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I am submitting herewith a thesis written by Carla S. Hall entitled "Phospholipid and Myoglobin Concentrations in Ground Bovine Extensor, Semitendinosus, and Gastrocnemius Muscles Heated to Three End Point Temperatures." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

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PHOSPHOLIPID AND MYOGLOBIN CONCENTRATIONS IN GROUND BOVINE
EXTENSOR, SEMITENDINOSUS, AND GASTROCNEMIUS MUSCLES
HEATED TO THREE END POINT TEMPERATURES

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ABSTRACT

A possible quantitative relationship between phospholipid and extractable myoglobin, or between their rates of change during the heating of beef was investigated. Three muscles--extensor, semitendinosus, and gastrocnemius--were chosen for study at three heating end points--55°, 66°, and 77°C.

The investigated relationship was not shown statistically across the muscles or age categories represented by the samples used. The effects of several variables on the muscle components studied were analyzed individually.

Extractable myoglobin decreased as internal end point temperature increased. Increasing end point temperature also resulted in increased phospholipid concentration, dry weight basis, and total lipid, wet weight basis. Neither myoglobin nor phospholipid concentrations differed significantly among muscles. Total lipid, on the wet and the dry weight basis, was significantly influenced by muscle type. Animal age did not appear to affect myoglobin concentration. Muscles from the older animal appeared to have a greater total lipid and a lower phospholipid content than those from the younger animal.

Phospholipids were fractionated into classes and analyzed for lecithin, cephalin, and sphingomyelin. Heat treatment, muscle, or animal did not affect phospholipid fractions.

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CHAPTER I

INTRODUCTION

The impact of phospholipids on other cellular components in biological systems is tremendous in relation to their prevalence (Green and Perdue, 1966). Although comprising less than one percent of the muscle, phospholipids are involved in enzyme activity and regulation, triglyceride transport, and movement of charged and uncharged molecules through biological membranes (Parks, 1971). Research involving phospholipids, because of their diversity of reactions and functions, might aid in understanding interactions in the intact and excised muscle.

Reports of studies of the chemistry and relationships of phospholipids with other muscle components have involved mainly raw muscle (Ruff, 1970). Increased consumer preference for prepared meat products has created a need for research to alleviate problems underlying the processing and marketing of cooked meats. Data on the qualitative and quantitative changes of phospholipids during heating are lacking (Keller and Kinsella, 1973) although phospholipids are among the more labile constituents of food (Lea, 1957).

Color of meat is a factor in judging the degree of cookery to which the meat has been subjected. The major component of muscle color, myoglobin, although the subject

of intense research, still presents qualitative and quantitative control problems in meat cookery. The existence of a relationship between myoglobin and phospholipids in raw beef was suggested by Beecher et al. (1965). Melton (1973) obtained evidence of myoglobin-phospholipid interactions during the heating of reaction systems.

Research has not been reported concerning interactions between myoglobin and phospholipids in cooked meat. However, from the raw to the done stage of cookery myoglobin is rendered less extractable (Draudt, 1969) whereas phospholipid concentration has been reported to increase (Roberts, 1966; Campbell and Turkki, 1967; Lackey, 1973). Although heat-induced changes in phospholipid concentration and in water-extractable myoglobin have been studied individually, they have not been reported for the same samples.

A possible quantitative relationship between phospholipid and extractable myoglobin, or between their rates of change, during the heating of beef was investigated in this study.

CHAPTER II

REVIEW OF LITERATURE

A. MYOGLOBIN

Consumers buy meat by its color (Jeremiah et al., 1972) which is directly related to the amount and form of myoglobin in the muscle (Draudt, 1969). In well-bled muscle myoglobin and its derivatives may be responsible for 90% of the tissue color (Watts, 1954).

Structure and Chemistry

Myoglobin is a protein of the sarcomere belonging to the tetrapyrrole family of which it is a heme derivative. The isolated heme group of myoglobin consists of an iron atom and four heterocyclic pyrrole rings linked by methene bridges (Clydesdale and Francis, 1971). A protein, globin, consisting of 153 amino acid residues is compacted into a space $45 \times 35 \times 25 \text{ \AA}$ by folding around the heme group (Kendrew, 1963). The amino acid composition of myoglobin differs from that of hemoglobin in that myoglobin contains isoleucine but no cystine (Schweigert, 1956). The complex of heme and globin gives stability to the total myoglobin molecule which neither moiety could retain alone.

In the live animal myoglobin contains 10% of the total iron. Oxymyoglobin (MbO_2) is formed by oxygenation, the

complexing of myoglobin with the oxygen required for cellular metabolism. In this way myoglobin acts as a retaining site for oxygen in the body (Clydesdale and Francis, 1971). The cyclic conversion of myoglobin to MbO_2 and metmyoglobin (MMb) is described by Fox (1966).

Post-Mortem Changes

Post-mortem changes in the pigment depend on a number of factors related to pre- and post-slaughter treatment of the animal (Munns and Burrell, 1966). Initially after the muscle is cut, MbO_2 may be converted to MMb by oxidation of iron from the ferrous to the ferric state (Fox, 1966). For acceptable retention of color, an equilibrium must be established between red myoglobin and brown MMb (Hutchins et al., 1967).

Several factors are important to the establishment of such an equilibrium. In 1938, Brooks related the rate of color change to reduced respiratory activity of the meat. Lowering oxygen pressure below 6mm Hg increases the rate of meat discoloration (Ledward, 1970). Oxygen also can cause lipid oxidation producing free radicals which oxidize the heme protein of MMb (Greene et al., 1971).

Brooks (1938) and Ledward (1971) have suggested that oxygen pressure is not as important to color change as is excessive loss of water from the meat. Loss of water causes a proportional increase in the salt concentration. When

the salt concentration is increased the rate of meat discoloration increases (Hutchins et al., 1967).

Factors affecting meat discoloration will alter meat color only to the extent that they affect total pigment content of the muscle (Hegesh and Avron, 1967). Initial pigment concentration, therefore, must be considered. Wide variation in the amount of myoglobin has been reported for samples subjected to the same treatments (Ledward, 1971). The breed and sex of the animal tested may influence initial pigment concentration (Briskey and Kauffman, 1971; Satterlee and Zachariah, 1972). DuFresne (1964) found differences in myoglobin concentration among individual animals. Rickansrud and Henrickson (1967) found initial levels of myoglobin to differ among four bovine muscles from the same animal. Franke and Solberg (1971) reported quantitative differences in myoglobin among samples taken from the same muscle. Pigment concentration also varies with species (Lawrie, 1950; Brown et al., 1961), size (Brabkin, 1950), age and level of exercise (Shenk et al., 1934; Romans et al., 1965).

Although myoglobin in meat is in free solution (Snyder, 1965), protein solubility and, therefore, amount of extractable myoglobin, is dependent on muscle composition (Aberle and Merkel, 1966). The molecular weight of myoglobin often is found to be greater than 17,000 (Kendrew, 1963) when estimated from water extracted myoglobin solutions. The

sedimentation constants for extracts of fresh meat myoglobin also have been found to be large in comparison to those for crystalline beef metmyoglobin. Large sedimentation constants may result from association of myoglobin with other proteins in the fresh meat extract, causing an apparent increase in molecular weight (Bernofsky et al., 1959).

Other changes in the amount and characteristics of myoglobin in raw muscle were attributed to unnatural treatments or improper handling of the meat (Solberg and Franke, 1971). Extreme lighting conditions may affect myoglobin, though the pigment in muscle is not changed in the normal range of illumination (Stewart et al., 1965). In the pH range 4.8 to 6.5 characteristic of most muscle tissue, myoglobin is stable (Satterlee and Zachariah, 1972). The effect of temperature on myoglobin is not evident in the range from -3°C to 30°C (Siedler and Schweigert, 1959). Changes in pigment characteristics unlike those caused by low oxygen pressure, lack of oxygen, lipid oxidation, loss of water, or inherent characteristics of the muscle indicate improper handling or treatment of the meat (Draudt, 1969).

Heat Treatment

Color changes, occurring continuously during application of heat, are caused by denaturation of the protein component of myoglobin (Bernofsky et al., 1959; Hamm, 1966). As a protein coagulates with heating it no longer can be extracted

(Laakkonen et al., 1970a). As the temperature is increased the amount of extractable myoglobin decreases (Draudt, 1969; Brown and Tappel, 1958). Along with globin denaturation iron is oxidized from the ferrous to the ferric state forming denatured globin hemichrome (Schweigert, 1956).

The temperature at which conversion of myoglobin to denatured hemichrome is completed has not yet been established. Irreversible denaturation of myoglobin has been reported by Hofman and Hamm (1969) to occur between 30° and 70°C, and by Siedler and Schweigert (1959) from 50° to 80°C. However, Quinn et al. (1964) stated that myoglobin is not noticeably altered by heating at 55°C for 5 minutes. Using disc gel electrophoresis, Laakkonen et al. (1970b) found that extractable myoglobin was not altered significantly at 60°C unless held for long periods of time.

Pigment denaturation in cooked meat has been attributed partially to coprecipitation of myoglobin with other proteins (Hamm, 1966; Draudt, 1969). The temperature range of coprecipitation is lower than the temperature of myoglobin precipitation in pure medium (Ledward, 1971). Hamm (1966) points out that such findings indicate a need for study of the effects of heat on myoglobin in the muscle tissue itself so that possible interactions among myoglobin and other muscle constituents may be studied.

Measurement

Franke and Solberg (1971) reported a method for measuring the amount of myoglobin on the surface of intact meat samples by use of reflectance spectrophotometry. The fractionation of myoglobin into electrophoretically mobile subfractions was reported by Quinn (1973). However, for the purpose of measuring total myoglobin in muscle tissue water extraction has been reported to be the most suitable method (Melton, 1973).

B. PHOSPHOLIPIDS

Phospholipids are thought to occur in every cell of the living organism (MacLean, 1918). They react both hydrophobically and hydrophilically in the performance of metabolic functions in the live animal (Lea, 1957; Parks, 1971). Their chemistry, function, and distribution have been investigated extensively in biological systems (Green and Perdue, 1966; Green and Tzagsloff, 1966).

After investigating several animal species, Bloor (1937) reported that tissues of a given category contained a relatively specific proportion of phospholipids. Phospholipids for a given muscle were determined to be relatively constant in four beef animals (Hornstein et al., 1961). In three test muscles O'Keefe et al. (1968) reported concentrations approximating 500 mg of phospholipid in 100 g of beef tissue.

Campbell and Turkki (1967) obtained similar results in raw beef and recorded concentrations of 680 mg of phospholipid in 100 g of pork tissue. Examination of beef, lamb, pork, and fish have revealed that phospholipid concentration ranges from 0.3 to 1.0% of the wet tissue (Dawson, 1968; Peng and Dugan, 1965; Harvey, 1965; Kuchmak and Dugan, 1963). As percentage of lipid increases in raw muscle the proportion of phospholipid in raw muscle lipid decreases (O'Keefe et al., 1968; Campbell and Harrill, 1971).

Type of finish was not found to influence concentrations of lipid components in grass or grain finished beef when phospholipids were expressed as a percentage of lipids (Nutt, 1963). Keller and Kinsella (1973) found phospholipid content of hamburger meat to increase as total lipid decreased. Link et al. (1967) found that increasing age and increasing total body weight did not influence phospholipids when expressed as percentage of meat, wet weight basis.

Heat Treatment of Phospholipids

Among the environmental factors which operate to change phospholipid concentration and composition, heat treatment is of major importance to the consumers of meat products.

Phospholipid concentration in cooked meat products varies with the method of heat application. Pan frying beef patties in a teflon skillet tended to increase total phospholipids but charcoal broiling or pan frying in a metal

skillet tended to decrease them (Keller and Kinsella, 1973).

Apparent effects of cooking on phospholipid concentration may vary with the method of expressing data, as well as with total lipid content of the meat. When beef containing large amounts of adipose tissue was heated, the total phospholipid concentration was larger in oven cooked patties than in raw ground beef whether expressed on a wet or a dry weight basis or as a percentage of total lipids (Campbell and Turkki, 1967). When very lean beef was studied, phospholipid concentration expressed on the dry weight basis or as a percentage of total lipid showed no change attributable to cooking; whereas expression on the wet weight basis showed phospholipid to increase with cooking (Roberts, 1966). Smith et al. (1972) stated that differences between raw and cooked phospholipid concentration apparently reflect changes in moisture and neutral lipids more than quantitative changes in phospholipids.

C. MYOGLOBIN-PHOSPHOLIPID INTERACTIONS

Lipid-protein complexes occur naturally in mammalian tissue (Giam and Dugan, 1965) with phospholipids being cited as one of the major links between biological lipids and proteins (Dawson, 1968). Phospholipids also have been demonstrated to interact with flour proteins (Pomeranz et al., 1966; Fullington, 1969) and with flavor constituents

of meat (Scheide, 1969) although the mechanisms of these reactions have not been investigated thoroughly.

A relationship between myoglobin and phospholipids was suggested by Beecher et al. (1965). In heated emulsions Melton (1973) found that interactions between phospholipids and myoglobin affected the heat precipitation of myoglobin. Phospholipids interacted with isolated myoglobin to destabilize the myoglobin to heat precipitation. Phospholipid-myoglobin interactions in sarcoplasmic protein extract resulted in destabilization of myoglobin at 65°C but protection against further destabilization at 77°C. Research on phospholipid-myoglobin interactions in meat tissue has not been reported.

CHAPTER III

PROCEDURE

Selection and Preparation of Meat Samples

Semitendinosus, gastrocnemius, and extensor carpi radialis muscle pairs were excised from two beef animals, one at a time at a local meat packing plant. Breed and pre-slaughter history were unknown. However, there was a noticeable difference in the age of the animals as indicated by overall carcass size, external fat cover, and relative amount of muscle mass. Selection of muscles was based on differences in initial phospholipid concentration (Turkki, 1965). The muscles were excised approximately 24 hr after slaughter and placed immediately in crushed ice.

The muscles were trimmed of external fat, large deposits of intramuscular fat, and loose connective tissue. Each muscle pair was ground in the grinding attachment of a Hobart N-50 mixer. Discs with $3/8$ and $3/16$ in. openings were used once each and a disc with $1/8$ in. openings was used for the two final grindings. Crushed ice for the meat containers and dry ice for the grinding equipment were used to keep the meat temperature below 10°C .

Ground meat was divided into 135 g portions, double wrapped in medium weight aluminum foil, and flushed with nitrogen during closure. Samples were stored at -20°C

Cooking of Meat

Frozen meat samples were thawed in the refrigerator (4°C) for 18 hr and thaw drip was reincorporated into each sample. Meat then was shaped into 130 g patties, 8.4 cm in diameter and 1.25 cm high. Three patties from each muscle sample were cooked, each on a wire rack over a small glass pie pan, in a Despatch electric oven equipped with a rotating hearth and heated to 177°C. One patty was removed when it reached an internal temperature of 55°C, another at 66°C, and the third at 77°C.

Cooked patties were allowed to cool on the wire racks for 1 hr. The cooking drip was reincorporated into each patty and homogeneity of samples obtained by blending for 2 min. in a Waring Blendor operated through a rheostat set at 35.

Moisture Determination

Triplicate 3-5 g samples from each ground patty were dried in weighed aluminum pans for 18 hr in an air oven at 100°C (AOAC, 1965). Dried samples were covered, cooled for 1 hr in a desiccator, and weighed. Samples were returned to the oven for 1 hr, re-cooled, and reweighed to ascertain that constant weight had been attained. Percentage dry weight was calculated (Table V, Appendix).

Separation of Water Soluble Proteins

Duplicate 20 g samples of each ground patty were transferred to 50 ml Erlenmeyer flasks and extracted with 10 ml of 0.2M potassium chloride and 0.01M potassium phosphate (pH 7.4) for 24 hr (Dikeman et al., 1971). All samples then were transferred to 50 ml polyethylene centrifuge tubes and centrifuged for 20 min at 4500 x G at 4°C in a Lourdes Model A-2 Beta-fuge refrigerated centrifuge with a 9RA head. The supernatant fluid was filtered through Whatman #42 filter paper and saved. The muscle residue was re-extracted for 2 hr with 10 ml of the same buffer, then recentrifuged. The supernatant fluids from the two extractions were combined.

Myoglobin Extraction and Determination

The pH of the extract was adjusted to 7.0 with a pH 7.4 phosphate buffer (50 ml of 0.1M KH_2PO_4 plus 32.1 ml of 0.1M NaOH) (Weast, 1966). A basic lead acetate solution¹ equal to 0.25 the volume of the supernatant fluid was added to precipitate proteins other than myoglobin and hemoglobin. The precipitate was removed by centrifuging (2000 x G at 4°C for 15 min) and filtering through Whatman #1 filter paper

¹Fisher Scientific basic lead acetate (stated PbO content 32.7%) was added in the amount of 5.55g/100ml of distilled, demineralized water; the mixture was stirred for 1 hr, then allowed to stand for 24 hr and restirred before use. Specific gravity of the solution was determined to be 1.0395. Enough solution was made at one time for the entire experiment. The solution was agitated, allowed to stand 24 hr, and restirred before each use.

(Rickansrud and Henrickson, 1967).

Hemoglobin was separated from the myoglobin by combining one volume of the hemoglobin-myoglobin solution with two volumes of 4.5M phosphate² to bring the pH to 6.6 and phosphate concentration to 3M. The precipitate formed on stirring was removed by centrifuging at 12,800 x G at 4°C for 15 min. The resulting solution was filtered through Whatman #1 filter paper (Melton, 1973).

Potassium ferricyanide (2 mg) and sodium cyanide (0.4 mg) were added to the reagent blanks and the myoglobin samples, forming cyanometmyoglobin (Drabkin, 1950). The absorbance (A) of the samples was determined in a Bausch and Lomb Spectronic 20 colorimeter (a direct reading grating spectrophotometer with a cell of 1.17 cm light path length) set at 540 nm. The instrument was zeroed with the reagent blanks.

Concentration of myoglobin was calculated from the photometric data by a method described by Nocito (1972)³.

$$\frac{A \times \text{Vol extract (liters)} \times 1000 \text{ mg/g} \times 17,000 \text{ g/Mole}}{\frac{11.3 \text{ mM/l}}{1.17 \text{ cm}}} \times \text{Weight of meat samples (g)}$$

²393 g of K₂HPO₄ and 306 g of KH₂PO₄ were added to 1000 ml of demineralized, distilled water, heated to 50°C with stirring then cooled to 35°C before use. The same solution was used throughout the experiment with heating and cooling before each use.

³The cyanometmyoglobin extinction coefficient of 11.3 mM/l using a light path of 1.00 cm (Drabkin, 1950) was adjusted since the Bausch and Lomb Spectronic 20 has a light path of 1.17 cm. The molecular weight of myoglobin was assumed to be 17,000 as suggested by Kendrew (1963).

Lipid Extraction

Forty grams of each of the muscle samples from each heating temperature were extracted by the method of Bligh and Dyer (1959) as modified by Ostrander and Dugan (1961). The extraction mixture separated into layers during a holding period of 24 hr at 4°C. After the volume of the chloroform extract (lower layer) was recorded, the extract was transferred to a 500 ml separatory funnel and again allowed to separate into layers. The lower chloroform layer was drained into an evaporating flask.

After thorough mixing of the chloroform-lipid solution, triplicate 10 ml portions were pipetted into three preweighed 30 ml beakers and allowed to evaporate for 24 hr. The solvent was evaporated in a vacuum desiccator under reduced pressure until constant weight was attained.

$$\begin{array}{l} \% \text{ lipid} \\ \text{(wet} \\ \text{weight)} \end{array} = \frac{10 \text{ (ml chloroform extract} \times \text{g lipid/10 ml extract)}}{\text{grams of muscle extracted}}$$

The remaining extract was concentrated on a rotary evaporator to a volume of 30-50 ml, transferred to a 125 ml Erlenmeyer flask with chloroform and stored under nitrogen at -20°C until used for phosphorus determination.

Simultaneously with sampling for phosphorus determination, the concentrated extract was sampled for determination of total lipid in the concentrated extract. This permitted determination of phospholipid as a percentage of the

total lipid. Triplicate 1 ml samples of each concentrated lipid extract were pipetted into preweighed 30 ml beakers. Excess chloroform was evaporated under the hood for 24 hr. Samples then were placed in a vacuum desiccator under reduced pressure until constant weight was attained. Weight of lipid in 1 ml concentrated extract was determined by difference.

Phosphorus Determination

The method of phosphorus analysis for estimation of phospholipids was that of Bartlett (1959) as modified by Marinetti (1962). Glass ware was acid washed and rinsed with distilled water then demineralized water before each use.

A preliminary standard curve was prepared with 0.2-1.0 ml of dibasic potassium phosphate solution ($5\text{ }\mu\text{g}$ of P/ml). Within the phosphorus range the curve was a straight line and only one concentration of phosphorus standard was carried through the sample analyses. For each pair of muscles all samples from the three internal temperatures were analyzed at once. One milliliter of each concentrated lipid extract was diluted with chloroform to contain 1-5 μg of phosphorus per 10 μl . Triplicate 10 μl portions of the diluted extract, duplicate aliquots of phosphorus standard solution, each containing 5 μg of P, and duplicate reagent blanks were analyzed. Demineralized water was added to

bring the total volume to 1 ml in 30 ml micro-Kjeldahl flasks; then 1.2 ml of 70% perchloric acid and two glass beads were added. Samples were digested for 30 min on a micro-Kjeldahl digestion rack then allowed to cool for 20 min at room temperature. After the inner walls of the flasks were washed down with 7 ml of demineralized water, 1.5 ml of 2.5% ammonium molybdate solution and 0.2 ml of Fiske Subba Row reagent⁴ were added. The flasks were heated in a boiling water bath for 7 min then cooled for 20 min at room temperature. Absorbance of the samples was determined with a Bausch and Lomb Spectronic 20 colorimeter equipped with a red filter and a red sensitive phototube used at a wavelength of 830 nm. The instrument was zeroed with the reagent blanks. Phospholipid concentration was calculated as percentage of extracted lipid, of wet tissue, and of dry matter according to the equations in the Appendix.

Lipid Fractionation

Phospholipids were separated from neutral lipids and fractionated into three major classes by thin layer chromatography (TLC). Procedures for preparation of TLC plates were those of Wood and Kinsell (1963). Acid washed glass plates, 20 x 20 cm, were coated with 0.3mm layers of silica

⁴Fiske Subba Row Reagent: Combine 3.75 g sodium bisulfite with 25 ml demineralized water; add 0.0635 g 1-NH₂ naphthol-4-sulfonic acid; add 0.1250 g anhydrous Na₂SO₃; filter and store at 4°C. Make fresh weekly.

gel G with a Camag applicator. Thirty grams of silica gel mixed with 59 ml of distilled water in a mortar were sufficient to coat four plates. The plates were air dried for 10 min then activated at 120°C for 1 hr in an air oven. TLC plates were stored in a Camag drying rack and used within 48 hr.

The plates were divided into 2.1 cm channels for sample application. Concentration of the lipid extracts under nitrogen allowed sufficient spotting with 10-15 μ l of the extract. The extract was applied as several drops dried under nitrogen between applications. Two lipid extracts were spotted in triplicate on each plate leaving three channels for use as blanks. Initially plates were prepared for sphingomyelin, phosphatidyl-choline (lecithin), and cephalin standards in order to determine their TLC-densitometric patterns. Spotted plates were placed in a stainless steel developing chamber which had been saturated with a mixture of chloroform-methanol-water in the ratio of 80:35:5 (v/v/v). The TLC plates were removed from the chamber when the solvent had moved to within 2.5 cm of the top. Plates air dried for 10 min were sprayed with 50% (v/v) sulfuric acid. Spots were charred by heating the plates for 25 min at 180°C in an air oven.

Analysis of Phospholipid Classes

Determination of the concentrations of the three phospholipid classes studied was accomplished by a densitometric

procedure described by Morrison and Campbell (1971). The charred spots in each channel of the TLC plates were scanned with a Photovolt densitometer equipped with a Varicord recorder and integrator. Peaks for the three phospholipid classes and corresponding integrator counts under the peaks were graphically recorded. Peaks representing phospholipid classes in the samples were identified by comparison with curves for sphingomyelin, lecithin, and cephalin standards. Integrator counts of the areas under the peaks for the three phospholipid classes were totaled for each sample. The percentage of each phospholipid class present in the sample was obtained by dividing specific integrator counts by the total integrator counts for the phospholipids in each spotted sample. Neutral lipids and the traces of lysolecithin were not analyzed.

Statistical Procedure

Relationships between myoglobin and phospholipids at three test temperatures were to have been determined for each muscle by three dimensional coordinate point mapping (Franzblau, 1958). A diagrammed three axis plan was designed with which data were to be analyzed. One of the mapping axes was defined by fixing internal end point temperature-- 55°, 66°, and 77°C-- based on research by Melton (1973) and Hofman and Hamm (1969). A second axis was designated for phospholipid concentration. In order to map a large

surface area, muscles were selected which were expected to have noticeable differences in initial phospholipid concentration (Turkki, 1965). Myoglobin concentration, represented by the third axis, was not a basis for muscle selection in order to provide a random variable for surface contour definition.

After data collection, analysis by a special computer mapping program would have selected the proper surface to describe changes in myoglobin and phospholipid concentrations over the three end point temperatures. Such a map also would have postulated statistically the actions and interactions of the two muscle components between the end point temperatures selected. As will be discussed in the succeeding chapter, the data obtained indicated that a complexity of relationships exists, preventing mapping under the conditions of this study.

In an attempt to identify possible sources of variation three-way analyses of variance were performed to separate muscle, animal, temperature, and interactions components for each of the muscle constituents studied.

CHAPTER IV

RESULTS AND DISCUSSION

A. RESULTS

Extractable Myoglobin

The concentration of myoglobin extracted from the three muscles cooked to varying internal temperatures is shown in Table I. The analysis of variance indicated that heat was the only factor that influenced extractable myoglobin concentration ($P < 0.005$, Table VI, Appendix). Extractable myoglobin decreased as end point temperature increased for each individual muscle (Table I). Whereas Hamm (1966) reported decreased myoglobin solubility primarily between 40° and 60°C, the data of the present study reflect major denaturation above 66°C as was observed by Hofman and Hamm (1969).

Muscle, animal, and muscle-animal interactions did not affect myoglobin in this study. However, Rickansrud and Henrickson (1967) found variation among muscles and Knoch (1970) and Briskey and Kauffman (1971) reported significant increases in myoglobin with increasing age. DuFresne (1964) and Stewart et al. (1965) also found differences of 4% or more between muscles of individual animals.

Total Lipid

Total lipid concentration, disregarding end point temperature, fell in the range of 1 to 5%, wet weight basis

Table I

Percent Myoglobin, Dry Weight Basis, Extracted from Ground Extensor,
Semitendinosus, and Gastrocnemius Muscles
Heated to Three End Point Temperatures^a

Muscle Name	Animal Number ^b	Internal Temperature, °C		
		55	66	77
Extensor	1	0.60	0.53	0.05
	2	0.40	0.30	0.05
Semitendinosus	1	0.30	0.14	0.05
	2	0.41	0.27	0.04
Gastrocnemius	1	0.48	0.35	0.09
	2	0.38	0.22	0.05

^aEach value is an average of two determinations.

^b1 represents the older animal; 2 represents the younger animal.

(Table II). Data are similar to results obtained with cooked lean beef by Campbell and Turkki (1967). Smith et al. (1972) found similar concentrations of total lipid in raw lean meat with extensor ranging from 1.6 to 2.4%, semitendinosus 3.4 to 4.7%, and gastrocnemius 3.0 to 5.2%.

Total lipid concentration was significantly affected by animal ($P<0.01$) and by muscle ($P<0.01$) (Table VI, Appendix). The analysis of variance indicated an interaction between muscle and animal ($P<0.01$, Table VI, Appendix). For each individual muscle, total lipid, expressed on the wet weight basis, appeared to increase as internal end point temperature increased. However, the analysis of variance indicated that heat did not affect total lipid concentration significantly. When total lipid was expressed on the dry weight basis, significant differences were not found between animals, among muscles, or among end point temperatures.

Phospholipids

The phospholipid concentrations expressed as a percentage of total lipid and on the dry weight basis are presented in Table III. Analysis of variance indicated no significant variation in phospholipid concentration among the muscles. However, an interaction between muscle and animal was shown ($P<0.01$, Table VI, Appendix).

Phospholipid concentration, on the dry weight basis, was affected significantly by internal end point temperature

Table II

Percent Total Lipid Extracted from Ground Extensor, Semitendinosus,
and Gastrocnemius Muscles Heated to Three End Point Temperatures^a

Muscle Name	Animal ^b Number	Internal Temperature, °C					
		Wet Weight Basis			Dry Weight Basis		
		55	66	77	55	66	77
Extensor	1	1.4	1.7	1.9	5.7	5.8	5.5
	2	2.0	2.3	2.6	7.7	5.8	8.0
Semitendinosus	1	3.0	4.2	4.8	11.2	15.5	13.6
	2	1.8	1.9	2.3	8.9	8.8	7.5
Gastrocnemius	1	2.0	2.4	3.2	8.1	9.9	11.1
	2	1.7	1.8	2.3	6.9	6.9	7.4

^a Each value is an average of three determinations.

^b 1 represents the older animal; 2 represents the younger animal.

Table III

Percent Phospholipid Extracted from Ground Extensor, Semitendinosus, and Gastrocnemius Muscles Heated to Three End Point Temperatures^a

Muscle Name	Animal Number ^b	Percent of Extracted Lipid			Internal Temperature, °C			Percent of Muscle, Dry Weight	
		55	66	77	55	66	77	55	66
Extensor	1	12.4	20.7	23.7	0.7	1.1	1.3		
	2	28.6	30.3	32.0	2.2	2.4	2.6		
Semitendinosus	1	4.8	7.0	10.2	0.5	1.1	1.4		
	2	26.1	31.9	32.8	1.8	2.2	2.4		
Gastrocnemius	1	16.4	17.0	23.8	1.3	1.6	2.6		
	2	13.7	20.0	19.6	0.6	1.3	1.5		

^aEach value is an average for three determinations.

^b1 represents the older animal; 2 represents the younger animal.

($P < 0.005$, Table VI, Appendix). Data indicate an apparent increase in amount of phospholipid as internal temperature increased from 55° to 66° to 77° C. These data are in agreement with increases in phospholipid concentration with heating reported by other workers (Roberts, 1966; Campbell and Turkki, 1967).

Animal also affected phospholipid concentration, on the dry weight basis and as a percentage of total lipid, ($P < 0.01$, Table VI, Appendix). Except in gastrocnemius, meat from the younger animal had a higher phospholipid content, dry weight basis, than that from the older animal at each heating temperature. This difference may reflect level of muscular activity (Bloor, 1937) since age has not been shown to affect phospholipid concentration (Link et al., 1967).

Phospholipid concentrations in total lipid from 4.8 to 32.8% were similar to values reported by Smith et al. (1972) for raw lean semitendinosus, gastrocnemius, and extensor. Expressed as a percentage of tissue on the dry weight basis, data were similar to those reported by Smith et al. (1972) for all three muscles and by O'Keefe et al. (1968) for semitendinosus.

Phospholipid Classes

Phospholipid class data are presented in Table IV. Lecithin ranged from 51 to 67%, cephalin 22 to 38%, and sphingomyelin 8 to 18% of phospholipids analyzed. Similar

Table IV

Percent Distribution of Phospholipids among Lecithin, Cephalin, and Sphingomyelin in Ground Extensor, Semitendinosus, and Gastrocnemius Heated to Three End Point Temperatures^a

Muscle Name	Animal Number ^b	Internal Temperature, °C								
		Lecithin			Cephalin			Sphingomyelin		
		55	66	77	55	66	77	55	66	77
Extensor	1	53.2	58.5	66.7	38.4	29.6	23.4	8.5	11.9	10.0
	2	52.4	57.6	52.1	38.5	39.7	32.5	9.2	9.9	15.4
Semitendinosus	1	64.9	65.1	64.6	21.7	22.6	27.0	13.4	12.3	8.6
	2	65.4	58.6	62.5	21.7	31.0	23.4	12.9	10.4	14.2
Gastrocnemius	1	58.2	60.4	51.0	25.6	27.2	33.5	16.2	12.4	15.5
	2	54.1	58.3	47.2	28.2	25.1	37.1	17.7	16.6	15.7

^aEach value is an average of three determinations.

^b1 represents the older animal; 2 represents the younger animal.

values were found in other studies (Parks, 1971; Keller and Kinsella, 1973). Phospholipid classes were unrelated to heat treatments, muscles, or animals.

Higher lecithin and lower cephalin levels reported in the studies of Roberts (1966) and Lackey (1973) for cooked meat in comparison to raw meat might have been caused by loss of cephalin in the cooking drip. However, since drip was reincorporated into the meat before sampling in this research, cooking did not appear to affect relative proportions of lecithin and cephalin over the three end point temperatures studied.

B. DISCUSSION

Originally the study was planned to investigate the possibility of a heat response relationship between myoglobin and phospholipid concentrations. When milligrams of myoglobin were plotted against milligrams of phospholipid in one gram of meat tissue, dry weight basis, decreases in myoglobin concentration, indicated by decrease in height of the temperature curves, occurred with increasing internal temperature (Figure I). Increases in phospholipid concentration are indicated by shift to the right of the temperature curves. For the 55° and 66° C curves, there was a tendency for myoglobin to decrease as phospholipid concentration increased with increasing end point temperature. However, when the attempt was made to analyze

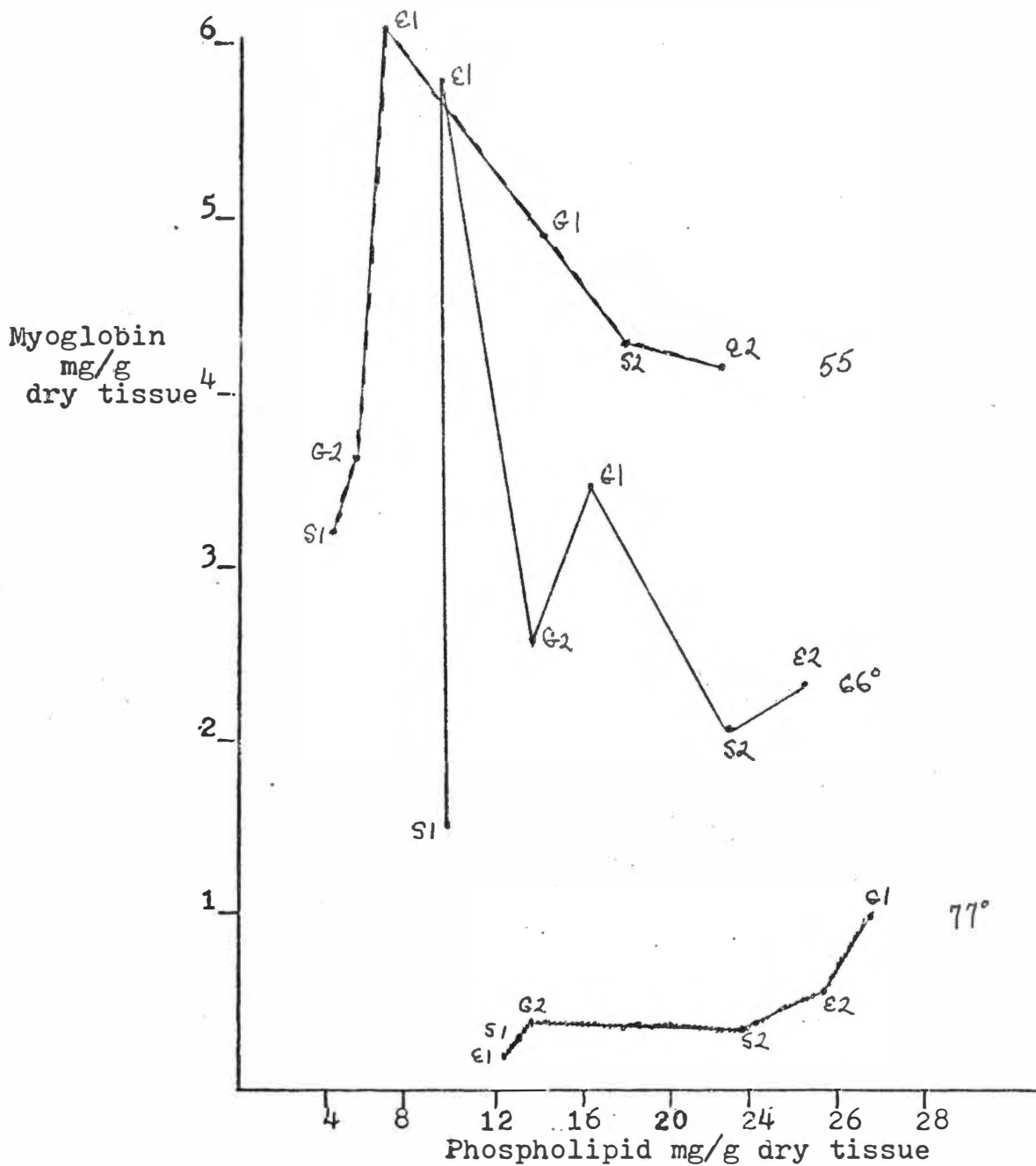


Figure I

Myoglobin, Dry Weight Basis, and Phospholipid, Dry Weight Basis, in Ground Extensor, Semitendinosus, and Gastrocnemius Heated to Three End Points

1 represents older animal; 2 represents younger animal.

the data by coordinate point mapping, no relationship could be shown. Therefore, the investigated relationship between myoglobin and phospholipid does not hold statistically across the muscles or age categories represented by the samples used in this research.

Age was not being considered so there was no intentional selection of animals of different ages. However, muscles were removed from a young and an older animal because theirs were the carcasses available at the times the muscles were excised. Differences in animal age may have been a major reason for failure to establish any relationship. The amount of extractable myoglobin increases with age (Briskey and Kauffman, 1971; Clydesdale and Francis, 1971) but phospholipid concentration remains relatively unchanged (Link et al., 1967).

Probably, specific muscles from a limited age range of animals with known previous histories should be selected for future study. Failure to establish a statistically significant relationship between myoglobin and phospholipid over the three end point temperatures studied may have occurred because stress was placed on emphasizing differences in the samples studied. Results of this study, therefore, indicate a need for selecting the samples so that only one of the measured constituents varies initially.

CHAPTER V

SUMMARY

A possible quantitative relationship between phospholipid and extractable myoglobin, or between their rates of change during heating of beef was investigated. Three muscles--extensor, semitendinosus, and gastrocnemius--were chosen for study at three heating end points--55°, 66°, and 77°C.

The investigated relationship was not shown statistically across the muscle or age categories represented by the samples used. The effects of several variables on the muscle components studied were analyzed individually.

Extractable myoglobin decreased as internal temperature increased. Increasing end point temperature also resulted in increased phospholipid concentration, dry weight basis, and total lipid, wet weight basis. Neither myoglobin nor phospholipid concentrations differed significantly among muscles. Total lipid, on the dry and the wet weight basis, was significantly affected by muscle. Animal age did not appear to affect myoglobin concentration. Muscles from the older animal tended to have a greater total lipid and a lower phospholipid content than those from the younger animal.

Phospholipids were fractionated into classes and analyzed for lecithin, cephalin, and sphingomyelin. Phospholipid classes were unrelated to heat treatment, muscle, or animal.

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APPENDIX

Table V

Percent Dry Matter in Ground Extensor, Semitendinosus, and Gastrocnemius Muscles
Heated to Three End Point Temperatures^a

Muscle Name	Animal Number ^b	Internal Temperature, °C		
		55	66	77
Extensor	1	24.0±0.0	28.9±0.6	34.0±0.2
	2	26.1±0.1	28.6±0.0	31.9±1.5
Semitendinosus	1	27.0±0.4	27.0±1.3	34.9±0.3
	2	26.2±0.2	27.9±0.3	30.2±0.2
Gastrocnemius	1	25.4±0.1	25.8±0.1	29.1±0.6
	2	24.8±0.4	25.8±0.3	30.7±0.0

^aEach value is an average for three determinations ± the standard deviation.

^b1 represents the older animal; 2 represents the younger animal.

CALCULATION OF PERCENT PHOSPHOLIPID IN EXTRACTED LIPID

1. Determine dilution of extract to be used for analysis.
2. Calculate μg phosphorus (P) in volume of diluted extract used for analysis.

$$\mu\text{g P in x ml extract} = \frac{(\text{absorbance of sample})(\mu\text{g P/ml standard})}{\text{absorbance of standard}}$$

3. Convert μg P to μg phospholipid (PL).
 $\mu\text{g PL in x ml diluted extract} = (\mu\text{g P}) (25)$
4. Convert to grams phospholipid.

$$\text{g PL in x ml diluted extract} = \frac{\mu\text{g PL}}{10^6}$$

5. Calculate g phospholipid per ml concentrated extract.

$$\frac{\text{g PL in 1 ml concentrated extract}}{\text{ml extract in the dilution}} = \frac{\text{g PL in x ml diluted extract}}{\text{ml extract in the dilution}}$$

6. Percent phospholipid in total lipid.

$$\% \text{ PL in total lipid} = 100 \frac{\text{g PL in 1 ml extract}}{\text{g lipid in 1 ml extract}}$$

7. Conversion to phospholipids as percent of meat.

$$\text{PL as \% of meat, wet weight basis} = \frac{(\% \text{ total lipid wet weight})(\% \text{ PL in lipid})}{100}$$

$$\text{PL as \% of meat, dry weight basis} = 100 \frac{\% \text{ PL in meat, wet weight basis}}{\% \text{ dry matter}}$$

(Lackey, 1973)

Table VI

F Values^a from Analyses of Variance for Myoglobin, Total Lipid, and Phospholipids Extracted from Ground Extensor, Semitendinosus, and Gastrocnemius Muscles Heated to Three End Point Temperatures

Source	Degrees of Freedom	Myoglobin Percent DWB ^b	Total Lipid Percent		Phospholipids Percent	
			DWB	WWB	Of Lipid	DWB
Muscle (A)	2	3.1	13.6	24.0*	11.5	2.8
Animal (B)	1	1.8	8.5	27.5*	86.5*	48.0*
Heat(C)	2	36.0**	1.0	16.1	10.8	26.4**
AxB	2	2.9	13.1	37.2*	35.1*	58.9*
AxC	4	1.0	1.3	0.8	0.1	1.5
BxC	2	0.3	1.1	2.2	0.9	1.8
AxBxC	4	1.0	1.0	1.0	1.0	1.0
Total	17					

^a* = $P < 0.01$; ** = $P < 0.005$.

^bDWB = percent of meat, dry weight basis; WWB = percent of meat, wet weight.

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