cattle and pigs. He pointed out that there was a parallel increase in fiber diameter for both species, especially at early ages or light weights. However, the diameter increase was more marked in the pig with up to a 6-fold increase. Similar enlargements of the muscle fibers have been reported in various muscles of humans (MacCallum, 1898; Bowden and Goyer, 1960), sheep, (Joubert, 1956a), rabbits (Meara, 1947), chicken (Moss, 1968a) and cattle (Joubert, 1956b).

The longissimus muscle of cattle exhibited a wide range of fiber diameters depending on the location in the muscle (Swanson et al., 1965). This relatively late developing muscle showed, however, the same trends as the other muscles of the bovine (Robertson and Baker, 1933; Joubert, 1956b). Also, no difference was shown when this muscle was compared between species for the fiber diameter changes with time or animal live weight (Janeba, 1933; Elson et al., 1963). Adametz (1888) was one of the first to report a breed difference for the muscle fiber diameter of cattle of similar age. Joubert (1956b) described some factors affecting postnatal growth of the muscle fiber of cattle. He reported that the Holstein-Friesian castrated males and their crosses had larger longissimus fibers than the pure and crossbred dairy Shorthorn castrated males of the same age, with the largest difference existing between purebred animals. Joubert (1956b) added that the Hereford and Angus bred castrated males had intermediate size fibers. Bendall and Voyle (1967) reported that the Holstein-Friesian castrated males had larger muscle fibers per cross-section area of muscle than

those of the Hereford breed.

Hammond and Appleton (1932) reported that the muscle fiber of mature semi-wild Shetland rams were appreciably smaller than those of the mature Suffolk rams. Live weight differences between these two breeds followed the same trend but with a much greater advantage for the Suffolk breed. Comparisons between wild and domestic pigs agreed with the observations made with sheep (Mauch and Marinesco, 1934). Staun (1963) stated in his review of factors influencing fiber diameter that meatier or larger breeds of pigs had the largest muscle fibers. Mehner's work (1938) could be used to summarize the previous results. In an experiment that included 12 strains and crosses of chickens, he found that the distinct breed differences for fiber diameter almost paralleled the differences in body size.

Most of the work done to estimate possible sex differences on muscle fiber diameter has been done between intact males and females. Eliot et al. (1943) reported only slight male to female differences in 375 day old rats. Their findings were confirmed in cattle (Janeba, 1933; Joubert, 1956b), chickens (Moss, 1968a) and pigs (Janeba, 1933; Staun, 1963) but are in opposition to the results of Adametz (1888) with cattle and Mehner (1938) with fowl. In a few reports (Joubert, 1956b; Moss, 1968a), it has been since demonstrated that females have slightly larger muscle fibers at birth but that the males have larger ones at maturity. So the age-sex interaction should not be overlooked and might explain the differences cited.

Female cattle seemed to have larger muscle fibers than castrated males (Brady, 1937; Satorius and Child, 1938; Hiner et al., 1953), but these results were confounded with breed or weight differences. Adametz (1888) and Yeates (1964) showed that mature castrated male cattle have slightly larger fibers than the females. Moody et al. (1970) found slight, if any, advantage of 50 kg male sheep over the castrated males for muscle fiber diameter of longissimus muscle. This was also reported in longissimus muscle of mature cattle (Adametz, 1888) and with 90 kg male pigs (Staun, 1963). Thus, intact male cattle may have muscle fibers of similar diameter as females and also very similar in size to those of castrated males.

Testosterone and Androgens

True growth occurs when the protein content of tissue increases. Testosterone and androgens have been shown to have a protein anabolic action (Kochakian, 1946, 1950, 1964). Kochakian (1964) showed that injection of testosterone propionate on male urine extract to rats and dogs produced a decrease in urinary nitrogen excretion within 24 hours. If these injections are continued, the level was further decreased for two or three days, then are maintained for several days and then gradually returned to the basal level in spite of the continued injections. This anabolic action of androgens was not mediated thru any other endocrine secretions (Kochakian, 1950, 1964).

The administration of testosterone propionate to castrated male

guinea pigs resulted in improved live weight gains (Scow and Hagen, 1955). This had previously been achieved with androgens in adult mice (Kochakian and Stettner, 1948). The response to androgens by the skeletal muscles in the rat was uniform but was quite variable between muscles in the guinea pig (Kochakian, 1966). Scow and Hagen (1955) reported a positive effect of testosterone injection on weight of temporal muscle, but no effect was seen on the biceps femoris or thigh muscles (Scow and Hagen, 1957). Castration reduced the weight of all muscles of the young guinea pig, but sometimes 10 weeks were needed before the difference could be measured in certain muscles (Kochakian et al., 1956a,b). The changes in live weight produced by the androgens did not alter the chemical composition of the muscles (Scow and Hagen, 1955; Kochakian, 1966).

Studies in the rat illustrate the importance of dose and duration of injection of androgen as well as the influence of live weight of the rat. Rubinstein and Salomon (1941) showed that physiological doses of androgens would promote the rate of growth of young rats, while larger doses as used in earlier experiments (Rubinstein et al., 1939) would inhibit gains. Testosterone proprionate injection resulted in a progressive increase in the live weight. As the concentration of the dose reached 1.0 mg per day, the maximum increase in gain became gradually less and was followed by an increasingly larger decrease in live weight with the continued injection of testosterone (Kochakian and Endahl, 1959). Andrews, Beeson and Johnson, (1950) treated

castrated male calves with 180 mg testosterone propionate and found no live weight improvement due to the treatment. The same findings were reported in lambs with 20 mg injection (O'Mary et al., 1951), 12 mg pellets (O'Mary et al., 1952), 175 mg per two weeks in castrated male lambs (McDonald and Slen, 1959) and in swine with 1 mg per kg live weight twice a week (Sleeth et al., 1953).

The overall anabolic effects of testosterone have been shown to be correlated to a general increase of RNA and an increase in protein synthesis (Kochakian et al., 1964). Florini (1970), with the aid of isotopes, confirmed a quantitative but not qualitative effect of testosterone on protein synthesis. Florini and Breuer (1966) presented evidence that testosterone propionate caused an increase in priming activity of the genetic material, chromatin, without influencing the RNA polymerase enzyme metabolism responsible for RNA synthesis. This sex hormone has a different mode of action than growth hormone and so their effects were additive (Florini and Breuer, 1966). In fact, testosterone action was not mediated thru any other endocrine secretions (Kochakian, 1950).

Muscle Biopsy

An accurate evaluation of muscle tissue excised from a live animal could offer means for studying the life development of this animal. Everitt and Carter (1961) studied the suitability of three muscles for biopsy work. They used a local anesthetic and collected

several 10 gram samples from semimembranosus, and biceps femoris muscles of sheep and cattle. The biopsy operations had no effect on live weight gains. Everitt and Carter (1961) did not observe any detrimental effect of the operation on fiber diameter or number of fibers per fasciculus of cattle. Wilson et al. (1955) stated that a biopsy was a satisfactory technique while collecting samples of 200 grams from thigh muscles of castrated male cattle.

Livingstone et al. (1966) used the biopsy technique on pigs ranging from 19 to 57 kg live weight. The biopsies did not interfere with live weight gains and the wounds healed well. Harris and Bennett (1970) designed a muscle tissue extractor for cattle in an attempt to have a quick, uniform, reliable and safe procedure. Their technique based on a stunning gun proved to be without harmful effects on yearling cattle but requires great skill in lighter animals.

Link et al. (1967) observed a fatty degeneration in portions of biopsied bovine longissimus muscle. They postulated that this was due to accidental denervation during the operation. At the biopsy sites where the fatty degeneration was not observed, only a band of scar tissue was present, filling the space where the tissue had been extracted. Link et al. (1970a,b) used a biopsy technique to take six samples of 35 grams from the longissimus muscle of mature Angus cattle at 60-day intervals. Their results agreed with the general values in the literature for lipid deposition and solubility of proteins and they suggested no effect by the biopsy operation.

Thus a biopsy technique could be used to take successive samples from the longissimus muscle of cattle, especially if the 10 to 15 gram samples were taken at intervals of 100 kg live weight gains. Local anesthesia has been shown to be necessary but sufficient (Link et al., 1967; Harris and Bennett, 1970), and a post-operative spray of an antibacterial solution on the wound is recommended.

Measure of Fiber Diameter

Estimation of the dimensions of a muscle fiber was often used as a criteria of growth in the skeletal muscle of various species. The histological techniques commonly used made it difficult to interpret the results because of factors like fixatives (Joubert, 1956b; Hegarty and Naude, 1969), embedding media (Drury and Wallington, 1967) and probably mounting media which cause shrinkage of muscle tissues. Most early workers used those techniques especially with 10 percent formaldehyde as the fixative (Hammond and Appleton, 1932; McMeekan, 1940a; Meara, 1947; Joubert, 1956b). Their results should thus be interpreted as relative differences between treatments rather than absolute values or differences. Staun (1968) found that a 10 percent salt-formaldehyde solution caused variable shrinkage of swine muscle fibers depending on the fiber types and the muscle themselves.

Swanson et al. (1965) reported considerable difficulty in cutting accurate axial cross-sections of mammalian muscle. In several muscles, the muscle fibers did not extend from one end to the other and thus a

problem arose to differentiate between a small fiber and the tapered end of a large fiber (Hegarty and Naude, 1970). Some workers (Hammond and Appleton, 1932; McMeekan, 1940a; Hiner et al., 1953; Joubert, 1956b) teased out the muscle fibers to eliminate some of the variation due to the tapered sections but the fixation of tissues still caused a lot of shrinkage. Brady (1937) studied fresh, aged and cooked muscles using a micro-dissection approach. They used the triceps brachii, abductor, semitendinosus and longissimus muscles of yearling cattle. It was probably the first study using unfixed fresh muscle fibers. They observed that the fibers were larger in fresh muscle tissues than in aged or cooked muscle samples.

Hegarty and Naude (1969) developed a technique for the separation of unfixed fibers by homogenization in physiological saline. They showed that the shrinkage due to freezing of muscle fibers was less important than that caused by fixation in 10 percent formaldehyde or formalin. So, a small sample of muscle could be frozen and stored until needed. The sample was then thawed and the fibers separated in saline solution. The homogenate was then placed under a light microscope and the width of the fibers is estimated by comparison to a micrometer. This method gave results that were repeatable (Hegarty and Naude, 1970). Also, because this technique could be applied to unfixed frozen samples, it did not present the problem of heterogenous fixation (Hegarty, 1970; Hegarty and Hooper, 1971).

MATERIALS AND METHODS

Animals

Five pairs of twin male dairy calves and five pairs of twin male beef calves were bought from different farmers in Iowa. These calves were purchased at approximately one week of age and selected for their resemblance within pairs. The dairy calves were of the Holstein breeding while the average breeding of the crossbred beef calves was 33 percent Hereford, 33 percent Angus and 33 percent Holstein. Within a few days of their arrival at the university research facilities, one animal of each pair was surgically castrated. All calves were fed one cup a day of milk replacer[1] and had access to a starter feed mixture (Table 1) until 45 kg live weight.

The animals were reared from 45 to 445 kg on dry rations (Table 1) with 0.5 cup of milk replacer[1] per day for the first two weeks on trial. At weights of 45, 145, 245, 345, 445 kg, muscle biopsies of 10 to 15 grams were taken from the longissimus muscle. The location and sequence of each biopsy was selected at random between each side of the animal and between locations immediately over the 11^{th} thoracic, 1^{st} and 4^{th} lumbar vertebrae. Because of the small size of the longissimus muscle at the initial weight of 45 kg, one biopsy of five grams was obtained from each of two sides at corresponding locations.

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[1] KAFF-A (Kraft, Chicago).

Table 1. Analysis of rations

	Pre-trial	Trial	
Weight range		45 to 145 kg	145 kg to 445 kg
Ration			
Milk replacer	1 cup/day	1/4 cup/day x 2 weeks	--
Feed	1.5 kg/day	ad libitum	ad libitum
Ground corn		182	216
Corn cob (ground)		-	154
Oats		123	-
Soybean		91	60
Molasses		45	18
Dicalcium phosphate		9	1.95
Limestone		-	3.3
Salt		4.5	1.2
Vit A		1140 I.U./kg feed	0.4 kg or 1140 I.U./kg feed
Vit D		135 I.U./kg feed	--
Chlor + oxytet.[a]		4.5 mg/kg feed	--
Mineral mix		-	0.15
Analysis (Dry matter)	Milk	Feed	Feed
TDN percent	--	82.8	73.5
Protein percent	22 +	18.2	10.9
Ca percent	--	.58	.456
P percent	--	.76	.322

[a]Chlortetracycline + oxytetracycline.

The animals were weighed at intervals corresponding to one of the biopsy weights.

Biopsy Procedure

The animal was isolated, weighed and directed into a chute with collapsible sides and some head restraint. The area chosen on the back of the animal for surgery was shaved with a small electric animal clipper[1] and cleaned with 90 percent alcohol. Local anesthesia was accomplished with 10 to 15 ml of 2 percent xylocaine. Ten minutes later, a five to seven cm incision of the skin was cut with a scalpel blade and underlying fat and membranes were teased apart. A one inch stainless steel cylinder was applied against the muscle tissue and with a rotational action, the steel core was pressed into the longissimus muscle. After removing the instrument, a small blade separated the whole muscle from the sample core. The sample was placed in a plastic bag lying on dry ice. An antibacterial[2] solution was sprayed into the wound and three to four discontinuous sutures with 000 Surgilon thread closed the incision. An antibacterial gel[3] was spread on the skin near the sutures and the animal was sent back to the pen. No post-operative complications were observed and healing was rapid. The sutures were removed three weeks later.

[1]Model A-2, Oster Co., Miluaukee, Wisconsin.

[2]TOPAZONE, Eaton Laboratories, Norwich, New York.

[3]FURACIN, Eaton Laboratories, Norwich, New York.

The sample was taken to the laboratory and stored in a freezer at -20°C until further analyses.

Laboratory Analysis

The sample was stored for 14 days or more. Then it was trimmed of visual excess fat and the outside connective tissue corresponding to the membrane of the longissimus muscle was cut away. The sample was divided into smaller units of approximately two grams to be used in the different analyses.

Muscle proteins

The technique used to determine sarcoplasmic and myofibrillar protein concentrations was based on the procedure of Helander (1957). Duplicate two gram samples were weighed, minced finely with a razor blade and put into 100 ml polyethylene bottles. The samples were then suspended in 10 volumes of 0.03M K-phosphate buffer, pH 7.4. The sarcoplasmic proteins were extracted at 2°C for two hours with a gentle stirring using a Garver[1] shaker. The solution was then centrifuged in 50 ml centrifuge tubes at 10000 X G for 15 minutes. The supernatant was decanted into a 50 ml volumetric flask and stored at 2°C. The extraction was repeated on the precipitate. Both supernatants were combined and brought to a constant volume of 50 ml with the pH 7.4 buffer solution. Protein concentration was determined using the

[1]Model 240, Garver Co., Union City, Indiana.

biuret reaction (Gornall, Bardawill and David, 1949) using bovine serum albumin as the standard.

The myofibrillar protein fraction was extracted from the precipitate of the first duplicate by suspending in 10 volumes of 0.6 M KCl, 0.1M K-phosphate buffer at pH 7.4. By stirring more vigorously, the myofibrillar proteins were extracted for three hours at 2°C. At the end of a centrifugation at 10000 X G for 15 minutes, the supernatant was decanted and stored at 2°C. The extraction was repeated. Both supernatants were combined and brought to a constant volume of 50 ml. The biuret analysis (Gornall et al., 1949) was used to determine the protein concentration of the solution where the same standard curve was used as for the sarcoplamic proteins.

The precipitate of the second duplicate was used in measuring the collagen content of the sample. The precipitate was suspended in 10 volumes of 1.1 M KI, K-phosphate buffer, and then processed as with the first duplicate but saving only the precipitate. This precipitate was then transferred into glass centrifuge tubes. Twenty ml of 6 N HCl was added and the tubes were covered with inverted 50 ml beakers. The suspension was heated at 121°C and 6.8 kg pressure in an autoclave. Then, a few drops of phenolphtalein indicator were added and the solution was neutralized with 6 N NaOH solution. The solution was diluted to 1000 ml with distilled water and then analyzed for collagen (Goll et al., 1963) using hydroxyproline as the standard, since hydroxyproline concentration is 12.8 percent of the collagen protein (Lawrie, 1966).

Calculation of protein content

The protein content of each fraction was determined in duplicate thus providing two optical density readings for myofibrillar protein and collagen and four for the sarcoplasmic protein. Stock solutions of 10 mg/ml of bovine serum albumin[1] and 10 mg/ml of hydroxyproline were prepared and stored at 2°C. Standard curves of 0, 2, 4, 8 and 10 mg/ml for BSA and 0, 1.375, 2.75, 5.5 μg/ml for hydroxyproline were prepared from the appropriate stock solution. The concentration per gram of fresh muscle was calculated from these equations:

$$\text{Protein concentration (mg/ml)} \times \frac{50 \text{ ml}}{\text{weight sample}} = \text{mg sarcoplasmic or myofibrillar protein/gram fresh muscle}$$

$$\text{Hydroxyproline concentration } (\mu\text{g/ml}) \times \frac{100 \text{ ml} \times 10 \text{ ml}}{\text{weight sample}} \times \frac{7.25}{1000} = \text{mg collagen/gram fresh muscle}$$

DNA concentration

Moss (1968b) recommended a procedure for the determination of nucleic acid content of animal tissue. The procedure used in this study was a modified form of that technique.

A two gram sample of muscle was weighed, minced with a razor blade

[1]General Biochemicals Co., Chagrin Falls, Ohio.

and scissors and suspended into 10 volumes of ice cold water. The solution was homogenized in a 30 ml pyrex flask with a Virtis 45 tissue grinder set at a medium speed for 90 seconds. Then 1 ml aliquots of the homogenized tissue were placed in duplicate test tubes with 1 ml of cold 0.2M KOH. The DNA content of the two samples was determined by the indole method of Ceriotti (1952).

Purified calf thymus DNA obtained from Calbiochem International Co. was used as standard. Stock solution of 25 mg per ml was carefully prepared and stored at 2°C. Dilutions of 0, 10, 20, 40 and 80 μg/ml of DNA in 0.1M KOH solution were prepared from the stock solution and and used as standards. The concentration of DNA per gram of fresh muscle was determined by using the equation:

$$\text{DNA concentration } (\mu g/ml) \times \frac{10 \text{ ml}}{1 \text{ g}} \times \frac{2 \text{ ml}}{1 \text{ ml DNA}} = \text{mg DNA/g fresh muscle}$$

Muscle fiber diameter

The estimation of muscle fiber diameter was based on a technique developed by Hegarty and Naude (1970). A transverse muscle "chip" of about 25 mg was cut and placed in a 1 ml stainless steel tube which contained approximately 0.5 to 0.8 ml of 0.85 percent (w/v) NaCl solution. The fibers were teased apart by homogenization with a Virtis 45. The shaft contained two dull aluminium blades. The shaft was placed in the stainless tube and a top was lowered. The blades were rotated at the slowest possible speed for five seconds.

Then, 0.3 ml of the slurry was placed with a pipette in the deep well of a culture slide[1] for immediate observation under microscope. Measurement of the width of the muscle fibers was made by means of an ocular micrometer, inserted into the eye-piece (10X) of the microscope, and a 10X objective lens. By means of a stage micrometer[2], the calibration of the ocular micrometer was determined to be 17 units per mm. The same micrometer and microscope were used all the time and illumination provided artificially.

The mean diameter was calculated by measuring the cross-section of 50 fibers that were observed while moving the slide across from left to right and then, at a slightly lower level, back again to the left. This procedure was repeated until the required 50 measurements were recorded. Occasionally, a second slide had to be prepared to get enough measurements. The single measurement of each fiber was done at its most central point.

Statistical Design

This factorial experiment was analyzed as a split-plot of two cattle breeds, two male types and five live weight treatments, replicated five times (Table 2). All the animals were kept together. Based on the limitations of sequential weights, the male type and breed treatments were combined as the whole plot while the weights constituted the split-plot. The castration within each pair was done at random.

[1]Matheson Scientifics 50680-10, 1 inch cylindrical concavity.

[2]A. H. Thomas Co., No. 6852-A.

Table 2. Analysis of variance

Source	Degrees of freedom
Total	99
Breed type	1
Male type	1
Breed · male	1
Animal/breed · male	16
Live weight	4
Breed · weight	4
Male · weight	4
Breed · male · weight	4
Animal/breed · male · weight	61
Missing data	3

Homogeneity of variance between treatments was expected. Several correlations were calculated.

An animal died at 200 kg as a result of pneumonia. The missing data technique of Steel and Torrie (1960) was used to supply the missing values of the three lost observations.

RESULTS AND DISCUSSION

The experiment was designed to take a muscle biopsy at every 100 kg of live weight gain, with the first sample being obtained at 45 kg. The live weight of the cattle, on the day of sampling, was not significantly ($P > 0.05$) different between breed and male types, while highly ($P < 0.01$) different for the live weight groups (Table 3). The mean values for the live weight of each treatment group is shown in Table 4. The difference in the mean of the five calves in each of the four treatment groups rarely exceeded 10 kg within a weight class and corresponded closely to the desired weights. It had been relatively difficult to get the 20 calves young enough to sample at 45 kg, and so the average live weight was slightly higher than wanted.

While most of the growth parameters measured in this study were correlated with live weight changes (Appendix), these relations will be pointed out later as each parameter is individually discussed.

DNA Concentration

There was no significant ($P > 0.05$) difference observed between breed and male types in concentration of DNA in the longissimus muscle within each weight group from 45 to 445 kg (Table 3). The interaction of breed · male cattle was statistically significant ($P < 0.05$) with or within live weight classes. Since no real trend could be shown, it was assumed that a variation caused by the small number of 20 samples

Table 3. Mean squares for growth-related parameters of the *longissimus* muscle of male cattle[a]

Trait	Breed	Male	Weight
Degrees of freedom	1	1	4
Absolute weight	282.2	4928.0	2250527.3**
DNA	.0276	.0013	.7336**
Fiber diameter	120.0	223.4	11759.2**
Myofibrillar pro.	4634.2**	0.1	4995.8**
Myo pro./DNA	17442.5	139.0	189179.4**
Sarcoplasmic pro.	1338.5	869.6	953.6**
Sarco pro./DNA	22949.2	9244.8	70681.4**
Collagen	1.86	0.44	236.9**
Total protein	845.5	903.4	5602.4**
Total pro./DNA	701.7	12185.9	475535.6**

[a]F values required for significance are as follows:
(a) with one and 16 degrees of freedom
*$P < 0.05$ 4.49 required
**$P < 0.01$ 8.53 required.
(b) with four and 61 degrees of freedom
*$P < 0.05$ 2.53 required
**$P < 0.01$ 3.65 required.

[b]Error term used in F test for male, breed and male-breed interaction.

[c]Error term used in F test for weight and weight interactions.

Breed X male	Breed X weight	Male X weight	Male X breed X weight	Error[b]	Error[c]
1	4	4	4	16	61
174.2	2071.9	714.2	1770.4	8912.3	3254.6
.0829*	.0348	.0111	.1128*	.0177	.0318
107.9	75.7	33.9	67.5	89.1	149.7
0.9	166.1	212.8	99.4	341.8	278.6
1796.9	9075.4	1038.1	14649.6	10999.4	10271.2
6.6	301.1	140.8	260.7	403.8	191.2
72.1	1814.9	1388.2	8499.6	9993.4	6600.4
8.17	9.64	2.87	13.83	11.4	16.20
.2	538.5	432.3	329.3	753.9	478.2
3705.2	13658.2	3011.1	44519.9	39240.3	30709.5

Table 4. Mean values of the absolute live weight (kg) at the time of the biopsies

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	53.6	148.4	262.0	346.6	445.7
Beef Cattle	65.5	157.7	251.1	344.3	445.2
Intact male	59.3	154.5	260.5	349.5	452.3
Castrated male	59.9	151.6	252.7	341.4	438.6
All	59.5	153.1	256.6	345.5	445.5

at each weight might have caused this apparent difference. In fact, the various cattle type combinations followed no special pattern at each weight and all of the final DNA values at 445 kg were essentially equal to 0.375 mg/g of fresh muscle tissue (Figure 1). The interactions of male·breed and male·breed·live weight were not specific enough to emphasize their importance. So no strong conclusions will be based on this difference.

The DNA concentration in fresh muscle tissue decreased from a high of 0.812 mg/g to a low of 0.375 mg/g as the live weights increased from 45 kg to 345 kg (Table 5). This linear decrease had been previously shown in several species by Kochakian et al. (1964), Moss et al. (1964), Cheek et al. (1965a), Harbison (1970), Norton and Walker (1970). These workers also reported that DNA concentration in skeletal muscle would eventually reach a constant level. This plateau was

Figure 1. Changes in muscle DNA concentration in *longissimus* muscle of the various breed and male cattle types

Code on figure:

O	Dairy cattle
X	Beef cattle
___	Intact male
---	Castrated male

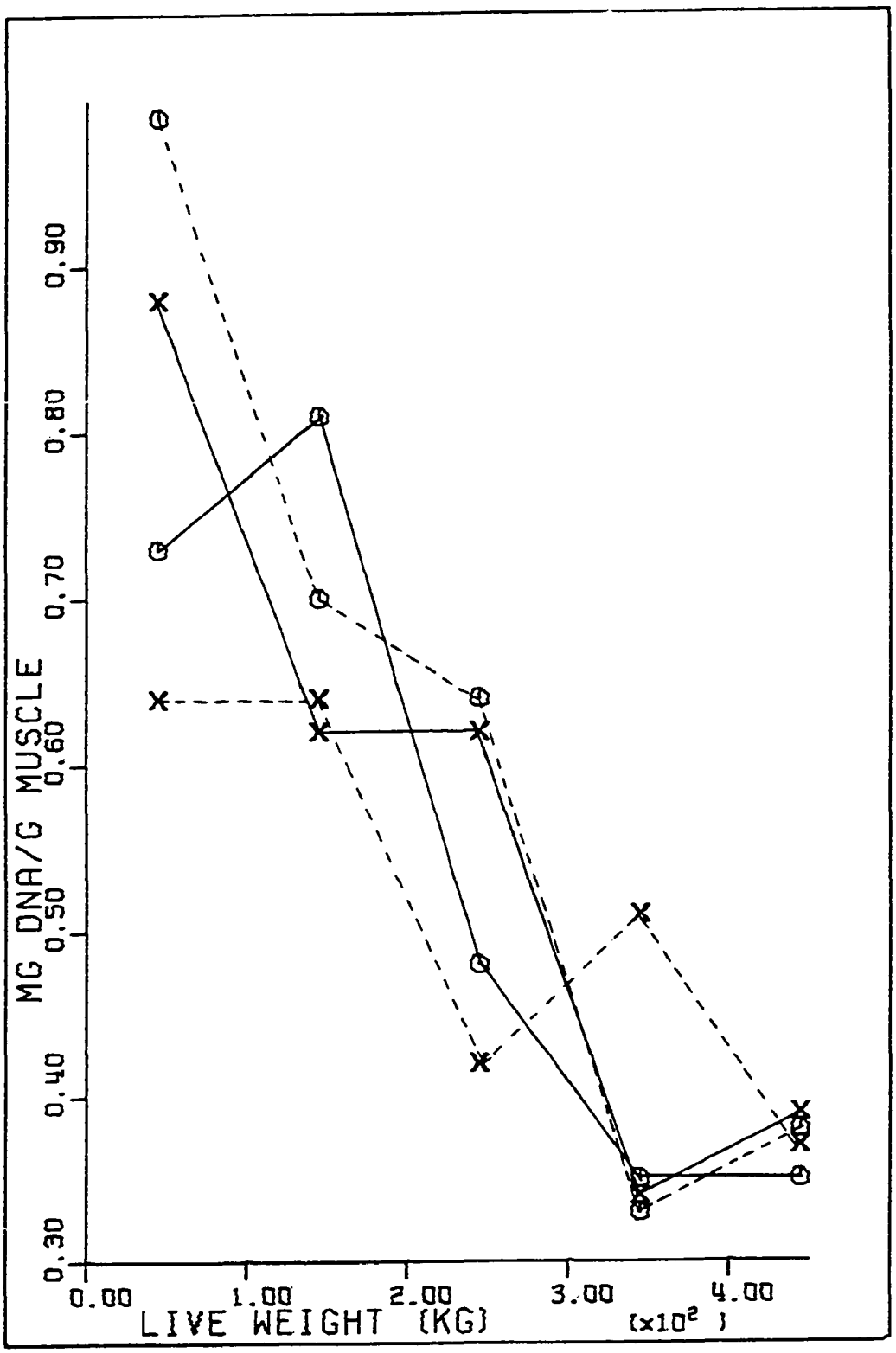

MG DNA/G MUSCLE
0.30
0.40
0.50
0.60
0.70
0.80
0.90
0.00
1.00
2.00
3.00
4.00
LIVE WEIGHT (KG)
(x10²)

Table 5. Mean values of the DNA concentration (mg/g tissue) of *longissimus* muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	.860	.751	.562	.340	.369
Beef cattle	.764	.629	.518	.425	.380
Intact males	.809	.712	.548	.341	.371
Castrated males	.815	.668	.532	.424	.378
All	.812	.690	.540	.383	.375

observed in this experiment when the cattle weighed 345 kg. At this time the DNA concentration was approximately 0.375 mg/g tissue. This value was in agreement with the 0.3 mg/g DNA concentration value in thigh muscle reported by Munro and Gray (1969) for a castrated male calf weighing 450 kg. A constant concentration of DNA in the *longissimus* muscle of pig was also reported by Harbison (1970).

Winick and Noble (1965) suggested that the constant concentration of DNA could occur with cessation of DNA synthesis and growth in rat muscle. Since the *longissimus* muscle of cattle can grow in length by adding new sarcomeres, and new DNA molecules (Bendall and Voyle, 1967) a constant concentration of DNA may not indicate cessation of DNA synthesis. It has been repeatedly shown that the total DNA content of a muscle increases with live weight (Enesco and Puddy, 1964; Cheek *et al.*, 1965a) and thus this plateau of DNA concentration might be

regarded more as an equal increment in muscle mass and DNA synthesis.

It was of great interest to observe how little effect the breed and male types had on the decrease in DNA concentration. The values had a large deviation of 45 kg but were quite close at 445 kg, suggesting that the cattle had reached a similar stage of muscle cell maturity. The decrease in DNA concentration that seemed to taper off between 345 kg and 445 kg live weight also suggested that the muscle may have been approaching cellular maturity. This hypothesis of maturity, however, could be confirmed only if the total muscle could be analyzed and shown to have reached its final length and composition. Thus, the plateau in the muscle DNA curve could have been brought about by maturity, end of DNA synthesis and end of elongation process.

The DNA concentration had a significant ($P < 0.01$) negative correlation with the fiber diameter and the total protein concentration (Appendix). Using the longissimus muscle of cattle, Herold and Nelms (1964) have also reported a negative relationship between DNA concentration and the growth of calves. The decrease in DNA concentration during growth indicates that hypertrophy of muscle cells is more important than hyperplasia during post natal growth. If the hyperplasia was dominant the DNA concentration changes would be less than observed and there would be a much lesser negative relation with fiber diameter and total protein concentration.

The DNA concentration has been shown to have a high negative relationship to the total protein concentration. The concentration of the collagen fraction decreased with live weight and thus contributed less

to the total protein concentration in the heavier animals. The relation of DNA concentration to total protein concentration was therefore slightly depressed by changes in collagen. However, collagen represents a very small percentage of the total protein concentration of muscle.

Since there was a negative relation between fiber diameter and DNA concentration and since an enlargement of fibers results in more myofibrillar protein, it follows that there should be a negative relationship between DNA and myofibrillar protein (Appendix). The sarcoplasmic protein concentration increased during growth but at a much slower rate, and much more inconsistently than the myofibrillar protein, so the correlation of DNA to sarcoplasmic protein was not significant ($P > 0.05$) (Appendix).

It has been suggested by Cheek et al. (1971) that the ratio of protein to DNA is an index of physiological cell size. The physiological cell size as measured by the total protein per unit of DNA was strongly dependent on DNA concentration in longissimus muscle and showed the same trends as the relation between the fiber diameter and the DNA content. Both sarcoplasmic and myofibrillar protein fractions per mg of DNA also showed a high negative correlation with DNA since this relation was done between highly related parameters.

Fiber Diameter

The mean muscle fiber diameter was estimated by the width of muscle teased out in a saline solution. The only treatment that had any

significant ($P < 0.01$) effect on the fiber diameter of longissimus muscle was the live weight of the cattle (Tables 3 and 6). There was

Table 6. The mean values of the fiber diameter (microns) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	31.69	60.21	73.33	80.20	92.52
Beef cattle	31.02	62.44	71.39	88.23	95.82
Intact male	32.53	61.75	74.99	84.39	97.25
Castrated male	30.19	60.89	69.74	84.05	91.09
All	31.36	61.32	72.36	84.22	94.17

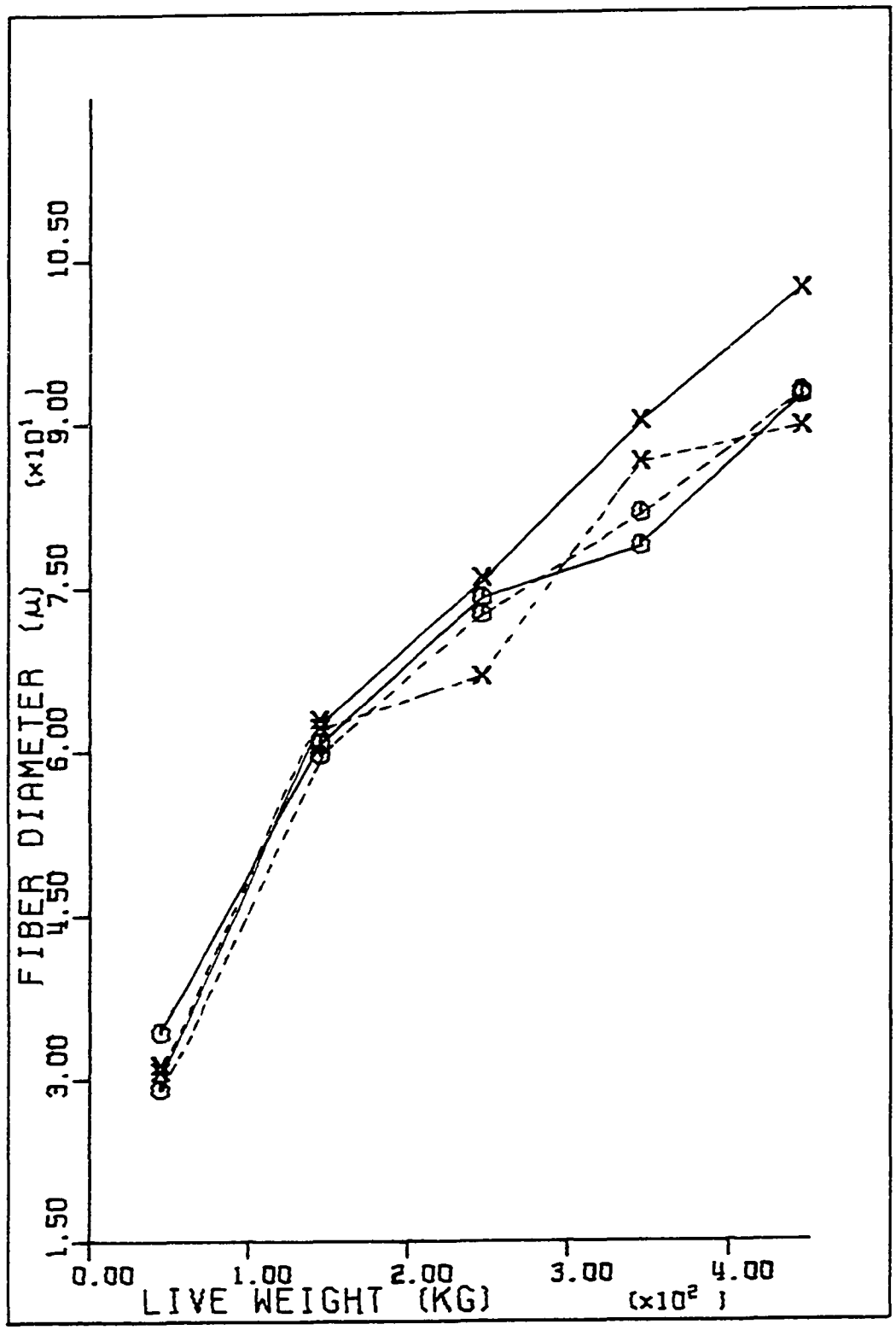
no significant ($P > 0.05$) interaction between any of the treatments. The various breed and male types had similar mean values at the 45 kg live weight and followed each other closely within each live weight class (Figure 2).

The lack of difference between the fiber diameter of the dairy and beef breeds was in opposition with the findings of Hammond and Appleton (1932), Joubert (1956b) and Bendall and Voyle (1967). A possible explanation is that in this experiment the weight differences at the time of sampling between dairy and beef cattle were not significant ($P > 0.05$) while differences in weight were often large in most of the literature cited. These authors recognized the fact that muscle

Figure 2. Changes in muscle fiber diameter of longissimus muscle of the various breed and male cattle types

Code on figure:

O	Dairy cattle
X	Beef cattle
____	Intact male
----	Castrated male


FIBER DIAMETER (μ) (x10¹)
1.50
3.00
4.50
6.00
7.50
9.00
10.50
0.00
1.00
2.00
3.00
4.00
LIVE WEIGHT (KG) (x10²)

fiber diameter increased with live weight and their analyses showed that only the breeds with large weight differences had large differences in fiber diameter. They did their comparison on an age basis, deleting the effect, on fiber size, of the live weight of their animals. Large differences in muscle fiber diameter were shown between wild and domesticated animals by Adametz (1888), Mauch and Marinesco (1934) but the live weights were also extremely variable between animals compared. Mehner (1938) showed with chickens that the differences in fiber diameter almost paralleled the differences in body size.

Between 45 and 145 kg, the muscle fiber diameter increased by 100 percent in size, and this increase was repeated between 145 and 445 kg live weight (Table 6). In fact, the fiber diameter mean went from 31μ to 61μ and finally to 94μ for these three live weights. These values were somewhat higher than the 70μ suggested by Joubert (1956b), Swanson et al. (1965), Henrickson and Gillis (1968). The present experiment used unfixed fiber rather than the commonly studied fixed fibers. The fixed muscle sample were generally smaller due to the shrinkage caused by the fixative and the heat treatment of the fibers incompletely fixed (Joubert, 1956b). The muscle fibers studied in the present experiment were allowed also to contract and enlarge since they were taken from live animals and stored without being restrained to a certain length.

The muscle fiber diameter increased first very sharply and then tapered off into a gradual linear increase up to 445 kg live weight. This doubling in size was in agreement with Goldspink (1962a,b) and

Goldspink and Rowe (1968) who proposed the biphasic pattern of growth in muscle fiber. However, the fibers continued to increase in size and this was in agreement with Hegarty (1970) who proposed a gradual increase. Hegarty (1970) proposed that there was a mixture of pre- and post-rigor fibers in the Goldspink's work. In the present trial, all samples were unfixed and were measured in a post-rigor state. Thus, the samples should not have contained that mixture of fibers. The highly negative correlation of fiber diameter with DNA concentration and the highly positive relationship with the various protein fractions (Appendix) suggested that the fiber size increased by gradual hyper-. trophy during the experiment. A more intense growth gradient in longissimus muscle between 45 and 145 kg live weight is possible and in agreement with the literature since the impetus had previously been reported by Elson et al. (1963) and Chrystall et al. (1969) in this muscle of pigs ranging from birth to adolescence. Goldspink's (1962a, b) work would not explain such gradual change after the growth impetus and is in opposition to our findings.

The changes in fiber diameter were positively correlated with the protein concentration and especially the myofibrillar protein concentration (Appendix). While the fiber size increased, the concentration of collagen decreased since collagen served as an envelope for the muscle fiber. The sarcoplasmic protein concentration was also positively correlated with the fiber diameter changes but to a lesser degree than with the two other protein fractions or the total protein concentration.

The scarcoplasmic protein concentration changed directly with fiber

diameter but at a slow rate. This might be regarded as a relative increase in sarcoplasmic protein concentration within a muscle fiber. This explanation was very unlikely in view of some peculiar changes during growth in the sarcoplasmic protein concentration as discussed later.

The fiber diameter was significantly ($P < 0.01$) correlated with the physiological cell size or total protein per unit of DNA. The increase in cell size was thus highly dependent upon an increase in muscle fiber diameter but the possibility of an increase in length of muscle fibers should not be ruled out. Neither of the two main protein fractions per unit of DNA seemed to have a higher correlation coefficient than total protein per unit of DNA with diameter. The physiological cell went thru a general size increase by affecting the main composition of that cell. However, it was unlikely that the muscle cell would change drastically its relative proportion of each protein in the muscle cell during growth.

Myofibrillar Protein Concentration

The myofibrillar protein concentration in longissimus muscle was not significantly ($P > 0.05$) different between castrated and intact males within any of the live weight groups (Table 3). The overall average value of 84 mg/g of muscle tissue was slightly lower than the 100 mg/g reported by Lawrie and Kirton (1961) and Lawrie (1966).

The breed types had a significant ($P < 0.01$) effect on the

myofibrillar protein concentration of the longissimus muscle (Table 3). The dairy breed had a similar concentration to the beef breed at 45 kg live weight but increased rapidly thereafter to maintain higher values at the heavier weights (Table 7). This difference was in opposition

Table 7. Mean values of the myofibrillar protein concentration (mg/g tissue) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	66.4	83.6	93.6	96.8	112.2
Beef cattle	61.7	66.7	77.5	77.7	101.0
Intact male	62.7	76.6	90.6	84.9	103.7
Castrated male	65.4	73.8	80.5	89.6	109.5
All	64.0	75.2	85.5	87.2	106.6

with the lack of effect of this treatment on DNA concentration and fiber diameter. The breed difference was difficult to explain since it would have to be supposed that the composition of the muscle fibers was different between the two breed types. No reports in the literature are known to support such an hypothesis. Figure 3 shows an important depression of the myofibrillar protein concentration in the intact beef males of 345 kg. If these values were omitted or corrected to equal the value of the dairy cattle, the breed difference would probably

Figure 3. Changes in muscle myofibrillar protein concentration in *longissimus* muscle of the various breed and male cattle types

Code on figure:

O	Dairy cattle
X	Beef cattle
____	Intact male
----	Castrated male

MYØ PRØT CØNC (MG/G) (×10¹)
4.00
5.50
7.00
8.50
10.00
11.50
13.00
LIVE WEIGHT (KG) (×10²)
0.00
1.00
2.00
3.00
4.00

not be statistically significant. Also, the results would correlate better with the fiber size and the DNA concentration of the two breed types. The unusually low values at 345 kg had a great influence on the mean value of the beef breed at 345 kg as well as on the overall mean value of all the cattle of that live weight (Table 7).

The live weight of the cattle had a significant ($P < 0.01$) effect on the concentration of myofibrillar protein of the longissimus muscle (Table 3). The increase observed at every 100 kg was positive and nearly linear, corresponding to an increase of approximately 10 mg/g muscle tissue for every 100 kg of live weight gain (Table 7). This sharp increase was in agreement with the findings of Helander (1957) in cattle, Dickerson and Widdowson (1960) in pigs and Gordon et al. (1966) in rats. The correlation between live weight and concentration of this protein fraction was high and positive (Appendix) and suggested that a good proportion of the increase in the size of muscle fiber and of the decrease in DNA concentration resulted from the growth changes in the myofibrillar protein concentration.

The myofibrillar protein fraction was positively correlated ($P < 0.01$) to the total protein concentration and it may be concluded that this protein fraction constituted a major portion of the total protein. The changes in this protein fraction were also responsible for a large proportion of the changes in the concentration of the total protein in the longissimus muscle.

The sarcoplasmic protein concentration poorly correlated with the concentration of myofibrillar protein. Thus it was assumed that these

two proteins varied independently. However, it is proposed that this relation was much more important than suggested here since the sarcoplasmic protein curve was very unexpected and seemed to be affected by a large variation often found within the 20 samples at each weight. The collagen concentration decreased with an increase in fiber diameter and a similar decline was seen when correlated with the concentration of myofibrillar protein. A significant ($P < 0.01$) negative correlation was shown between these two protein fractions (Appendix).

The ratio of myofibrillar protein concentration per mg of DNA could be a sign of the composition of the physiological muscle cell, if it was accepted that total protein per unit of DNA corresponded to the physiological cell size. An increase in the myofibrillar protein concentration would lead to an increase of this protein within the cell since it was normally accepted that the myofibrils are the main basic structure of the muscle fibers. The mean values of this protein in the cell varied with live weight but were not affected by either breed or male types (Table 3). Since the DNA concentration decreased linearly and the protein concentration generally increased, the ratio between them increased over the live weights studied (Table 8).

It has been shown that there was a breed type difference in the concentration of myofibrillar protein. The ratio of this protein to to DNA, however, was not significantly ($P > 0.05$) different between breeds. The muscle fiber diameter, the DNA concentration, and the myofibrillar protein content of the muscle cell were shown to be equal between

Table 8. Mean values of the ratio between myofibrillar protein concentration (mg/g) and DNA (mg/g) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	83.5	115.8	190.2	323.9	326.4
Beef cattle	91.8	106.0	193.1	228.4	288.4
Intact male	79.9	108.1	192.1	286.6	301.2
Castrated male	95.4	113.7	191.3	265.8	313.6
All	87.7	110.9	191.7	276.2	307.4

dairy and beef cattle (Appendix). Because these parameters were shown to be not different between breed types, it is now possible to think that the differences in concentration of myofibrillar protein concentration in muscle tissue were mainly due to an unusually large variation at 345 kg for one of the treatments made of five samples.

Sarcoplasmic Protein Concentration

The mean values for the changes in sarcoplasmic protein concentration in longissimus muscle are shown in Table 9. There was no significant ($P > 0.05$) difference in this parameter due to breed and male types, even though a large variation was observed within certain live weights (Table 3). One of the treatment combination curves had a very different shape than the others and so will be somewhat disregarded. The general

Table 9. Mean values of the sarcoplasmic protein concentration (mg/g tissue) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	55.2	78.3	56.8	62.7	64.7
Beef cattle	60.1	73.2	70.0	76.3	74.6
Intact male	52.5	77.0	58.9	65.0	67.8
Castrated male	62.8	74.6	67.9	74.0	71.5
All	57.6	75.8	63.4	69.5	69.7

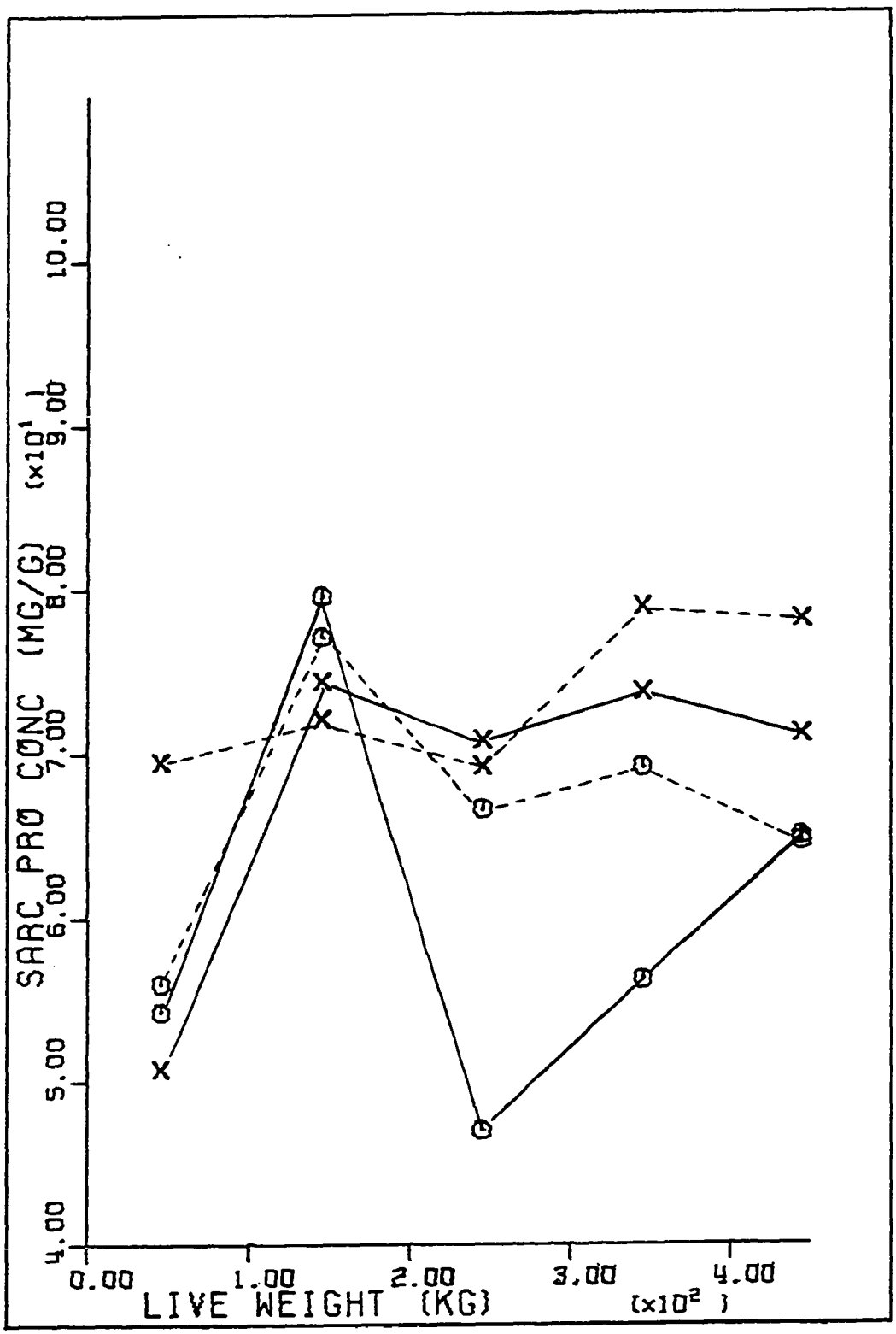
curves were not significantly ($P > 0.05$) different. However, it must be pointed out that the dairy cattle type tended to have lower sarcoplasmic protein content, especially the mean value at 245 kg live weight (Figure 4).

The effect of live weight on the sarcoplasmic protein concentration was significant ($P < 0.01$) but some of the variation was larger than others. Overall, the protein concentration increased with the increase live weight. This was in agreement with the findings of Helander (1957) and Lawrie and Kirton (1961). The increase with live weight was much less and not as gradual as the one seen with myofibrillar protein concentration, as also reported by Dickerson and Widdowson (1960) in pigs and Gordon *et al.* (1966) in rats. The values reported in this work were larger than those found in the literature and might be partially due to the presence of blood in the sample. The samples were taken from

Figure 4. Changes in muscle sarcoplasmic protein concentration in longissimus muscle of the various breed and male cattle types

Code on figure:

0	Dairy cattle
X	Beef cattle
____	Intact male
----	Castrated male


SARC PRO CONC (MG/G) (x10¹)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
0.00
1.00
2.00
3.00
4.00
LIVE WEIGHT (KG) (x10²)

live animals and were not treated before storage. The exceptionally high sarcoplasmic protein concentrations in the 145 kg animals could not be explained but it was thought that the first biopsy of cattle could have caused some damage due to denervation (Link et al., 1967) and thus maybe have caused an increased blood supply in some areas of the muscle of the very young calves. It is, however, important to recognize the overall gradual increase in the concentration of this protein with live weight.

The high concentrations at 145 kg and some of the low concentrations at 245 kg might explain the lack of correlation between the sarcoplasmic protein and some other parameters studied such as DNA, myofibrillar proteins and collagen (Appendix). The positive correlation between total protein and sarcoplasmic protein was significant ($P < 0.01$) since this protein fraction represented slightly more than 40 percent of the total protein. Muscle fiber diameter was not related to sarcoplasmic protein as expected from the same trends observed with the constitutents of the muscle fiber.

Sarcoplasmic protein concentration per unit of DNA may be indicative of cell size but would refer to the portion of the cell that contains the more soluble proteins. No difference ($P > 0.05$) in sarcoplasmic protein per unit of DNA was observed due to breed or male types. (Table 3). A change in live weight was the major factor in increasing this ratio, with a comparable increment within each type of cattle (Table 10). Since DNA concentration tended to taper off with the heavy live weights, and since the same plateau was apparent with sarcoplasmic

Table 10. Mean values of the ratio between sarcoplasmic protein concentration (mg/g) and DNA (mg/g) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	71.4	108.7	106.2	208.9	190.8
Beef cattle	96.7	118.2	167.3	233.1	222.7
Intact male	68.2	111.9	115.6	215.0	202.8
Castrated male	99.2	115.0	157.9	227.0	210.7
All	83.7	113.5	136.8	221.0	206.8

protein, the ratio of these two parameters was constant at 345 and 445 kg.

The means of sarcoplasmic protein per unit of DNA correlated highly ($P < 0.01$) with most growth parameters as DNA was highly dependent on growth or live weight changes (Appendix). Therefore, physiological cell size, live weight, DNA and total protein concentrations had high correlation coefficients with sarcoplasmic protein in the muscle cell, while others like collagen and myofibrillar protein were less well related to sarcoplasmic protein changes during growth.

Collagen Concentration

The collagen mean values are plotted in Figure 5. The concentration was relatively high at 45 kg and decreased rapidly to a low level

Figure 5. Changes in muscle collagen concentration in *longissimus* muscle of the various breed and male cattle types

Code on figure:

0	Dairy cattle
X	Beef cattle
_____	Intact male
----	Castrated male

COLLAGEN CONC (MG/G)
1.00
3.00
5.00
7.00
9.00
11.00
13.00
LIVE WEIGHT (KG)
0.00
1.00
2.00
3.00
4.00
(x10²)

of 3.5 mg/g at 345 kg live weight (Table 11). The differences due to

Table 11. Mean values of the collagen concentration (mg/g tissue) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	11.1	7.1	3.9	2.8	3.2
Beef cattle	12.1	5.1	4.4	4.5	3.4
Intact male	11.1	6.5	4.6	3.6	3.4
Castrated male	12.1	5.7	3.8	3.7	3.1
All	11.6	6.1	4.2	3.6	3.3

breed and male types, within each live weight class, were not significant ($P > 0.05$), and these differences became smaller during growth. The live weight was the only factor that markedly affected the concentration of collagen in the longissimus muscle (Table 3) and this was further shown by a high correlation between these two variables (Appendix). This decrease followed by a constant concentration of collagen is in agreement with other findings in cattle (Mitchell et al., 1928; Yeates, 1964; Bendall and Voyle, 1967).

High positive coefficients of correlation were found between the collagen concentration, myofibrillar protein concentration, fiber diameter, physiological cell size or protein fractions within the cell (Appendix).

Since collagen constituted a main part of the support material of the longissimus muscle fiber, any factor related to growth and muscle fiber size would be expected to be highly related to collagen. Very little relation existed between collagen content and sarcoplasmic protein. A poor correlation existed between collagen and the total protein concentration since 40 percent of the total protein could be accounted for by the sarcoplasmic protein. The collagen concentration represented a very small proportion of the total protein concentration and could not really influence the protein content.

The collagen concentration decreased by 50 percent in the first 100 kg live weight gain, followed by another 50 percent decrease by 445 kg (Table 11). The same rate of change has been observed earlier with the fiber diameter (Table 6). It appeared that larger fibers resulted in less connective tissue per surface area as the fiber represented more and more of the weight of the sample rather than collagen. On a total basis, Yeates (1964) showed that the collagen content increased in the muscle with an increase in live weight.

Total Protein Concentration

The total protein concentration was calculated as the sum of the myofibrillar, sarcoplasmic and collagen concentrations. The analysis of variance in Table 3 shows no significant ($P > 0.05$) difference due to breed and male types, or their interactions. Since this was the sum of mean values that were generally not different, it was reasonable

to expect that the sum would show no difference either. This finding was in agreement with the lack of variation in diameter fiber due to the same treatments.

Florini (1970) showed that testosterone had a quantitative effect on protein synthesis. Scow and Hagen (1955, 1957) found that this hormone had no effect on the composition of muscle but they added that the response to that hormone could vary with muscles for rats and guinea pigs. The effect of male cattle types was very small on muscle composition and appeared to be minimum in longissimus muscle.

The mean values for the total protein concentration are expressed in Table 12. The mean was around 15 percent protein which was slightly

Table 12. Mean values of the total protein concentration (mg/g tissue) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	132.6	169.0	154.3	162.3	180.1
Beef cattle	133.9	145.0	151.9	158.4	180.0
Intact male	126.3	160.0	154.0	153.5	175.0
Castrated male	140.2	154.0	152.2	167.3	185.1
All	133.3	157.0	153.1	160.4	180.1

less than some other values found in cattle longissimus muscle (Lawrie, 1961; Lawrie and Kirton, 1961). The mean value of 18 percent total

protein at 445 kg live weight was quite comparable to the values for a mature weight class.

The live weight change during growth tended to increase the total protein concentration in longissimus muscle (Figure 6, Table 12). This significant increase was around 33 percent with a range of 133 to 180 mg protein/g tissue as the live weight increased. There was no weight interactions with breed or male types. These increases were in agreement with similar findings by Spray and Widdowson (1950), Usborne et al. (1968), Robinson and Bradford (1969) in various species and also Lawrie (1961) in male cattle.

A high peak in total protein was reached at 145 kg and this was mainly caused by the peak of sarcoplasmic protein concentration at that live weight (Figure 4). Because of the peak of this protein fraction, and because of the gradual increase of the myofibrillar protein concentration, the total protein concentration showed a very slow rise between 145 and 345 kg. However, if the idea of a lesser increase in the concentration of sarcoplasmic protein was accepted, then the upward curve of total protein concentration would be regular and more gradual. The collagen concentration was quite small except at 45 kg live weight and thus had limited effect on the curve of the total protein.

The increase in total protein concentration was highly correlated ($P < 0.01$) with the direct growth parameters like the decrease in DNA concentration, and the increase in fiber diameter and physiological cell size (Appendix). Since the majority of the total protein could be accounted for by the myofibrillar and sarcoplasmic protein fractions,

Figure 6. Changes in muscle protein concentration in longissimus muscle of the various breed and male cattle types

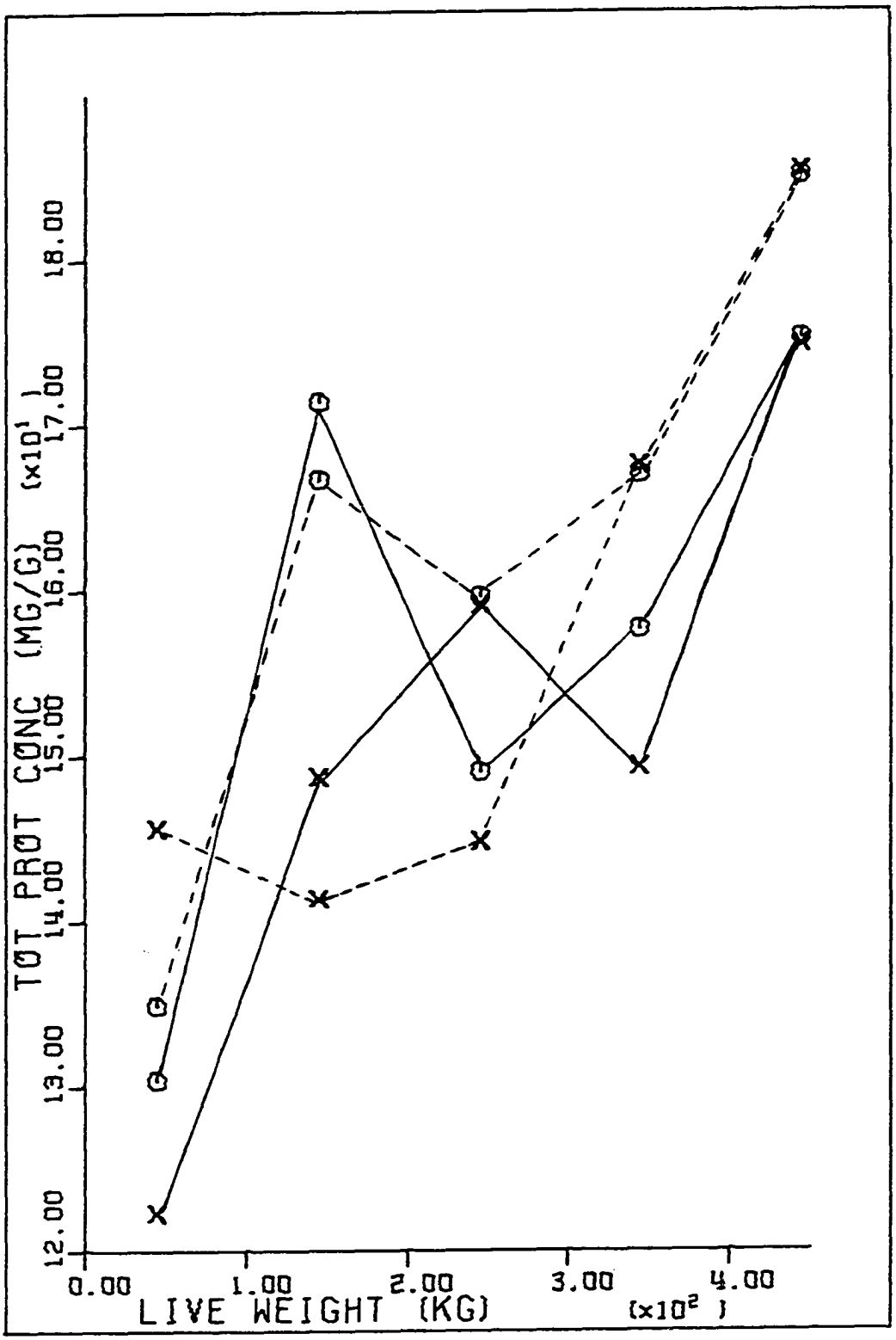
Code on figure:

O Dairy cattle

X Beef cattle

____ Intact male

---- Castrated male


TOT PROT CONC (MG/G) (x10¹)
12.00
13.00
14.00
15.00
16.00
17.00
18.00
0.00
1.00
2.00
3.00
4.00
LIVE WEIGHT (KG)
(x10²)

there was a strong positive coefficient of correlation between these parameters and total protein concentration. The collagen content was, however, poorly correlated representing less than five percent of the total protein concentration.

Cell size has been defined by Cheek et al. (1971) as the ratio of protein per unit of DNA in a cell, since DNA concentration is constant at 6.2 pg per nucleus (Vendrely and Vendrely 1948, 1949). In muscle, this ratio has a special meaning since a muscle fiber is multinucleated. Such a ratio is more descriptive of the physiological muscle cell rather than the physical size of the cell.

The DNA and total protein concentration did not differ within breed and male types and thus their ratio did not show any difference ($P > 0.05$) (Table 3). The interaction breed•male seen in DNA concentration was somewhat masked in the ratio and no difference was reported for the physiological cell size. Thus, it appeared that the rearing of the different types of cattle had no effect on the cell size of the longissimus muscle between these types.

The cell size increased by 100 percent for each increase of 200 kg in live weight between 45 and 445 kg. There seemed to be a plateau level at the heavier weights since the increase was only five percent for the last 100 kg (Table 13). This was especially true in view that DNA, collagen and sarcoplasmic concentrations were also tapering off at those heavier weights. The increase in cell size with live weight was also reported in pigs (Harbison, 1970), mice (Robinson and Bradford, 1969), lambs (Norton and Walker, 1970). Munro (1969) suggested that a

Table 13. Mean values of the ratio total muscle protein concentrations (mg/g) and DNA (mg/g) of longissimus muscle during growth

Cattle type	Live weights (kg)				
	45	145	245	345	445
Dairy cattle	169.2	234.1	303.3	543.6	527.5
Beef cattle	206.9	232.4	371.1	472.7	521.2
Intact male	162.2	229.2	316.8	512.8	514.8
Castrated male	213.8	237.3	357.6	503.5	533.9
All	188.0	233.2	337.2	508.1	524.3

constant cell size was a sign of tissue maturity and thus the cattle of this experiment could have been mature at 345 kg or so. However, this hypothesis cannot hold for the polynucleated muscle cell because new sarcomeres could be synthesized within this cell without affecting DNA content. Constant protein per unit of DNA may be a sign of maturity of mononucleated cells.

The cell size was highly correlated ($P < 0.01$) with the various protein fractions of that cell. The cell size was also related to the other parameters that changed with growth like fiber diameter and the protein concentration per gram of muscle tissue (Appendix).

Cheek et al. (1965b, 1971) proposed a cubic equation for the increase in physiological cell size of male rats. This equation could not be checked in the present work since only five values were obtained

during the growth. Any equation could ultimately fit these five points and give a good fitting line.

General Growth

Moss et al. (1964) suggested from his work on breast muscle of chickens that there was a linear relationship between the logarithm of muscle weight and the logarithm of the total number of nuclei in this muscle. This has been confirmed in rat leg muscle and longissimus muscle of cattle (Trenkle, 1972, unpublished data).

Most workers used 6.2 pg DNA per nucleus to determine the number of nuclei of a sample. Thus, the DNA concentration·muscle weight is equivalent to 6.2·number of nuclei. Since it is thought that the longissimus muscle grows at the same rate as the body, it was decided to plot the logarithm of live weight against the logarithm of DNA concentration·live weight. A linear relationship would indicate a parallel growth gradient in live weight of the body and longissimus muscle. The data of this trial showed a correlation coefficient of 0.97 between the mean at each live weight, 0.92 between the four means of the treatment groups at each live weight and 0.79 if all the values from individual animals were used in the calculations. This strong linear relationship pointed out a parallel growth between the longissimus and the body.

The correlation coefficient of 0.79 between the logarithms was greatly decreased from 0.92 by the variation in DNA concentration

(Table 5) and live weight at the time of the first biopsy (Table 4). However, it was apparent that the values at 45 kg tended to be below the regression line. It appeared that the growth of the longissimus muscle was initiated slightly later than general body growth since the relationship between DNA content of the muscle and weight is linear when the muscle weight rather than body weight is used (Trenkle, 1972, unpublished data). The longissimus muscle has been reported to be average developing or even slightly late maturing (Butterfield, 1963a, b; Butterfield and Berg, 1966). The findings of the present trial agreed with the conclusion of these workers. The strong relation agreed with the concept that absolute size was the only factor in determining form of an animal (Huxley, 1932). The present data also agreed with Berg (1968) and Mukhoty and Berg (1971) who found no difference in growth between male types. These results were in opposition to the idea that castrated males grow differently from intact males (Bonsma, 1967).

Since other workers showed no variation in the linear relation due to nutrition (Moss et al., 1964) and species (Trenkle, 1972, unpublished data) and since no cattle breed or male types effects were observed in the present experiment, it may be postulated that this linear relationship between DNA content and weight is a basic characteristic of normally growing muscle.

GENERAL DISCUSSION

The growth pattern studied in this trial referred only to the differences in composition of the muscle at identical weights and so did not measure the rate at which a change was brought about. The study tried to determine if composition of the muscle was the same between dairy and beef male cattle and if the changes due to live weight were similar in each of the treatments.

The results of this study confirmed several findings and conclusions reported in the literature concerning the changes in the longissimus muscle during growth of cattle from 45 to 445 kg live weight.

First, it appeared that male cattle types had no significant ($P > 0.05$) effect on the growth of the longissimus muscle as defined by the various parameters used in this study. The comparison of intact and castrated males showed no difference in fiber diameter, physiological cell size and cell protein composition, DNA concentration and various muscle protein fraction. It was apparent that any difference between these two male types did not reside in the quantitative aspect of these parameters. There was no evidence in this study to suggest that the characteristics of the meat from the longissimus muscle of these male types would be different at 445 kg. The collagen and fiber diameter of these breeds would produce the same intrinsic meat toughness. The other measures of structure had also similar values. The fact that there was no difference in the growth as seen from the present work on the longissimus muscle was in agreement with the literature but this was

not to say that the same findings would occur in other muscles.

Testosterone has been shown to be effective at the level of transcription of protein synthesis (Kochakian et al., 1964). So it seems there might be a difference between castrated and intact males. However, no difference in composition of the longissimus muscle was observed in the present study with animals of similar body weights. It may be that this muscle is less affected by testosterone since it is essentially a support muscle for the body. The role of the longissimus muscle may be quite passive compared with the muscles involved in reproduction or general body strength which may be more affected by male sex hormones.

The muscle fiber diameter was the same in both male types since basically the same body weight had to be supported. At birth, the group of calves that were eventually castrated would have had a similar number of fibers as the group of calves that were left intact. Since only hypertrophy has been shown after birth and since each muscle had basically the same composition, the pattern of changes of the longissimus muscle with live weight should be the same in both male groups. So the characteristics of the longissimus muscle were the same for both male cattle types.

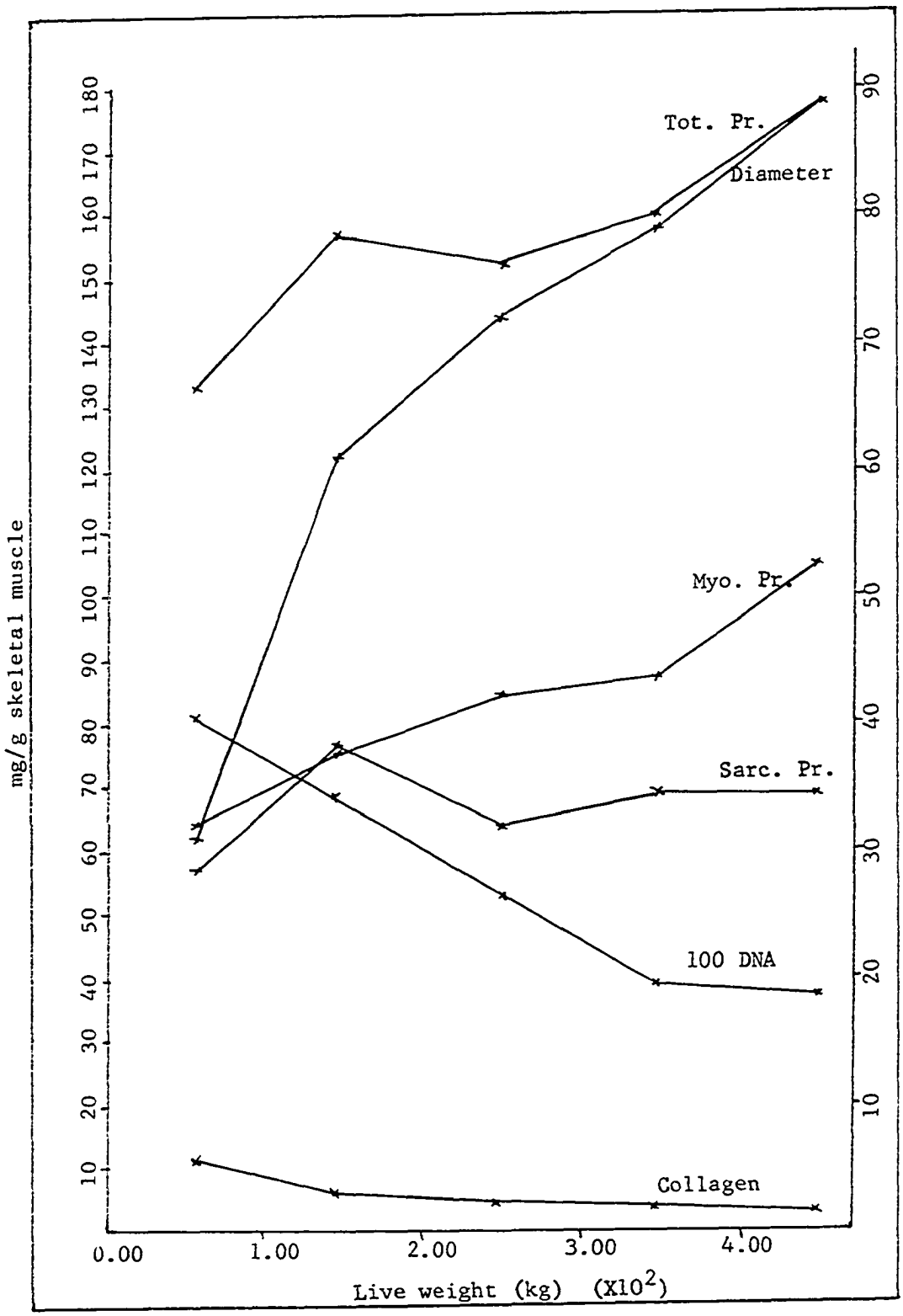
The effect of breed type was negligible in most growth related parameters studied. Since physiological cell size and composition, fiber diameter and DNA concentration analyses revealed no significant ($P > 0.05$) difference, it was concluded that there were minimum effects of breed type on the growth pattern of the longissimus muscle of male

cattle. It might be reasonable to extrapolate that the selection over the years for meat or milk production did not change the growth processes of male calves as seen in the longissimus muscle. The dairy and beef breeds had an identical growth while quite different in conformation.

A marked difference during growth was observed between the two breed types for the protein concentration. The dairy breed type tended to have a larger myofibrillar and smaller sarcoplasmic protein concentration in the longissimus muscle. However, there were no significant differences between the two breed types in total protein, collagen, myofibrillar protein per unit of DNA and fiber diameter. No evidence of a difference in concentration of myofibrillar proteins in muscle from dairy and beef breeds has been reported in the literature. It is difficult to explain how some muscle fibers of similar diameter could have such a different composition. Any definite conclusions based on these protein components should be made very carefully in view of the unusually large variation for these protein parameters in the present study.

Changes with live weight in the growth of the longissimus muscle was marked for all the parameters studied (Figure 7). Generally, the fiber diameter size increased with live weight. This increase was paralleled by an increase in myofibrillar protein concentration that constituted the bulk of the muscle structure, by a lesser increase in sarcoplasmic protein, and by an increase in the total protein concentration since the major percent of the total was accounted for by sarcoplasmic and myofibrillar proteins. As the muscle fiber got larger, the

Figure 7. Changes in the different parameters studied in *longissimus* muscle of the various breed and male cattle types


mg/g skeletal muscle
Diameter (microns)
Live weight (kg) (X10²)
Tot. Pr.
Diameter
Myo. Pr.
Sarc. Pr.
100 DNA
Collagen
0.00
1.00
2.00
3.00
4.00
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180

the collagen concentration decreased proportionally. The same decrease was apparent in the DNA concentration in the longissimus muscle. The changes due to growth were important with an increase to 300 percent in fiber diameter, an increase to 150 percent in myofibrillar concentration, a decrease to 30 percent in collagen and 50 percent in DNA concentration, and an increase to 300 percent in cell size (Figure 7). These relative changes were in agreement with the findings in the literature. The fact that the muscle got stronger or larger was in agreement with a better support needed by a larger animal.

In the present work, most parameters changed during growth by factors of 1.5x to 3x. However, the sarcoplasmic protein changes were poorly correlated with the changes in fiber size. While that protein fraction went upward rapidly first and stayed constant thereafter, the overall increase was only slightly affected by changes in live weight. That increase distorted the total protein concentration changes in longissimus muscle. Also, a slight negative effect on total protein concentration was obtained from the decrease in the collagen fraction.

If the fiber diameter increased by three fold, it means that the area of that muscle would increase by nine fold rather than three fold, since the area ratio between a circle of three "d" diameter and one of "d" diameter is 9.0, based on the formula $1/4\,\pi d^2$. The DNA concentration decreased only by 2.5 fold during the same time. The DNA concentration should have decreased by at least nine fold if the muscle fiber length stayed the same. This apparent discrepancy suggested that there was an increase in the total DNA content of the longissimus

muscle. The increase in the DNA content is especially large if we also consider the lengthening of the muscle which occurs during growth. A large increase in total DNA content of muscle after birth has also been reported by Enesco and Puddy (1964), Moss et al. (1964), Robinson and Bradford (1969).

The heavier live weights seemed to bring maturity in the animals. Since DNA concentration, physiological cell size, collagen, and sarcoplasmic protein concentration were tapering off, it was interpreted as a sign of maturity in the longissimus muscle. There was still an increase in diameter size and myofibrillar protein concentration over the last 100 kg but this increase could be expected to taper off soon. This would be in agreement with Moulton (1923) who proposed as chemical maturity the time of no muscle composition change. It appeared that cattle of all types would act in the same way since no difference has been shown between these types at 445 kg live weight. It is possible that a later maturity in the intact male or dairy breed would have allowed these two groups to continue to grow for a longer time than used in this study. However, there was no evidence of such a trend at 445 kg.

The growth of the longissimus muscle has often been accepted as parallel to the live weight growth of the body. Most of the work done to support this hypothesis has been based on dissection techniques where the animals were sacrificed. Some workers have shown that the muscle growth could be expressed in a linear relation between the logarithm of muscle weight and the logarithm of number of nuclei of the muscle.

Since this linearity was unchanged by species and nutrition level (Trenkle, 1972, unpublished data), it was reasonable to expect that breed or male types would not affect this curve. Based on the idea that the body weight changes correlated well (Appendix) with longissimus muscle growth, the logarithm of the total live weight was plotted against the logarithm of DNA concentration·total body weight. The coefficient of correlation was 0.79 and it showed that this muscle grew at the same rate as the body. The significant ($P < 0.001$) linear relationship suggested that breed and male types were without effect on this correlation. This linearity also refuted the concept of a possible growth differential in male types for the longissimus muscle (Bonsma, 1967). Selection for a production characteristic also did not affect the growth pattern of the longissimus muscle in cattle.

SUMMARY

An experiment was designed to study the changes in the longissimus muscle during growth of different types of male cattle up to a live weight of 445 kg. Male calves representing dairy or beef breeding were kept intact or castrated within three weeks of birth. Each animal was biopsied at 100 kg intervals from 45 to 445 kg live weight. From the findings of this experiment, the following conclusions were drawn:

1). The castration of male calves had no effect on the concentration of protein and DNA, or on the fiber diameter of the longissimus muscle. No differences were observed when the protein concentrations were expressed per unit of DNA. It was evident from this study that the composition of the muscle was not influenced by male type at similar live weight. Also, it was suggested that the intrinsic tenderness of the meat supplied by the longissimus muscle would not be different between the males.

2). The breed type of the calves had no effect on the growth of the longissimus muscle. While definite differences were measured in the concentration of the major muscle proteins, differences were absent when the protein concentrations were calculated per mg DNA. The differences were explained by large variation within the small number of samples. It appeared that selection for meat or milk production had very limited effect on the growth of this muscle in cattle as seen in the parameters studied.

3). The increase in live weight had a profound effect on most parameters studied in the longissimus muscle. The fiber diameter of the muscle increased in size during growth with correlated changes in protein and DNA concentrations. Collagen and DNA concentrations decreased with growth while the other parameters increased. Live weight was the only factor studied which brought about consistent changes in the various parameters.

4). The correlation between logarithm of live weight and logarithm of DNA concentration·live weight was high. This relation was linear and similar to the reported relation between the logarithms of muscle weight and number of nuclei in the muscle. Based upon this linear relation it was proposed that the longissimus muscle grew at a similar rate as the total body.

5). The physiological cell size, as measured by the quantity protein per unit of DNA, appeared to enlarge at a faster rate than the area of the muscle fiber. Fat deposition was thought to be at least a partial reason for this discrepancy.

6). Maturity and elongation of the muscle cell were discussed only briefly since no measurements were done or these parameters.

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APPENDIX

Table 14. Relationships among all measurements taken on the 20 bovine animals 45 to 445 kg live weight

	Real weight	DNA	Myo-fibrillar protein	Sacro-plasmic protein
Real weight	1.00	-0.70 **	0.62**	0.15
DNA	-0.70**	1.00	-0.38**	-0.15
Myofibrillar protein	0.62**	-0.38**	1.00	0.00
Sarcoplasmic protein	0.15	-0.15	0.00	1.00
Collagen	-0.56**	0.43**	-0.40**	0.01
Total protein	0.51**	-0.33**	0.77**	0.62**
Fiber diameter	0.85**	-0.59**	0.47	0.32
Sarcopl. prot./DNA	0.52**	-0.77**	0.25*	0.51**
Myofibr. prot./DNA	0.68**	-0.80**	0.66**	0.09*
Total prot./DNA	0.63**	-0.83**	0.50**	0.29**

[a]98 degrees of freedom for all figures.

*$P < 0.05$ .197 required for significance.

**$P < 0.01$ .257 required for significance.

Collagen	Total protein	Fiber diameter	Sarcopl. prot./DNA	Myofib. prot./DNA	Total prot./DNA
-0.56**	0.51**	0.85**	0.52**	0.68**	0.63**
0.43**	-0.33**	-0.59**	-0.77**	-0.80**	-0.83**
-0.40**	0.77**	0.47**	0.25*	0.66**	0.50**
0.01	0.62**	0.32**	0.51**	0.09	0.28**
1.00	-0.15	-0.54**	-0.24*	-0.38**	-0.31**
-0.15	1.00	0.49**	0.49**	0.54**	0.55**
-0.54**	0.49**	1.00	0.47**	0.50**	0.50**
-0.24*	0.49**	0.48**	1.00	0.78**	0.93**
-0.38**	0.54**	0.50**	0.78**	1.00	0.96**
-0.31**	0.55**	0.50**	0.93**	0.96**	1.00