March 2, 1967

To the Graduate Council:

I am submitting herewith a thesis written by Sarah Alice Smitherman entitled "Relation of Phospholipids to DNA in Breast and Thigh Muscle Tissue of Chicken." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

Ada Marie Campbell
Major Professor

We have read this thesis and recommend its acceptance:

Grayce E. Bentz

John T. Smith

Accepted for the Council:

Vice President for
Graduate Studies and Research

RELATION OF PHOSPHOLIPIDS TO DNA IN BREAST AND THIGH MUSCLE TISSUE OF CHICKEN

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Sarah Alice Smitherman

March 1967

ACKNOWLEDGEMENT

Without the aid, guidance, and encouragement of others, the author would not have been able to conduct this investigation. Dr. Ada Marie Campbell assisted with the planning of the study and with the preparation of the manuscript. Dr. John Smith and Dr. Grayce E. Goertz also made numerous helpful suggestions. Joyce Ostrander and Kitty Roberts rendered invaluable assistance in the establishment of the experimental procedure.

In addition, the writer is deeply indebted to her parents, Mr. and Mrs. Dwight Smitherman, for their encouragement throughout her graduate studies.

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CHAPTER I

INTRODUCTION

The phospholipid content of animal tissues has been studied extensively in recent years. Expressed as per cent of total lipid, the phospholipid concentration tends to vary inversely with the total lipid content (Turkki, 1965). Lecithins account for the greatest proportion of the tissue phospholipids, followed by cephalins, sphingomyelin, and traces of lysolecithin (Masoro et al., 1964). The quantitative and qualitative distribution of these substances varies considerably with like tissues from different animals of the same species and in various tissues of the same animal. Even greater differences exist between animal species. The significance of the observed differences and similarities is difficult to interpret because the exact functions of phospholipids in biological tissues have not been defined.

The phospholipid content tends to be relatively high in the more physiologically active muscles (Bloor and Snider, 1934). Red muscles often are more extensively used than white ones and tend to have a higher phospholipid content (Olley et al., 1962). The observed high phospholipid content in red, active muscles was long thought to result from greater usage (Bloor and Snider, 1934); however, these muscular variations in phospholipid content may result from differences in the fiber content rather than from differences in the extent of usage.

Muscles are composed of mixtures of red and white fibers. The red fibers may have an inherently higher phospholipid content than the white ones. The higher ratio of red to white fibers in the red than in the white muscles may account for the higher phospholipid concentration in red muscles.

In studies of the phospholipid content of red and white muscle tissue, the data have been expressed in milligrams of phospholipid per unit weight of tissue. Red fibers are considerably smaller in diameter than white ones (Lasiewski and Galey, 1965). A given weight of red muscle tissue will contain more fibers than the same weight of white tissue. As a result, the finding that a given weight of red tissue has a higher phospholipid content than a similar amount of white tissue gives no clue to the relative phospholipid content of individual red and white muscle fibers.

The mean desoxyribonucleic acid (DNA) content per nucleus in adult, somatic cells of all tissues from a given species has been shown to fall within comparatively narrow limits (Frazer and Davidson, 1953; Gray and DeLuca, 1956; Leuchtenberger et al., 1951; Alfert and Swift, 1953; and Pollister et al., 1951). Consequently, the DNA content often can be employed as a reference standard for tissue analyses of adult subjects. In an attempt to utilize DNA as a standard for striated muscles, the problem of multinucleation is encountered. However, each nucleus has a definite amount of cytoplasm associated with it. If each of these units is considered a "cell," the average number

of "cells" per unit weight of tissue can be determined and used as a reference (Gray and DeLuca, 1956; Turkki, 1965).

In this study, the phospholipid content per unit of DNA in chicken breast and thigh muscle was ascertained to determine whether in red muscle fibers the "cells," as described above, might tend to have a higher phospholipid content than those in white muscle fibers. The total lipids and the proportions of cephalins, lecithins, and sphingomyelin were determined also.

CHAPTER II

REVIEW OF LITERATURE

Early workers in the field of lipid research suggested that animal tissue contained two types of lipid material, the élément constant and the élément variable. Neutral fat, the élément variable, was considered as a reserve source of fuel for energy metabolism, fluctuating in amount with modifications in the diet and energy expenditures. The lipids classified as the élément constant were regarded as essential cell constituents, present in characteristic amounts and types in all organic tissue and not subject to major alterations with extreme physiological variations (Bloor et al., 1930; Terroine, 1936; Kaucher et al., 1943).

This early observation currently is accepted with reservations. All body components, including the lipids, are believed to be in a state of fluxion (Cantarow and Schepartz, 1963). Certain of the lipids, however, are less subject to change than other lipids. Phospholipids comprise the bulk of these essential lipids in both organ and muscle tissues (Acosta et al., 1966).

Through the years, considerable variation in the phospholipid content of animal tissues has been reported. Differences occur both between muscles within an animal and between like muscles from different animals of a species. It was long thought that the variations reflected differences

in the amount of physiological activity. Increased phospholipid content appeared to be associated with increased physiological activity.

During the late twenties and early thirties, Bloor and his associates conducted a series of experiments in an attempt to establish a positive relationship between physiological activity and phospholipid content. Initially, the voluntary muscles of beef were studied. The phospholipid concentration was greatest in the heart, followed by jaw, diaphragm, neck, and round. The preceding order was believed to coincide with the order of decreasing physiological activity (Bloor, 1927). A similar tendency for the phospholipid concentration in the vital organs of beef animals to parallel their degree of metabolic activity was observed (Bloor and Snider, 1930). The fact that the phospholipid concentration in the active corpus luteum was considerably greater than that in the developing or retrograding organ was cited as further evidence of a positive correlation between phospholipid content and physiological activity (Bloor et al., 1930).

Operating on the premise that muscle tissue that had been actively exercised should have a higher phospholipid content than less used muscles, Bloor and Snider (1934) determined the phospholipid content of various muscles tissues in wild and laboratory rabbits. As postulated, the muscles concerned with locomotion had a much higher phospholipid content in the wild animals than the same muscles in the laboratory specimens. Muscles such as jaw, heart, neck, and diaphragm, which would be expected to have similar usage in different animals, had similar phospholipid concentrations.

In answer to the criticism that the observed differences in phospholipid concentration had not been produced experimentally but had occurred naturally (Ludewig and Chanutin, 1936), Bloor (1937) attempted to experimentally induce variations in the amount of physical activity of rats and pigeons. One group of rats was placed in small individual cages for one to two months. A second group was placed in exercise cages, each equipped with a running wheel. As compared with the non-exercised group, the exercised animals had a higher phospholipid concentration in all muscles except the diaphragm, as well as considerable hypertrophy of all muscles. The differences were particularly marked in the locomotor muscles.

In a second phase of the experiment, Bloor bred female rats that had proven to be especially good runners. The offspring were exercised for a month after they reached the experimental weight. Some of the rats were sacrificed and others were bred. The latter's progeny were exercised for a month. The second generation had a higher phospholipid concentration in all muscles except heart and diaphragm than the first generation, but little difference was observed between the second and third generations (Bloor, 1940).

Pigeons that had been permitted to fly freely in a barn had a higher muscle phospholipid concentration than pigeons confined in a cage (Bloor, 1937). The increases were especially marked in the pectoralis major, pectoralis minor, and other muscles concerned with flying as well as in the heart. No hypertrophy was observed in any of the muscles examined.

Bloor suggested that the normal response of a muscle to an increased work load is an "increase in quantity of the muscle" and an "improvement in quality." In some instances, only one response is elicited (Bloor, 1937; 1940).

Kaucher et al. (1943) also observed that an increased phospholipid content coincided with increased physiological activity in beef organs. In addition, these workers reported that the light meat of chicken contained 2.72 per cent dry weight of phospholipid and the dark 4.36 per cent. This was further evidence of the parallelism of phospholipid concentration and physiological activity since the dark muscle is more active than the white.

There has long been a consensus that phospholipids play a major role in the metabolic activities of the body; yet the exact nature of this role has not been defined. Consequently, it has been difficult to account for the apparent correlation between physiological activity and phospholipid concentration.

In a review article, Artom (1952) enumerated three possible functions of phospholipids: essential structural materials of the cells; intermediate compounds in the absorption, transport, and metabolism of fatty acids; and active participants in biological oxidation reactions. Phospholipids apparently do make a major contribution to cellular structure (Kreps et al., 1966; Cantarow and Schepartz, 1963). It is likely that they are present in the lipoprotein complexes that structure the matrix of cell walls and membranes, the myelin sheath, and such bodies

as mitochondria and microsomes (Cantarow and Schepartz, 1963). Undoubtedly, they contribute to the properties of these structures. Membrane permeability is known to be affected by the phospholipid content (Green and Fleischer, 1964). Phospholipids also aid in the active transport of ions across the cell membrane and within the cell (Cantarow and Schepartz, 1963; Kreps et al., 1966; Green and Fleischer, 1963).

Although the role of phospholipids as structural components of body tissues should not be minimized, it seems evident that the observed correlation between physiological activity and phospholipid concentration must result from a more direct phospholipid involvement in energy metabolism. The idea that phospholipids might serve as intermediaries in fat catabolism is old (Artom, 1952). Until recently it was discounted to a great extent because there was considerable question as to whether any lipid material could serve as a direct energy source in the cells. Glycogen was thought to be the primary, if not exclusive, fuel of muscle metabolism. In order for lipids to be utilized, they supposedly had to be converted to glycogen by the liver (Fritz et al., 1958, and Tepperman et al., 1956).

In 1949, Geyer et al. reported that rat tissues had metabolized aerobically trilaurin and octanoic acid in vitro. There was no indication that it was requisite for these fatty acids to be altered either physically or chemically by the liver in order to be utilized by extrahepatic tissues in vivo. Tepperman and associates (1956) found that rat diaphragm segments could oxidize palmitic acid-1-^{14}C as well as shorter chained fatty acids.

Experiments conducted by Fritz et al. (1958) confirmed that rat muscle tissue was capable of directly oxidizing both long and short chain fatty acids. Fritz (1961) suggested that lipids might serve as the major source of muscular fuel during periods of prolonged activity and carbohydrate during intense, short exertions.

In 1960, Neptune and co-workers reiterated the possibility that phospholipids might serve as intermediaries in fat catabolism. They noted that the diaphragms of rats fasted forty-eight hours prior to sacrifice contained 22 per cent less triglyceride and 90 per cent less phospholipid fatty acids than the diaphragms of non-fasted subjects. A four hour incubation further reduced the level of both substances in the fasted tissues, but the triglyceride decrease was somewhat greater. After a similar incubation period, tissue from the non-fasted rats had greater reduction in the total phospholipid fatty acid. The total decrease in fatty acids during incubation was considerably larger in the non-fasted than in the fasted animals. Labeled palmitic acid was incorporated more rapidly into triglycerides than into phospholipids. It was suggested that fatty acids might enter triglycerides initially, subsequently be incorporated into phospholipids, and then be transferred to oxidative enzyme systems for oxidative degradation.

Marion (1965) observed that the phospholipid content in chicken breast muscle decreased with age. At age twenty-one days, the phospholipid content averaged 0.54 per cent of the wet tissue. Six weeks

later the phospholipid content was 0.41 per cent. The author suggested that the observed decrease in total phospholipid content with age might signify that the phospholipids required for normal function of tissue change with age or that not all phospholipids present in the body are needed for metabolic purposes. It is possible that some phospholipids function solely as depot reserves.

The possibility that intracellular muscle lipids functioned as an energy source was investigated further by Masoro and co-workers (Masoro et al., 1966; Masoro et al., 1965). After eighteen hours of fasting, monkeys were anesthetized and the motor nerve to gastrocnemius and soleus muscles of the left leg was isolated. The nerve was stimulated electrically once per second to cause maximum contraction in these muscles. In a second group of animals, the same muscles were made to contract against a load five times per second. A third group of animals received the same treatment as the second group except that they were fasted sixty-five rather than eighteen hours. All subjects were sacrificed after five hours of stimulation and the lipid content of the gastrocnemius and soleus muscles in the right and left legs was determined. Because it was found in a previous study (Masoro et al., 1964) that the intracellular composition of bilateral muscles essentially did not differ, the inactive right muscles were used as controls for the active left ones. Contrary to expectations, there was no significant difference in the phospholipid or total lipid content of the exercised and non-exercised muscles. The animals were fed palmitate-1-^{14}C at the

beginning of the stimulation period in order to ascertain whether exercise increased the rate of muscle lipid turnover. The failure to observe differences in the specific activity of the muscles indicated that there was no apparent acceleration of lipid turnover. The authors concluded that *in vivo* the intracellular lipids apparently are not involved in augmented energy metabolism of contracting muscle. Evidently the lipids used by muscle are derived from the blood.

Contrary to the earlier work of Neptune *et al.* (1960), Masoro and Spitzer (1966) did not observe a reduced amount of phospholipid in the *gastrocnemius* of rats fasted as long as seventy-two hours. The free fatty acid level rose markedly during the test period, but the level of triglycerides remained the same as that in the non-fasted subjects. The free fatty acid level remained low in the non-fasted animals. Apparently, the lipids of the rat *gastrocnemius* do not serve as an energy reservoir to be drawn upon during periods of food deprivation.

On the basis of present evidence, it seems unlikely that the intracellular lipids are utilized for muscular energy or that phospholipids are intermediaries in fat catabolism.

Energy released by the oxidative processes is coupled to the synthesis of adenosine triphosphate (ATP) by the mitochondrion. Acetone extraction of the lipid, over 90 per cent of which is phospholipid, destroys the ability of the mitochondrion to transfer electrons. The addition of coenzyme Q and phospholipid restores the activity. A need for phospholipid has been shown in at least three segments of the

electron-transfer chain: succinate--> Q; reduced Q (QH_2) to cyctochrome C; and cytochrome C--> O_2 (Green and Fleischer, 1963; Tzagoloff and MacLennan, 1965). The functions of phospholipid may be to provide a medium of low dielectric constant and to solubilize the mitochondrial proteins. Most mitochondrial proteins are pronouncedly hydrophobic. Phospholipids protect the hydrophobic faces of the proteins from water, but the polar ends of the phospholipid molecule solubilize the entire lipid-protein complex in water. It is believed that a high-energy bond is destroyed in an aqueous medium. Phospholipids may provide the non-aqueous medium requisite for electron flow between protein components of the transfer chain (Green and Fleischer, 1963). In addition, phospholipids are known to be necessary for the active transport of ions across the mitochondrial membranes (Green and Fleischer, 1964) and for ATP-induced membrane contraction (Vignais et al., 1964a; Vignais et al., 1964b).

The apparent correlation between phospholipid content and extent of physiological activity might be an indirect result of an inherent difference in the type of fibers present in the muscles. Striated muscles have long been classified as either red or white muscles. Szent-Gyorgyi (1953) noted that the red skeletal muscles contracted slowly and tonically whereas the white muscles contracted rapidly and intensely. Red muscles tended to be used for prolonged activity and the white for immediate, intense, but brief activity.

The histochemical studies by Ogata (1960) revealed that mammalian skeletal muscles actually contain three types of fibers: red, white, and intermediate. Each muscle contains different ratios of the fibers. Within a muscle, there is considerable variance in the distribution of the three types of fibers. The color of the muscle depends on the dominant type of fiber (Ogata, 1960; Beecher et al., 1965).

There have been several attempts to elucidate the biochemical and histological differences between the fibers. Red fibers tend to have a smaller diameter and a proportionately larger amount of surface area (George and Naik, 1958b). Ascompared with the white fibers, the red fibers frequently have higher lipid contents (Drummond and Black, 1960), more sarcoplasmic granules (George and Naik, 1958a), and similar myofibrillar protein contents (Gergely et al., 1965).

Myoglobin, the pigment primarily responsible for the red color of muscle fibers, serves as a storage mechanism for oxygen in the cells (Giffee et al., 1960). Lawrie (1952) noted that a high myoglobin content tended to be associated with high enzymic activity in the muscle cytochrome system of numerous animal species. In addition, the rate of pH decrease under anaerobic conditions and the content of high energy compounds were inversely related to the myoglobin content. He suggested that the higher the myoglobin content (i.e., the darker the color), the greater is the capacity of a muscle for respiratory metabolism and the less its ability for glycolytic processes, and conversely.

Ogata (1960) studied the enzymatic activities and the content of certain labile constituents in red and white rabbit muscles. The red fiber preparation was obtained from the central part of the M. soleus and was completely free of white fibers. The white fiber preparation, secured from the posterior edge of M. adductor magnus, contained no more than 3 per cent red fibers. White muscles contained nearly four times as much glycogen as the red muscles and twice the concentration of high energy phosphate compounds. After a five minute incubation period in a medium containing adenosine triphosphate, more than three times as much inorganic phosphate was present in the white muscle homogenate as in the red muscle homogenate. Glycolysis was considerably greater in the white fibers than in the red ones. At the end of a thirty minute incubation period in a medium containing glucose, the red fibers produced 25.69 µg. of lactic acid per milligram of tissue under anaerobic conditions. The white fibers produced 42.33 µg. per milligram of tissue under similar conditions. Succinic dehydrogenase activity and the pyruvate oxidation rate were approximately six times greater in the red than in the white muscle fibers. These results indicated that the red muscle fibers had higher tricarboxylic acid activity than the white fibers. The latter appeared to have the greater capacity for glycolytic metabolism. Greater ATP-ase activity and greater stores of glycogen and high energy phosphate compounds suggested that a limited amount of energy is more readily available in muscles containing predominately white fibers than in those containing predominately red fibers.

The work of Beatty et al. (1963) on rat adductor muscles suggested similar conclusions. Groups of predominantly red or white fibers were obtained from animals that had been fasted twenty hours. Initially the glycogen content was 5.00 mg./g. wet weight of tissue in the white fibers and 4.40 mg./g. in the red fibers. Two hours later, the level was 2.59 mg./g. in the white fibers and 3.51 mg./g. in the red. No difference was detected in the glucose uptake of the two types of fibers, but the lactate production was greater in the white fibers. This was considered evidence that muscles composed predominantly of white fibers rely on glycolytic metabolism more than do the muscles comprised primarily of red fibers. The 30 to 35 per cent greater uptake of acetoacetic acid, the 32 per cent greater oxygen consumption, and the greater β-hydroxy butyrate dehydrogenase activity of the red fibers circumstantiate greater tricarboxylic acid activity in these fibers.

Contrary to the widely accepted viewpoint, the white fibers may not have a greater capacity for glycolytic activity than the red fibers (Beatty et al., 1963; Ogata, 1960; Beecher et al., 1965). Bocek et al. (1966b) studied the relative importance of the glycolytic pathway in the adductor muscles of rats. Tissue preparations, consisting primarily of white or red fibers, were incubated in Krebs bicarbonate medium plus glucose-^{14}C for periods of 0, 10, 20, 30, 45, 60, 90, and 120 min. The initial total glycogen content was lower in the red muscle fibers (2.92 mg./g. wet tissue as opposed to 3.42 mg./g. in the white). Little change in the glycogen level of either fiber preparation occurred during

the first hour of incubation. During the second hour, the glycogen level decreased markedly in both types of fibers, but the decrease was more marked in the white fibers. (Final content was 2.15 mg./g. tissue in the red and 1.89 mg./g. in the white.) The red contained three times as much radioactivity as the white fibers at the end of the 120 min. incubation period. The amount of $^{14}CO_2$ produced from labeled glucose was consistently higher in the red fibers. The authors concluded that the glucose to glycogen pathway was more active in muscle tissue containing predominantly red fibers than in tissue composed primarily of white fibers. There was some indication that glycogen might be metabolized differently in red and white fibers.

In a second series of experiments, Bocek et al. (1966a) further investigated the comparative glycolytic processes in red and white fibers of the rat adductor muscle. In agreement with previous results, the total glycogen concentrations and the percentage of glucose-^{14}C present in the glycogen after a two hour incubation period were greater in red muscle fibers than in white fibers. The total glucose uptake was larger in the red muscle fibers. Glycolysis, as measured by pyruvate and lactate production, was approximately two times as great in the white fibers. The authors pointed out, however, that the pyruvate and lactate content could not be considered a measure of the extent of glycolysis. Because the experiment was conducted under aerobic conditions, lactate and pyruvate would have been oxidized via the tricarboxylic acid cycle. The difference in rate of oxidation known to exist between

red and white fibers would have affected the final lactate and pyruvate content.

In another phase of the experiment, the red and white fibers were labeled by incubation with glucose-^{14}C and then transferred to a fresh, glucose-free medium for an additional sixty minute incubation period. Because the per cent decrease and absolute decrease in the glycogen content of the fiber preparations were the same, there appeared to be no difference in the glycogen catabolic rate. However, the per cent decrease in specific activity of glycogen was 24 per cent for the red fibers and 67 per cent for the white. It is possible that the newly added glucosyl units of glycogen in the white fibers were more labile than those in the red.

The comparative ability of dark and white trout muscle tissue to metabolize carboxyl labeled fatty acids was investigated by Belinski (1963). Dark muscles oxidized all three acids studied, hexanoate, octanoate, and myristate, at a considerably faster rate than the white tissue. The magnitude of difference increased with the length of the fatty acid chain. Hamosh et al. (1966) observed a similar greater capacity for metabolizing fatty acid in the brown muscle than in white tissue of carp.

George and Scaria (1958) demonstrated histochemically that the lipase present in the pectoralis major muscle of pigeons occurred primarily in the narrow, red fibers. The small amount of lipase present in some white fibers might have been the result of diffusion from the narrow fibers.

On the basis of present evidence, it seems likely that red muscle fibers have a greater capacity for aerobic metabolism of both carbohydrate and fat. Whether or not white muscle fibers have a correspondingly greater glycolytic capacity is subject to question. It is possible that the rate and mode of glycogen synthesis and degradation vary in the two fiber types.

Both red and white fibers are found in the avian pectoralis major, the principal muscle of flight. The ratio of red to white fibers varies considerably. In pigeons, the ratio is sixteen red per one white (George and Naik, 1958a). The parakeet, bee-eater (George and Naik, 1958a), and humming bird (Lasiewski and Galey, 1965) contain only the red fibers whereas only white fibers are found in the pectoralis major of the domestic fowl (George and Naik, 1958a). Red fibers appear to be dominant in the muscle from active fliers and white fibers are dominant in the soaring or non-flying species (Lasiewski and Galey, 1965).

In birds, the extent of muscle usage tends to be positively correlated with the red fiber content. It is possible that a similar relationship exists in other species.

George and Naik (1958b) demonstrated histochemically that the mitochondria were found primarily in the red fibers of the pigeon breast muscle. Paul and Sperling, as cited by Perry (1956), also observed that the mitochondria tended to be concentrated in these fibers. Mitochondria are known to contain a certain amount of phospholipid (Green and Fleischer, 1963). The observed positive correlation

between phospholipid content and physiological activity may be the indirect result of a positive correlation between mitochondrial content in red fibers and extent of muscular usage.

It was observed previously that large numbers of mitochondria arranged between myofibrils are characteristic of highly active muscle tissue. The energy expenditure of the humming bird during flight is extremely high, and the mitochondrial enrichment of the muscular tissues is particularly marked. In many sections of the pectoralis major, the ratio of mitochondria to myofibrils is as high as 1:1 by volume. Although the average diameter of mitochondria rarely exceeds 1 μ, some mitochondria as large as 1.5 μ by 10 μ are present in this tissue; the myofibrils range from 0.5 μ to 0.9 μ in diameter. The close apposition of the large mitochondria to myofibrils insures "the maximal apposition of . . . . the enzyme system of the sarcolemma, and the contractile proteins of the myofibrils" (Lasiewski and Galey, 1965). Mitochondrial enrichment appears to be an adaptive mechanism of muscle for meeting high energy needs. A correspondingly high phospholipid content is probable in tissues with a large mitochondrial content.

CHAPTER III

PROCEDURE

Meat Samples

Eight chickens were obtained from a local poultry processing plant on separate occasions over a three month period. All chickens were 5-6 wks. old and had a dressed and drawn weight of $2\frac{1}{4}$-$2\frac{1}{2}$ lb.

The carcasses were removed from the processing line within 4-5 min. after death and packed in crushed ice. After approximately 30 min., the left and right pectoralis major muscles were excised for use as the white muscle samples. As there was not a single muscle in the thighs or legs that would provide a sufficiently large sample of dark tissue, all thigh muscles were combined for a dark muscle sample. Following removal of visible fat and loose connective tissue, each sample was ground twice in an electric grinder, mixed thoroughly, and sampled for moisture determination, lipid extraction, and DNA analysis.

Moisture determinations and lipid extractions were begun on the same day. The samples for the DNA assay were stored at -20° C. for two days prior to analysis.

Moisture Determinations

Weighed triplicate samples of 3-5 g. each were dried in an air oven for 20 hr. at 110 $\pm$ 1° C. and then reweighed. The per cent moisture content of the wet tissue was considered to be the weight lost in drying

expressed as per cent of the initial weight.

Lipid Extraction

Total lipids were extracted from 50 g. samples of white and dark muscle tissue by the procedure of Ostrander and Dugan, Jr. (1961) as adapted by Turkki (1965). All filtrations were done under a stream of nitrogen. The filtrate was transferred quantitatively to a 500 ml. cylinder and stored overnight at approximately 1° C. The volume of the bottom layer, the chloroform-lipid extract, was recorded prior to transferral of the entire filtrate to a separatory funnel for final separation. The chloroform-lipid extract was drained into an evaporating flask and mixed thoroughly. Three 10 ml. aliquants were pipetted immediately into preweighed 30 ml. beakers. The remaining lipid extract was concentrated in a rotary evaporator to a volume of 30-50 ml. The concentrated extract was stored under nitrogen at -20° C. until fractionated by thin layer chromatography.

The solvent was evaporated from the aliquants under a hood and finally in a vacuum desiccator until constant weight was attained. The following equation was used to calculate the per cent total lipid in each sample extracted:

$$\text{per cent lipid} = \frac{\text{ml. } CHCl_3 \text{ extract x g. lipid in 10 ml. aliquant x 100}}{\text{g. of meat extracted x 10}} .$$

Thin Layer Chromatography

A thin layer chromatographic process was used to separate the triglycerides from the phospholipids and to fractionate the latter

compounds into their three major classes. The method outlined by Roberts (1966) for preparing, spotting, and developing the plates was followed.

When the chromatoplates were sprayed with 0.004 per cent aqueous fluorescein and observed under ultraviolet light, four major spots consistently appeared between the origin and solvent front. The lowest spot was identified as sphingomyelin. It reacted positively to fluorescein and negatively to ninhydrin. The second lowest spot was identified as lecithin. It gave the characteristic negative reaction to ninhydrin and positive reactions to fluorescein and Dragendorf reagent.* A commercial lecithin preparation traveled to the same height on the plates and reacted in a similar fashion. Both of the two upper spots were believed to be cephalins. The highest one gave positive responses to fluorescein and ninhydrin, but the second highest one responded positively only to fluorescein. Neither was visualized by Dragendorf reagent. A definite positive reaction to ninhydrin is characteristic of phosphatidyl ethanolamine. Phosphatidyl serine typically gives a very weak or no reaction to ninhydrin. Both phosphatidyl ethanolamine and phosphatidyl serine give positive reactions to fluorescein and negative ones to Dragendorf reagent.

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*Reagent was prepared by mixing 20 ml. of solution I (1.7 g. basic bismuth nitrate in 100 ml. of 20 per cent acetic acid) with 5 ml. of solution II (40 g. potassium iodide in 100 ml. distilled water) and 70 ml. of distilled water (Wagner et al., 1961).

After the identity of the phospholipid fractions and their relative order on the plates was established, they could be located by spraying the plates with fluorescein. The spots were demarcated with a stainless steel spatula. Duplicate samples of each phospholipid class and its corresponding silica gel blank were scraped into micro-Kjeldahl flasks with the straight edge of the spatula. Each lecithin sample contained only one spot, whereas each sphingomyelin contained two because the quantity of sphingomyelin in muscle is minute. For each cephalin sample, one phosphatidyl serine and the corresponding phosphatidyl ethanolamine spot were combined.

Phosphorus Determination

The phosphorus content of the phospholipid fractions was ascertained by the micromethod of Bartlett (1959) as adapted by Marinetti (1962). Duplicate samples of the lecithin, cephalin, and sphingomyelin fractions, their corresponding silica gel blanks, and duplicates of the standard* at one level were analyzed at a time. The concentration of phosphorus in the standard varied from one analysis to another within the limits of the curve.

Two glass beads and 1.2 ml. of 70 per cent perchloric acid were added to each digestion flask. Because the phosphorus standard solution was aqueous, distilled water was added as needed for a final volume

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*A stock solution of a phosphate standard was prepared with 4.3933 g. of monobasic potassium phosphate dissolved in a small quantity of distilled water and diluted to 1 l. Five milliliters of the stock solution were diluted to 1 l. for the operational standard. The operational standard was used in amounts ranging from 1 to 5 µg. in the preparation of a standard curve. The relationship between optical density and phosphorus content was linear over the entire curve.

of 1 ml. of water in each flask. The samples were digested for 30 min. on a micro-Kjeldahl digestion rack and cooled to room temperature.

The particles of silica gel adhering to the sides of the flask were washed down into the digestion mixtures by the careful addition of 7 ml. of distilled water to each. After 1.5 ml. of 2.5 per cent ammonium molybdate solution was added to each flask and all flasks thoroughly agitated, 0.2 ml. of Fiske-Subba Row reagent was added and the flasks heated in a boiling water bath for exactly 7 min. During the 10 min. cooling period, the samples were transferred into centrifuge tubes. The mixtures were centrifuged for 10 min. at 1000 x G. The clear solutions were decanted into cuvettes and their absorbance was read in a Bausch and Lomb "Spectronic 20" spectrophotometer at a wave length of 830 mμ. A reagent blank was used for zeroing the instrument. The readings for the silica gel blanks were used for correcting the corresponding sample readings.

The phosphorus content of the fractions was calculated by the equation:

$$\mu g.\ P \text{ in sample} = \frac{\text{corrected absorbance of sample} \times \mu g.\ P \text{ in standard}}{\text{absorbance of standard}}.$$

Phosphorus was assumed to constitute 4 per cent of the weight of the phospholipids; thus, the phosphorus values were multiplied by 25 for conversion to phospholipid values. The amounts of the three phospholipid classes in each tissue extract were calculated as percentages of the recovered phospholipids.

The total concentration of phospholipids in the lipid extract was determined in 20 µl. portions of extract having a lipid concentration no higher than 50 µg./ml. Duplicate samples were digested and analyzed for P in the manner described for the fractions. The lipid concentration of each extract at the time of the phospholipid assay was determined by drying two 1 ml. aliquants to constant weight.

DNA Determination

The extraction procedure of Schneider (1945) as adapted for use with muscle tissue by Turkki (1965) was used with certain modifications. Triplicate samples of 3.5-5.5 g. each were extracted. The meat samples, which had been weighed in individual 30 ml. beakers prior to freezing, were thawed at room temperature for 10 min. and then placed in crushed ice.

Each defrosted meat sample was macerated in a chilled mortar with 15 ml. of cold 10 per cent trichloroacetic acid (TCA) and 5 g. of sand. The residue and extract were transferred to a 50 ml. centrifuge tube with the aid of 25 ml. of cold TCA for rinsing the beaker, mortar, and pestle. The mixture was centrifuged at 6300 x G. for 30 min. The supernatant solution was removed by suction. The residue was resuspended in 15 ml. of 10 per cent TCA and recentrifuged at 6300 x G. for 25 min. The supernatant fluid was again discarded and the tissue re-extracted with 15 ml. of cold 5 per cent TCA. After 25 min. of centrifuging at the same force, the liquid was again removed with suction. The last few drops of the extract were eliminated by carefully inverting

the tube on paper toweling. Fifteen milliliters of 5 per cent TCA were added to the tissue residue with a pipet. The covered tubes were heated in a water bath for 15 min. at 90° C., cooled, and recentrifuged for 25 min. at 6300 x G. The nucleic acid extract was filtered through Whatman No. 44 acid washed filter paper.

The Dische diphenylamine reaction (Seibert, 1940) was employed to ascertain colorimetrically the desoxyribose content of the nucleic acid extracts. For each sample, a 3 ml. aliquot of the extract was placed in a test tube. A standard containing 50 µg. of pure desoxyribose dissolved in 3 ml. of 5 per cent TCA was placed in another test tube. After 6 ml. of diphenylamine reagent* had been added to each tube, the tubes were covered with marbles and heated in a boiling water bath for 10 min. Each cooled sample was transferred to a cuvette and the absorption was read at 600 mu in a Bausch and Lomb "Spectronic 20" spectrophotometer zeroed with a reagent blank.

The concentration of desoxyribose in each aliquot was calculated by the following equation:

$$\mu g.\ \text{desoxyribose/aliquot} = \frac{\text{absorbance of aliquot} \times 50}{\text{absorbance of standard}} .$$

The desoxyribose values were subsequently converted to DNA-P** as follows:

$$\text{mg. DNA-P/100 g. tissue} = \frac{\mu g.\ \text{desoxyribose in aliquot} \times 5 \times 100}{\text{g. of tissue extracted} \times 4.33^{***} \times 1000} .$$

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*1 g. of diphenylamine dissolved in 100 ml. of glacial acetic acid and 2.75 ml. of concentrated sulfuric acid.

**DNA-phosphorus.

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

The DNA-P weight represents approximately 10 per cent of the DNA weight; therefore, the DNA-P values were multiplied by 10 for conversion to DNA values.

Statistical Analysis

The t-test for paired comparisons (Steel and Torrie, 1960) was used to compare the sample means. The standard deviation was calculated by the following equation:

$$\text{standard deviation} = \sqrt{\frac{\sum x_i^2 - \frac{(\sum x_i)^2}{n}}{n-1}} \quad .$$

CHAPTER IV

RESULTS AND DISCUSSION

The lipid concentrations of breast and thigh muscles are shown in Table I. The lipid content was higher in the thigh muscles than in the breast. The mean lipid values of 1.14 per cent wet weight for the breast and 2.83 per cent for the thigh were slightly higher than other values that have been reported. Katz et al. (1966) found lipid contents of 1.0 per cent and 2.5 per cent in the breast and thighs of 14 wk. old fryers. In 9 wk. old broilers, the lipid content was 0.81 per cent in the breast and 2.16 per cent in the thighs (Marion, 1965). Marion and Woodroof (1965) reported values of 0.89 and 2.72 per cent for the same tissues in $2\frac{1}{4}$-$2\frac{1}{2}$ lb. broilers whereas the respective values were 0.90 and 1.89 per cent in one year old chickens (Peng and Dugan, 1965). Because the sex, age, and diets of the birds have varied in these studies, differences in the values would be expected (Mickelberry et al., 1964, 1966; Marion, 1965).

Total lipid contents of the breast and thigh muscles seemed to vary independently of each other. Although the thigh muscles consistently had a higher lipid content than the breast muscles, an animal with a comparatively high lipid content in one muscle did not necessarily have a correspondingly high lipid level in the other muscle. In a study of beef animals (Turkki, 1965), those with the highest and lowest

TABLE I

PER CENT LIPID EXTRACTED FROM BREAST AND THIGH MUSCLES OF CHICKEN

Bird	Wet Weight Basis		Dry Weight Basis[a]	
	Breast	Thigh	Breast	Thigh
A	1.09	2.40	4.4	10.6
B	1.16	2.63	4.5	11.3
C	1.09	2.61	4.4	11.2
D	1.41	3.11	5.5	13.9
E	1.07	2.38	4.3	10.4
F	1.01	3.58	4.0	15.3
G	1.07	2.85	4.1	11.8
H	1.26	3.10	4.9	13.2
Mean	1.14 ***	2.83	4.5 ***	12.2
Std. Dev.	0.04	0.14	0.12	0.62

[a]Based on moisture values presented in Table VII, Appendix.

***$P < .001$.

total lipid content in the psoas major were also the ones with the highest and lowest lipid levels in the extensor carpi radialis.

The concentrations of phospholipid in the two types of muscle tissue are presented in Table II. The mean phospholipid contents of the fresh tissue were 0.40 and 0.71 per cent in the breast and thigh respectively and the difference is significant ($P<.001$). These values are similar to those of Peng and Dugan (1965) for 1 yr. old chickens, 0.36 and 0.74 per cent respectively for breast and thigh. In the Marion and Woodroof (1965) study of broilers, the corresponding values reported were higher, 0.55 and 0.93 per cent, but the ratio of breast phospholipid to thigh phospholipid content was still approximately 1:2. In two other studies, a similar 1:2 relationship between phospholipid concentrations in breast and thigh muscles was not observed. Marion (1965) determined that breast muscle in 63 da. old broilers contained 0.35 per cent phospholipid and thigh 0.52 per cent. From the data given in the report of a study by Katz et al. (1966), the phospholipid content of the muscles of 14 wk. old fryers was calculated to be 0.48 per cent in the breast and 0.52 per cent in the thigh. The ratio of the phospholipid concentration in the two muscles was about 1:1.5 in the Marion study and 1:1 in the Katz investigation.

On both the dry weight and the non-neutral lipid bases (Table II), phospholipid concentrations differed significantly ($P<.001$) between white and red muscles. The difference was in the same direction as on the wet weight basis.

TABLE II

PER CENT PHOSPHOLIPID[a] IN CHICKEN BREAST AND THIGH MUSCLES

Bird	Wet Weight Basis		Dry Weight Basis		Nonneutral Lipid Dry Weight Basis		Total Lipid Basis	
	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
A	0.46	0.65	1.83	2.87	1.88	3.10	41.8	27.2
B	0.44	0.67	1.72	2.89	1.77	3.15	38.4	25.6
C	0.37	0.73	1.49	3.14	1.54	3.41	33.7	27.9
D	0.37	0.77	1.46	3.30	1.53	3.67	26.5	24.8
E	0.40	0.59	1.62	2.60	1.67	2.81	37.4	25.0
F	0.38	0.79	1.52	3.36	1.55	3.82	37.8	22.0
G	0.40	0.79	1.52	3.28	1.56	3.58	37.0	22.7
H	0.41	0.69	1.60	2.94	1.65	3.28	32.5	22.2
Mean	0.40 ***	0.71	1.60 ***	3.04	1.64 ***	3.35	35.6 ***	24.7
Std. Dev.	0.011	.025	0.045	0.102	0.045	0.118	1.64	0.79

[a]Phosphorus x 25.

***$P < .001$.

A common method for expressing phospholipid concentration is as per cent of the total lipid content. Turkki (1965) observed that the percentage of phospholipid tends to increase as the content of total lipid decreases in beef. A similar relationship was found in this study. The total lipid content was lower in breast than in the thigh muscles and the phospholipid content expressed as percentage of the total lipid was higher in the breast muscle. The difference in the means, 35.6 per cent for the breast and 24.7 per cent for the thigh, is significant ($P<.001$). In other investigations (Peng and Dugan, 1965; Katz et al., 1966; Marion, 1965; and Marion and Woodroof, 1965), the percentage of phospholipid expressed as per cent of total lipid has ranged from 39 to 62 per cent in the breast and from 21 to 40 per cent in the thigh. Because the total lipid content is not a constant, a meaningful comparison of phospholipid values from different studies is difficult. The ratio of phospholipid concentrations expressed as per cent of total lipid was almost 1:1 for the breast and thigh muscles in one study whereas two other groups reported a 2:1 relationship. In one study a 2:1 phospholipid ratio between breast and thigh was found when values were expressed as percentage of total lipid. The ratio was 1:1 when the phospholipid was expressed as percentage of the wet tissue. Variations in total lipid content resulted in this difference.

The relationship of the phospholipid content to DNA is presented in Table III. Significant differences in both the level of phospholipid ($P< .001$, Table II) and the level of DNA ($P< .01$, $P<.001$, Table IV).

TABLE III

RELATIONSHIP OF PHOSPHOLIPID TO DNA[a]

Bird	Wet Weight Basis		Dry Weight Basis		Nonneutral Lipid Dry Weight Basis	
	Breast	Thigh	Breast	Thigh	Breast	Thigh
A	89.4	84.6	89.8	84.1	89.8	84.1
B	68.8	52.5	69.7	52.7	69.7	52.7
C	40.4	65.5	40.6	65.4	40.6	65.4
D	40.2	52.8	40.1	52.8	40.1	52.9
E	32.3	34.6	32.5	34.6	32.5	34.6
F	33.5	36.0	33.6	36.0	33.6	36.0
G	39.6	65.8	39.7	65.6	39.7	65.6
H	47.0	46.3	47.0	46.2	47.0	46.2
Mean	48.9	54.8	49.1	54.7	49.1	54.7
Std. Dev.	7.06	5.92	6.95	5.87	7.13	5.87

[a]Both in mg. per 100 g. of tissue.

TABLE IV

CONCENTRATION OF DNA IN THE BREAST AND THIGH MUSCLES OF CHICKEN[a]

Bird	Wet Weight Basis		Dry Weight Basis		Nonneutral Lipid Dry Weight Basis	
	Breast	Thigh	Breast	Thigh	Breast	Thigh
A	5.1	7.7	20.4	38.1	21.0	36.9
B	6.4	12.8	24.7	61.7	25.4	59.8
C	9.1	11.1	36.8	54.1	37.9	52.2
D	9.3	14.6	36.5	72.0	38.0	69.4
E	12.4	17.2	50.0	83.6	51.4	81.3
F	11.4	21.9	45.1	110.0	46.2	105.9
G	10.0	12.0	38.3	56.6	39.3	54.6
H	8.7	14.9	34.0	73.4	35.2	71.0
Mean	9.0	** 14.0	35.7	** 68.7	36.8	*** 66.4
Std. Dev.	0.85	1.51	3.44	7.68	3.52	7.40

[a]Mg. of DNA per 100 g. of tissue.

**$P < .01$.

***$P < .001$.

in the tissues were observed. The thigh muscles had a higher content of phospholipid and DNA in all subjects than did the breast muscles; however, there appeared to be no consistent relationship between the DNA and phospholipid contents in this study. On the basis of the mean values alone, the postulated higher ratio of phospholipid to DNA in the thigh than in the breast was observed. The difference was not significant ($P > .2$) possibly because of the low DNA values for animals A and B. The basis for calculating the relationship of phospholipid to DNA did not affect the ratio.

The writer knows of no previous study in which the relationship of the phospholipid content to DNA in light and dark muscles has been explored. Turkki (1965) observed a significantly higher phospholipid/DNA ratio in the psoas major muscle of beef than in the extensor carpi radialis (51.8 mg. and 44.8 mg. phospholipid per mg. of DNA, respectively). Although the difference between muscles was slight in some animals, the psoas major consistently had the higher ratio. She, too, found marked differences between ratios of the same muscle from different animals.

It may be that the failure to observe a consistent relationship between phospholipid and DNA content in this study was attributable to the experimental procedure. Obtaining reproducible results in the DNA determination is difficult (Turkki, 1965). Considerable variation in the DNA content occurred among animals. On a wet weight basis, the values in the breast ranged from 5.1 to 12.4 mg. of DNA per 100 g. of

tissue and the thigh values from 7.7 to 21.9 mg. with respective means of 9.0 and 14.0 mg. (Table IV, page 34). Within an animal, the mean DNA content of the replicates was consistently higher for the thigh than for the breast, but the values for the replicates sometimes showed greater variation than the values between muscles. Turkki experienced similar difficulty in securing agreement between sample replicates. The variation among beef animals in her study was slightly less than occurred in this study. On a wet weight basis, the DNA content of the psoas major ranged from 11.2 mg, to 15.5 mg. per 100 g. of tissue and that of the extensor carpi radialis ranged from 9.6 mg. to 14.7 mg.

No other comparative studies of the DNA content in breast and thigh muscles of chickens were available. Shchesno (1965) determined the DNA content of the white and red muscles of the rabbit. On a dry weight basis, the content of DNA was 10.4-20.4 mg. in the white muscles and 22.8-30.9 mg. per 100 g. of tissue in the red. The respective means were 14.5 and 27.0 mg. Shchesno considered the difference in the DNA content to be characteristic of the two types of fibers. The occurrence of a similar greater DNA content in the red muscles of hens, cats, and guinea pigs was mentioned.

The DNA values in the present study are considerably higher than the corresponding values reported by Shchesno for rabbits. On a dry weight basis, the breast muscle fibers contained from 20.4 to 50.0 mg. DNA and the red contained 38.1-110.0 mg. per 100 g. of tissue. The respective means, 35.7 and 68.7, differed significantly ($P<.001$).

The distribution of the three major phospholipid classes, shown in Table V, was different in the two muscles. The breast muscle contained 25.6 per cent cephalins, 67.5 per cent lecithins, and 6.9 per cent sphingomyelin whereas the thigh contained 31.0 per cent cephalins, 56.4 per cent lecithins, and 12.7 per cent sphingomyelin. No lysolecithin was detected in either muscle. Breast muscle had a lower percentage of sphingomyelin and cephalins and a higher content of lecithins than the thigh muscles. The difference between muscles was most marked in the sphingomyelin fractions.

Peng and Dugan (1965) determined the relative distribution of the phospholipids in the breast and thigh muscles of two 1 yr. old chickens by gravimetric, infrared, and phosphorus analysis. The results of the three methods of analysis agreed fairly well. As determined by the phosphorus analysis, the breast contained 24.7 per cent cephalins, 58.0 per cent lecithins, 3.26 per cent sphingomyelin, and 14.0 per cent of a fraction containing both sphingomyelin and lecithins. The distribution in the thigh was: cephalins, 44.9 per cent; lecithins, 52.0 per cent; and sphingomyelin, 3.47 per cent.

In 1 yr. old turkey males, the distribution of phospholipids was 34.54 per cent cephalins, 51.64 per cent lecithins, and 14.31 per cent sphingomyelin in the breast and 36.47 per cent cephalins, 51.18 per cent lecithins, and 14.77 per cent sphingomyelin in the thigh when three combinations of chloroform and methanol were used to elute the lipids in column chromatography. The sphingomyelin content was reduced

TABLE V

DISTRIBUTION OF PHOSPHOLIPID AMONG CEPHALINS, LECITHINS, AND SPHINGOMYELIN IN CHICKEN BREAST AND THIGH MUSCLES[a]

Bird	Cephalin		Lecithin		Sphingomyelin	
	Breast	Thigh	Breast	Thigh	Breast	Thigh
A	21.3	28.1	72.9	57.9	5.8	13.9
B	26.9	24.0	65.8	60.4	7.3	15.6
C	19.5	29.6	77.2	57.2	3.2	13.3
D	27.2	31.5	60.3	55.5	12.5	13.0
E	24.0	36.8	70.9	50.8	5.0	12.4
F	35.7	27.6	56.7	61.2	7.5	11.2
G	32.5	36.9	62.4	53.8	5.1	9.3
H	17.6	33.2	73.6	54.2	8.8	12.6
Mean	25.6 *	31.0	67.5	56.4	6.9 **	12.7
Std. Dev.	2.22	1.50	2.04	2.66	1.01	0.66

[a]As per cent of the recovered phospholipid.

*$P < .05$.

**$P < .01$.

to approximately 2.0 per cent in both tissues and the other contents proportionately increased when seven combinations of chloroform and methanol were used for elution (Acosta et al., 1966).

In agreement with the above observations, the order of decreasing content in this study consistently was: lecithins, cephalins, and sphingomyelin. Sphingomyelin constituted a larger proportion of the thigh than of the breast phospholipid. This difference which did not occur in either of the previous studies might possibly have resulted from a more efficient isolation of the sphingomyelin in the breast than in the thigh.

The ratios of the content of phospholipid fractions to the DNA content of breast and thigh muscle tissue are shown in Table VI. In each bird, the ratio of sphingomyelin to DNA was consistently higher in the thigh than in the breast and the difference between the mean ratios was significant ($P < .01$). With two exceptions, the ratio of cephalins to DNA was also higher in the thigh than in the breast. The difference between the mean ratios was not significant. The lecithin-DNA ratio in the thigh was neither consistently higher nor lower than that of the breast and no significant difference occurred between the mean ratios of the two muscles. Because the specific metabolic roles of the phospholipid fractions have not yet been ascertained, the higher cephalin-DNA and sphingomyelin-DNA ratios in the thigh than in the breast cannot be explained.

TABLE VI

RELATIONSHIP OF CEPHALINS, LECITHINS, AND SPHINGOMYELIN TO DNA IN CHICKEN BREAST AND THIGH MUSCLES[a]

Bird	Cephalins		Lecithins		Sphingomyelin	
	Breast	Thigh	Breast	Thigh	Breast	Thigh
A	19.0	23.8	65.1	49.0	5.2	11.8
B	18.5	12.6	45.3	31.7	5.0	8.2
C	7.9	19.4	31.2	37.5	1.3	8.7
D	10.9	16.6	24.2	29.3	5.0	6.9
E	7.8	12.7	22.9	17.6	1.6	4.3
F	12.0	9.9	19.0	22.0	2.5	4.0
G	12.9	24.3	24.7	35.4	2.0	6.1
H	8.3	15.4	34.6	25.1	4.1	5.8
Mean	12.2	16.8	33.4	31.0	3.3	** 7.0
Std. Dev.	3.40	5.29	15.3	9.88	1.6	2.6

[a]Both in mg. per 100 g. of tissue.

**$P < .01$.

CHAPTER V

SUMMARY

The content of phospholipids per unit of DNA and the distribution of cephalins, lecithins, and sphingomyelin in the breast and thigh muscle tissues of eight chickens were studied. Nucleic acids were extracted from the muscle tissues with hot trichloroacetic acid and the content of desoxyribose, determined colorimetrically by the Dische diphenylamine reaction, was used as an index to the DNA concentration. Thin layer chromatography was used to separate the triglycerides from the phospholipids and to fractionate the latter compounds. Phosphorus analysis was used to estimate the quantity of phospholipids and of each phospholipid class.

The amounts of total lipid, phospholipid, and DNA in a given weight of tissue were lower in the breast than in the thigh on both a wet weight and a dry weight basis. Phospholipids constituted a greater proportion of the total lipids in the breast than in the thigh muscles. Although the mean amount of phospholipid per unit of DNA was higher in the thigh than in the breast, this relationship was not consistent.

In both tissues the phospholipid classes in order of decreasing abundance were lecithins, cephalins, and sphingomyelin. Concentrations of cephalins and sphingomyelin were lower in breast than in thigh phospholipids and the percentage of lecithin was correspondingly higher

in the breast phospholipids. Cephalin-DNA and sphingomyelin-DNA ratios were higher in thigh than in breast muscle.

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APPENDIX

TABLE VII

PER CENT MOISTURE IN CHICKEN BREAST AND THIGH MUSCLES

Bird	Breast	Thigh
A	75.1	77.2
B	74.2	76.7
C	75.4	76.8
D	74.5	76.6
E	75.3	77.1
F	74.8	76.6
G	74.0	75.9
H	74.4	76.6
Mean	74.7	76.7
Std. Dev.	0.19	0.14