June 5, 1970

To the Graduate Council:

I am submitting herewith a dissertation written by Marian Dianne Ruff, entitled "Relation of Phospholipids to Selected Tissue Components in Light and Dark Portions of Porcine Semitendinosus Muscle." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science.

Ada Marie Campbell
Major Professor

We have read this dissertation
and recommend its acceptance:

Grayce E. Goertz

Bernadine Meyer

John T. Smith

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

RELATION OF PHOSPHOLIPIDS TO SELECTED TISSUE COMPONENTS IN LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLE

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Marian Dianne Ruff

August 1970

ACKNOWLEDGMENTS

Without the assistance, guidance and encouragement of many people, this manuscript would not have been possible. The author wishes to express her sincere appreciation to Dr. Ada Marie Campbell for her aid in development of the experimental plan, suggestions during the course of the investigation and patient assistance in the preparation of the manuscript.

The author also wishes to thank Dr. Grayce Goertz, Dr. Bernadine Meyer and Dr. John Smith for the time expended in serving as members of the thesis committee and reviewing the manuscript. Appreciation is expressed also to Miss Amelia Brown, Miss Connie Lazure and Miss Patricia Read for their help in the laboratory.

Financial assistance through the General Foods Fund, the College of Home Economics, the Agricultural Experiment Station and the National Science Foundation has made this advanced degree possible.

The author wishes to acknowledge East Tennessee Meat Packing Company for providing the meat samples for this experiment.

For their support throughout her graduate work, the author is deeply indepted to her parents, Rev. and Mrs. W. H. Ruff.

ABSTRACT

The semitendinosus of pork contains a light and a dark portion that have physicochemical properties similar to those of uniformly white and red muscles, respectively. Although the two portions differ in metabolic activity they function as one muscle. The purpose of this study was to investigate the relation of phospholipid concentration to predominant fiber of the two portions of the porcine semitendinosus. The light and dark portions of the porcine semitendinosus were selected in order to minimize the possible effect of exercise on phospholipid content.

Phospholipids of the light and dark tissue of the porcine semitendinosus were studied in relation to total nitrogen (N), selected nitrogenous components, DNA and total lipid of the tissue. Phospholipids were separated into lecithin, cephalin and sphingomyelin fractions by thin-layer chromatography, and the percentage of each fraction was estimated by densitometry.

Semitendinosus light tissue contained more total and protein N than the dark. Sarcoplasmic and nonprotein N were higher in the light than dark portion on the basis of mg per g of tissue. Fibrillar and stroma N were similar in the two portions. No differences between light and dark portions in any of the nitrogenous compounds expressed as percent of total N were found.

Average DNA content was higher in the dark tissue than light regardless of means of expressing concentration. A wide variation among the animals was found.

The dark portion contained more moisture and less lipid than the light portion. Phospholipid content was higher in the dark than light tissue whether expressed in terms of wet weight, dry weight, nonlipid dry weight, total N or protein N of the tissue. No difference was found in the concentration of lecithin, cephalin or sphingomyelin fractions of the two portions. Lecithins accounted for about 60 percent of the total of the three fractions, cephalins for about 30 percent and sphingomyelin for about 10 percent.

Phospholipid content per unit weight of DNA was slightly higher on the average for the light portion than the dark, but the difference only approached significance. Further investigations of phospholipid-DNA ratio in red and white muscles would be of value if a more reproducible method for DNA determination were available.

From the results it would appear that the phospholipid content of the light and dark portions of the porcine semitendinosus muscle was related more closely to metabolic activity, as indicated by fiber content, than to physical exercise.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	4
	Red and White Skeletal Muscles	4
	Lipid Composition of Skeletal Muscle	14
	Means of Expressing Results of Tissue Analysis	23
III.	PROCEDURE	28
	Meat Samples	28
	Moisture Determination	28
	Total Nitrogen	29
	Protein Fractionation	29
	DNA Analysis	31
	Lipid Extraction	33
	Total Lipid and Phospholipid of the Lipid Extract	34
	Lipid Fractionation and Analysis of Phospholipid Classes	36
	Statistical Analysis	38
IV.	RESULTS AND DISCUSSION	39
V.	SUMMARY	60
LIST OF REFERENCES		62
APPENDIX		70
VITA		72

LIST OF TABLES

TABLE PAGE

I. Percent Moisture, Lipid and Protein of the Light and Dark Portions of Porcine Semitendinosus Muscles 40

II. Nitrogen Composition of the Light and Dark Portions of Porcine Semitendinosus Muscles 42

III. Distribution of Total Nitrogen in the Light and Dark Portions of Porcine Semitendinosus Muscles 44

IV. Concentration of DNA in the Light and Dark Portions of Porcine Semitendinosus Muscles 46

V. DNA as Related to the Nitrogenous Components of the Light and Dark Portions of Porcine Semitendinosus Muscles 47

VI. Percent Phospholipid in the Light and Dark Portions of Porcine Semitendinosus Muscles 49

VII. Relation of Total Phospholipid to DNA, Total Nitrogen and Protein Nitrogen in the Light and Dark Portions of Porcine Semitendinosus Muscles 52

VIII. Total Phospholipid in Relation to Fibrillar Nitrogen, Sarcoplasmic Nitrogen and Nonconnective Tissue Nitrogen in the Light and Dark Portions of Porcine Semitendinosus Muscles 54

IX. Percent Lecithin, Cephalin and Sphingomyelin of the Phospholipid Extracted from Light and Dark Portions of Porcine Semitendinosus Muscles 55

TABLE PAGE

X. Lecithin, Cephalin and Sphingomyelin in the Light and Dark Portions of Porcine Semitendinosus Muscles, Wet Weight Basis 57

XI. Ratios of Lecithin, Cephalin and Sphingomyelin to DNA in the Light and Dark Portions of Porcine Semitendinosus Muscles 58

XII. Individual Values for DNA Concentration in Light and Dark Portions of Porcine Semitendinosus Muscles 71

CHAPTER I

INTRODUCTION

Most of the early studies of phospholipids in meat were limited to their presence in organ tissue. Few studies have dealt with the phospholipid content of striated porcine muscles. Studies in the early 1960s suggested that phospholipids may have a role in accelerating flavor deterioration in cooked meats (Watts, 1962). Implications of effects of phospholipids on keeping quality of meats led to their investigation in muscle meats, especially pork. Phospholipids constitute between 0.5 and 0.9 percent of pork muscle tissue (Kuchmak et al., 1963; Hornstein et al., 1961). The lecithin fraction predominates in meat tissue phospholipids followed by cephalins (Kuchmak et al., 1963).

Phospholipids have been investigated in relation to muscle activity. The phospholipid content tends to be higher in more active muscles according to Bloor et al. (1934). Turkki (1965), on the other hand, found a higher phospholipid concentration in the psoas major muscle of beef than in the extensor carpi radialis, which is more heavily exercised. She suggested that the differences in phospholipid content of the two beef muscles might be attributed to differences in muscle fiber content rather than in physical activity of the muscles. Differences in fiber content reflect differences in metabolic activity. Mammalian skeletal muscles contain three types of fibers--red, white and intermediate--and are classified as red or white depending on the dominant

type of fiber (Ogata, 1960). Red muscles depend largely on aerobic metabolism to supply energy and white muscles have a greater capacity for anaerobic metabolism. Red muscles generally have a higher total lipid and phospholipid content than white muscles.

Among different muscles that are red in color considerable variability exists as to relative proportions of red and white fibers. The predominating fiber can be determined analytically. However, even though two muscles, one with predominantly red fiber and one with a high proportion of white fibers, are obtained from the same animal, those muscles receive differing amounts of exercise. Such samples, therefore, are not useful in attempts to relate phospholipid concentration to either exercise or metabolic activity.

The semitendinosus of pork has a light (white) and a dark (red) portion that have physicochemical properties similar to uniformly white and uniformly red muscles, respectively (Beecher et al., 1968). Although the two portions differ in metabolic activity, they function as one muscle. Thus, it should be possible with the light and dark portions of the porcine semitendinosus muscle to minimize the effect of exercise and investigate the relation of phospholipid concentration to predominant fiber content in striated muscles. A relationship between phospholipid concentration and predominant fiber type would indicate a relationship between phospholipid concentration and major type of metabolic activity. Phospholipid contents in relation to other tissue components were explored by Turkki (1965) for two beef muscles. Obtaining similar information for the light and dark portions of the semitendinosus muscle would seem to be in order.

In this investigation the relationship of phospholipids to deoxyribonucleic acid (DNA), total nitrogen (N), fibrillar N, sarcoplasmic N, nonprotein N (NPN) and stroma N were compared in the light and dark portions of the porcine semitendinosus muscle. The phospholipids of the lipid extract of the two portions were fractionated into lecithin, cephalins and sphingomyelin.

CHAPTER II

REVIEW OF LITERATURE

Red and White Skeletal Muscles

The variation in color of skeletal muscles even within the same animal has long been known (Needham, 1926). Many scientists of the eighteenth and nineteenth centuries sought to explain the degrees of red pigmentation in muscles (Needham, 1926). Ranvier in 1873 was the first to show experimental evidence of different behavior of the two types of muscle; he observed that the frequency of stimulation required for tetanization was higher for red muscles than for white (Needham, 1926; Gergely *et al.*, 1965). The striated muscles of many animals have since been classified as red or white muscles (Ogata, 1960), and numerous physiological and biochemical studies of the two muscle types have been made (Gergely *et al.*, 1965). Early studies presented evidence that the redness of a muscle depended upon the myoglobin content (Walls, 1960). Attempts to associate muscle color and activity led Paukul to the conclusion that slowly contracting muscles are always red, but red muscles are not always slowly contracting (cited by Needham, 1926). Grützner in 1883 stated that every muscle contains two types of fibers--one fine and dark, the other larger in diameter and bright (cited by Needham, 1926). The histological study of cat soleus and gastrocnemius muscles by Denny-Brown (1929) revealed that striated muscles of mammals are composed of a mixture of red and white muscle fibers and that the

predominance of one fiber over another results in the classification of the muscles as red or white.

Studies of striated muscles went through a slack period from about 1930 to 1950. In 1952 Lawrie reported an investigation of the relationship between enzyme activity in muscle preparations and myoglobin content of corresponding muscles in various animal species. He found that apparently the more myoglobin in skeletal muscle the greater is the activity of the cytochrome system within the muscle and its capacity for respiratory metabolism and the less is its ability to carry out glycolytic processes, and conversely. A study by Paul et al. (1952) revealed that red skeletal muscles generally have higher respiratory activities and contain more mitochondria than do white muscles. Mitochondria contain the respiratory enzymes and supply the myofibrils with ATP. Slater's (1960) finding of more mitochondria in red than in white muscles confirmed the earlier work of Paul et al. (1952). Szent-Györgyi (1953) found that white skeletal muscles are associated with short and powerful motion and dark muscles are associated with prolonged muscle function. Consequently, the energy supply for the two types of contraction varies. The energy for short violent contraction is supplied by a relatively high concentration of ATP and creatine phosphate and a highly developed glycolytic system; the energy for prolonged activity is supplied by a highly developed oxidative mechanism (Szent-Györgyi, 1953).

Histochemical studies as reported by Ogata (1960) showed that the striated muscles of mammals contained three types of muscle fibers--red, white and intermediate. A difference in the ratio of the three types of

fibers was noted for each muscle observed. The three types showed different activities of succinic dehydrogenase, cytochrome oxidase and several other enzymes. Red muscle fibers had the greatest concentration of mitochondria and higher activity of respiratory enzymes than the white muscle fibers. A biochemical study was made of practically pure preparations of red and white muscle fibers of rabbits (Ogata, 1960). White fibers contained almost four times as much glycogen and twice as much high energy phosphate as the red fibers. Aerobic oxidation was about six times higher in the red muscle fibers than in the white fibers, and glycolysis was about two times higher in the white than in the red fibers. Adenosine triphosphatase (ATPase) activity was about three times higher in the white fibers. These results again indicate that red muscle has higher activity of the tricarboxylic acid cycle than white muscle. The histochemical and biochemical studies by Ogata (1960) suggested that the variation in chemical constituents and metabolic activity of different striated muscles is attributable to the different ratios of red and white fibers in the muscles. Further histochemical study confirmed the findings that most mammalian striated muscle fibers fall into three types following histochemical demonstration of oxidative enzyme reactions (Ogata et al., 1964). The smaller red fibers showed high activity of oxidative enzymes as compared with that of the large white fibers. The "medium" fibers were intermediate in size and their oxidative enzymatic activity was between the activities found for the red and white fibers.

Three types of muscle fibers also have been described by Stein et al. (1962) from their histochemical classification of individual fibers of the rat gastrocnemius and soleus muscles. The three fiber types (A, B, and C) of the gastrocnemius were identifiable by the characteristic pattern of the distribution of succinic dehydrogenase activity. The evidence suggested that type A is the classical white fiber whereas B and C are two types of red fibers. Variation of fiber ratios within the gastrocnemius was noted; the deep interior portion of the muscle was composed principally of the classical white fiber. The soleus, which is generally considered homogeneous, actually was composed of the two types of red fiber that were found also in the gastrocnemius. Henneman et al. (1965) histologically distinguished three types of muscle fibers in pale muscles such as the gastrocnemius of the cat. They described these types as the large, pale fiber with few mitochondria, the small, dark fiber with intense ATPase activity and filled with small mitochondria, and the fiber that is intermediate in size and ATPase activity with peripheral accumulation of mitochondria. The cat soleus was reported to have a section that contained only the intermediate fiber, which is a type of red fiber.

Red fiber content of muscles often is established histochemically by the affinity of the red fiber for Sudan Black B, which stains intracellular lipid (Beecher et al., 1965a). A comparison of this histochemical method and the biochemical method in which high succinic dehydrogenase activity is associated with red fibers was made, and the two methods showed similar trends for red fiber contents of the seven porcine

muscles studied. Only red and white fibers are distinguished by this method.

Henneman et al. (1965) in an attempt to relate the structure to function of cat gastrocnemius and soleus muscles found that the redness of the fiber was associated with economy of energy and resistance to fatigue. The red soleus and pale gastrocnemius apparently complement each other mechanically. In cold-blooded vertebrates and birds it is now well established that muscles may contain "slow" and "fast" fibers (Hoyle, 1967). This distinction has not yet been clearly established for mammals although the individual mammalian fibers do show different lipid staining reactions. The aerobic oxidation associated with the red fibers is usually, but not always, connected with slow contraction, which usually involves sustained activity. Fast fibers are generally used for short bursts of activity, and the energy for such activity can come from glycolysis. Peachey (1968) indicated that it has become clear that the classification of red and white muscles as slow and fast with respect to rate of contraction does not hold in all cases.

Metabolism of red and white muscles has been studied in rats by the use of ^{14}C-labeled compounds. Beatty et al. (1963) demonstrated differences in relative importance of various metabolic pathways in predominantly red or white fiber groups from the adductor muscle of fasted rats. Glycogen concentrations were higher in white than in red muscle immediately after sacrifice but were lower in the white muscle after 2-hr incubation of the excised muscle. Glucose uptake was similar in the red and white tissue, but lactate production was greater

in the white than in the red muscle. The high oxidative enzyme activity of red muscle has led to suggestions that fatty acids are oxidized extensively in red muscle via the tricarboxylic acid cycle. In this respect, Beatty et al. (1963) found acetoacetic acid-^{14}C uptake 30 to 35 percent higher in red fiber groups than in white groups. A lower percentage of the lable appeared in the lactate fraction of white fiber groups than in that of the red. White fiber groups contained a higher percentage of glycogen that was labled. Glycogen synthesis and breakdown was high in the white muscle preparations.

Bär et al. (1965) reported studies of the contribution of glucose to glycogen and CO_2 production in red and white muscle. Rat diaphragm and external oblique abdominal muscles were used as the red and white samples, respectively. Glycogen levels in fasted animals were higher in the white muscle than in red muscle. Glucose administered orally to fasted rats 3 hr before sacrifice increased glycogen values in red muscle preparations but did not influence glycogen levels in the white muscle. After a 2-hr incubation of the muscles in a physiological medium containing lactate and glucose, glycogen increased in all the white muscle samples. The increase in glycogen in the incubated red tissue preparations was progressively smaller as the in vivo glucose dose was increased. The net glycogen change appeared to be similar in red and white muscle samples that had similar initial glycogen concentrations. An inverse relationship was found between glycogen change and glucose-U-^{14}C incorporation into glycogen, both of which were greater in red than white muscle. Glucose-U-^{14}C and lactate-1-^{14}C were converted

to CO_2 in both red and white muscles; $^{14}CO_2$ production was greater in red than in white muscle.

Although the diaphragm has been used widely in studies of striated skeletal muscle, Bocek et al. (1966a) contended that the diaphragm is a highly specialized, constantly active muscle that is not typical of skeletal muscles. In their studies of glycogen and glucose metabolism in the two types of muscles, Bocek et al. (1966a,b) used red and white fiber groups from rat adductor muscles. Results of in vitro experiments with fiber groups from fasted rats showed that total glycogen concentrations were lower initially in red muscle than in white and were maintained during the first hour of a 2-hr incubation period (Bocek et al., 1966a). The glycogen concentration was significantly higher in the red fibers than in the white after incubation for 90 to 120 min in Krebs bicarbonate medium containing added glucose-^{14}C. During the first hour of incubation, the incorporation of the label from glucose-^{14}C in the medium was 10 times greater in red than in white muscle. The authors stated that the results indicate more active conversion of glucose to glycogen in red muscle fibers than in white and that there might be a difference in glycogen metabolism in red and white muscle. In the study of glucose metabolism, muscle samples were incubated for 2 hr in a medium containing glucose-U-^{14}C. Glycogen concentrations were again higher in red than in white muscle fiber groups at the end of the incubation period. Glucose uptake was greater in vitro in the red muscle portion. The percentage of the glucose label incorporated into glycogen and CO_2 was higher in red muscle than white; however, the percentage of the lable

appearing in lactate and pyruvate was higher in the white muscle than the red. The difference in the amount of the lable from glucose that was incorporated into the various metabolic fractions in the red and white muscles indicates a difference in the metabolism of the muscles.

Metabolism in red and white muscles is apparently different as indicated by the studies, cited above, of Beatty et al. (1963), Bär et al. (1965) and Bocek et al. (1966a,b). These investigations were in vitro studies employing the Warburg apparatus, which simulates conditions in the resting muscle. Bocek et al. (1966b) pointed out that the physiological state of the tissue may affect the results obtained from a study of metabolic processes.

Other components of red and white muscles have been investigated. A study of cell water, sodium and potassium in 12 different hind leg muscles and the diaphragm of the rat was made to determine the relationship of such biochemical data to the histological classification of the muscles as red or white (Sréter et al., 1963). A definite relationship between water content and ionic composition and the ratio of red to white fibers was shown. In muscles predominantly composed of white fibers, the sodium ion concentration was low and the potassium ion concentration high. As the numbers of red fibers increased, the sodium ion concentration increased and the potassium ion concentration decreased proportionately. The difference in concentration of the sodium and potassium ions may be a factor in the different speeds of contraction of muscles. The red muscles were found to have a significantly larger extracellular fluid compartment than white muscles. From histological

data it was obvious that red and white fibers were not uniformly distributed within the muscles studied; white fibers were reported to be mostly in the external portions and the red fibers in the innermost portions.

Most investigations of red and white muscles of mammals have involved small laboratory animals. The muscles of pigs have been classified as red and white, or light and dark, on the basis of visual variation in redness, histological and biochemical differences (Beecher et al., 1965a). The wide use of porcine muscles for food has stimulated research on the composition and metabolism of these muscles. A recent study by Beecher et al. (1969) was made to determine the concentration of several energy metabolites in red and white striated muscles and to investigate the effect of post-mortem metabolism on the concentration of the metabolites. Samples from six pigs were taken from two red and two white muscles immediately after exsanguination and at intervals from 15 to 1440 min post-mortem. As in small animals, muscle with high red fiber content had high myoglobin concentration and high succinic dehydrogenase activity, which indicates that red porcine muscles have a high capacity for aerobic metabolism. The muscles low in red fibers were considered white muscles, and they appeared to be more oriented toward anaerobic metabolism as succinic dehydrogenase activity was low. Initial glycogen concentrations were similar in the red and white muscles. The concentrations of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, pyruvate, ATP and citrate were not consistently higher or lower in the two red muscles than in the two white muscles.

As biochemical and histological studies of muscle fibers from different locations of the gastrocnemius of the cat and rat have shown, the internal or deep portion of the muscle contains a higher proportion of red fibers than does the external or superficial layer (Denny-Brown, 1929; Stein et al., 1962; Ogata et al., 1964; Sréter et al., 1963; Beecher et al., 1968). Beecher et al. (1965a) classified the dark portion of the semitendinosus and the inside portion of the biceps femoris from pigs as red muscles on the basis of red fiber content as established by affinity for Sudan Black B and high succinic dehydrogenase activity. In the semitendinosus, the dark portion lies near the femur and the light portion is near the subcutaneous fat layer. Further study of the porcine semitendinosus revealed that the red portion had a significantly higher glycogen content initially, but that there was no significant difference in the lactic acid concentration at death (Beecher et al., 1965b). The rate of glycogen disappearance was similar in both portions. In attempting to further characterize the two portions of the porcine semitendinosus, Beecher et al. (1968) found the myoglobin content, red fiber content and succinic dehydrogenase activity to be more than twice as high in the dark portion as in the light. Glycogen concentrations immediately post-mortem were similar in the two portions. The reported variations in the initial glycogen content between red and white muscles have been attributed to a greater acceleration of glycolysis in the white muscle caused by physiological stresses at the time of death (Beecher et al., 1965a,b; Bocek et al., 1966a). Lactic acid concentrations were higher in the lighter portion than in the dark portion; ADP and inorganic

phosphate levels were similar in the two portions. The light portion of the porcine semitendinosus was of lower moisture and higher lipid content than the dark portion. Higher lipid content generally has been associated with red muscles. Myofibrillar nitrogen was similar in the light and dark portions, but the sarcoplasmic nitrogen values were higher in the semitendinosus light portion than in the dark portion. The data from the studies suggest that although the light and dark portions of the porcine semitendinosus constitute the same muscle, i.e. they have the same origin and insertion and are thought to function uniformly as a single muscle, they possess marked differences in physical and chemical properties (Beecher et al., 1965b, 1968). The physicochemical properties of the light and dark portions are similar to those of uniformly white and red muscles, respectively (Beecher et al., 1968). Some of the recent interest in red and white porcine muscles can be attributed to the recognition of a condition known as pale, soft and exudative (PSE) pork muscles in the early 1960s. White porcine muscles are highly susceptible to the PSE condition whereas red muscles are resistant (Briskey, 1964). The condition is associated with an accelerated post-mortem glycolysis and has been found to occur more frequently in certain breeds of hogs than in others.

Lipid Composition of Skeletal Muscle

MacLean (1918) reviewed some of the early work on phospholipid composition of plant and animal tissues. Phospholipids were found to occur in every cell of the living organism, but reliable methods for

quantitating the concentration of the phospholipds were not available in the early part of the century (MacLean, 1918). From observations of phospholipids in tissues from various animal species, the phospholipid content of living tissue was thought to be constant in amounts characteristic of the tissue (Bloor et al., 1930). In 1919 Terroine noted that the concentration of phospholipids was not affected by prolonged fasting (Bloor, 1943; Terroine, 1936). Terroine (1936) suggested that the lipid material of animal tissue is of two types, the élément constant that is an essential cell component and the élément variable that is an energy reserve. The élément constant included the phospholipids, which were not considered subject to alteration by physiological variation; the élément variable was primarily neutral fat that had the physiologically passive function of storing energy.

Bloor (1927) suggested that the phospholipid content of a tissue was related to the physiological activity of the tissue. In his study of the phospholipid fatty acids in beef muscles, the heart had the highest phospholipid content followed by the jaw, diaphragm, neck and round; he considered the physiological activity to decrease in the same order.

A series of experiments to substantiate his hypothesis was initiated. A study of the corpus luteum of the sow revealed that the total phospholipid content varied markedly with the activity of the gland (Bloor et al., 1930). The concentrations of phospholipids in locomotor muscles of wild and laboratory rabbits were found to differ (Bloor et al., 1934). Higher phospholipid per unit weight of dry tissue was associated with those muscles of motion in the wild rabbit than in the

laboratory animals. Ludewig et al. (1936) criticized the hypothesis on the basis that the findings were a result of differences that occur naturally between animals and not differences produced experimentally. From a study of exercised and nonexercised rats, Bloor (1937) found that the animals that were forced to run on an exercise wheel for one to two months had higher phospholipid concentrations for all muscles studied except the diaphragm than the animals housed in small cages for the same period of time. This new evidence supported the hypothesis that phospholipid content is related to physiological activity.

Two recent studies have dealt with the effect of exercise on the lipid content of muscles. Shchesno (1965) analyzed pairs of red and white rabbit muscles for phospholipid content after one muscle in each pair had been subjected to strenuous exercise by application of electrical shock. Changes in the red muscle phospholipids were slight after exercise; however, about 50 percent of the white muscles showed an increase in phospholipid content after exercise. Morgan et al. (1969) studied the effect of long-term exercise on the lipid composition of the vastus lateralis muscle in 10 human subjects. The quadriceps muscle of one thigh was trained by daily exercise for a period of 39 days; muscles of the opposite leg served as an untrained control. Muscle biopsies were performed at the end of the training period, and total phospholipid, lecithin and triglyceride concentrations were higher in the trained than in the untrained muscles. The authors suggested than an increase in the phospholipid-rich cell organelles, particularly an increase in the number and size of the mitochondria, might have occurred.

The phospholipid contents of skeletal muscles of mammals were reported in a few studies before 1960. Bloor (1943) reviewed studies of 12 mammalian species in which the phospholipid per unit weight of dry tissue ranged from 8.75 percent for the jack rabbit to 1.70 percent for the laboratory rabbit. A study was made by Kaucher et al. (1943) of the distribution of lipids in animal tissues from several species. Pork contained 3.06 percent phospholipid by dry weight; two beef samples, 3.08 and 3.39 percent phospholipid; and lamb, 4.74 percent phospholipid.

Fractionation of the phospholipids was difficult in early studies because of lack of sophisticated techniques for separation. In general muscle phosphatides contained little sphingomyelin and approximately equal amounts of lecithin and cephalins, although there often was slightly more lecithin (Bloor, 1943; Wittcoff, 1951). Kaucher et al. (1943) estimated the amount of each phospholipid class by fractionating the extract on the basis of solubility differences of the components. The phospholipids in each fraction were determined by analysis of the extract for various components such as phosphorus, glycerol, fatty acids, amino nitrogen and the different bases and quantitating the phospholipid classes through ratios and differences of the compounds. The concentration of phospholipids and the distribution of lecithin, cephalin and sphingomyelin were estimated in the muscles of many meat animals. Dry tissue of pork contained 1.69 percent lecithin, 1.25 percent cephalin and 0.12 percent sphingomyelin, or 55, 41 and 4 percent of the total phospholipid, respectively. In all of the mammalian skeletal muscles

studied, lecithin was 50-60 percent of the total, cephalins 30-45 percent and sphingomyelin less than 10 percent.

Advances in lipid research in the past decade have been related largely to the development of chromatographic techniques for separation of the lipid components into pure fractions. Column, gas-liquid and thin-layer chromatography have been applied in numerous methods for the separation and identification of phospholipid components. Continued work is being carried out on finding new methods and perfecting established ones.

Animal cells have been found to contain three types of lipid: phospholipids, glycolipids and sterol, generally cholesterol (Rouser et al., 1968). The phospholipids identified as components of the animal cells are phosphatidyl choline (lecithin), phosphatidyl ethanolamine, phosphatidyl serine, sphingomyelin, phosphatidyl inositol and phosphatidic acid. The cephalin fraction includes both phosphatidyl ethanolamine and phosphatidyl serine. Most reports of phospholipid composition do not include phosphatidyl inositol or phosphatidic acid (Rouser et al., 1968). With improved techniques more complete characterization of the phospholipid components should be possible.

Few studies have dealt with the phospholipid content of striated skeletal muscles. The lipids, especially phospholipids, have been discussed with regard to keeping quality and flavor of meat (Lea, 1957; Younathan et al., 1960; Hornstein et al., 1961). Younathan et al. (1960) found that phospholipids in cooked pork that had been refrigerated for a week were more susceptible to oxidation than the neutral lipids of the

cooked refrigerated pork. Hornstein et al. (1961) reported that rancid odors developed more quickly in the total and phospholipid extracts of raw beef and pork on prolonged exposure to air than in the neutral fat. Watts (1962) described the oxidation of the muscle lipids in cooked meats as heme catalyzed with rancidity resulting. Such implications of the phospholipids in accelerating flavor deterioration led to their investigation in muscle meats, especially pork.

The lipid composition of aged lean beef and pork was studied by Hornstein et al. (1961). The lipid extract was fractionated on silicic acid columns and phosphorus, total nitrogen, amino nitrogen, reducing sugars, infrared spectra and fatty acid determinations were made to identify the fractional components. The total phospholipid content of beef was 0.8-1.0 percent of the wet tissue and pork was 0.7-0.9 percent. In a study by Campbell et al. (1967) the average phospholipid content of raw ground pork was 0.68 percent of the meat. Hornstein et al. (1961) found that the phospholipids of both beef and pork were composed of about 40-45 percent lecithin, 40-45 percent cephalin and 10-15 percent sphingomyelin. Unsaturated fatty acids accounted for only about 10 percent of the fatty acids of the triglycerides but about 50 percent of the fatty acids of the phospholipids.

Two studies of phospholipids isolated from pork muscle tissue from four locations on a hog carcass were reported by Kuchmak et al. (1963, 1965). The phospholipids were fractionated on silicic acid columns and the fractions quantitated by three methods: gravimetric analysis, phosphorus content and infrared spectra (Kuchmak et al., 1963). Total

concentration of phospholipid varied from 0.45 to 0.58 percent of the fresh tissue. Lecithin accounted for 54-63 percent of the total phospholipids, cephalins for 31-42 percent, and sphingomyelin for 1-6 percent. Slight differences were noted in the total phospholipid and fraction concentrations in the muscles of the belly, ham, loin and rib; however, the data represent only one animal and are not a sufficient basis for general statements concerning compositional differences. The fractions of the phospholipids extracted from the four locations on the hog carcass were analyzed to determine their fatty acid composition (Kuchmak et al., 1965). From the gas-liquid chromatographic data, the cephalins were shown to contain a higher percentage of polyunsaturated fatty acids than lecithin, and the composition of the phospholipids varied with carcass location.

In studies of phospholipid fractions in skeletal muscles of other vertebrates, lecithin generally was found in the largest amount, followed by cephalin (Turkki, 1965; Smitherman, 1967; Rouser et al., 1968). When phosphatidyl inositol was determined, it was reported in larger amounts than sphingomyelin by some authors (Rouser et al., 1968; Kryzywicki et al., 1967). Krzywicki et al. (1967) reported total lipid phosphorus of pork longissimus dorsi to be 48.1-62.1 percent lecithin, 18.1-30.4 percent cephalin, 7.8-14.7 percent inositide and 4.3-8.6 percent "early" fraction.

The functions of phospholipids in animal tissue are still being investigated. Adipose tissue is known to contain very little phospholipid, but phospholipids are found in larger amounts in other body

tissues including skeletal muscles (Deuel, 1957; Lea, 1962). The primary function of cellular phospholipids is as an essential structural component of the cell (Deuel, 1957). The cellular membranes contain the bulk of the phospholipids of the cell (Ansell et al., 1964). Most lipids of animal cell membranes occur in five different organelles, which are plasmalemma, endoplasmic reticulum, Golgi complex, mitochondria and nuclei (Rouser et al., 1968). The knowledge of the arrangement of lipids and other components in cell membranes is limited. The most popular theory is the trilaminar arrangement, in which the membrane structure is as a lipid bilayer between two layers of protein. An interesting observation is that the lipid composition of normal cells and cellular organelles appears to be constant, which might indicate that membranes are composed of regular repeating units (Rouser et al., 1968). The quantitative data on phospholipids of subcellular particles appear to be limited primarily to organ tissue. Mitochondria of beef heart muscle have been shown to contain 25 percent lipid by weight and approximately 95 percent of the lipid is phospholipid (Green et al., 1964).

Krzywicki et al. (1967) attempted to determine the phospholipid content of subcellular fractions of porcine longissimus dorsi muscles. The following subcellular fractions were obtained by differential centrifugation: "myofibrillar," "mitochondrial," "reticular" and "final supernatant." The lipid-phosphorus expressed as percent of total lipid-phosphorus of the unfractionated meat was distributed as follows: "myofibrillar," 40-78 percent; "mitochondrial," 1-6 percent; "reticular," 4-12 percent; and "final supernatant," 7-10 percent. The

unrecovered phospholipids amounted to 5-40 percent of the total lipid-phosphorus. The authors attributed the high percentage of lipid-phosphorus associated with the "myofibrillar" fraction to the presence of sarcoplasmic reticulum with the fibrils.

As has been discussed, more mitochondria are found in the red than in the white muscle fibers (Slater, 1960). No reports of studies of other organelles in red and white muscles have been found in the literature. With higher mitochondrial content, higher phospholipid concentration would be expected in the red muscles than in the white. An observation of high phospholipid content in red muscles was made in 1915 by Quagilarello in rabbit muscles (Needham, 1926). Bloor et al. in 1934 found no close correlation between redness and phospholipid content in the muscles of wild and laboratory rabbits, but they did report that in general redness varied with total lipid content. Red muscle fibers frequently have been shown to contain more lipid material than white muscle fibers (Drummond et al., 1960; Swift et al., 1959; Beecher et al., 1965a). Allen et al. (1967) appear to have confirmed the findings of higher phospholipid and total lipid in red muscles than white in a study of three porcine muscles. The diaphragm contained higher levels of total lipid and lipid-phosphorus than the longissimus dorsi. The average lipid-phosphorus per 100 g of muscle was 78.3 and 39.6 mg for the above two muscles, respectively. The diaphragm is the classic red muscle and the porcine longissimus dorsi has been classified as a white muscle (Beecher et al., 1965a).

Sudan Black B, the stain often used to establish red fiber content in order to differentiate red and white muscles for histological studies, is thought to stain phospholipids in preference to neutral lipids; the implication is that the phospholipid content should be higher in the red than white muscle fiber (Beecher et al., 1965a). Studies on the phospholipid content of chicken muscles have revealed that the thigh muscle may contain as much as twice the amount of phospholipid as the breast (Smitherman, 1967; Marion et al., 1965; Marion, 1965). Shchesno (1965) reported higher phospholipid in the red muscles of rabbit than in the white. Turkki (1965) suggested that variation in phospholipid content of two beef muscles, the extensor carpi radialis and psoas major, might result more from differences in fiber content than from differences in the extent of usage.

Means of Expressing Results of Tissue Analysis

In tissue analysis it is necessary to relate the results to a standard characteristic of the tissue (Thomson et al., 1953). The reference standard must be constant under the conditions of the investigation, or reported change in the variable actually may be attributable to a change in the standard characteristic. The most obvious method of expressing the results of tissue analysis is in the form of concentrations per 100 g of the wet weight of the tissue; however, changes in the concentration of the constituent of interest might be only a reflection of a change in the moisture content (Munro et al., 1969). Concentration per unit weight of dry or nonlipid dry tissue frequently

is selected as the method of reporting data to avoid the variation associated with water content or water and fat concentrations. Analytical data for lipid constitutents often are expressed as percent of the total lipid. Rouser et al. (1968) preferred to express total lipid values for organelles on the basis of weight of lipid per weight of protein. Total N and protein N often are used as the basis for expressing data from tissue analysis.

The constancy of DNA per cell nucleus for a given species has led to the use of DNA content of tissue as a reference standard in tissue analysis (Glick, 1961). The first published results indicating that DNA content of somatic cells of a given species is constant were obtained by Boivin et al. (1948). By analysis of isolated nuclei that were extracted in bulk, they showed that nuclei of various bovine organs contained about 6.5×10^{-6} μg of DNA each and that the haploid nuclei of sperm contained approximately half the DNA of the diploid somatic nuclei. Almost simultaneously Mirsky et al. (1949) demonstrated cytochemically that the DNA content of individual nuclei is approximately constant per set of chromosomes for a given species and that the DNA values are characteristic of the species. The constancy of DNA per nucleus and the haploid-diploid relationship has since been demonstrated in many species (Brawerman et al., 1962; Vendrely, 1955; Vendrely et al., 1956).

Mirsky et al. (1951) first noted that the DNA content varies from one class of animals to another in a manner that is not obviously related to the complexity of the organism. The DNA content of nondividing

cells in various tissues of mammals is close to 7×10^{-12} g per diploid set of chromosomes (Munro et al., 1969; Vendrely, 1955; Brawerman et al., 1962). The DNA content per nucleus is higher in tissues containing polyploid cells than in those having solely diploid cells (Vendrely, 1955). Harrison (1951) found a direct relationship between the amount of DNA per nucleus and the number of chromosomes per nucleus in the livers of adult rats. Appropriate multiples of the DNA content of the haploid nucleus are known to occur in polyploid cells (Moses, 1964). Epstein (1967) found cell size directly proportional to cell ploidy in Swiss mice. These findings indicated that the ratio of cytoplasm to DNA is not disturbed by polyploidy and the ratio of cytoplasmic components to DNA still is meaningful (Munro et al., 1969).

Through the use of isotopic and autoradiographic techniques, it became apparent that DNA is not turned over in the normal cell and that it remains intact once synthesized (Moses, 1964). The DNA of the resting nuclei is remarkably stable in normal tissues as well as in tissues undergoing physiological and pathological changes (Vendrely, 1955; Brachet, 1957).

The generally accepted concept of the presence of cellular DNA exclusively in the nucleus was challenged by Brawerman et al. (1962). Typical mitochondria have been shown to contain small amounts of DNA (DuPraw, 1968), but the combined DNA content of all the mitochondria per cell is small compared to the amount found in the nucleus (Nass, 1969). As most of the cellular DNA is concentrated in the nucleus, the assumptions based on the above-stated concept are still valid.

The constancy of DNA content per somatic cell led Boivin et al. (1948) to suggest that DNA might serve as a reference standard for analyses of organ tissue. A more detailed discussion of the use of DNA as a standard in analysis of organ tissue is given by Thomson et al. (1953). Gray et al. (1956) extended the use of DNA as a reference standard to muscle tissue analyses. Skeletal muscle contains multinucleated fibers rather than discrete cells; however, a finite volume of cytoplasm is associated with each nucleus (Cheek, 1968). Gray et al. (1956) arbitrarily termed each of the units of nucleus and cytoplasm a cell. The number of such cells in the weight of tissue studied can be calculated from DNA content for diploid somatic cells of the same species.

DNA was used as a reference standard in numerous studies cited by Leslie (1955) and Turkki (1965). Some studies have related phospholipid content to DNA content. Morrill et al. (1964) used the DNA-phosphorus content as a reference standard for a study of phospholipid and plasmalogen changes in adipose tissue of newborn rats. In studying changes in phospholipids of rabbit epidermis after the application of nonionic surfactants, Mezei et al. (1967) calculated the changes on the basis of wet weight and of DNA content. Although there was a slight increase in the DNA content, the authors concluded that the DNA might be the more reliable reference standard. Phospholipids of beef and chicken muscles were related to DNA content by Turkki (1965) and Smitherman (1967), respectively. Turkki (1965) found the DNA content essentially the same in fresh tissue of the extensor carpi radialis and psoas major from beef; the range for both muscles was 9.6-15.5 mg of DNA in 100 g of fresh tissue. The

concentration of phospholipid per unit weight of DNA was significantly higher in the psoas than in the extensor. Smitherman (1967) found the DNA content to be significantly higher in the thigh than in the breast on a wet weight basis. Considerable variation among birds was noted as the DNA content of the breast ranged from 5.1 to 12.4 mg per 100 g of tissue and the DNA of the thigh was from 7.7 to 21.9 mg per 100 g of tissue. No significant difference was noted between the phospholipid-DNA ratio of the breast and thigh.

CHAPTER III

PROCEDURE

Meat Samples

Semitendinosus muscles from eight sows were obtained, one muscle at a time, from a local meat packing company. Breed and preslaughter history were not known. Only animals whose carcass weights ranged from 240 to 260 lbs were used. Maturity of the animal as indicated by live weight was shown to affect total N and protein solubility (Sayre et al., 1966). The muscles were excised approximately 1 hr after slaughter and placed immediately in crushed ice. After 1 hr the muscles were trimmed of external fat and connective tissue and divided into light, intermediate and dark portions (Beecher et al., 1968). The three portions were weighed and the intermediate portion was discarded. Each portion was ground four times in a precooled meat grinder and the ground meat was mixed well. Samples for moisture determination, total N, protein fractionation, DNA analysis and lipid extraction were weighed on an analytical balance and immediately refrigerated. The lipid extraction samples were flushed with nitrogen and stored at -20°C. All equipment used in the preparation of the meat samples was precooled, and samples were kept on ice whenever possible.

Moisture Determination

Triplicate 4-6 g meat samples were weighed into preweighed aluminum moisture pans and dried, uncovered, to constant weight in an air oven at

100°C (AOAC, 1965). After replacement of the lids, the pans were cooled 1 hr in a desiccator and reweighed. The loss in weight, expressed as percent of the original weight of the sample, is reported as moisture concentration.

Total Nitrogen

Meat samples weighing 300-400 mg were analyzed in duplicate for total N content by a modification of the AOAC (1965) micro-Kjeldahl procedure. The samples were weighed on small pieces of filter paper and dropped into micro-Kjeldahl flasks containing 1.9 ± 0.1 g K_2SO_4 and 40 ± 10 mg HgO. After addition of 3.0 ± 0.1 ml conc. H_2SO_4, samples were digested about 4 hr. The completely digested samples were transferred to the distillation apparatus, with the aid of distilled water, and 15 ml of a NaOH-$Na_2S_2O_3$ solution was added to the still. The distillate was collected in 20 ml of saturated boric acid solution that contained methyl red-bromcresol green indicator. The ammonia was titrated with 0.1 N HCl. Blank determinations of the filter paper were made, and the amount of nitrogen was calculated by the equation:

$$\text{mg N/g of tissue} = \frac{(\text{ml HCl}_{\text{sample}} - \text{ml HCl}_{\text{blank}}) \times \text{N of HCl} \times 14.01}{\text{g of tissue extracted}}$$

Protein Fractionation

Duplicate 5-g samples of dark and light portions of muscle were fractionated into sarcoplasmic protein N, fibrillar protein N, stroma protein N and nonprotein N (NPN) by the procedure of Hegarty *et al.* (1963) as adapted by Turkki (1965). Each sample was ground with 5 g of

sand in a chilled mortar with 60 ml of phosphate buffer at pH 7.6 and ionic strength of 0.05 M. The ground sample was transferred to a 250-ml polyethylene centrifuge bottle and allowed to stand 1 hr in a refrigerator at 4°C. The ground sample was centrifuged at 2°C for 15 min at 4100 x G; the supernatant fluid was decanted into a 250-ml Erlenmeyer flask, which then was covered and refrigerated. The residue was resuspended in 100 ml of the buffer and after standing 1 hr at 4°C was centrifuged as above. The second supernatant fluid was added to the first, and the combined supernatant portions were filtered through gauze into a 250-ml graduated cylinder. The volume of the filtrate, designated fraction A, was recorded and the filtrate was refrigerated until analyzed for N content.

The residue from the second centrifugation and the gauze used to filter the supernatant fluid were extracted at room temperature with 200 ml of 0.1 M NaOH for 4 hr and the extract was filtered through gauze as described for fraction A. The filtrate was labeled fraction C and refrigerated until analyzed. Two 15-ml samples of fraction A were mixed with 5 ml of 10 percent trichloroacetic acid (TCA) and allowed to stand 15 min. After filtration through Whatman No. 1 filter paper, the precipitated protein was discarded and the filtrate was designated fraction B.

Nitrogen content of 15-ml samples of fraction B and 10-ml samples of fractions A and C was determined by the AOAC (1965) micro-Kjeldahl method. In analysis of the protein fractions, 2.0 ± 0.1 ml H_2SO_4 was used in digestion, and 10 ml NaOH-$Na_2S_2O_3$ solution was used in distillation. The distillate was collected in 5 ml of a saturated boric acid

solution and titrated with 0.02 N HCl. With these exceptions, the method was as described for total N. Blanks of the buffer, 0.1 M NaOH and buffer plus 10 percent TCA were analyzed and the appropriate corrections made. The amounts of nitrogen in fractions A and C were calculated as follows:

mg N/g of tissue =

$$\frac{\text{ml of fraction} \times (\text{ml HCl}_{\text{sample}} - \text{ml HCl}_{\text{blank}}) \times N \times 14.01}{\text{g of tissue extracted} \times 10}$$

The N content of fraction B, NPN, was calculated by the following equation:

mg NPN/g of tissue =

$$\frac{\text{ml of fraction A} \times (\text{ml HCl}_{\text{sample}} - \text{ml HCl}_{\text{blank}}) \times N \times 14.01 \times 1.33}{\text{g of tissue extracted} \times 15}$$

The nitrogen content of the various protein fractions was derived as follows:

B = NPN

C = fibrillar protein N

A-B = sarcoplasmic protein N

A+C = non connective tissue N

Total N-B = protein N

Total N - (A+C) = connective tissue (stroma) protein N

DNA Analysis

The DNA contents of the light and dark portions of the semitendinosus muscles were determined by the method of Schneider (1946) as adapted for use with muscle tissue by Turkki (1965). Triplicate

4.5-5.5 g samples of fresh meat were ground with 5 g of sand and 25 ml of cold 10 percent TCA in a chilled mortar. The contents of the mortar were transferred to a 50-ml stainless steel centrifuge tube, and the mortar and pestle were rinsed with 15 ml of the 10 percent TCA. After centrifugation at 6300 x G for 15 min at 2°C, the supernatant was decanted and discarded. The residue was extracted with 25 ml of 10 percent TCA twice under the conditions described above. After a final cold extraction with 15 ml of 5 percent TCA, the liquid was decanted and the tubes were inverted on a paper towel to remove all the liquid. The above series of extractions with cold TCA removed the nucleosides and nucleotides; hot extraction of the residue then was used to remove the nucleic acids. The residue was suspended in 15 ml of 5 percent TCA and heated in a loosely covered tube for 15 min in a water bath at 90°C. After cooling and centrifugation, the supernatant fluid was filtered through Whatman No. 44 acid washed filter paper.

The deoxyribose content of the extract was determined colorimetrically by the Dische diphenylamine reaction (Seibert, 1940). The diphenylamine reagent consists of 1 g of diphenylamine dissolved in 100 ml of glacial acetic acid and 2.75 ml H_2SO_4. Duplicate 3-ml aliquots of the extract and 6-ml portions of the diphenylamine reagent were mixed in 30-ml test tubes, which were covered with marbles and placed in a boiling water bath for 10 min. After cooling, the contents of each tube were transferred to a cuvette. A deoxyribose standard of 50 µg deoxyribose in 3 ml of 5 percent TCA and a 5 percent TCA blank were analyzed with the extracts from each muscle. A Bausch and Lomb "Spectronic 20"

spectrophotometer was used to measure abosrbance of the solutions at 600 mμ with the instrument zeroed with a reagent blank. The following equation was used for calculation of the amount of deoxyribose in each aliquot:

$$\mu g \text{ of deoxyribose / aliquot} = \frac{\text{absorbance of aliquot x 50}}{\text{absorbance of standard}}$$

The DNA-phosphorus (DNA-P) was derived from amount of deoxyribose in each aliquot in the following equation:

$$\text{mg DNA-P/100 g tissue} = \frac{\mu g \text{ deoxyribose in aliquot x 5}}{\text{g of tissue extracted x 4.33* x 10}}$$

As DNA-P is approximately 10 percent of the DNA, the concentration of DNA in the tissue was obtained by multiplication of DNA-P by 10.

Lipid Extraction

Lipids of 40 g samples of the light and dark portions of the semitendinosus muscle were extracted by the procedure described by Turkki (1965) from the Ostrander et al. (1961) modification of the method of Bligh et al. (1959). The frozen sample was thawed overnight in a refrigerator. The entire sample was transferred into a blender jar with the aid of 1/2 facial tissue and 130 ml of methanol and was blended. Two subsequent blendings after additions of 65 ml of chloroform followed by one with 65 ml of added water containing 1.5 g of zinc acetate were performed. The extract was transferred to a Büchner funnel and filtered through Whatman No. 1 filter paper under suction. Residue and filter paper were

---

*$\frac{\text{mol. wt. of deoxyribose}}{\text{mol. wt. of phosphorus}}$

reblended with 100 ml of chloroform, and the extraction mixture was filtered as above with 100 ml of chloroform used for rinsing. Both filtrations were carried out under a stream of nitrogen. The filtrate was transferred to a 500-ml graduated cylinder with 50 ml of chloroform and stored overnight at approximately 1°C. The volume of the chloroform layer was recorded, and the chloroform-lipid extract was separated from the water-methanol layer by means of a 500-ml separatory funnel.

Total Lipid and Phospholipid of the Lipid Extract

Immediately after removal of the chloroform layer from the separatory funnel, three 10-ml samples of the extract were pipetted into preweighed beakers for total lipid determination. The chloroform was evaporated first under the hood and then under reduced pressure in a vacuum desiccator until the sample reached constant weight (Turkki, 1965). Total lipid extracted from the tissue was derived as follows:

$$\text{percent lipid in tissue} = \frac{\text{ml } CHCl_3 \text{ extract x g lipid in 10 ml x 10}}{\text{g of tissue extracted}}$$

The remaining chloroform extract was concentrated in a rotary evaporator and transferred to a 125 ml Erlenmeyer flask by rinsing with chloroform. The concentrated extract was stored under nitrogen at -20°C until analysis of total phospholipid and phospholipid fractionation were carried out.

The concentration of phospholipids in the lipid extract was determined by Marinetti's (1962) modification of the micro-phosphorus analysis of Bartlett (1959). All glassware was acid washed initially and rinsed thoroughly with distilled water and finally with demineralized water between samples. Two 1-ml samples of the concentrated lipid extracts were

pipetted into preweighed beakers for determination of the lipid concentration. In preliminary work absorbance for the method was linear in the range of 1-6 µg of phosphorus. The concentrated lipid extracts were diluted to contain 1-6 µg phosphorus per 20 µl, and two 1-ml portions of the diluted extract were taken for the determination of lipid concentration. The range for dilutions was established on the basis of phospholipid content of other red and white muscle tissue and preliminary analysis of semitendinosus light and dark tissue. The lipid concentration of the lipid extract diluted for phosphorus analysis was from 19 to 35 mg/ml and from 10 to 14 mg/ml for the extracts of the light and dark portions, respectively. Triplicate 20-µl samples of the lipid extracts of the light and dark portions of the muscle, duplicate phosphorus standard solutions each containing 5 µg of phosphorus and duplicate demineralized-water blanks were analyzed for each muscle. The samples were digested in 30-ml micro-Kjeldahl flasks that contained 1 ml of demineralized water, 1.2 ml of 70 percent perchloric acid and two glass beads. Digestion was carried out on a micro-Kjeldahl digestion rack for 30 min. After flasks had cooled at room temperature for 20 min, 7 ml of demineralized water followed by 1.5 ml of 2.5 percent ammonium molybdate was added to each flask and the contents were mixed well. Following the addition of 0.2 ml Fiske-Subba Row reagent*, the flasks were heated in a

---

*The reagent was prepared fresh weekly by adding 0.5 g of 1-amino-2-napthol-4-sulfonic acid to 200 ml of freshly prepared 15 percent $NaHSO_3$ (anhydrous) solution followed by 1.0 g anhydrous Na_2SO_3. The solution was mixed on a magnetic stirrer and filtered into a dark bottle for storage.

boiling water bath for 7 min. When the samples had cooled 20 min at room temperature, the absorbance was read at 830 mμ in a Bausch and Lomb "Spectronic 20" spectrophotometer, which was adapted for near infra-red wavelengths through the use of a red filter and specified phototube. The reagent blank was used to zero the instrument.

The phosphorus in the 20-μl samples of the extracts was obtained from the equation:

$$\mu g \text{ P in } 20\ \mu l = \frac{\text{absorbance of sample x } 5}{\text{absorbance of standard}}$$

Phospholipids were assumed to contain 4 percent phosphorus, and the phospholipid content of the sample thus was obtained by multiplication of the phosphorus value by 25. The concentration of phospholipid in the total lipd was calculated from the equation:

$$\text{percent phospholipid} = \frac{\mu g \text{ phospholipid in } 20\ \mu l \text{ of extract x } 5}{\text{mg lipid / ml of extract}}$$

Lipid Fractionation and Analysis of Phospholipid Classes

Thin-layer chromatography (TLC) was used to separate neutral lipids from the phospholipids and to fractionate the phospholipids into three major classes. The preparation of the TLC plates was similar to the method described by Roberts (1966). Glass plates, 20 x 20 cm, were coated with 0.3 mm layers of silica gel G with a Camag applicator. Six plates were prepared each time from a slurry of 30 g of the silica gel and 60 ml of distilled water. After air drying for 10 min, the plates were activated at 120°C for 1 hr in an air oven. The TLC plates then were stored in a Camag drying rack and used within 48 hr.

Lipid extracts were concentrated under nitrogen so that 10-15 ml of the extract was required per spot. Initially the extent of concentration required trial and error; estimation on the basis of early samples became possible. The samples were applied to plates that had been divided into 2.1 cm channels. A plate was prepared for each muscle extracted with the two lipid extracts spotted in triplicate and one spot each of sphingomyelin, phosphatidyl-choline (lecithin), and cephalin standards. The method of developing the plates was outlined by Turkki (1965). The stainless-steel developing chamber was allowed to become saturated 1 hr with the mixture of chloroform-methanol-water in the ratio of 80:35:5 (v/v/v). The solvent was allowed to ascend the TLC plate to within 2.5 cm of the top. After air drying 10 min, the plate was sprayed lightly with a saturated solution of $K_2Cr_2O_7$ in 80 percent H_2SO_4 and heated in an air oven at 180°C for 25 min to char the spots (Blank et al., 1964).

The Photovolt densitometer with Varicord recorder and integrator was used in the analysis of the charred spots. Scanning of the lanes containing the fractionated lipid samples gave densitometer tracings that included peaks for the phospholipid classes and a record of the integrator counts. Peaks for lecithin, cephalin and sphingomyelin fractions of the samples were identified by comparison of locations of sample and standard spots on the plate. The areas under the peaks were expressed as counts by the integrator and were corrected for uneven layers of silica gel by subtraction of counts for corresponding portions of the blank plate; the counts for the blank plate were obtained by a

scanning prior to spotting. The percentages of lecithin, cephalin and sphingomyelin in the samples were estimated from the corrected areas of the individual peaks in relation to the combined corrected area of the three peaks. The expression of phospholipid fractions in terms of the phospholipids determined is recommended by Rouser et al. (1968). No attempt was made to analyze for other phospholipid classes.

Statistical Analysis

The means for the light and dark samples of each muscle were compared by use of the t-test for paired comparisons, and standard error was calculated (Steel et al., 1960). An Olivetti Programma 101 was used for analysis of the means.

CHAPTER IV

RESULTS AND DISCUSSION

The proximate composition of the light and dark portions of the eight semitendinosus muscles of pork is shown in Table I. According to Lawrie (1966) the typical adult mammalian muscle contains 75.5 percent water, 18.0 percent protein and 3.0 percent lipid; however, muscle composition is known to vary with breed, sex, age, anatomical location of the muscle, training or exercise, nutritional status, inter-animal variation and pre-slaughter conditions. The muscles in this study were from animals of the same sex and the carcass weights were within a 20 lb range. The muscles weighed from 398 to 476 g after trimming of external fat and connective tissue, which is a rather narrow range considering the lack of control over background characteristics.

The moisture content was higher ($P<0.01$) and the lipid content was lower ($P<0.05$) in the dark portion of the semitendinosus than the light. The semitendinosus light portion was more variable than the dark portion in both percent moisture and percent lipid. The light portion of samples VI and VII had markedly higher lipid and lower moisture values than the light portion of the other six muscles. The light portion is near the subcutaneous lipid area of the hog (Beecher *et al.*, 1968), and the two muscles of high fat content appeared to have large amounts of fat distributed among the muscle fibers. Such fat cannot be readily removed from the muscles prior to lipid extraction. A similar observation of high fat content was made by Taylor (1964) in a beef semitendinosus muscle.

TABLE I

PERCENT MOISTURE, LIPID AND PROTEIN OF THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

	Moisture		Lipid		Protein[a]	
Muscle	Light	Dark	Light	Dark	Light	Dark
I	74.3	77.5	6.0	3.8	18.2	15.6
II	72.2	76.7	7.9	4.4	17.9	17.2
III	74.3	76.6	3.3	3.4	21.0	18.7
IV	74.0	76.0	3.9	3.3	21.1	19.5
V	73.2	75.5	7.4	4.8	18.1	17.7
VI	67.8	75.0	12.0	4.9	18.4	15.4
VII	68.5	74.0	10.6	6.4	19.2	18.2
VIII	72.6	74.2	6.3	4.6	19.4	19.6
Mean	72.1 **	75.7	7.2 *	4.4	19.2 **	17.7
Std. Error	0.9	0.4	1.1	0.4	0.5	0.6

[a](Total N - NPN) x 6.25

**P<0.01

* P<0.05

In general the moisture content varied inversely with fat content in both portions.

The findings with respect to moisture and lipid are in agreement with those of earlier workers (Lawrie et al., 1963, 1964; Beecher et al., 1968). Beecher et al. reported the moisture content of the semitendinosus light portion to be 70.2 percent and the dark portion 75.7 percent. Considerable variation in the light was reflected by the high standard error reported. In the study by Beecher et al., the lipid content as determined by ether extraction also was higher ($P<0.05$) in the light portion than in the dark.

Higher lipid content was associated with the red skeletal muscle in previous studies of mammalian tissue (Swift et al., 1959; Bocek et al., 1966a). In a study by Allen et al. (1967), the porcine diaphragm, a red muscle, contained 10.0 percent intramuscular lipid and the longissimus dorsi, a white muscle, contained 4.6 percent lipid. The uniqueness of the semitendinosus with respect to higher lipid content in the light portion was noted by Beecher et al. (1968).

A higher ($P<0.01$) protein content was found in the light portion of the semitendinosus than in the dark portion. Protein concentration ranged from 17.9 to 21.1 in the light and 15.4 to 19.6 in the dark. The variability possibly was attributable to the nutritional history of the animals as muscle protein is affected by dietary protein depletion (Waterlow, 1969).

The amounts of nitrogen in the N-containing fractions of the semitendinosus light and dark portions are given in Table II. The lower

TABLE II

NITROGEN COMPOSITION OF THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

	Nitrogen, mg / g of meat									
	Total		Fibrillar		Sarcoplasmic		Nonprotein		Stroma	
Muscle	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
I	32.9	28.6	11.1	12.1	6.4	5.9	3.8	3.6	11.6	7.0
II	32.6	31.3	11.6	7.3	6.2	5.8	3.9	3.8	10.9	14.4
III	37.8	33.2	15.8	15.0	8.0	7.2	4.2	3.3	9.8	7.7
IV	37.6	34.8	13.9	8.1	7.6	7.3	3.8	3.6	12.3	15.8
V	32.6	31.5	13.7	13.1	6.1	6.6	3.7	3.2	9.1	8.6
VI	33.3	28.2	14.3	13.4	7.4	6.6	3.8	3.6	7.8	4.6
VII	34.8	32.6	15.9	15.2	7.6	6.6	4.1	3.4	7.2	7.4
VIII	35.5	35.2	16.4	14.2	7.3	7.2	4.5	3.9	7.3	9.9
Mean	34.6 **	31.9	14.1	12.3	7.1 *	6.6	4.0 **	3.6	9.5	9.4
Std. Error	0.8	0.9	0.7	1.1	0.3	0.2	0.1	0.1	0.7	1.4

**$P<0.01$

*$P<0.05$

($P<0.01$) total N content in dark than in light muscle reflects a difference in protein content. Lawrie et al. (1963) also reported lower total-N-values for the darker portion of the semitendinosus than the lighter portion for three weight groups of pigs, but the differences were not significant.

As seen in Table II, there was no significant difference in the fibrillar-N or stroma-N contents of the semitendinosus light and dark portions. The light portion had a higher ($P<0.05$) concentration of sarcoplasmic protein N and ($P<0.01$) NPN than did the dark portion.

The N-components expressed in terms of percent of total N are presented in Table III. There is no significant difference in any of the fractions of the light and dark samples when the N-fractions are expressed in terms of total N, which indicates that the differences observed between the light and dark portions in terms of concentration were quantitative rather than qualitative.

The stroma protein N varied greatly in both the light and dark portions whether expressed as mg per g of N (Table II) or as percent of the total N (Table III). On the basis of percent of total N the values for stroma N ranged from 20.6 to 35.3 for the light portion and 16.3 to 46.0 for the dark portion. Turkki (1965) pointed out that the variation in stroma N, which she also observed in beef, may be attributable to the method used for estimating stroma N, which is by difference. In the use of the difference method for estimation of stroma N, the errors in analysis of the other fractions contribute to the variation among the stroma values. Lawrie et al. (1963), who used hydroxyproline concentration as an

TABLE III

DISTRIBUTION OF TOTAL NITROGEN IN THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

	Nitrogen-containing fraction, percent of total nitrogen							
	Fibrillar		Sarcoplasmic		Nonprotein		Stroma	
Muscle	Light	Dark	Light	Dark	Light	Dark	Light	Dark
I	33.7	42.3	19.5	20.6	11.5	12.6	35.3	24.5
II	35.6	23.3	19.0	18.5	12.0	12.1	33.4	46.0
III	41.8	45.2	21.2	21.7	11.1	9.9	25.9	23.2
IV	37.0	23.3	20.2	21.0	10.1	10.3	32.7	45.4
V	42.0	41.6	18.7	21.0	11.3	10.2	27.9	27.3
VI	42.9	47.5	22.2	23.4	11.4	12.8	23.4	16.3
VII	45.7	46.6	21.8	20.2	11.8	10.4	20.7	22.7
VIII	46.2	40.3	20.6	20.5	12.7	11.1	20.6	28.1
Mean	40.6	38.8	20.4	20.9	11.5	11.2	27.5	29.2
Std. Error	1.6	3.5	0.5	0.5	0.3	0.4	2.1	3.8

indication of connective tissue content, reported somewhat higher values for the darker than for the lighter portion of porcine semitendinosus muscles. Both portions of the semitendinosus had higher concentration of hydroxyproline than all but one of the other six muscles they studied.

In Tables IV and V the DNA content of the semitendinosus light and dark portions is expressed as mg per 100 g of tissue and mg per g of N, respectively. Regardless of the means of expressing results, the DNA concentration was higher in the dark than in the light portion of the porcine semitendinosus. In spite of considerable variation in DNA content of each muscle portion, the difference between the light and dark portions was consistent (Table XII, Appendix).

On the basis of wet weight DNA averaged 5.8 ± 0.6 mg per 100 g of tissue in the light portion of the muscle and 8.9 ± 0.9 mg per 100 g of tissue for the dark portion (Table IV). Turkki (1965) reported average values of 13.3 and 12.8 mg of DNA per 100 g of fresh tissue for beef extensor and psoas major muscles, respectively; the difference was not significant. Smitherman's (1967) average values for chicken breast and thigh muscles, respectively, were 9.0 and 14.0 mg of DNA per 100 g of wet tissue. Both Smitherman and Turkki found wide ranges of values for the same muscle from different animals as well as for replicates of the same sample. Shchesno (1965) reported the DNA content of red muscles to be higher than the DNA content of white muscles in studies of rabbits, guinea pigs, cats and hens. Search of the literature revealed no reports of previous study of DNA content of pork muscle.

TABLE IV

CONCENTRATION OF DNA IN THE LIGHT AND DARK PORTIONS OF PORCINE SEMINTENDINOSUS MUSCLES

	DNA, mg / 100 g tissue					
	Wet weight basis		Dry weight basis		Nonlipid dry weight basis	
Muscle	Light	Dark	Light	Dark	Light	Dark
I	7.2	8.2	28.0	36.4	36.5	43.9
II	3.4	5.1	12.2	21.9	17.1	27.0
III	5.1	7.9	19.8	33.8	22.8	39.5
IV	3.4	6.3	13.1	26.2	15.4	30.4
V	6.2	9.8	23.1	40.0	32.0	49.7
VI	7.3	11.2	22.7	44.8	36.1	55.7
VII	6.2	12.2	19.7	46.9	29.7	62.2
VIII	7.4	10.6	27.0	41.1	35.1	50.0
Mean	5.8 ***	8.9	20.7 ***	36.4	28.1 ***	44.8
Std. Error	0.6	0.9	2.0	3.1	3.0	4.3

***P<0.001

TABLE V

DNA AS RELATED TO THE NITROGENOUS COMPONENTS OF THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

	DNA, mg / g of nitrogen									
	Total		Nonconnective tissue		Protein		Fibrillar		Sarcoplasmic	
Muscle	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
I	2.2	2.9	3.4	3.8	2.5	3.3	6.5	6.8	11.2	13.9
II	1.0	1.6	1.6	3.0	1.2	1.9	2.9	7.0	5.5	8.8
III	1.3	2.4	1.8	3.1	1.5	2.6	3.2	5.3	6.4	11.0
IV	0.9	1.8	1.3	3.3	1.0	2.0	2.4	7.8	4.5	8.6
V	1.9	3.1	2.6	4.3	2.1	3.5	4.5	7.5	10.2	14.8
VI	2.2	4.0	2.9	4.7	2.5	4.6	5.1	8.4	9.9	17.0
VII	1.8	3.7	2.2	4.8	2.0	4.2	3.9	8.0	8.2	18.5
VIII	2.1	3.0	2.6	4.2	2.4	3.4	4.5	7.5	10.1	14.7
Mean	1.7 ***	2.8	2.3 ***	3.9	1.9 ***	3.2	4.1 ***	7.3	8.2 ***	13.4
Std. Error	0.2	0.3	0.2	0.2	0.2	0.3	0.5	0.3	0.9	1.3

***P<0.001

Some of the variation in DNA can be attributed to the method. Both Turkki (1965) and Smitherman (1967) reported difficulties in obtaining reproducible results. Munro et al. (1969) cautions that the measurement of DNA in mammalian tissue can present certain difficulties. With the Schneider (1946) procedure, the conditions of extraction are critical. Mild conditions may leave some of the DNA in the tissue and vigorous extraction results in a risk of destruction of deoxyribose (Munro et al., 1969). In the present study it is possible that the conditions were too mild to remove all of the DNA. Large variations among animals have been reported in other studies.

The concentrations of phospholipid extracted from the two portions of the porcine muscle are presented in Table VI. The dark portion contained more ($P<0.001$) phospholipid than the light portion whether expressed on a wet weight, dry weight, nonlipid-dry weight or total lipid basis. The phospholipid content of fresh tissue ranged from 0.55 to 0.64 percent in the light portion and from 0.69 to 0.90 percent in the dark portion; the respective means were 0.61 and 0.80 percent. These values fall within the range of 0.5-0.9 for the percent phospholipid in raw pork reported in the literature (Hornstein et al., 1961; Kuchmak et al., 1963).

The average phospholipid concentration was 2.2 percent of the dry tissue in the semitendinosus light portion and 3.3 percent in the dark portion. Kaucher et al. (1943) found pork muscle to contain 3.06 percent total phospholipid on a dry weight basis, which falls between the means for the light and dark semitendinosus. On a nonlipid-dry weight basis the light semitendinosus contained 2.9 percent phospholipid and the dark 4.0 percent.

TABLE VI

PERCENT PHOSPHOLIPID OF THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

Muscle	Wet weight basis		Dry weight basis		Nonlipid dry weight basis		Total lipid basis	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
I	0.58	0.70	2.3	3.1	2.9	3.7	9.7	18.4
II	0.55	0.73	2.0	3.1	2.8	3.9	7.0	16.6
III	0.64	0.80	2.5	3.4	2.9	4.0	19.3	23.5
IV	0.64	0.69	2.5	2.9	2.9	3.3	16.3	20.7
V	0.58	0.90	2.2	3.7	3.0	4.6	7.8	19.0
VI	0.64	0.88	2.0	3.5	3.2	4.4	5.3	17.8
VII	0.62	0.82	2.0	3.2	3.0	4.2	5.8	12.7
VIII	0.60	0.86	2.2	3.3	2.8	4.1	9.5	18.5
Mean	0.61 ***	0.80	2.2 ***	3.3	2.9	***4.0	10.1 ***	18.4
Std. Error	0.01	0.03	0.1	0.1	0.05	0.1	1.8	1.1

[a]P x 25

***P< 0.001

Phospholipid content expressed as percent of total lipid was inversely related to total lipid concentration of the muscle tissue as depicted in Figure 1. The curvilinear relationship of the pork samples is similar to that of Turkki (1965) for the beef muscles although the values differ in magnitude because of the inherent differences in beef and pork. Similar relationships for beef were reported by Roberts (1966) and O'Keefe et al. (1968) and for chicken muscles by Smitherman (1967).

The higher phospholipid content of red muscles than white was reported in early studies of muscles (Needham, 1926) and was supported in more recent work (Shchesno, 1965). Allen et al. (1967) found the porcine diaphragm to contain 78.3 mg of lipid-phosphorus per 100 g of muscle whereas the white longissimus dorsi contained 39.6 mg; the two muscles differed ($P<0.01$) with respect to lipid-phosphorus. There appeared to be considerable variation in the lipid-phosphorus contents of the 10 animal carcasses even though feeding and handling were controlled.

Although both DNA and phospholipid concentrations were lower ($P< 0.001$) in light than in dark portions of the semitendinosus (Table IV, page, 46, and Table VI, page 49), when phospholipid content was expressed in relation to DNA, values were higher for the light than for the dark (Table VII). The latter difference only approached significance ($0.05< P<0.10$). With both the phospholipid and DNA content of the light portion lower than the dark portion (Table IV, page 46, and Table VI), the lack of difference between the mean ratios is not surprising.

Turkki (1965) reported the phospholipid-DNA ratio for the bovine extensor to be lower ($P<0.05$) than for bovine psoas. Whereas the

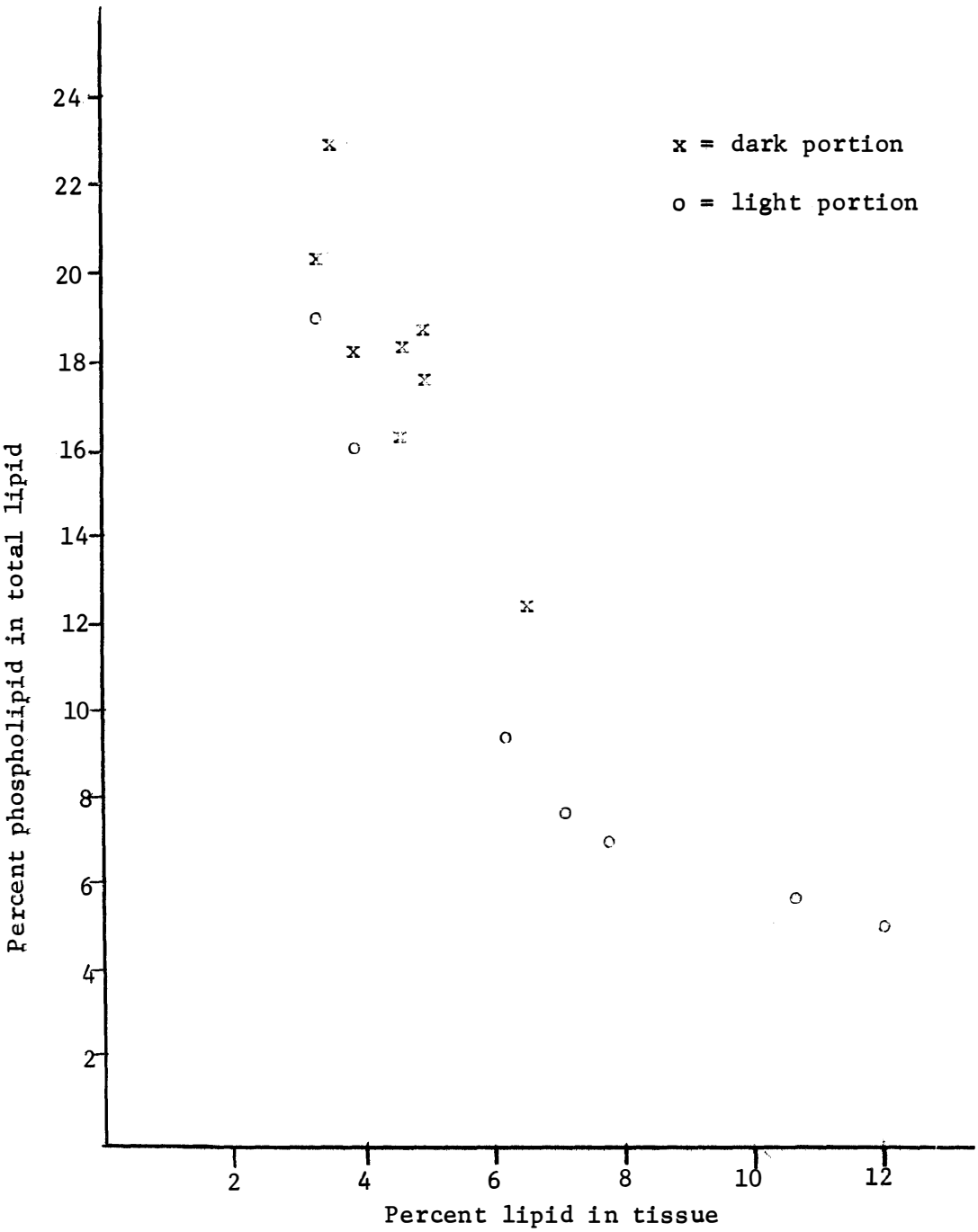

Figure 1. Phospholipid as Related to Total Lipid of Light and Dark Portions of Porcine Semitendinosus Muscles.

TABLE VII

RELATION OF TOTAL PHOSPHOLIPID[a] TO DNA, TOTAL NITROGEN AND PROTEIN NITROGEN[b] IN THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

Muscle	Phospholipid-DNA Ratio		Phospholipid, mg / 100 mg N			
			Total		Protein	
	Light	Dark	Light	Dark	Light	Dark
I	80.6	85.4	17.6	24.5	19.9	28.0
II	161.8	143.1	16.9	23.3	19.2	26.5
III	125.5	101.3	16.9	24.1	19.0	26.8
IV	188.2	109.5	17.0	19.8	18.9	22.1
V	93.5	91.8	17.8	28.6	20.1	31.8
VI	87.7	78.6	19.2	31.2	21.7	35.8
VII	100.0	67.2	17.8	25.2	20.2	28.1
VIII	81.1	81.1	16.9	24.4	19.4	27.5
Mean	114.8	94.8	17.5 ***	25.1	19.8 ***	28.3
Std. Error	14.3	8.3	0.3	1.2	0.3	1.4

[a] P x 25

[b] N precipitated by TCA

***P<0.001

phospholipid content of the two beef muscles differed ($P<0.01$), the DNA content of the two muscles was similar; therefore, differences in the phospholipid-DNA ratio would be expected.

The phospholipid content of the light portion of the semitendinosus was lower ($P<0.001$) than that of the dark portion when expressed in terms of total and protein N (Table VII). In Table VIII the phospholipid content is given in relation to some of the protein fractions. Total phospholipid was lower in the light portion whether expressed in terms of fibrillar ($P<0.01$), sarcoplasmic ($P<0.001$) or nonconnective tissue N ($P<0.001$).

Table IX shows the percentage distribution of lecithin, cephalin and sphingomyelin fractions of the light and dark portions of the semitendinosus. Differences were not found in the percentage of each fraction in the two portions; considerable variation among the eight samples is noted. The lecithin averaged 62.1 percent of the total for the light portion and 57.9 percent in the dark portion. The cephalin content averaged 27.2 percent of the total in the light samples and 31.7 percent in the dark. The average sphingomyelin content of the two muscle portions was similar with 10.7 percent in the light and 10.3 percent in the dark. Lecithin and cephalin values were within the ranges reported in the literature for porcine muscles; however, the sphingomyelin content was relatively high (Kuchmak et al., 1963; Krzywicki et al., 1967).

The phospholipid content of the fresh tissue was higher in the dark sample than the light (Table VI, page 49); thus, when expressed on the basis of wet tissue the lecithin and cephalin contents were higher

TABLE VIII

TOTAL PHOSPHOLIPID IN RELATION TO FIBRILLAR NITROGEN, SARCOPLASMIC NITROGEN AND NONCONNECTIVE TISSUE NITROGEN IN THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES [a]

	Phospholipid, mg / 100 mg N					
	Fibrillar		Sarcoplasmic		Nonconnective tissue	
Muscle	Light	Dark	Light	Dark	Light	Dark
I	52.2	57.9	90.6	118.6	27.2	32.4
II	47.4	100.0	88.7	125.9	25.3	43.2
III	40.5	53.3	80.0	111.1	22.9	31.4
IV	46.0	85.2	84.2	94.5	25.3	36.3
V	42.3	68.7	95.1	136.4	24.7	39.3
VI	44.8	65.7	86.5	133.3	25.1	37.3
VII	39.0	53.9	81.6	124.2	22.5	32.5
VIII	36.6	60.6	82.2	119.4	21.3	34.0
Mean	43.6 **	68.2	86.1	***120.4	24.3 ***	35.8
Std. Error	1.8	5.8	1.8	4.7	0.7	1.4

***P<0.001

**P<0.01

TABLE IX

PERCENT LECITHIN, CEPHALIN AND SPHINGOMYELIN OF THE PHOSPHOLIPID EXTRACTED FROM LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

Muscle	Lecithin		Cephalin		Sphingomyelin	
	Light	Dark	Light	Dark	Light	Dark
I	71.2	61.0	19.8	28.4	9.0	10.5
II	61.3	61.5	27.9	27.2	10.9	11.2
III	55.1	59.9	34.2	29.9	10.6	10.2
IV	59.8	58.2	28.3	32.8	12.0	9.0
V	63.8	47.4	26.1	38.2	10.0	14.5
VI	64.6	56.6	27.6	37.4	7.8	5.9
VII	65.3	58.6	21.2	28.4	13.5	13.0
VIII	55.4	60.2	32.7	31.4	12.0	8.3
Mean	62.1	57.9	27.2	31.7	10.7	10.3
Std. Error	1.9	1.6	1.8	1.5	0.6	1.0

[a]Based on the totals of the three fractions

($P<0.01$) in the dark than in the light tissue (Table X). The sphingomyelin content did not differ when expressed in terms of wet tissue. The table does not represent absolute concentrations within the two portions as the assumption that the phospholipid fraction contains only three components is not valid; however, the data do give some indication of the relationship between the total phospholipid concentration and the relative percentages of the major fractions.

Lecithin was the only component that was different ($P<0.05$) in the two portions when expressed in terms of DNA content (Table XI). The phospholipid-DNA ratio for lecithin of the light portion averaged 70.3 and that of the dark 55.2

Turkki (1965) in a study of two beef muscles of different anatomical location suggested that the differences in phospholipid content of the two muscles might be attributed to the distribution of red and white fibers in the muscles. From a comparison of the two beef muscles and the light and dark portions of the porcine semitendinosus, there is no clear evidence that one beef muscle corresponds to either portion of the porcine semitendinosus with respect to the attributes studied. The beef psoas and the light portion of porcine semitendinosus had lower moisture and higher lipid contents than the extensor and dark portion, respectively. The extensor and the semitendinosus light portion had higher protein contents, higher total N, lower phospholipid contents on the basis of wet weight, total N and protein N than the paired sample. The DNA content was higher in the dark porcine sample than the light, but no difference was noted between the two beef muscles.

TABLE X

LECITHIN, CEPHALIN AND SPHINGOMYELIN IN THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES, WET WEIGHT BASIS[a]

Muscle	Lecithin		Cephalin		Sphingomyelin	
	Light	Dark	Light	Dark	Light	Dark
I	0.41	0.43	0.11	0.20	0.05	0.07
II	0.34	0.45	0.15	0.20	0.06	0.08
III	0.35	0.48	0.21	0.24	0.07	0.08
IV	0.38	0.40	0.18	0.23	0.08	0.06
V	0.37	0.43	0.15	0.34	0.06	0.13
VI	0.41	0.50	0.18	0.34	0.05	0.05
VII	0.40	0.48	0.13	0.23	0.08	0.11
VIII	0.33	0.52	0.20	0.27	0.07	0.07
Mean	0.37 **	0.46	0.16 **	0.26	0.06	0.08
Std. Error	0.01	0.01	0.01	0.02	0.004	0.01

[a]In mg/100 mg tissue

**P<0.01

TABLE XI

RATIOS OF LECITHIN, CEPHALIN AND SPHINGOMYELIN TO DNA IN THE LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

Muscle	Lecithin		Cephalin		Sphingomyelin	
	Light	Dark	Light	Dark	Light	Dark
I	56.9	52.4	15.3	24.4	6.9	8.5
II	100.0	88.2	44.1	39.2	17.6	15.7
III	68.6	60.8	41.2	30.4	13.7	10.1
IV	111.8	63.5	52.9	36.5	23.5	9.5
V	59.7	43.9	24.2	34.7	9.7	13.3
VI	56.2	44.6	24.7	30.4	6.8	4.5
VII	64.5	39.3	21.0	18.9	12.9	9.0
VIII	44.6	49.1	27.0	25.5	9.5	6.6
Mean	70.3 *	55.2	31.3	30.0	12.6	9.6
Std. Error	8.2	5.6	4.6	2.4	2.0	1.3

*P<0.05

The semitendinosus functions as one muscle but has two portions of markedly different physical and chemical properties (Beecher et al., 1965b). Previous studies by Beecher et al. (1965a,b; 1968) showed some physicochemical properties of the light and dark portions of porcine semitendinosus to be similar to uniformly white and uniformly red muscle, respectively. Phospholipid and DNA concentrations were not included in the studies by Beecher et al. In the present study the light portion had lower phospholipid and DNA concentrations than the dark protion; these findings supplement the available information concerning the light and dark portions of porcine semitendinosus muscles.

Phospholipid concentration in the dark portion of the semitendinosus muscle clearly was higher than in the light portion. On the basis of these results, it would appear that phospholipid content of the light and dark portions of the porcine semitendinosus muscle was related more to metabolic activity, as indicated by fiber content, than to physical activity.

The question as to whether phospholipid content is similar in the "cells" of red and white muscles remains unsolved. Phospholipid-DNA ratios possibly would constitute more reliable evidence with the use of a method for determination of DNA that gives more reproducible results than the method used in this study. Work on methodology should be conducted.

CHAPTER V

SUMMARY

Phospholipids of the light and dark portions of porcine semitendinosus muscles were studied in relation to selected other tissue components. The semitendinosus muscles of eight sows were divided into light, dark and intermediate portions, and the light and dark portions were analyzed for total N, total lipid, total phospholipid, DNA and moisture content. The fibrillar N, sarcoplasmic N, stroma N and NPN were estimated. Phospholipids were separated into lecithin, cephalin and sphingomyelin fractions by thin-layer chromatography. The thin-layer plates were charred and the percent of each fraction in terms of the total of the three was estimated by means of a densitometer with recorder and integrator.

The average DNA content was higher in the semitendinosus dark portion than in the light regardless of the means of expressing the concentration. A wide variation among the animals was noted. The DNA content averaged 5.8± 0.6 mg per 100 g of wet tissue in the light portion and 8.9± 0.9 mg per 100 g in the dark portion. More DNA was associated with the nitrogenous components of the dark tissue than of the light.

Fresh semitendinosus light tissue contained more protein and total N than the dark tissue. Sarcoplasmic protein N and NPN were higher in the light portion than the dark when expressed in terms of mg per g tissue. Similar amounts of fibrillar and stroma protein N were associated with the two muscle portions. When the N containing fractions were

expressed in terms of total N no difference in any of the components was found between the light and dark portions of the muscle.

The dark portion of the semitendinosus had more moisture and less lipid than the light portion. The average total lipid content of the wet tissue was 7.2 percent for the light portion and 4.4 percent for the dark portion. The phospholipid content was higher in the dark than in the light portion whether expressed in terms of wet weight, dry weight, nonlipid-dry weight, total lipid, total N or protein N of the tissue.

Differences in phospholipid content per unit weight of DNA only approached significance. The ratio of phospholipid to DNA varied greatly among samples. This was the only means of expressing phospholipid content that resulted in average values slightly higher for the light portion than for the dark.

No difference was found in the percent lecithin, cephalin and sphingomyelin of the phospholipids extracted from the two portions. Lecithins accounted for about 60 percent of the total of the three fractions, cephalins for about 30 percent and sphingomyelin for about 10 percent in both portions of the semitendinosus.

From the results of this study it would appear that the phospholipid content of the light and dark portions of the porcine semitendinosus muscle was related more closely to metabolic activity, as indicated by fiber content, than to physical activity. Further investigation of phospholipid-DNA ratio in red and white muscles would be of value if a more reproducible method for DNA determination were available.

LIST OF REFERENCES

LIST OF REFERENCES

Allen, E., Cassens, R. G. and Bray, R. W. 1967. Comparative lipid composition of three porcine muscles. J. Animal Sci. 26, 36-40.

Ansell, G. B. and Hawthorne, J. N. 1964. "Phospholipids." Elsevier Publishing Co., New York.

AOAC. 1965. "Official Methods of Analysis," (10th ed.), pp. 346, 744. Assoc. Official Agric. Chemists, Washington.

Bär, U. and Blancher, M. C. 1965. Glycogen and CO_2 production from glucose and lactate by red and white skeletal muscle. Am. J. Physiol. 209, 905-909.

Bartlett, B. R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234, 466-468.

Beatty, C. H., Peterson, R. D. and Bocek, R. M. 1963. Metabolism of red and white muscle fiber groups. Am. J. Physiol. 204, 939-942.

Beecher, G. R., Cassens, R. G., Hoekstra, W. G. and Briskey, E. J. 1965a. Red and white fiber content and associated post-mortem properties of seven porcine muscles. J. Food Sci. 30, 969-976.

Beecher, G. R., Briskey, E. J. and Hoekstra, W. G. 1965b. A comparison of glycolysis and associated changes in light and dark portions of the porcine semitendinosus. J. Food Sci. 30, 477-486.

Beecher, G. R., Kastenschmidt, L. L., Cassens, R. G., Hoekstra, W. G. and Briskey, E. J. 1968. A comparison of the light and dark portions of a striated muscle. J. Food Sci. 33, 84-88.

Beecher, G. R., Kastenschmidt, L. L., Hoekstra, W. G., Cassens, R. G. and Briskey, E. J. 1969. Energy metabolites in red and white striated muscles of the pig. J. Agr. Food Chem. 17, 29-33.

Blank, M. L., Schmit, J. A. and Privett, O. S. 1964. Quantitative analysis of lipids by thin-layer chromatography. J. Am. Oil Chemists' Soc. 41, 371-376.

Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Bloor, W. R. 1927. Distribution of unsaturated fatty acids in tissues. II. Voluntary muscles of beef. J. Biol. Chem. 72, 327-343.

Bloor, W. R. 1937. Effect of activity on the phospholipid and cholesterol content of muscle. J. Biol. Chem. 119, 451-465.

Bloor, W. R. 1943. "Biochemistry of the Fatty Acids." Reinhold Publising Corporation, New York.

Bloor, W. R., Okey, R. and Corner, G. W. 1930. The relation of lipids to physiological activity. I. The changes in the lipid content of the corpus luteum of the sow. J. Biol. Chem. 86, 291-305.

Bloor, W. R. and Snider, R. H. 1934. Phospholipid content and activity of muscle. J. Biol. Chem. 107, 459-470.

Bocek, R. M., Peterson, R. D. and Beatty, C. H. 1966a. Glycogen metabolism in red and white muscle. Am. J. Physiol. 210, 1101-1107.

Bocek, R. M., Basinger, G. M. and Beatty, C. H. 1966b. Comparison of glucose uptake and carbohydrate utilization in red and white muscle. Am. J. Physiol. 210, 1108-1111.

Boivin, A., Vendrely, R. and Vendrely, C. 1948. L'acide désoxyribonucléique du noyau cellulaire, despositaire des caractéres hereditaires; arguments d'ordre analytique. Compt. Rend. Acad. Sci. (Paris) 226, 1061-1063.

Brachet, J. 1957. "Biochemical Cytology," pp. 85-89. Academic Press Inc., New York.

Brawerman, G. and Shapiro, H. S. 1962. Nucleic acids. In "Comparative Biochemistry," Vol. IV, eds. Florkin, M. and Mason, H. S., pp. 107-183. Academic Press, New York.

Briskey, E. J. 1964. Etiological status and associated studies of pale, soft, exudative porcine musculature. Adv. Food Res. 13, 89-178.

Campbell, A. M. and Turkki, P. R. 1967. Lipids of raw and cooked ground beef and pork. J. Food Sci. 32, 143-146.

Cheek, D. B. 1968. A new look at growth. In "Human Growth," ed. Cheek, D. B., p. 9. Lea and Febiger, Philadelphia.

Denny-Brown, D. E. 1929. The histological features of striped muscle in relation to its functional activity. Proc. Roy. Soc., London, Ser. B. 104, 371-411.

Deuel, H. J., Jr. 1957. "The Lipids," Vol. III. Interscience Publishers, Inc., New York.

Drummond, G. I. and Black, E. C. 1960. Comparative physiology: Fuel of muscle metabolism. Ann. Rev. Physiol. 22, 169-190.

DuPraw, E. J. 1968. "Cell and Molecular Biology," pp. 152-153. Academic Press, New York.

Epstein, C. J. 1967. Cell size, nuclear content and development of polyploidy in the mammalian liver. Proc. Nat. Acad. Sci. 57, 327-334.

Gergely, J., Pragay, D., Scholz, A. F., Seidel, J. C., Sréter, F. A. and Thompson, M. M. 1965. Comparative studies on white and red muscle. In "Molecular Biology of Muscular Contraction," eds. Ebashi, S., Oosawa, F., Sekine, T. and Tonomura, Y., pp. 145-159. Elsevier Publishing Company, New York.

Glick, D. 1961. "Quantitative Chemical Techniques of Histo- and Cytochemistry," pp. 58-59. Interscience Publishers, New York.

Gray, D. E. and DeLuca, H. A. 1956. Use of deoxyribonucleic acid as reference standard in metabolic experiments. Am. J. Physiol. 184, 301-303.

Green, D. E. and Fleischer, S. 1964. Role of lipid in mitochondrial functions. In "Metabolism and Physiological Significance of Lipids," eds. Dawson, R. M. C. and Rhodes, D. N., pp. 581-618. John Wiley and Sons Ltd., London.

Harrison, M. F. 1951. Relation between polyploidy and the amount of deoxynucleic acid per nucleus in the liver and kidney of adult rats. Nature 168, 248-249.

Hegarty, G. R., Bratzler, L. J. and Pearson, A. M. 1963. The relationship of some intracellular protein characteristics to beef muscle tenderness. J. Food Sci. 28, 525-530.

Henneman, E. and Olson, C. B. 1965. Relations between structure and functions in the design of skeletal muscles. J. Neurophysiol. 28, 581-598.

Hornstein, I., Crowe, P. F. and Heimberg, M. J. 1961. Fatty acid composition of meat tissue lipids. J. Food Sci. 26, 581-586.

Hoyle, G. 1967. Diversity of striated muscle. Am. Zoologist 7, 435-449.

Kaucher, M., Galbraith, H., Button, V. and Williams, H. H. 1943. The distribution of lipids in animal tissues. Arch. Biochem. 3, 203-215.

Krzywicki, K. and Ratcliff, P. W. 1967. The phospholipids of pork muscle and their relation to the post-mortem rate of glycolysis. J. Sci. Food Agric. 18, 252-257.

Kuchmak, M. and Dugan, L. R., Jr. 1963. Phospholipids of pork muscle tissues. J. Am. Oil Chemists' Soc. 40, 734-736.

Kuchmak, M. and Dugan, L. R., Jr. 1965. Composition and positional distribution of fatty acids in phospholipids isolated from pork muscle tissues. J. Am. Oil Chemists' Soc. 42, 45-48.

Lawrie, R. A. 1952 Biochemical differences between red and white muscle. Nature 170, 122-123.

Lawire, R. A. 1966. "Meat Science." Pergamon Press, London.

Lawrie, R. A., Pomeroy, R. W. and Cuthbertson, A. 1963. Studies on the muscles of meat animals. III. Comparative compositon of various muscles in pigs of three weight groups. J. Agric. Sci. 60, 195-208.

Lawrie, R. A., Pomeroy, R. W. and Cuthbertson, A. 1964. Studies on the muscles of meat animals. VI. Comparative composition of various muscles in boars of two weight groups in relation to hogs. J. Agric. Sci. 63, 385-386.

Lea, C. H. 1957. Deteriorative reactions involving phospholipids and lipoproteins. J. Sci. Food Agric. 8, 1-13.

Lea, C. H. 1962. Adipose tissue and animal lipids. In "Recent Advances in Food Science," Vol. I, eds. Hawthorn, J. and Leitch, J. M. Butterworths, London.

Leslie, I. 1955. The nucleic acid content of tissues and cells. In "The Nucleic Acids," Vol. II, eds. Chargaff, E. and Davidson, J. N., pp. 1-50. Academic Press, New York.

Ludewig, S. and Chautin, A. 1936. The lipid phosphorus content of hypertrophied hearts and kidneys in the rat. J. Biol. Chem. 115, 327-332.

MacLean, H. 1918. "Lecithin and Allied Substances: The Lipins." Longsmans, Green and Company, London.

Marinetti, G. V. 1962. Chromatographic separation, idenficiation and analysis of phosphatides. J. Lipid Res. 3, 1-20.

Marion, J. E. 1965. Effect of age and dietary fat on the lipids of chicken muscle. J. Nutr. 85, 38-44.

Marion, J. E. and Woodroof, J. G. 1965. Lipid fractions of chicken broiler tissue and their fatty acid composition. J. Food Sci. 30, 38-43.

Mezei, M. and Sager, R. W. 1967. Dermatic effects of nonionic surfactants. II. Changes in phospholipid and in deoxyribonucleic acid content of rabbit epidermis in vivo. J. Pharmaceutical Sci. 56, 1604-1608.

Mirsky, A. E. and Ris, H. 1949. Variable and constant components of chromosomes. Nature 163, 666-667.

Mirsky, A. E. and Ris, H. 1951. The deoxyribonucleic acid content of animal cells and its evolutionary significance. J. Gen. Physiol. 34, 451-462.

Morgan, T. E., Short, F. A. and Cobb, L. A. 1969. Effect of long-term exercise on skeletal muscle lipid composition. Am. J. Physiol. 216, 82-86.

Morrill, G. A. and Rapport, M. M. 1964. Phospholipid and plasmalogen changes during functional differentiation of adipose tissue in the newborn rat. J. Biol. Chem. 239, 740-742.

Moses, M. J. 1964. The nucleus and chromosomes: A cytological perspective. In "Cytology and Cell Physiology," ed. Bourne, G. H., pp. 423-558. Academic Press, New York.

Munro, H. N. and Fleck, A. 1969. Analysis of tissues and body fluids for nitrogenous constituents. In "Mammalian Protein Metabolism," ed. Munro, H. N., pp. 423-525. Academic Press, New York.

Nass, M. M. K. 1969. Mitochondrial DNA: Advances, problems, and goals. Sci. 165, 25-35.

Needham, D. M. 1926. Red and white muscle. Physiol. Rev. 6, 1-27.

Ogata, T. 1960. The differences in some labile constituents and some enzymatic activities between the red and the white muscle. J. Biochem., Tokyo, 47, 726-732.

Ogata, T. and Mori, M. 1964. Histochemical study of oxidative enzymes in vertebrate muscles. J. Histochem. Cytochem. 12, 171-182.

O'Keefe, P. W., Wellington, G. H., Mattick, L. R. and Stouffer, J. R. 1968. Composition of bovine muscle lipids at various carcass locations. J. Food Sci. 33, 188-192.

Ostrander, J., and Dugan, L. R., Jr. 1961. A rapid quantitative lipid extraction method. Am. Meat Inst. Foundation Bull. 50.

Paul, M. H. and Sperling, E. 1952. Cyclophorase system. XXIII. Correlation of cyclophorase activity and mitochondrial density of striated muscle. Proc. Soc. Exp. Biol. Med. 79, 352-354.

Peachey, L. D. 1968. Muscle. Ann. Rev. Physiol. 30, 424.

Roberts, K. L. 1966. The effect of cooking on the phospholipid content of lean ground beef. M. S. thesis, The University of Tennessee, Knoxville.

Rouser, G., Nelson, G. J., Fleischer, S. and Simon, G. 1968. Lipid composition of animal cell membranes, organelles and organs. In "Biological Membranes," ed. Chapman, D., pp. 5-69. Adacemic Press, New York.

Sayre, R. N., Para, J., and Briskey, E. J. 1966. Protein alterations and associated changes in porcine muscle as influenced by maturity, genetic background and post-mortem muscle temperature. J. Food Sci. 31, 819-824.

Schneider, W. C. 1946. Phosphorus compounds in animal tissues. III. A comparison of methods for the estimation of nucleic acids. J. Biol. Chem. 164, 747-751.

Seibert, F. B. 1940. Removal of the impurities, nucleic acid and polysaccharide from tuberculin protein. J. Biol. Chem. 133, 593-604.

Shchesno, T. V. 1965. Effect of strenuous exercise on content of nucleic acids and other compounds in functionally distinct rabbit muscles. Federation Proc. 24, T90-T92.

Slater, E. C. 1960. Biochemistry of sarcosomes. In "Structure and Function of Muscle," Vol. II, ed, Bourne, G. H., pp. 105-140. Academic Press, New York.

Smitherman, S. A. 1967. Relation of phospholipids to DNA in breast and thigh muscle tissue of chicken. M. S. thesis, The University of Tennessee, Knoxville.

Sréter, F. A. and Woo, G. 1963. Cell water, sodium, and potassium in red and white mammalian muscles. Am. J. Physiol. 205, 1290-1294.

Steel, R. G. D., and Torrie, J. H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Company, New York.

Stein, J. M. and Padykula, H. A. 1962. Histochemical classification of individual skeletal muscle fibers of the rat. Am. J. Anat. 110, 103-115.

Swift, C. E. and Berman, M. D. 1959. Factors affecting the water retention of beef. I. Variation in composition and properties among light muscles. Food Technol. 13, 365-370.

Szent-Györgyi, A. 1953. "Chemical Physiology of Contraction in Body and Heart Muscle," pp. 104-107. Academic Press, New York.

Taylor, L. E. 1964. Comparison of two fractionation methods in the study of muscle lipids from grain- and grass-finished beef. M. S. thesis, The University of Tennessee, Knoxville.

Terroine, E. F. 1936. Fat metabolism. Ann. Rev. Biochem. 5, 227-246.

Thomson, R. Y., Heagy, F. C., Hutchinson, W. C., and Davidson, J. N. 1953. The deoxyribonucleic acid content of the rat cell nucleus and its use in expressing results of tissue analysis, with particular references to the composition of liver tissue. Biochem. J. 53, 460-474.

Turkki, P. R. 1965. Relation of phospholipids to other tissue components in two beef muscles. Ph. D. thesis, The University of Tennessee, Knoxville.

Vendrely, R. 1955. The deoxyribonucleic acid content of the nucleus. In "The Nucleic Acids," Vol. II, eds. Chargaff, E. and Davidson, J. N., pp. 155-180.

Vendrely, R. and Vendrely, C. 1956. The results of cytophotometry in the study of deoxyribonucleic acid (DNA) content of the nucleus. In "International Review of Cytology," Vol. V, eds. Bourne, G. H. and Danielli, J. F., pp. 171-197. Academic Press Inc., New York.

Walls, E. W. 1960. The microanatomy of muscle. In "Structure and Function of Muscle," Vol. I, ed. Bourne, G. H., pp. 40-41. Academic Press, New York.

Waterlow, J. C. 1969. The assessment of protein nutrition and metabolism in the whole animal, with special reference to man. In "Mammalian Protein Metabolism," ed. Munro, H. N., pp. 326-329. Academic Press, New York.

Watts, B. M. 1962. Meat products. In "Symposium on Foods: Lipids and their Oxidation," ed. Schultz, H., pp. 202-214. The AVI Publishing Company, Inc., Westport, Connecticut.

Wittcoff, N. 1951. "The Phosphatides." Reinhold Publishing Corporation, New York.

Younathan, M. T. and Watts, B. M. 1960. Oxidation of tissue lipids in cooked pork. Food Res. 25, 538-543.

APPENDIX

TABLE XII

INDIVIDUAL VALUES[a] FOR DNA CONCENTRATION[b] IN LIGHT AND DARK PORTIONS OF PORCINE SEMITENDINOSUS MUSCLES

Muscle	Sample	Light		Dark	
		A	B	A	B
I	1	7.4	7.1	8.3	8.4
	2	7.7	7.4	7.9	8.0
	3	6.4	7.2	8.4	8.5
	av	7.2		8.2	
II	1	3.5	3.4	4.8	4.6
	2	3.5	3.3	6.5	6.2
	3	3.2	3.4	4.2	4.2
	av	3.4		5.1	
III	1	4.0	4.0	8.8	8.2
	2	5.0	5.1	6.8	6.5
	3	6.1	6.3	8.7	8.6
	av	5.1		7.9	
IV	1	2.5	2.1	6.1	6.2
	2	3.7	4.4	7.1	7.3
	3	3.7	3.9	5.1	6.1
	av	3.4		6.3	
V	1	6.6	6.9	10.5	10.5
	2	5.8	6.5	9.4	9.4
	3	5.9	5.7	9.4	9.5
	av	6.2		9.8	
VI	1	7.7	7.6	10.7	10.7
	2	7.0	6.9	11.2	10.4
	3	7.2	7.5	11.9	12.1
	av	7.3		11.2	
VII	1	6.0	6.2	12.2	11.9
	2	6.7	---	12.1	11.8
	3	6.1	6.2	13.3	11.7
	av	6.2		12.2	
VIII	1	7.2	7.2	9.9	9.8
	2	7.8	8.0	10.5	10.7
	3	7.1	7.3	11.2	11.2
	av	7.4		10.6	

[a]Triplicate tissue samples (1, 2, 3); duplicate tissue-extract samples (A, B)

[b]In mg / 100 g of wet tissue

VITA

Marian Dianne Ruff was born in Atlanta, Georgia, on June 9, 1945. She attended public schools in DeKalb, Upson and Clarke counties in Georgia and was graduated from Athens High School, Athens, Georgia, in 1963. In June, 1963, she entered the University of Georgia and received the degree Bachelor of Science in Home Economics in June, 1966.

She began graduate study at The University of Tennessee, Knoxville, in June, 1966, and received the Doctor of Philosophy degree with a major in Food Science in August, 1970. She was a graduate teaching assistant, 1966-1967; a General Foods Fellow, 1967-1969; and a National Science Foundation Summer Trainee, 1969. She is a member of Phi Kappa Phi, Phi Upsilon Omicron, Omicron Nu, the American Home Economics Association and the Institute of Food Technologists.