THE RELATIONSHIPS AMONG TENDERNESS, WATER RETENTION, CALCIUM AND PHOSPHORUS OF BOVINE *LONGISSIMUS DORSI* MUSCLE

BY

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INTRODUCTION

Numerous studies have shown that tenderness is the most important palatability factor in the acceptance of beef. However, consumer evaluation of tenderness is not uniform. The consumer appears to desire a maximum of tenderness as long as the meat retains its "customary" characteristics. In addition, each meat consumer has a particular ideal for juciness, flavor, texture and aroma of beef. These desired palatability characteristics are often referred to as "meat quality" which has been defined as "the summation of distinctive traits or special features that determine the ultimate acceptability of the product to the consumer" (Doty, 1959). Meat quality is a complex entity and may be influenced by the physical, structural or chemical characteristics of a particular cut. In turn, these characteristics may be affected by other factors; some which have been elucidated and many which are probably unknown.

The purpose of this study was to investigate three possible influential components of meat tenderness; namely, water retention, calcium and phosphorus content. Although, it has been found that water retention of meat is directly related to tenderness and that significant relationships exist between water retention and mineral content of muscle,

knowledge with regard to the influence of minerals on meat tenderness is still somewhat limited. Thus, it was theorized that investigations, regarding the role that mineral content may play in affecting protein hydration and meat tenderness, might further the understanding of the nature of meat tenderness.

The first objective of this study was to develop a laboratory procedure for the chemical determination of calcium and phosphorus in meat. The second objective was to determine the content and the variation of calcium and phosphorus in the *longissimus dorsi* muscle at various designated locations. In addition, variation in *longissimus dorsi* water-holding capacity was determined at these respective locations. The third objective was to observe the relationships among tenderness, water-holding capacity, calcium and phosphorus content of bovine *longissimus dorsi* muscle.

REVIEW OF LITERATURE

This review includes some of the work relative to (1) the mineral composition of bovine muscle, (2) water retention of muscle, and (3) tenderness as it is affected by minerals and water retention properties.

Mineral Composition of Muscle

Minerals in muscle have a two fold role. They serve as a dietary nutrient in muscle prepared for consumption and have effects on meat quality.

Mineral Content

A study of the nutritive value of cooked meat by Leverton and Odell (1958) revealed that phosphorus and potassium contents of meat were relatively high, being approximately 200 and 435 mg. per 100 grams of cooked meat, respectively. Minerals found to be less abundant in meat were calcium, sodium and magnesium which were present in quantities of approximately 8, 48 and 23 mg. per 100 grams of cooked meat, respectively. A large amount of variation in mineral content was found between different carcasses and different cuts within carcasses. Mineral content was found to vary with fat.

Swift and Berman (1959) measured the mineral contents of eight selected beef muscles. They analyzed the variation in the *longissimus dorsi*, *psoas major*, *semimembranosus*, *latissimus dorsi* and *trapezius* muscles for calcium, magnesium, zinc, sodium, potassium, iron and phosphorus content. Average mineral values expressed as mg. per 100 grams of muscle tissue for all eight muscles were as follows: calcium, 4.0; magnesium, 21.1; zinc, 4.2; sodium, 38.5; potassium, 395; iron, 2.7 and phosphorus, 171.

Mineral values of separable lean from T-bone steaks of choice grade beef carcasses were found to be 11.9, 197.1, 3.2, 65.0 and 35.49 mg. per 100 grams of meat respectively for calcium, phosphorus, iron, sodium and potassium (Watt and Merrill, 1963). Similar mineral values of beef muscle have been reported by Mittledorf and Landon (1952) and Schweigert and Payne (1956).

Mineral Status

The state in which minerals are found in muscle is dependent upon certain metabolic reactions and conditions.

In rigor and post-rigor some of the bivalent ions are bound to the structural proteins so tightly that it is impossible to remove them completely by treatment with ion exchangers or chelating reagents. (Bozler, 1955; Hamm, 1960). It is known that polyvalent cations decrease the hydration of proteins. They link together the peptide chains by forming cross linkages which cause a tightening of the protein

structure (Hamm, 1960). The source of energy for muscle contraction comes directly from splitting adenosinetriphosphate (ATP) which is present as a magnesium-complex of ATP (Davies et al., 1959). Falk (1956) reported that ATP forms complexes with calcium during contraction of the living muscle. In the live animal and at the time of slaughter almost all the calcium in muscle is bound to protein, whereas 90 percent of the magnesium is unbound.

Numerous studies have been made of the interactions between alkali ions and muscle proteins. Some experimental evidence exists for the binding of potassium and sodium to myosin (Conway and Carey, 1955; Lewis and Saroff, 1957; Saroff, 1957). Other investigations indicate that potassium ions are adsorbed by myosin and actomyosin, but not by actin. It was further stated that these ions influence the solubility and extractability of these proteins (Szent-Gyorgi, 1951, 1953; Weber and Portzehl, 1952). Arnold et al. (1956) studied the post-mortem movements of sodium, potassium, magnesium and calcium in beef muscle. They found that during the aging process, sodium and calcium were continously released by the muscle proteins, potassium was absorbed after the first twenty-four hours, and magnesium was released during the first twenty-four hours and also between six and thirteen days followed by a decrease in released magnesium.

Phosphorus is found in meat as phosphates or as various organic phosphate complexes such as sugar phosphates, nucleic

acids, proteins through serine phosphate linkage, phosphatidic acid derivatives and high energy phosphate compounds.

Water Retention of Muscle

Water retention of muscle is an important factor of meat quality as it is closely related to taste, tenderness, pH and color (Hamm, 1960).

"Water-holding capacity" as stated by Hamm (1960) refers to the ability of meat to hold fast to its own or added water during application of any force such as grinding, heating and pressing. "Swelling" was defined as the uptake of water by meat from any surrounding fluid which causes an increase in volume or weight of muscle. Methods employed in determining water-holding capacity measure the liberated water which has been termed "loose water" and that which is retained by the tissue termed as "bound water" (Hamm, 1960). Water-holding capacity of meat can be expressed in terms of the amount of loose water relative to the total moisture content of the muscle; or on a positive basis, it can be expressed in terms of the amount of bound water relative to the total moisture content of the muscle.

Methods of Determining Water-holding Capacity

(a) Sedimentation

For this method, used by Mohler and Kiermeier (1953), ground meat is suspended in a calibrated cylinder. After twenty hours the volume of the tissue sediment is read.

This method was used in observing the effect of certain salts on the swelling of meat.

(b) Centrifugation

Several centrifugation methods for determining water-holding capacity have been established; however, the most common method used today is that presented by Wierbicki *et al*. (1957a). This method involved placing a sample of meat on a fritted glass disk in a special glass centrifuge tube and separated the loose water from tissue, by centrifuging at 1000 r.p.m., into a smaller graduated section of the tube. The meat sample was heated to 70° C and then cooled to 30-35° C before centrifuging. In experiments studying drip loss after thawing, the meat samples were heated only to 40° C and then cooled before centrifuging.

(c) Filtration

Lloyd and Morgan (1933) devised a method of ultrafiltration for determining bound water in gelatin. It was pointed out that this particular method might be applicable for homogenates of tissue.

(d) Mechanical Press

The primary pressing method used today is one designed by Grau and Hamm (1953, 1957) or slight modifications of this method. In this method, meat tissue is placed on filter paper between two plexiglas plates and pressed to a thin film by use of a hand operated hydraulic press. The water pressed out is absorbed by the filter paper. After a definite period of time, the pressure is released and the meat film is traced

before being removed from the filter paper. The inside meat area which contains practically no moisture is then subtracted from the total area to give the moisture area. These areas can be measured by the use of a planimeter. The moisture area or expressed juice absorbed by the filter paper is proportional to the amount of loose water in the meat sample. In working with this method, Urbin et al. (1962) obtained more consistant results by using an electrically driven centrifugal pump in place of the hand operated pump.

Binding of Water to Muscle

Water, being a dipolar substance, is attracted to the electrically charged polar groups of muscle protein (Hamm, 1959a). He describes two ways by which water may be bound. First described is the "net charge effect" which is due to the net charge of the protein. Groups adding to the net charge could be free carboxyl, amino, hydroxyl or sulfhydryl groups. The "stereo effect" is another method by which water is bound by protein. This is described as changes of meat hydration which are not due to changes in net charge. Certain cross chains, such as salt linkages, bivalent metal linkages, disulfide bonds and hydrogen bonds, are not available to water because of "steric" and spatial reasons. By cleavage of the cross linkages the peptide chains become more flexible and water can then attach to the polar groups.

The direct electrostatic binding of water to protein is very firm. Some loosely bound water is also present (Hamm,

1959a). There is now reason to believe that several layers of water may be bound to the protein charges. Some water is attracted by the primary bound water dipoles while other water is taken up by capillary forces.

Factors Affecting Water-holding Capacity

pH

Water-holding capacity is directly related to pH (Wierbicki and Deatherage, 1958; Swift and Berman, 1959; Hamm, 1959a). Known causes of variation in the pH values of meat include differences in lactic acid formation after slaughter, age at slaughter and degree of aging of meat (Swift and Berman, 1959). The pH and water-holding capacity of muscle are highest at the time of slaughter. For the next twenty-four to forty-eight hours the pH drops due to the formation of lactic acid, loss of ATP and decrease in electrical activity (Hamm, 1960; Wilson, 1959). At the same time the sarcolemma looses some of its semi-permeable characteristics and a freer interchange of electrolytes takes place. Hamm (1959a) stated that one-third of the pH change is due to the breakdown of ATP. With further aging, autolysis will bring about cleavage of some bonds and water-holding capacity will be increased by the stereo effect (Hamm, 1959a) or redistribution of ions within the muscle.

Protein has its lowest water-binding capacity when its positive and negative charges are equal. This condition is referred to as the "isoelectric" point which in muscle is

found to be in the pH range of 5.0-5.4 (Hamm, 1959b; Arnold et al., 1956; Wismer-Pedersen, 1960). The addition of an acid or base at this point will increase the water-holding capacity of meat. Briskey et al. (1958) and Wismer-Pedersen (1959) found that as the pH of meat approached the isoelectric point there was a definite increase in the amount of expressible water. A similar relationship was shown for eight different muscles studied by Briskey et al. (1960).

ATP

Hamm (1960) stated that two-thirds of the hydration drop in beef post-mortem is due to the breakdown of ATP, and one-third to the fall of pH. It was concluded that ATP imparts to muscle the state of high hydration. Hydration decreases to the extent that ATP is broken down. Additions of ATP to fresh muscle increased water-holding capacity. Additions to rigor or post-rigor muscle causes further contraction. An explanation of this is that the source of energy for muscle contraction comes directly from splitting ATP (Davies et al., 1959). The splitting of a terminal phosphate from ATP is activated by ATP-ase in the presence of magnesium ions. Also present is a relaxing factor when in an active state will prevent the splitting of ATP. This relaxing factor can inhibit the interaction of the contractile proteins, actin and myosin, because without energy from ATP splitting, the fibrils are incapable of contracting. However, this relaxing factor was described by Partmann (1963)

to be inhibited by the presence of calcium ions, thus preventing the relaxation of fibrils and resulting in contraction. Since most of the calcium in fresh muscle is bound to the proteins, the relaxing factor is not inhibited. However, as the pH of the muscle drops after death, the calcium ions are released and become available for inhibition of the relaxing factor. This results in ATP breakdown and muscle contraction due to contractile proteins, actin and myosin, forming the actin-myosin complex which causes stiffness of the muscle. So long as ATP is not broken down, it exerts a hydrating and softening effect on muscle; but while it is being decomposed, it causes dehydration and contraction.

It is possible to keep the hydrating effect of ATP in the case of stored meat by removing the free alkaline earth ions from the tissue by means of ethylene diamine tetraacetate (EDTA) (Hamm, 1960). Effects of other chelating agents were described by Carpenter _et al_. (1961), and Belenkij (1962). Sodium hexametaphosphate and "demotin" which contained diacetyl choline iodine were the two chelating agents studied, respectively. Both workers found that pre-rigor infusions of these chelating agents resulted in improved tenderness of muscle over controls. This was due to the fact that the chelating agents tied up the alkaline earth ions and prevented ATP breakdown. Thus, muscles did not become rigid.

Minerals

Hamm (1959a) found that despite their low concentration, magnesium, calcium and perhaps zinc have an effect on water-holding capacity. With the removal of calcium there is an increase in water-holding capacity. By removing metallic cross linkages more charged groups become available for water binding. Swift and Berman (1959) reported that water retention was significantly correlated with pH and zinc content. Calcium, magnesium and potassium revealed a negative relationship with water-holding capacity. This indicates that zinc may differ from the other ions in an important aspect. It was suggested by Swift and Berman (1959) that zinc may participate in the determination of pH as a component of an enzyme system. Berman (1961) confirmed that zinc was a component of lactic dehydrogenase. However, lactic dehydrogenase was inversely correlated with pH whereas zinc and pH revealed a positive correlation. Bozler (1955) found that a partial extraction of calcium by EDTA and of magnesium by polyphosphate, increased the water retention of muscle tissue.

Effects of potassium and sodium on water-holding capacity of muscle may be quite small in comparison with the effect of bound bivalent ions because the alkali ions are bound to muscle protein rather weakly (Hamm, 1960). Nevertheless, some relationship seems to exist between the content of alkali metals in the press juice and the amount of expressible water (Briskey *et al*., 1959).

The action of phosphorus in the various phosphate compounds is generally thought to: (1) increase the pH; (2) tie up bivalent cations in meat; and (3) resemble the action of ATP by splitting actomyosin to actin and myosin, resulting in a swelling action with the uptake of water (Hamm, 1959a; Hellendorn, 1962).

Tenderness as it is Affected by Minerals and Water Retention Properties

Arnold et al. (1956) reported that mineral content of muscle tissue had an influence on tenderness, but largely in an indirect way. They reported that the greater the mineral content of meat the greater the water-holding capacity, due to the minerals' great affinity for water which has been previously discussed. The more water that muscle holds, the greater the extent to which the collagen will be hydrolized; thus, generally improving tenderness. The more water that is held in the tissues, the thinner the muscle fiber membranes are stretched; consequently, requiring less force to tear them apart. As was previously stated, muscle has its lowest water-holding capacity at its isoelectric point. It was reported by Hamm (1960) that muscle is also least tender at its isoelectric point.

Results by DeFremery and Pool (1960) and Bate-Smith and Bendall (1949) indicated a correlation between rate of ATP breakdown and muscle tenderness. The effects of certain bivalent cations on ATP breakdown have been referred to

previously. This was illustrated by the use of chelating agents in tieing up certain ions which resulted in muscle not becoming rigid. It is known that relaxed muscles are more tender than partially contracted muscles (Locker, 1960).

This study was designed to investigate the interrelationships among calcium, phosphorus, water-holding capacity and tenderness of the longissimus dorsi muscle. It was thought that definite patterns with regard to mineral deposition, water-holding capacity and tenderness may be found. The degree of parallelism of patterns may clarify the association among these variables.

EXPERIMENTAL PROCEDURE

Materials

The data reported were collected from 9 Hereford steers of West Texas origin. Steers were obtained by the Oklahoma Agricultural Experiment Station and placed on a high concentrate ration plus 5 percent fat and 13 percent polyethylene fluff (Table I).

TABLE I

HIGH CONCENTRATE RATION

Ingredients	Percent[1]
Steam rolled milo	79.55
Cottonseed meal	5.00
Alfalfa meal	5.00
Molasses	3.00
Urea	1.50
Stabilized animal tallow	5.00
Salt	0.50
Limestone	0.40
Vitamin A premix	0.03
Trace mineral premix	0.02
Polyethylene fluff	(300 lbs./ton)

[1]Percentage figures listed do not include polyethylene as part of the total.

At the initiation of the feeding period, the average weight of the steers was 686 pounds. At the termination of 132 days on feed the cattle were slaughtered at the Oklahoma

State University abattoir. Average final weight of the steers was 1028 pounds. The animals were approximately 18 months of age at slaughter.

Methods

Slaughter Procedure

The experimental animals were weighed prior to slaughter after 12 hours without feed or water. Standard experimental slaughter procedures were followed. Cartilage tips from the dorsal spinous processes of designated thoracic vertebrae were removed at the time of slaughter. Carcasses were then placed in a 34-36° F cooler to chill for 72 hours before additional sampling.

Sampling Procedure

Cartilage tips from the 1st, 3rd, 5th, 7th and 9th thoracic spinous processes were obtained at the time of slaughter after the carcasses were split. The entire cartilage tips were removed from both the left and right sides. Cartilage tips were immediately frozen in sealed glass jars and later analyzed for phosphorus and calcium content.

At the end of the 72 hour chilling period, meat samples were secured from the left sides of the nine beef carcasses. *Longissimus dorsi* muscle samples were taken opposite the 5th, 7th, 9th, 11th and 13th thoracic vertebrae and opposite the 2nd and 4th lumbar vertebrae. At each of these designated vertebral locations, two 1 1/2 inch steaks were

procured. The first steak from each specified location was taken beginning at the anterior edge of the vertebral body, perpendicular to the long axis of the carcasses and continuing posteriorly for 1 1/2 inches. The longissimus dorsi muscle was removed from each steak and immediately frozen at -10° F for later determination of shear value. The second 1 1/2 inch steak was cut adjacent and posterior to the first steak and all fat trimmed from the muscle periphery. The muscle sample was then ground through a 1/4 inch mill plate, mixed, reground through a 1/8 inch mill plate and mixed again. Two 25 gram samples of the freshly ground muscle were obtained and immediately used in duplicate water-holding capacity determinations. Two additional 25 gram portions were utilized for duplicate phosphorus and calcium determinations. Moisture values of the meat were determined from these samples used for mineral analysis. Fat content of the ground muscle was also determined.

Chemical Analysis

The longissimus dorsi muscle and cartilage tips were analyized for calcium and phosphorus content at each specified location. In the case for longissimus dorsi muscle, duplicate meat samples were ashed and dissolved in an acid solution as described in Appendix A. Duplicate aliquots were procured from the solution for each muscle sample, thus, four determinations were conducted for each muscle location. When determining the phosphorus and calcium content of cartilage,

only one sample was ashed and two aliquots taken from this one sample since the tips were not sufficiently large to allow for two samples. Procedures developed for determining the calcium and phosphorus content of muscle and cartilage incorporated basic techniques designed by the Association of Official Agriculture Chemists (1955) and methods and reagents outlined by Dade Reagents, Inc. as prescribed by Ferro and Ham (1957a; 1957b). Complete detailed chemical analysis procedures for calcium and phosphorus are presented in Appendix A.

The method employed in determining the water-holding capacity of muscle was that described by Wierbicki et al. (1957a) with the exception that total grams of moisture in each sample were calculated from the percent moisture found in respective samples used for mineral analysis. Water-holding capacity values were expressed as percent moisture loss and converted to percent moisture retained.

Proximate analysis (moisture, ash and ether extract) was conducted according to the procedures outlined by the Association of Official Agriculture Chemists (1955).

Determination of Shear Values

The frozen steaks were removed from the freezer 12 hours prior to determining shear values and permitted to thaw in a 42-43° F cooler. Each steak was then weighed and cooked to an internal temperature of 150° F in deep fat. Fat cooking medium was held at 275° F during the cooking of

the steaks. Steaks were removed from the deep fat and reweighed when the internal temperature had decreased to 100° F. Percent cooking loss was then calculated.

Steaks were placed back in the 42-43° F cooler and allowed to chill 12 hours. At the end of this period each steak was removed from the cooler and five 3/4 inch cores were removed. Each core was sheared once by the Warner-Bratzler shearing device. Pounds of force were recorded for shearing each of the cores for each steak.

Analysis of Data

Statistical methods described by Snedecor (1956) were used to analyze the data. Simple correlations were determined between the specific variables to observe the magnitude of existing linear relationships. The analysis of variance was utilized to study the factors contributing to variation within a variable.

Statistical designs for the various selected traits under study are presented in Appendix B, Figures 3-6.

RESULTS AND DISCUSSION

Slaughter Data

Final live animal weights of the nine Hereford steers used ranged from 940 to 1100 pounds. All carcasses were of choice grade and possessed "A" maturity characteristics. Individual carcass data is presented in Table II.

TABLE II

CARCASS DATA FOR INDIVIDUAL ANIMALS

Animal No.	Slaughter Wt. (lb.)	Cold Carcass Wt. (lb.)	Dressing %	Ribeye[1] Area	Fat[2] Thickness
1	1005	598	59.5	9.18	0.87
2	1070	672	62.8	10.36	0.93
3	1030	612	59.4	9.00	0.80
4	1080	661	61.2	10.89	1.03
5	1100	668	60.7	12.29	0.73
6	1015	597	58.8	11.40	0.63
7	1045	626	59.9	11.11	0.67
8	975	578	59.3	10.19	0.73
9	940	586	62.3	10.42	0.83

[1]Expressed as square inches measured at the 12th rib.
[2]Expressed as inches over the 12th rib.

Chemical Analysis

<u>Longissimus</u> <u>dorsi</u> calcium was determined at each of seven designated muscle locations from nine animals. Calcium values expressed as mg. calcium per 100 gm. wet muscle tissue ranged from 2.93 to 17.35 mg.

The analysis of variance for calcium in the _longissimus dorsi_ muscle indicated that a highly significant (P <.01) animal by location interaction existed when calcium values were expressed on a wet muscle tissue basis (Table III).

TABLE III

ANALYSIS OF VARIANCE FOR _LONGISSIMUS DORSI_ CALCIUM[1]

Source	df	M.S.	F-Test
Total	251		
Animal	8	44.22	2.30*
Location	6	74.30	3.86**
Animal x Location	48	19.23	5.37**
Sample	63	3.58	5.96**
Determination	126	0.60	

[1]Calcium expressed as mg./100 gm. wet muscle tissue
* P <.05.
** P <.01.

The animal by location component of variance was the largest source of variation of calcium values expressed on a wet muscle tissue basis (Table IV). This factor alone accounted for 46.44 percent of the total variation. Sampling and location components were of a lesser magnitude accounting for 17.10 and 18.17 percent of the total variation, respectively. Variation due to animals and chemical determinations was relatively small. The various components of variance are shown in Table IV.

The animal by location interaction was found to be largely due to fat differences in samples from different

locations and animals. When sample weights were corrected for fat, and calcium content expressed as mg. calcium per 100 gm. of fat-free muscle tissue, the animal by location interaction was not statistically significant (Table V). The percent of the total variation due to the animal by location moiety was reduced 31.63 percent when sample weights were corrected for fat (Table VI).

TABLE IV

COMPONENTS OF VARIANCE FOR *LONGISSIMUS DORSI* CALCIUM[1]

Source	σ_i^2	% of Total Variation
Total	8.42	100.00
Animal	0.89	10.57
Location	1.53	18.17
Animal x Location	3.91	46.44
Sample	1.49	17.70
Determination	0.60	7.12

[1]Calcium expressed as mg./100 gm. wet muscle tissue.

Another factor contributing to the animal by location interaction may have been due to differences in amount of residual blood in the tissues among different animals and locations within animals. Blood vessels and blood are both higher in calcium content than is muscle (Dukes, 1947). Since the heart quits functioning prior to the complete drainage of all the blood from the carcass at the time of slaughter, different amounts of residual blood might be noted among different locations in the carcass and among

different animals since the rate of blood drainage is not constant from animal to animal. The area from which these muscle samples were procured, directly opposite thoracic and lumbar vertebrae, are characterized by the presence of major blood vessels. It is known that a major artery branches from the dorsal aorta at each vertebral transverse process and protrudes parallel to the transverse process (Sisson and Grossman, 1953).

TABLE V

ANALYSIS OF VARIANCE FOR *LONGISSIMUS DORSI* CALCIUM[1]

Source	df	M.S.	F-Test
Total	251		
Animal	8	49.92	3.63**
Location	6	82.87	6.56**
Animal x Location	48	12.64	1.16
Sample	63	10.85	16.19**
Determination	126	0.67	

[1]Calcium expressed as mg./100 gm. fat-free muscle tissue.
** $P < .01$.

Connective tissue differences in samples from different animals and locations within animals may be an additional contributing factor. Pure connective tissue contains two to four times as much calcium as does composite meat samples. This was observed in a pilot study conducted earlier in this work.

The analysis of variance for *longissimus dorsi* calcium

expressed on a fat-free muscle tissue basis (Table V), revealed highly significant (P <.01) differences among animals, locations within animals and samples within locations.

TABLE VI

COMPONENTS OF VARIANCE FOR LONGISSIMUS DORSI CALCIUM[1]

Source	σ_i^2	% of Total Variation
Total	9.35	100.00
Animal	1.19	12.73
Location	1.95	20.86
Animal x Location	0.45	4.81
Sample	5.09	54.44
Determination	0.67	7.16

[1]Calcium expressed as mg./100 gm. fat-free muscle tissue.

Sampling was found to be the major source of variation in calcium content (Table VI). This indicates that the grinding of the longissimus dorsi muscle through a 1/4 inch mill plate, then regrinding through a 1/8 inch mill plate and mixing was not sufficient to obtain a homogeneous mixture. Determination error contributed to a small portion of the total variation.

The percent of the total variation attributal to locations within animals was 20.86 percent. The differences in calcium content among different locations were highly significant (P <.01) as shown in Table V. The least amount of calcium deposition in the longissimus dorsi muscle was found to occur in the region opposite the 13th thoracic vertebra.

The most abundant amounts of calcium were found in the anterior locations. A progressive decrease in calcium content was noted from the anterior muscle locations to those procured opposite the 13th thoracic vertebra, and then an increase in content from areas opposite the 13th thoracic vertebrae to the 4th lumbar vertebrae. The pattern of calcium deposition in the longissimus dorsi muscle is represented graphically in Figure 1.

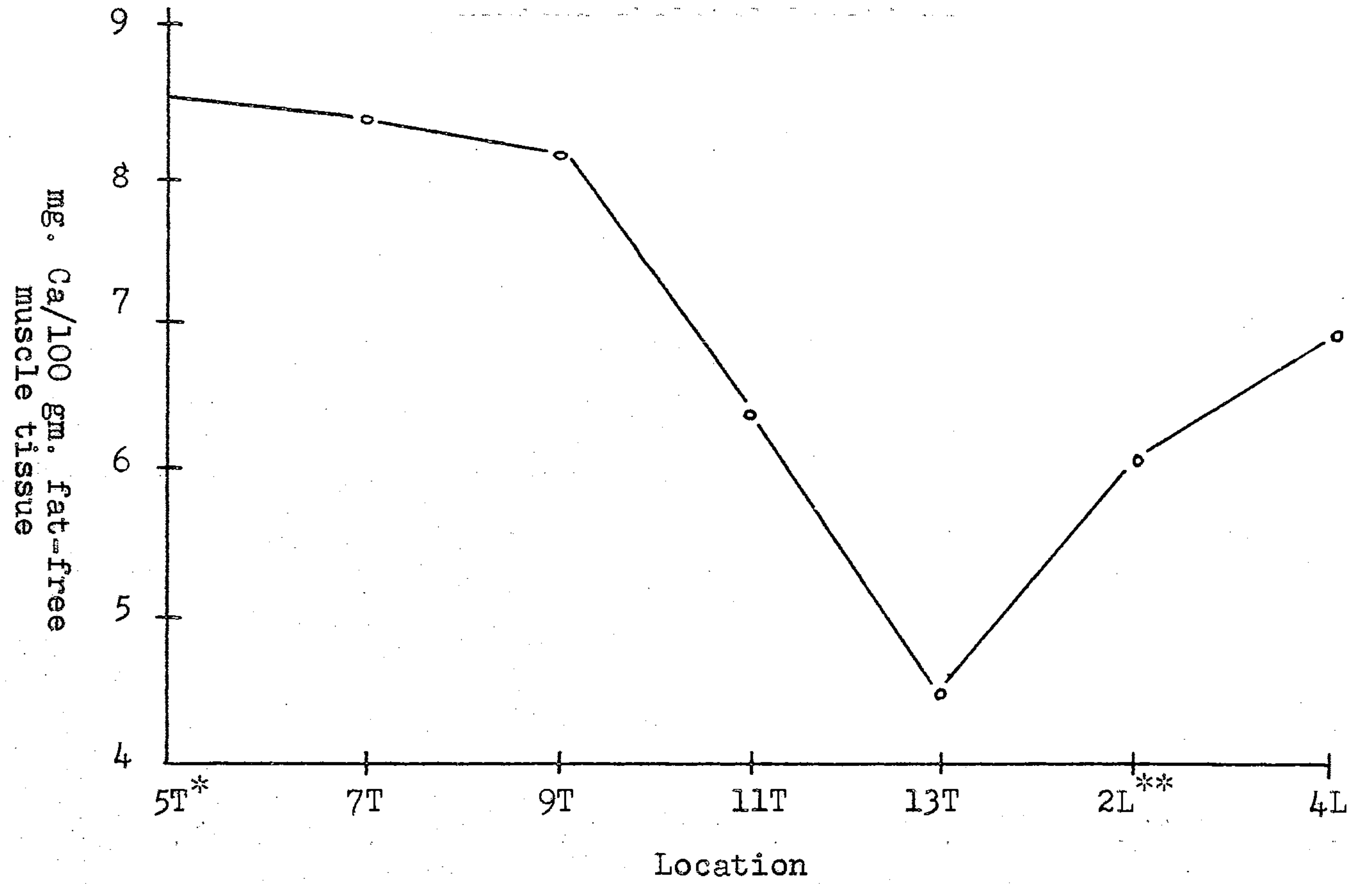
This pattern of calcium deposition observed in this study tends to parallel patterns of maturity and growth rates as reported by several workers. Joubert (1959) and Malkus (1964) stated that the final stages of carcass development occur near the junction of the thoracic and lumbar vertebrae and that this region is the last part of the carcass to attain its maximum growth rate. Similar results were reported by Palsson and Verges (1952) with sheep.

Calcium values expressed on a fat-free muscle tissue basis for the various locations and animals are found in Appendix C, Table XX.

Phosphorus content in the longissimus dorsi muscle ranged from 182.48 to 205.52 mg. per 100 gm. wet muscle tissue. The analysis of variance for longissimus dorsi phosphorus expressed on a wet tissue basis, exhibited highly significant ($P < .01$) differences due to animals, locations within animals and samples within locations as shown in Table VII.

FIGURE 1

<u>LONGISSIMUS DORSI</u> CALCIUM CONTENT OPPOSITE VARIOUS SKELETAL LOCATIONS



*T=Thoracic vertebra.
**L=Lumbar vertebra.

TABLE VII

ANALYSIS OF VARIANCE FOR *LONGISSIMUS DORSI* PHOSPHORUS[1]

Source	df	M.S.	F-Test
Total	251		
Animal	8	638.01	15.54**
Location	6	150.50	3.67**
Animal x Location	48	41.06	1.53
Sample	63	26.76	15.93**
Determination	126	1.68	

[1]Phosphorus expressed as mg./100 gm. wet muscle tissue.
**P <.01.

The primary source of variation in phosphorus values expressed on a wet tissue basis was found to be due to animal differences. This source contributed over one-half of the total variation (Table VIII). Samples were another large source of variation. Location, animal by location and determination components were relatively small.

TABLE VIII

COMPONENTS OF VARIANCE FOR *LONGISSIMUS DORSI* PHOSPHORUS[1]

Source	σ_i^2	% of Total Variation
Total	42.16	100.00
Animal	21.32	50.57
Location	3.04	7.21
Animal x Location	3.58	8.49
Sample	12.54	29.74
Determination	1.68	3.99

[1]Phosphorus expressed as mg./100 gm. wet muscle tissue.

Longissimus dorsi phosphorus content expressed on a wet tissue basis was found to be the greatest at the most posterior location and the least at the most anterior location. However, the pattern of phosphorus deposition was not linear with respect to progressive location designations (Figure 2).

Variation in phosphorus content due to animal differences was reduced 28.05 percent when sample weights were corrected for fat content. The largest source of variation of phosphorus content when expressed on a fat-free muscle tissue basis was due to differences between samples (Table IX).

TABLE IX

COMPONENTS OF VARIANCE FOR *LONGISSIMUS DORSI* PHOSPHORUS[1]

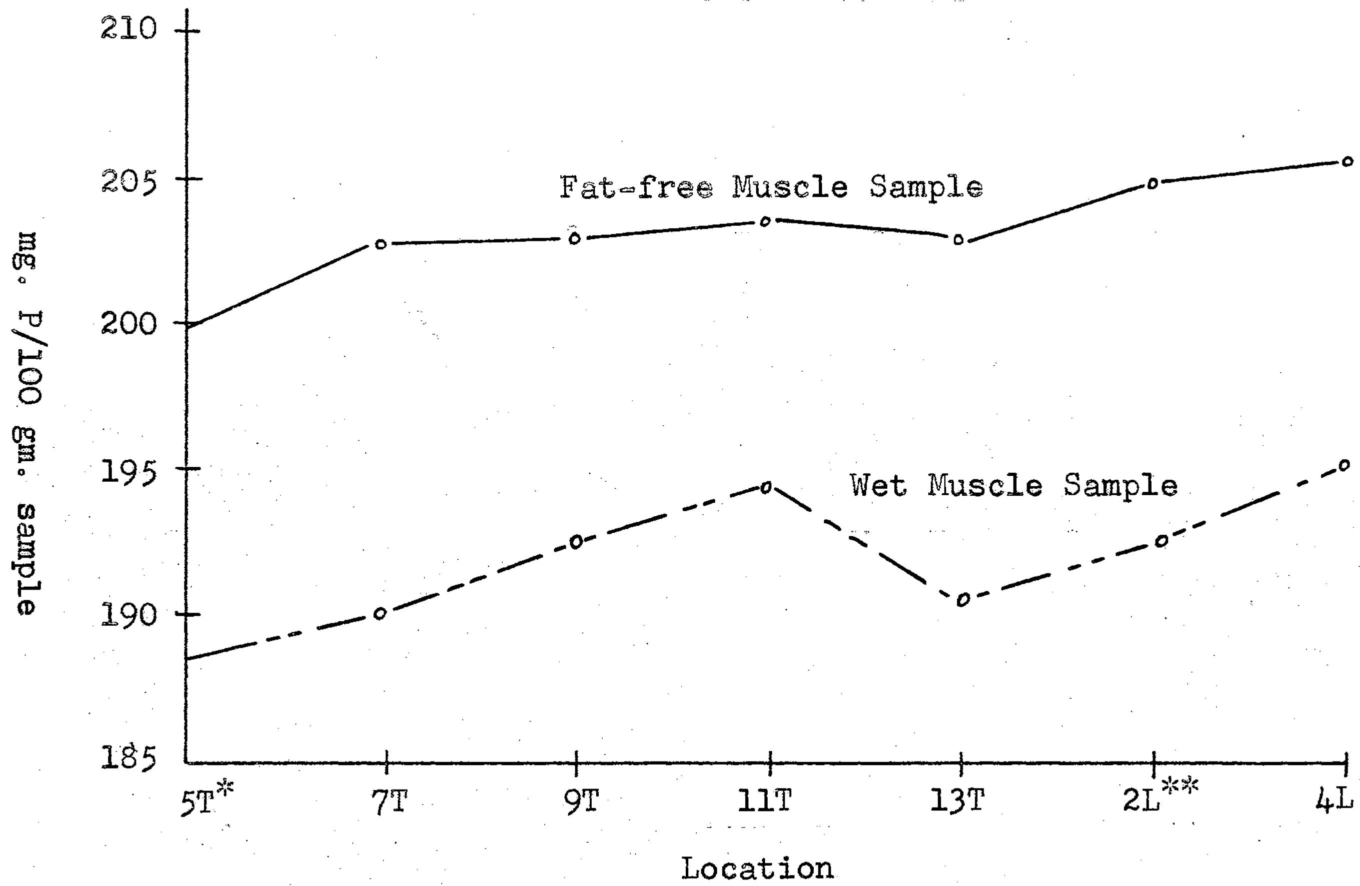
Source	σ_i^2	% of Total Variation
Total	29.04	100.00
Animal	6.54	22.52
Location	2.77	9.54
Animal x Location	3.80	13.09
Sample	14.07	48.45
Determination	1.86	6.40

[1]Phosphorus expressed as mg./100 gm. fat-free muscle tissue.

Location differences in phosphorus contents expressed on a fat-free basis were found to be highly significant ($P < .01$) as shown in Table X. The largest amount of phosphorus was found in the most posterior location and the least in the most anterior. The same results were deduced

FIGURE 2

LONGISSIMUS DORSI PHOSPHORUS CONTENT OPPOSITE VARIOUS SKELETAL LOCATIONS



*T=Thoracic vertebra.
**L=Lumbar vertebra.

when phosphorus content was expressed on a wet tissue basis.

TABLE X

ANALYSIS OF VARIANCE FOR *LONGISSIMUS DORSI* PHOSPHORUS[1]

Source	df	M.S.	F-Test
Total	251		
Animal	8	228.42	5.06**
Location	6	144.79	3.21**
Animal x Location	48	45.17	1.51
Sample	63	29.99	16.12**
Determination	126	1.86	

[1]Phosphorus expressed as mg./100 gm. fat-free muscle tissue.
**P <.01.

On a wet tissue basis, the increase in phosphorus content from anterior locations to posterior locations was not linear. When phosphorus content was viewed on a fat-free basis the increase in phosphorus content in the *longissimus dorsi* from anterior to posterior tended to be a linear function of location with the slope approaching zero. The total increase in phosphorus per 100 gm. fat-free tissue from the most anterior to the most posterior location was only 5.98 mg. Thus, the phosphorus content expressed on a fat-free basis was relatively constant from anterior to posterior regions of the *longissimus dorsi* muscle. Phosphorus values, both corrected and uncorrected for fat are illustrated in Figure 2. Phosphorus contents corrected and uncorrected for fat for various animals and locations are found in Appendix C, Tables XXI and XXII, respectively.

The correction of phosphorus values for fat is somewhat invalid. When sample weights are corrected for fat the resultant phosphorus values may be biased upwards due to the fact that fat contains phosphorus. Phosphorus in fat is present in the various phosphatides and phospholipids; however, the phosphate moiety relative to the total fat mass is relatively minute.

The water-holding capacity of the <u>longissimus</u> <u>dorsi</u> muscle when expressed as percent moisture retained in the muscle revealed values ranging from 54.6 to 71.2 percent for the various animals and locations within animals sampled. The analysis of variance for <u>longissimus</u> <u>dorsi</u> water-holding capacity divulged a highly significant ($P < .01$) animal by location interaction (Table XI).

The elucidation of the components of variance in water-holding capacity values indicated that the animal by location interaction component contributed 33.33 percent of the total variation (Table XII). The animal component accounted for 35.12 percent of the total variation in water retention values which was noted to be the largest source of variation. Variations attributal to determinations and locations were 23.60 and 7.95 percent, respectively.

When the <u>longissimus</u> <u>dorsi</u> muscle samples analyzed for water-holding capacity were corrected for fat content, an interesting diversion of results was noted. A highly significant ($P < .01$) animal by location interaction was still found to be present, but no significant difference in water

TABLE XI

ANALYSIS OF VARIANCE FOR *LONGISSIMUS DORSI* WATER-HOLDING CAPACITY[1]

Source	df	M.S.	F-Test
Total	125		
Animal	8	94.39	6.44**
Location	6	37.91	2.59*
Animal x Location	48	14.65	3.83**
Determination	63	3.83	

* P <.05.
** P <.01.
[1]Muscle sample weight uncorrected for fat.

TABLE XII

COMPONENTS OF VARIANCE FOR WATER RETENTION OF *LONGISSIMUS DORSI* MUSCLE[1]

Source	σ_i^2	% of Total Variation
Total	16.23	100.00
Animal	5.70	35.12
Location	1.29	2.95
Animal x Location	5.41	33.33
Determination	3.83	23.60

[1]Muscle sample weight uncorrected for fat.

retention values was noted between locations (Table XIII). The animal component of variance was reduced 11.36 percent from that present when sample weight was not corrected for fat content (Table XIV). Location and animal by location components were reduced 3.69 and 7.02 percent, respectively. A large increase was noted in the determination component of variance as it increased from 23.60 to 45.67 percent of the total variation in water retention values. Water retention values corrected and uncorrected for fat are found in Appendix C, Tables XXIII, and XXIV, respectively.

TABLE XIII

ANALYSIS OF VARIANCE FOR *LONGISSIMUS DORSI* WATER-HOLDING CAPACITY[1]

Source	df	M.S.	F-Test
Total	125		
Animal	8	93.00	4.38**
Location	6	37.84	1.78
Animal x Location	48	21.22	2.15**
Determination	63	9.86	

[1]Muscle sample weight corrected for fat content.
**P <.01.

The animal by location interactions observed in the analyses of variance (Tables XI and XIII) can be attributed partially to fat differences between animals and locations within animals. This was noted when muscle sample weights were corrected for fat content which resulted in a 7.02 percent reduction in variation of water retention values due

to the animal by location interaction component of variance. However, many other factors could induce this interaction. One possibility is that pH differences may exist between animal samples. The pH of muscle is directly related to water-holding capacity due to the electrical charge of protein and the attraction of water by polar groups as reported by Wierbicki and Deatherage (1958), Swift and Berman (1959) and Hamm (1959a). Known causes of variation in the pH values of meat include differences in lactic acid formation after slaughter (Swift and Berman, 1959) and rate of ATP breakdown post-slaughter (Hamm, 1959a). Animals differ in the rate of lactic acid formation after slaughter due to different pre-slaughter stress and environmental conditions. The rate of ATP breakdown in each animal is dependent upon ATP-ase in the presence of magnesium ions. The relaxing factor inhibits ATP breakdown and itself is inhibited by the presence of calcium ions as reported by Partmann (1963).

TABLE XIV

COMPONENTS OF VARIANCE FOR WATER RETENTION OF *LONGISSIMUS DORSI* MUSCLE[1]

Source	Oi^2	% of Total Variation
Total	21.59	100.00
Animal	5.13	23.76
Location	0.92	4.26
Animal x Location	5.68	26.31
Determination	9.86	45.67

[1]Muscle sample weight corrected for fat content.

The chemical analysis for calcium and phosphorus of cartilage tips of the dorsal spinous processes from designated thoracic vertebrae revealed an extreme variation in values for these two minerals. Phosphorus values were found to range from 10.77 to 296.31 mg. per 100 grams of cartilage. The value of 296.31 mg. of phosphorus per 100 grams of cartilage was 190.81 mg. higher than the next largest value. Cartilage calcium values were found to range from 33.11 to 393.64 mg. per 100 grams of cartilage. The value of 393.64 mg. calcium per 100 grams of cartilage was found at the same location and animal as was noted for the high phosphorus value. This high calcium value was 207.23 mg. higher than the next largest value observed. These extremely high mineral values for the cartilage tip taken at the 3rd thoracic vertebra from animal number five are unexplanable.

The analyses of variance for cartilage calcium and phosphorus revealed highly significant ($P < .01$) animal by location interactions for both minerals (Table XV).

The elucidation of the components of variance for cartilage calcium and phosphorus (Table XVI) indicate that location was not a major cause of mineral content differences, but rather an extremely large animal by location interaction component. The animal by location component of variance for cartilage calcium accounted for 72.85 percent of the total variation. The animal by location component of variance for cartilage phosphorus was of similar magnitude as it contributed 72.42 percent of the total variation.

TABLE XV

ANALYSES OF VARIANCE FOR CARTILAGE CALCIUM AND PHOSPHORUS

Source	df	Calcium M.S.	Phosphorus M.S.
Total	89		
Animal	8	14,663.77*	8,760.45*
Location	4	9,629.93	4,123.36
Animal x Location	32	5,995.52**	3,196.17**
Determination	45	40.69	0.47

* P <.05.
**P <.01.

TABLE XVI

COMPONENTS OF VARIANCE FOR CARTILAGE CALCIUM AND PHOSPHORUS

	σ_i^2		% of Total Variation	
Source	Ca	P	Ca	P
Total	4086.85	2206.26	100.00	100.00
Animal	866.83	556.43	21.21	25.22
Location	201.91	51.51	4.94	2.34
Animal x Location	2977.42	1597.85	72.85	72.42
Determination	40.69	0.47	1.00	0.02

The animal components of variance for cartilage calcium and phosphorus accounted for 21.21 and 25.22 percent of the total variation, respectively. The mineral differences due to animals may largely be explained by the fact that minerals of bone and cartilage are in a dynamic state continually being exchanged with minerals of the blood (Sherman, 1964). Calcium is drawn from the bones and cartilage to maintain a constant calcium level in the blood. Blood calcium is then

absorbed into the cells for metabolism purposes when required. Thus, the mineral content of cartilage from animal to animal can largely be affected by each animal's metabolic mineral requirement.

Determination components of variance for cartilage calcium and phosphorus were negligible in size. Cartilage calcium and phosphorus values for the various animals and locations within animals are found in Appendix C, Tables XXV and XXVI, respectively.

Shear Values

The analysis of variance for shear values revealed a highly significant ($P < .01$) animal by location interaction (Table XVII) even though this interaction component of variance accounted for only 0.08 percent of the total variation in shear values (Table XVIII). No significant difference was noted in shear values among different locations. Determinations by use of the Warner-Bratzler shearing device accounted for 51.53 percent of the total variation. The animal component of variance was the second largest source of variation in shear values. Variation in shear values attributal to location differences was found to be less than one percent. Shear values for the various animals and locations sampled are shown in Appendix C, Table XXVII.

Integral Relationships

It was found in this study that a highly significant relationship ($r = .92$; $P < .01$) existed between <u>longissimus</u>

TABLE XVII

ANALYSIS OF VARIANCE FOR *LONGISSIMUS DORSI* SHEAR VALUES

Source	df	M.S.	F-Test
Total	314		
Animal	8	136.50	14.80**
Location	6	13.46	1.46
Animal x Location	48	9.22	1.89**
Determination	252	4.88	

**P <.01.

TABLE XVIII

COMPONENTS OF VARIANCE FOR *LONGISSIMUS DORSI* SHEAR VALUES

Source	σ_i^2	% of Total Variation
Total	9.47	100.00
Animal	3.64	38.44
Location	0.09	0.95
Animal x Location	0.86	9.08
Determination	4.88	51.53

dorsi calcium and phosphorus when both were expressed on a wet muscle tissue basis or $r = .90$ ($P < .01$) when both were expressed on a fat-free muscle tissue basis (Table XIX). Phosphorus was much more abundant in the longissimus dorsi muscle than calcium. The ratio of calcium to phosphorus in this muscle was 1:33.42 ± 1.68. Swift and Berman (1959) found this ratio to be somewhat higher as they reported it to be 1:42. In a study by Watt and Merrill (1963), this ratio was shown to be as low as 1:16. It is well to point out that both of these studies cited used several different muscles in their analyses for calcium and phosphorus.

The relationship between calcium and phosphorus content from cartilage tips of designated dorsal spinous processes in the thoracic region was highly significant ($r = .96$; $P < .01$). The calcium to phosphorus ratio in the cartilage tips analyzed was 1:0.430 ± .005. The amount of calcium in the cartilage tips was more than twice that of phosphorus.

No significant relationships were observed between the amount of calcium or phosphorus present in the longissimus dorsi muscle and that in the cartilage tips at corresponding locations. Since the most anterior cartilage tip samples procured were from the dorsal spinous processes of the 1st thoracic vertebrae and the most anterior muscle samples commenced opposite the 5th thoracic vertebrae, only those cartilage and muscle samples keyed at the 5th, 7th and 9th thoracic vertebrae were correlated.

TABLE XIX

CORRELATIONS BETWEEN VARIOUS SELECTED TRAITS OF THE LONGISSIMUS DORSI MUSCLE

	Phosphorus (W)	Phosphorus (FF)	Shear Value	Cooking Loss	Water Retention(W)	Water Retention(FF)
Calcium(W)[1]	.92**		-.03	-.18	-.22	-.13
Calcium(FF)[2]		.90**	-.06	-.19	-.23	-.16
Phosphorus(W)			.45**	-.20	.02	.01
Phosphorus(FF)			.17	-.16	.22	.20
Shear Value				.18	-.15	-.10
Cooking Loss					.21	.20

[1]W=Wet muscle tissue basis.
[2]FF=Fat-free muscle tissue basis.
**P <.01.

A highly significant correlation (r = .45; P <.01) was noted between muscle phosphorus expressed on a wet muscle tissue basis and shear value. If shear value is assumed to be an accurate indicator of muscle tenderness, which some say it is not, phosphorus expressed on a wet muscle tissue basis and tenderness are negatively related in this study. The relationship between phosphorus expressed on a fat-free muscle basis and shear value was lower (r = .17) and not statistically significant at the five percent level of probability.

No statistically significant correlations were noted between either muscle calcium or phosphorus and water-holding capacity expressed on a wet or fat-free muscle tissue basis (Table XIX). Contrary to this, Swift and Berman (1959) found a significant negative correlation (r = -.78; P <.05) between calcium content and water-holding capacity of muscle. Hamm (1959a) also reported that a negative relationship existed between muscle calcium and water-holding capacity. A positive correlation (r = .54) was found between phosphorus content and water-holding capacity of muscle as reported by Swift and Berman (1959). The correlations between phosphorus and water-holding capacity, expressed on a fat-free or wet tissue basis or combinations, were all positive. All relationships between calcium and water retention were negative. Negative relationships were noted between cooking loss and the minerals studied. They were not statistically significant, however. Shear value and cooking loss were not

significantly related in this study. The relationship between these two variables was positive. Contrary to the results reported by Wierbicki *et al*. (1957) and Arnold *et al*. (1956), water retention and shear were not significantly related in this study.

SUMMARY

The *longissimus dorsi* muscles from the left sides of nine Hereford steer carcasses were used as experimental material. Calcium and phosphorus contents, water-holding capacities and shear values were determined at *longissimus dorsi* locations opposite the 5th, 7th, 9th, 11th and 13th thoracic and 2nd and 4th lumbar vertebrae. Cartilage tips from the 1st, 3rd, 5th, 7th and 9th thoracic dorsal spinous processes were analyzed for calcium and phosphorus content.

The results indicate that patterns of calcium deposits in the *longissimus dorsi* muscle were quite similar to those observed for growth and maturity reported by other workers. The most abundant quantities of calcium were observed in the utmost anterior and posterior *longissimus dorsi* locations. The least amount of calcium deposition was noted opposite the junction of the thoracic and lumbar vertebrae. Phosphorus content was relatively constant in the portion of the *longissimus dorsi* muscle analyzed. Chemical procedures developed for the determination of calcium and phosphorus in muscle contributed to very little error.

A highly significant ($P < .01$) relationship was observed between calcium and phosphorus in the *longissimus dorsi* muscle. The muscle calcium to phosphorus ratio was found

to be 1:33.42 ± 1.68. Calcium to phosphorus ratio in the cartilage tips was 1:0.430 ± .005. The correlation between calcium and phosphorus in the cartilage tips was highly significant ($P < .01$).

No significant relationships were observed between shear and water retention values or between water retention and mineral content. However, a highly significant ($P < .01$) correlation existed between muscle phosphorus expressed on a wet muscle weight basis and shear value.

LITERATURE CITED

Arnold, N., E. Wierbicki and F. E. Deatherage. 1956. Postmortem changes in the interactions of cations and proteins of beef and their relation to sex and diethylstilbestrol treatment. Food Technol. 10:245.

Association of Official Agriculture Chemists. 1955. Official Methods of Analysis. 8th ed. Association of Official Agriculture Chemists, Washington, D.C.

Bate-Smith, E. C. and J. R. Bendall. 1949. Factors determining the time course of rigor mortis. J. Physiol. 110:47.

Belenkij, G. 1962. Demotation of slaughter livestock. 8th European Conf. of Meats Res., Moscow.

Berman, M. D. 1961. Factors affecting the water retention of beef. V. Variation of the zinc-containing enzymes. J. Food Sci. 26:422.

Bozler, E. 1955. Binding of calcium and magnesium by the contractile elements. J. Gen. Physiol. 38:735.

Briskey, E. J., R. W. Bray, W. G. Hoekstra, P. H. Phillips and R. H. Grummer. 1958. The effect of high protein, high fat and high carbohydrate rations on the water-binding properties of pork muscle. J. Animal Sci. 17:1149.

_______, R. W. Bray, W. G. Hoekstra, P. H. Phillips and R. H. Grummer. 1959. The chemical and physical characteristics of various pork ham muscle classes. J. Animal Sci. 18:146.

_______, W. G. Hoekstra, R. W. Bray and R. H. Grummer. 1960. A comparison of certain physical and chemical characteristics of eight pork muscles. J. Animal Sci. 19:214.

Carpenter, J. S., K. L. Saffle and L. O. Kamstra. 1961. Tenderization of beef by pre-rigor infusion of a chelating agent. Food Technol. 15:197.

Conway, E. J. and M. J. Carey. 1955. Muscle sodium. Nature. 175:773.

Davies, R. E., D. Cain and A. M. Delluva. 1959. The energy supply for muscle contraction. Annals of the New York Academy of Sci. 81(2):468.

DeFremery, D. and M. F. Pool. 1960. Biochemistry of chicken muscles as related to rigor mortis and tenderization. Food Res. 25(1):73.

Doty, D. M. 1959. Relationships between beef quality, grades and standards. National Beef Industry Conf.

Dukes, H. H. 1947. The Physiology of Domestic Animals. Comstock Publishing Co., Ithaca, New York.

Falk, G. 1956. Nonspecificity of ATP-contraction of living muscle. Science. 123:632.

Ferro, P. V. and A. B. Ham. 1957a. A simple spectrophotometric method for the determination of calcium. Amer. J. of Clinical Path. 28:208.

_______. and A. B. Ham. 1957b. A simple spectrophotometric method for the determination of calcium. II. A semimicro method with reduced precipitation time. Amer. J. of Clinical Path. 28:689.

Grau, R. and R. Hamm. 1953. Eine einfache Methode zur Bestimmung der Wasser-bindung im Muskel. Naturwissenschaften. 40:29.

_______. and R. Ham. 1957. Uber das Wasserbindungsvermogen des Saugetiermuskels. II. Mitt. Uber die bestimmung der Wasserbindung des Muskels. Z. Lebensm. Untersuch. u. Forsch. 105:446.

Ham, R. 1959a. Biochemistry of meat hydration. Proceedings of 11th research conference, Amer. Meat Inst. Found. p. 17.

_______. 1959b. The isoelectric point of muscle proteins and its relationship to solubility and dispersibility. Twelfth annual Reciprocal Meat Conf. p. 102.

_______. 1960. Biochemistry of meat hydration. Advances in Food Res. 10:355.

Hellendorn, E. W. 1962. Influence of NaCl and phosphates on the water retention of comminuted meat at various pH values. Food Technol. 16:119.

Joubert, D. M. 1959. The influence of age on the body conformation and carcass composition of cattle. S. Afr. J. of Sci. 55:139.

Leverton, Ruth M. and George V. Odell. 1958. The nutritive value of cooked meat. Okla. Agr. Exp. Sta., Misc. Pub. No. MP-49.

Lewis, M. S. and H. A. Saroff. 1957. The binding of ions to muscle proteins. Measurements of the binding of potassium and sodium ions to myosin A, myosin B and actin. J. Am. Chem. Soc. 79:2112.

Lloyd, D. J. and R. Morgan. 1933. Bound water of gelatin gels. Nature. 132:515.

Locker, R. H. 1960. Degree of muscular contraction as a factor in tenderness of beef. Food Res. 25:304.

Malkus, Louis A. 1964. Changes in carcass composition from 9 to 18 months in heifers fed a fattening ration. M. S. Thesis. Okla. State Univ.

Mittledorf, A. J. and D. O. Landon. 1952. Spectrochemical determination of the mineral-element content of beef. Anal. Chem. 24:469.

Mohler, K. and F. Kiermeiser. 1953. Die Wirkung anorganischer phosphate auf tierisches Eiweiss. III. Mitts. Der Einfluss auf die Konsistenz von Fleischbrat. A. Lebensm. Untersuch. u. Forsch. 96:90.

Palsonn, H. and J. B. Verges. 1952. Effects of the plane of nutrition on growth and development of carcass quality in lambs Part I. The effects of high and low planes of nutrition at different ages. J. Agr. Sci 42:1.

Partmann, W. 1963. Post-mortem changes in chilled and frozen muscle. J. Food Sci. 28:15.

Saroff, H. A. 1957. The binding of ions to the muscle proteins. A theory for K+ and Na+ binding based on a hydrogen bonded and chelated model. Arch. Biochem. Biophys. 71:194.

Schweigert, B. S. and Barbara J. Payne. 1956. A summary of the nutritive content of meat. Amer. Meat Inst. Found. Bull. No. 30.

Sisson, S., and J. D. Grossman. 1953. _Anatomy of the Domestic Animals_. 4th ed. W. B. Saunders Co., Philadelphia.

Sherman, William C. 1964. Calcium. Food and Nutrition News. 36(1):3.

Snedecor, G. W. 1956. Statistical Methods. 5th ed. The Iowa State College Press, Ames, Iowa.

Swift, C. E. and M. D. Berman. 1959. Factors affecting the water retention of beef. I. Variations in composition and properties among eight muscles. Food Technol. 13:365.

Szent-Gyorgyi, A. 1951. Chemistry of Muscular Contraction. 2nd ed. Academic Press, New York.

_______. 1953. Chemical Physiology of Contraction in Body and Heart Muscle. Academic Press, New York.

Urbin, M. C., Darrel A. Zessin and G. D. Wilson. 1962. Observations on a method of determining the water binding properties of meat. J. Animal Sci. 21:9.

Watt, Bernice K. and A. L. Merrill. 1963. "Composition of Foods -- Raw, Processed, Prepared." U. S. Dept. Agr. Handbook, No. 8.

Weber, H. H. and H. Portzehl. 1952. Muscle contraction and fibrous muscle proteins. Advances in Protein Chem. 7:162.

Wierbicki, E., V. R. Cahill and F. E. Deatherage. 1957b. Effects of added sodium chloride, potassium chloride, calcium chloride, magnesium chloride, and citric acid on meat shrinkage at 70° C and of added sodium chloride on drip losses after freezing and thawing. Food Technol. 11:74.

_______. and F. E. Deatherage. 1958. Determination of water-holding capacity of fresh meats. J. Agr. Food Chem. 6:387.

_______, L. E. Kunkle and F. E. Deatherage. 1957a. Changes in the water-holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technol. 11:69.

Wismer-Pedersen, J. 1959. Quality of pork in relation to rate of pH change post mortem. Food Res. 24:711.

_______. 1960. Effect of cure on pork with watery structure; binding of salt and water to the meat. Food res. 25:789.

Wilson, G. E. 1959. Physical characteristics of muscle tissue as related to imbibed water. Proceedings of the 12th annual Reciprocal Meat Conf. p. 97.

APPENDIX A

A METHOD FOR DETERMINING CALCIUM AND PHOSPHORUS IN MUSCLE AND CARTILAGE

I. Preparation of sample for calcium and phosphorus analysis

<u>Reagents</u>:

1. Dilute HCl (1:1)-- add 500 ml. concentrated HCl to 500 ml. distilled-demineralized water and mix thoroughly.

<u>Procedure</u>:

1. Weigh the sample material (25 gm. meat or 5 gm. cartilage) into a crucible and dry at 105° C for 12 hours.

2. Weigh dry sample and place in ash oven at 500° C for 12 hours.

3. To the crucible containing the ash, add enough distilled-demineralized water at the upper edge of the dish to moisten the ash.

4. Add 10 mg. 1:1 HCl in the same manner described in step 3. After the reaction has ceased, rinse the ash into a 250 ml. beaker. Rerinse the crucible with 5 ml. 1:1 HCl and pour contents into the 250 ml. beaker.

5. Wash the crucible carefully with distilled-demineralized water and pour into the 250 ml. beaker making sure to wash all the ash from the side of the crucible into the beaker.

6. Set the beaker on a hot plate and bring the solution to a slow boil. Let the solution evaporate until only approximately 10 ml. of solution are left in the beaker.

7. Add 40 ml. distilled-demineralized water and heat until solution starts to boil.

8. Filter the solution through an ashless filter paper (Whatman No. 42) into a 100 ml. volumetric flask.

9. Wash the paper and residue 5 times with hot, distilled-demineralized water.

10. Cool to room temperature, dilute to volume and pour into an erlenmeyer flask and mix.

II. Calcium determination

Reagents:

1. Chloranilic acid solution -- via Dade Chemical Co.

2. Isopropyl alcohol solution -- via Dade Chemical Co.

3. Ethylene diamine tetraacetate solution -- via Dade Chemical Co.

4. Standard calcium solution (10 mg. Ca per 100 ml.)-- weigh out 0.2497 gm. reagent grade calcium carbonate and transfer to a 1 liter volumetric flask. Add 9 ml. dilute HCl (1 ml. concentrated HCl in 8 ml. distilled-demineralized water) and allow to stand until all of the $CaCO_3$ has dissolved. Add distilled-demineralized water to 1000 ml. and mix.

5. Lab-trol -- via Dade Chemical Co.

Procedure:

1. Unknown -- pipet 4 ml. of ash solution into a heavy-walled centrifuge tube. Then adjust pH to 5-7 by adding 2 drops of p-nitrophenol, enough 0.2N NaOH until solution just shows a tinge of yellow and then 1 drop of 0.1N acetic acid.

2. Standard -- into a second tube, pipet 2 ml. standard solution (1 ml. = 0.1 ml. Ca) and adjust pH as before.

3. Control -- into a third tube, pipet 2 ml. Lab-trol and adjust pH as before.

4. To each tube add 1 ml. of chloranilic acid solution. The tubes containing protein should be constangly agitated by twirling to redissolve any precipitated protein.

5. Allow the tubes to stand for approximately 30 minutes.

6. Centrifuge at 1800 r.p.m. for 10 minutes. Decant the supernatant and allow the tubes to drain for 2 or 3 minutes on some absorbent filter paper.

7. Wipe mouth of tube dry with filter paper or cotton gauze.

8. Wash each percipitate with 6 or 7 ml. of isopropyl alcohol solution. A fine stream from a polyethylene

wash bottle should be used. The precipitate should be broken up and resuspended in isopropyl alcohol.

9. Centrifuge and drain as before. The supernatant may be cloudy due to the presence of a small amount of protein. This will not affect the results.

10. Add 2 drops (0.1 ml.) of distilled-demineralized water to each packed precipitate.

11. Break-up the precipitate by striking bottom of tube sharply against palm of hand until the mat breaks loose and precipitate is suspended in the water.

12. To each tube add 6 ml. of ethylene diamine tetra-acetate.

13. Stopper tubes and invert several times until precipitate is completely dissolved. Avoid vigorous shaking.

14. Read solutions in colorimeter at 520 mu. Adjust reading of a blank for 100 percent transmittance. The pink solutions can be read immediately or up to 5 days.

15. Use the same matched cuvets for all readings. Drain cuvet momentarily on a piece of gauze between readings. Estimate readings to the nearest 1/4 division percent transmittance. Convert percent transmittance to optical density (optical density = 2.0000 - log percent transmittance).

Calculations:

$$\text{mg. Ca/100 gm. sample} = \frac{(\text{O.D.u})}{(\text{E})}\;\frac{(100\text{ gm.})}{(\text{sample wt.})}\;\frac{(100\text{ ml.})}{(4\text{ ml.})}$$

$$= \frac{(\text{O.D.u})}{(\text{E})}\;\frac{(2500\text{ gm.})}{(\text{sample wt.})}$$

$$= \frac{(\text{O.D.u})\quad(\text{K})}{(\text{sample wt.})}$$

$$E_{\text{mg/ml}} = \frac{\text{O.D.s}}{\text{Cs}}$$

$$K = \frac{2500}{E}$$

C = concentration
E = extinction coefficient
O.D. = optical density
u = unknown
s = standard
K = constant

III. Phosphorus determination

Reagents:

1. Ammonium meta vandate and nitric acid -- dissolve 2.350 gm. ammonium meta vandate in 500 ml. hot distilled-demineralized water, add 290 ml. concentrated nitric acid, cool, and dilute to 1000 ml.

2. Ammonium molybdate -- dissolve 100 gm. of molybdic acid in 400 ml. of distilled-demineralized water and 80 ml. concentrated ammonium hydroxide, boil for 20 minutes, filter, dilute to 1000 ml.

3. Standard phosphorus solution -- dissolve 0.2197 gm. KH_2PO_4 dried for 1 hour at 105° C in 500 ml. distilled-demineralized water (1 ml. = 0.1 mg.P).

Procedure:

1. Measure out 2 ml. of the dissolved ash solution in the case of meat phosphorus analysis or 25 ml. of the dissolved ash solution in the case of cartilage phosphorus analysis and transfer to a 100 ml. volumetric flask.

2. Add about 40 ml. of distilled-demineralized water.

3. Add 10 ml. ammonium meta vandate solution, shaking during the addition.

4. Add 10 ml. ammonium molybdate and shake thoroughly.

5. Dilute to volume and transfer to an erlenmeyer flask and mix well.

6. Prepare standards in the same manner.

7. Prepare a blank of all reagents.

8. Read the solutions in a colorimeter at 450 mu. Adjust reading of the blank for 100 percent transmittance.

9. Use the same matched cuvets for all readings. Drain cuvet momentarily on a piece of gauze between readings. Estimate readings to the nearest 1/4 division of percent transmittance. Convert percent transmittance to optical density (optical density = 2.0000 - log percent transmittance).

<u>Calculations</u>:

$$\text{mg. P/100 gm. sample} = \frac{(\text{O.D.u})\ (100\ \text{gm.})\ (100\ \text{mg.})}{(\text{E})\ (\text{sample wt.})\ (\text{V})}$$

$$= \frac{(\text{O.D.u})\ (10{,}000)}{(\text{E})\ (\text{sample wt.})\ (\text{V})}$$

$$= \frac{(\text{O.D.u})\ (\text{K})}{(\text{sample wt.})}$$

$$E_{\text{mg/ml}} = \frac{\text{O.D.s}}{\text{Cs}}$$

$$K = \frac{10{,}000}{(\text{E})\ (\text{V})}$$

C = concentration
E = extinction coefficient
O.D. = optical density
u = unknown
s = standard
K = constant
V = mg. of aliquot

APPENDIX B

FIGURE 3

STATISTICAL DESIGN FOR LONGISSIMUS DORSI MINERAL ANALYSIS

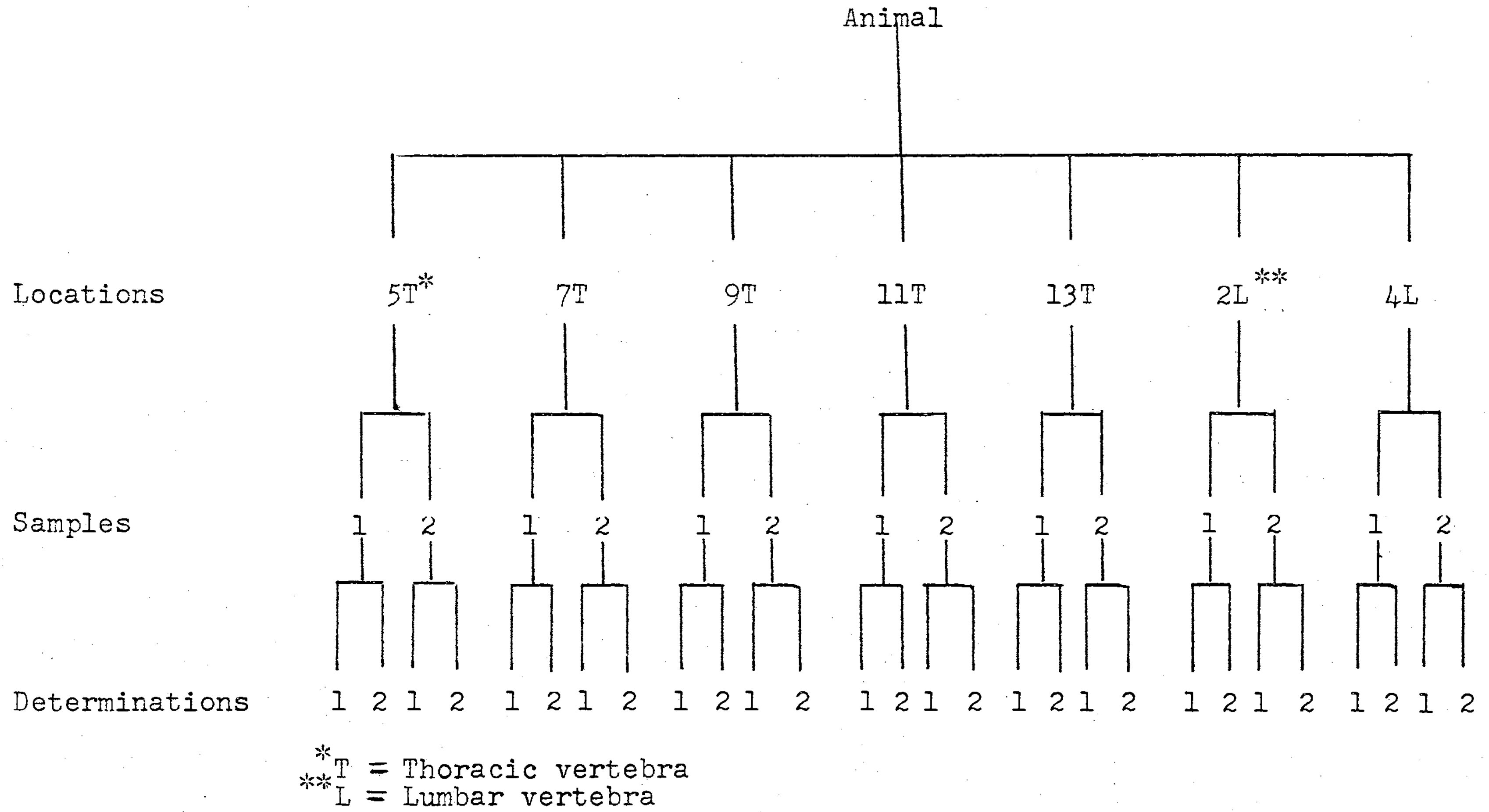
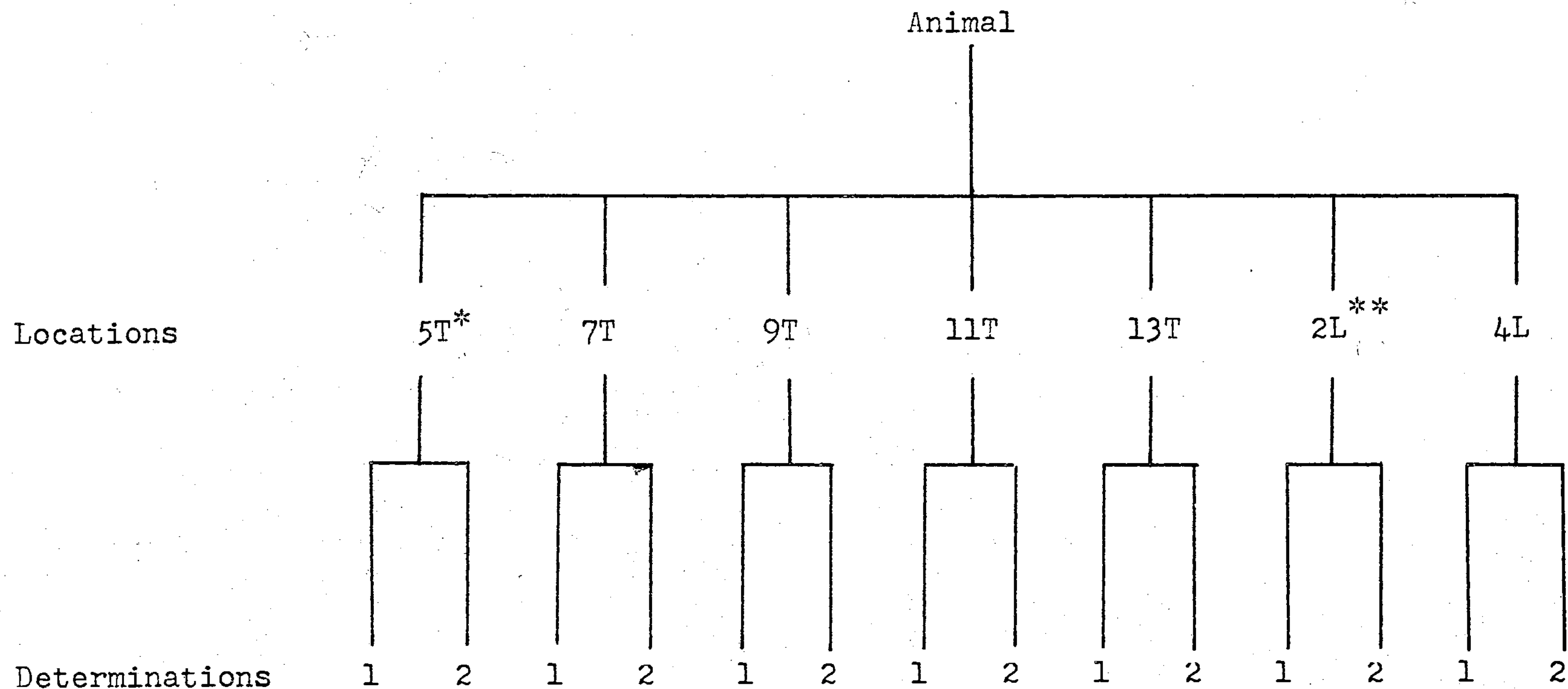


FIGURE 4

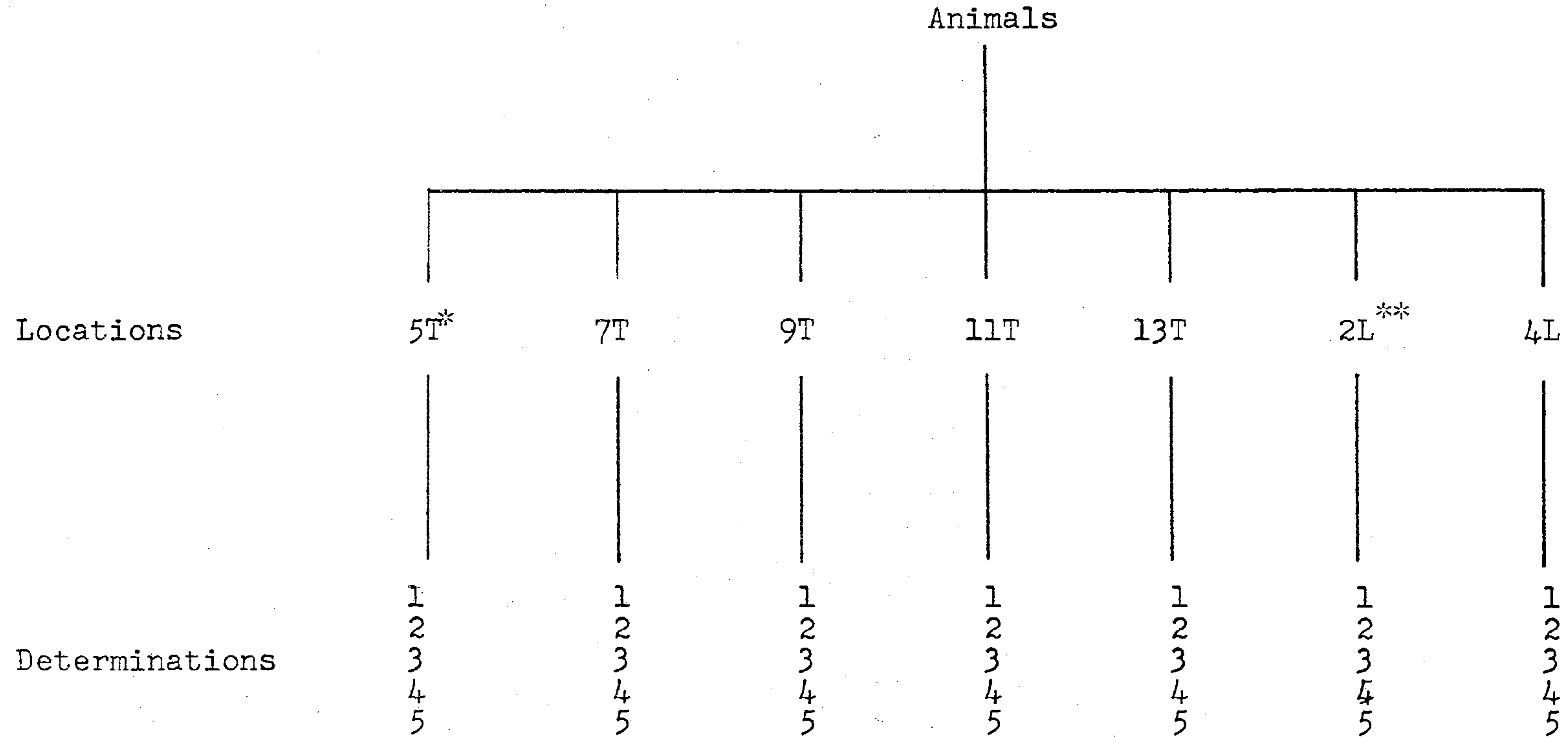
STATISTICAL DESIGN FOR LONGISSIMUS DORSI WATER-HOLDING CAPACITY



*T = Thoracic vertebra
**L = Lumbar vertebra

FIGURE 5

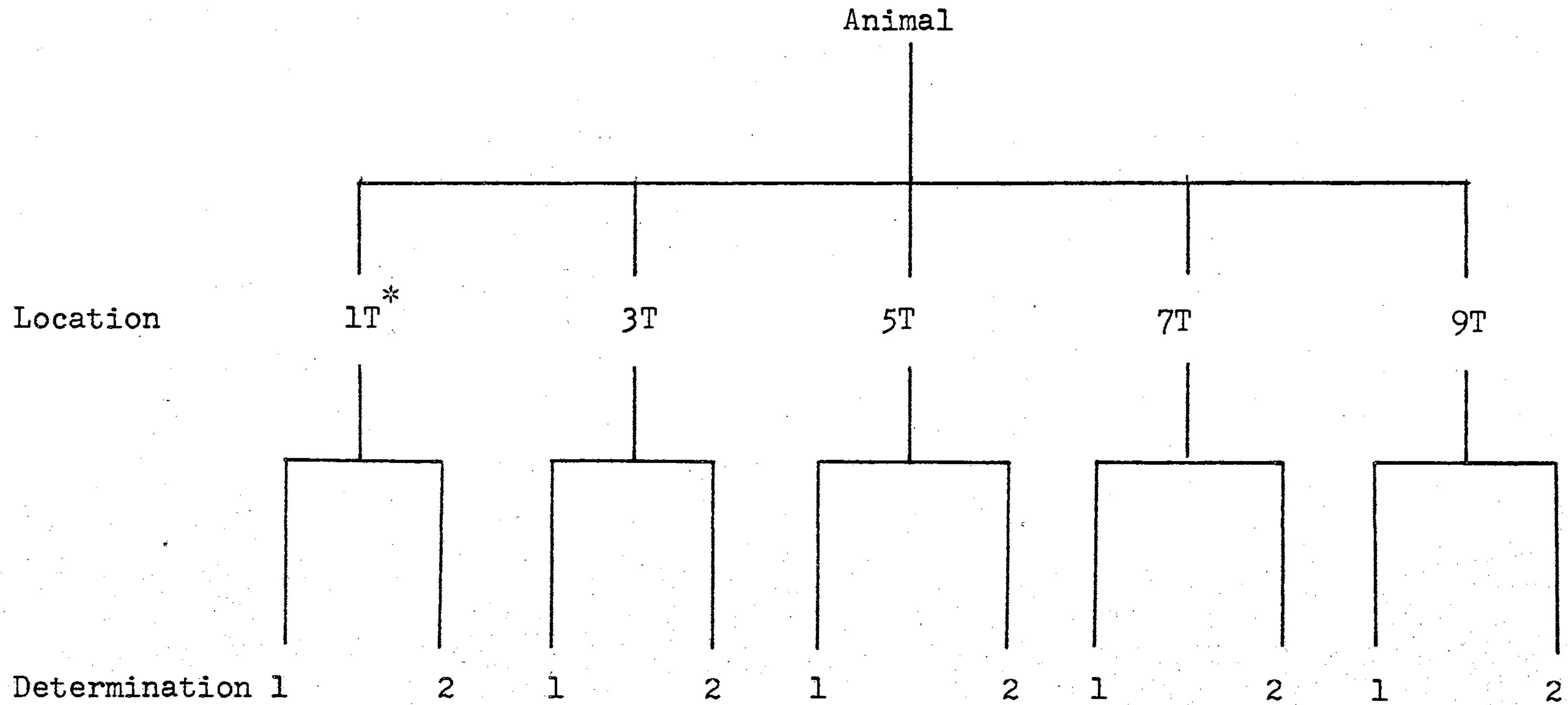
STATISTICAL DESIGN FOR LONGISSIMUS DORSI SHEAR VALUES



*T = Thoracic vertebra
**L = Lumbar vertebra

FIGURE 6

STATISTICAL DESIGN FOR CARTILAGE MINERAL ANALYSIS



*T = Thoracic vertebra

APPENDIX C

TABLE XX

LONGISSIMUS DORSI CALCIUM CONTENT[1]
ON A FAT-FREE TISSUE BASIS

Locations

Animals	5T*	7T	9T	11T	13T	2L**	4L
1	7.03	5.91	7.57	3.05	3.02	3.96	9.31
2	6.58	5.52	4.83	8.36	5.88	6.66	7.46
3	6.14	6.73	7.26	7.43	6.74	6.95	11.45
4	10.38	10.99	9.83	6.99	6.30	6.87	6.51
5	6.37	3.69	6.60	5.29	3.27	4.54	7.44
6	7.77	13.84	6.65	5.30	3.37	4.79	5.52
7	5.80	9.85	10.78	5.94	3.26	6.53	3.80
8	8.17	7.49	10.18	7.74	3.27	8.06	5.20
9	18.29	11.93	10.96	7.77	4.48	7.12	5.81

[1]Calcium expressed as mg./100 gm. fat-free tissue.
* T = Thoracic vertebra.
**L = Lumbar vertebra.

TABLE XXI

LONGISSIMUS DORSI PHOSPHORUS CONTENT[1]
ON A FAT-FREE TISSUE BASIS

Locations

Animals	5T*	7T	9T	11T	13T	2L**	4L
1	204.99	211.43	206.16	205.16	209.04	214.88	210.90
2	194.38	203.28	200.16	205.78	202.30	204.20	203.74
3	192.57	198.91	201.94	206.33	196.46	207.17	204.22
4	199.10	199.01	200.76	201.84	203.10	204.01	297.10
5	199.74	202.97	202.35	196.63	202.70	200.83	199.74
6	203.89	208.91	203.09	208.17	201.94	204.35	206.04
7	196.87	201.23	201.18	200.33	199.98	206.15	207.34
8	197.99	197.03	208.60	206.90	204.77	207.16	211.18
9	206.95	205.11	207.75	207.17	207.17	201.24	210.02

[1]Phosphorus expressed as mg./100 gm. fat-free tissue.
* T = Thoracic vertebra.
**L = Lumbar vertebra.

TABLE XXII

LONGISSIMUS DORSI PHOSPHORUS CONTENT[1] ON A WET TISSUE BASIS

Animals	5T*	7T	9T	11T	13T	2L**	4L
1	196.44	200.44	199.19	199.95	202.85	204.26	205.52
2	178.71	187.42	156.42	192.82	188.02	190.52	185.17
3	181.29	185.56	192.08	195.20	173.19	193.26	190.94
4	193.48	190.55	195.38	197.40	198.42	195.68	188.52
5	182.48	187.94	188.15	184.82	189.32	185.92	188.02
6	192.26	192.07	192.45	196.60	192.97	190.90	186.78
7	183.17	184.60	186.92	191.12	188.86	189.32	193.45
8	189.28	188.08	197.96	198.01	193.72	195.48	195.62
9	195.69	199.00	196.50	193.28	196.38	190.65	199.04

[1]Phosphorus expressed as mg./100 gm. wet muscle tissue.
* T = Thoracic vertebra.
**L = Lumbar vertebra.

TABLE XXIII

PERCENT WATER RETAINED FOR FAT-FREE LONGISSIMUS DORSI SAMPLES

Animals	5T*	7T	9T	11T	13T	2L**	4L
1	61.1	62.7	63.8	57.7	59.8	57.7	64.0
2	52.0	55.1	59.0	61.5	58.8	60.2	59.1
3	58.4	58.6	58.9	58.3	60.7	58.2	53.8
4	56.4	59.1	59.6	55.8	58.4	61.1	53.8
5	64.8	60.1	69.2	64.7	63.5	66.0	67.8
6	63.5	59.5	62.2	66.9	63.9	61.8	66.0
7	58.5	72.1	60.9	60.5	60.7	62.6	60.1
8	53.2	58.5	65.9	65.8	61.5	59.4	60.4
9	54.8	53.3	65.4	62.6	60.8	57.4	62.2

* T = Thoracic vertebra.
**L = Lumbar vertebra.

TABLE XXIV

PERCENT WATER RETAINED FOR LONGISSIMUS DORSI SAMPLES

Locations

Animals	5T*	7T	9T	11T	13T	2L**	4L
1	62.70	64.51	65.02	58.76	60.88	59.66	64.84
2	55.58	58.37	61.44	63.75	61.53	62.66	62.38
3	60.71	60.93	59.84	60.36	62.72	60.82	56.67
4	57.61	60.76	60.68	56.71	59.33	62.62	55.70
5	67.56	62.18	71.15	66.69	65.78	68.29	69.51
6	65.42	62.45	64.05	68.64	65.37	63.58	68.87
7	61.28	61.74	63.50	62.20	62.74	65.37	62.55
8	55.15	60.26	67.53	67.22	63.41	61.60	63.07
9	57.09	54.54	67.18	64.44	62.77	59.45	64.06

* T = Thoracic vertebra.
**L = Lumbar vertebra.

TABLE XXV

CARTILAGE CALCIUM CONTENT[1]

Locations

Animals	1T*	3T	5T	7T	9T
1	63.46	105.77	165.48	51.48	39.66
2	49.58	77.27	150.14	89.14	62.78
3	145.72	87.18	165.61	74.27	33.11
4	55.57	185.04	63.55	81.49	47.44
5	135.15	393.64	165.67	141.23	186.41
6	48.22	77.45	165.07	160.60	51.23
7	63.00	121.76	58.49	129.39	134.83
8	68.45	57.44	89.34	79.04	104.08
9	159.90	69.96	177.97	87.98	50.63

[1]Calcium expressed as mg./100 gm. cartilage.
*T = Thoracic vertebra.

TABLE XXVI

CARTILAGE PHOSPHORUS CONTENT[1]

Locations

Animals	1T*	3T	5T	7T	9T
1	16.16	44.86	76.39	18.86	12.26
2	17.76	30.84	77.62	40.49	16.45
3	63.44	17.20	73.90	32.00	18.68
4	12.08	83.16	19.20	33.57	18.30
5	56.21	296.34	84.91	105.50	81.17
6	12.55	25.57	78.61	79.46	9.54
7	10.77	45.70	15.90	51.80	66.50
8	24.07	15.15	42.68	29.65	44.76
9	68.67	21.34	49.58	36.96	12.20

[1]Phosphorus expressed as mg./100 gm. cartilage.
*T = Thoracic vertebra.

TABLE XXVII

<u>LONGISSIMUS</u> <u>DORSI</u> SHEAR VALUE[1]

Locations

Animals	5T*	7T	9T	11T	13T	2L**	4L
1	18.43	18.02	15.94	17.92	18.97	18.42	17.04
2	16.17	13.83	14.24	14.13	13.58	14.45	11.27
3	12.62	13.55	12.04	14.29	13.13	12.89	10.90
4	12.48	18.76	16.03	15.43	14.81	18.15	14.13
5	13.15	13.56	13.67	11.91	11.49	11.92	12.97
6	12.87	12.36	14.61	12.44	12.94	11.70	12.13
7	12.77	12.58	11.19	10.71	9.98	10.06	9.74
8	14.18	14.11	13.28	16.71	13.83	13.18	11.07
9	12.37	12.19	13.23	15.83	13.10	16.08	15.51

[1]Shear values expressed as pounds force required to shear a 3/4 inch steak core.
* T = Thoracic vertebra.
**L = Lumbar vertebra.

VITA

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Master of Science

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