

TENDERNESS AND CHANGES IN pH AND
PROTEIN EXTRACTABILITY OF TURKEY BREAST
MUSCLE EXHIBITING DIFFERENT RATES OF
POST - MORTEM GLYCOLYSIS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
DOELAS RANDY LANDES

1969



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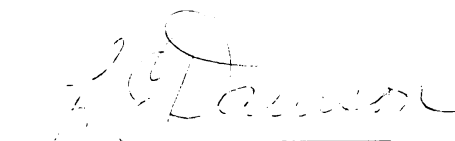
TENDERNESS AND CHANGES IN pH AND PROTEIN
EXTRACTABILITY OF TURKEY BREAST MUSCLE
EXHIBITING DIFFERENT RATES OF POST-MORTEM GLYCOLYSIS

presented by

DOELAS RANDY LANDES

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Food Science



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Major professor

Date November 17, 1969

ABSTRACT

TENDERNESS AND CHANGES IN pH AND PROTEIN EXTRACTABILITY OF TURKEY BREAST MUSCLE EXHIBITING DIFFERENT RATES OF POST-MORTEM GLYCOLYSIS

By

Doelas Randy Landes

Rate of pH decline and protein extractability of turkey breast muscle was determined from birds exhibiting different rates of post-mortem glycolysis. Muscle tenderness values were determined and related to the post-mortem changes. This investigation was approached in the following manner.

Different rates of post-mortem glycolysis were produced by injecting one group of birds (AN birds) with pentobarbital before slaughter to prevent the death struggle while a second group of birds (N-AN birds) was allowed to struggle freely during slaughter.

Quantitative changes in sarcoplasmic and fibrillar proteins of the breast muscle were observed at 0, 1/4, 1/2, 1, 3, 6, 12, 24, 48 and 72 hours post-mortem. This was done by extracting muscle samples with KCl-phosphate buffer, pH = 7.5 and $I^{1/2} = 1.0$. Portions of the extract were diluted to $I^{1/2} = 0.25$, $I^{1/2} = 0.05$, or treated with

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trichloroacetic acid and the actomyosin-, sarcoplasmic protein- and non-protein- nitrogen, respectively, were determined. Data from these fractionations were also used to determine total extractable- and total fibrillar protein- nitrogen. Residue of the KCl-phosphate buffer extraction was treated with a pyrophosphate containing buffer in an attempt to cause dissociation of the unextracted actomyosin. Myosin and actin nitrogen released by dissociation of the unextracted actomyosin was determined. The remaining residue was extracted with a sodium hydroxide solution in order to determine the unextracted soluble protein nitrogen.

Changes in pH during post-mortem aging was monitored by blending muscle samples in 0.001 M sodium iodoacetate solution and determining pH of the homogenates.

Kramer shear values of muscle from all of the birds were determined as an estimate of tenderness. Correlation of these tenderness data with changes in pH and protein extractability was determined.

The rate of pH decline during the first 6 hours post-mortem was faster in muscle of the N-AN birds than in the muscle of the AN birds. Statistical minimum pH levels of 5.85 - 5.66 and 5.87 - 5.77 were reached at 6 and 12 hours post-mortem in the N-AN and AN birds respectively.

Non-protein nitrogen did not change significantly in muscle from either group of birds during the 72 hour experimental period. However, it did appear to increase during post-mortem aging in the AN birds. This fraction was essentially the same in both groups of birds.

The amount of sarcoplasmic protein extracted from muscle was significantly greater from the AN birds than from the N-AN birds, but there were no significant changes in extractability during aging in either group.

Extractability of total extractable nitrogen, total fibrillar protein nitrogen and actomyosin nitrogen fractions closely paralleled each other in muscle from both groups of birds. Extractability of these fractions began to increase steadily from zero time to statistical maximum levels at 1, 1/2 and 1 hour respectively in the N-AN birds. However, extractability of these three fractions remained fairly constant at a low level in the AN birds during the first hour post-mortem, then it began to increase to statistical maximum levels at 12 hours for all three fractions.

Very little dissociation of the unextracted actomyosin occurred in muscle from either group of birds when it was extracted with the pyrophosphate containing buffer. The released residual myosin and actin nitrogen extracted as well as the unextracted soluble protein nitrogen were fairly constant during the first hour post-mortem in the AN birds. The level of extractability of these three fractions then began to decline similar to the declining levels of extractability of these fractions from muscle of the N-AN birds which started at zero time. The statistical minimum levels of extractability of these three fractions from muscle were reached at 1/4, 1 and 3 hours and 6, 6 and 12 hours post-mortem in the N-AN and AN birds respectively.

Breast muscles of the AN birds were found to have significantly

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lower shear values than those of the N-AN birds. Correlation analysis revealed that as pH declined in the AN birds during the first 6 hours post-mortem, shear values decreased, however, pH decline after 6 hours resulted in higher shear values. No similar trends were observed in the N-AN birds. No relationship was found between tenderness and protein extractability in muscle from either group of birds.

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PROTEIN EXTRACTABILITY OF TURKEY BREAST
MUSCLE EXHIBITING DIFFERENT RATES OF
POST-MORTEM GLYCOLYSIS**

By

Doelas Randy Landes

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1969

THE UNIVERSITY OF CHICAGO
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THEORY OF MATHEMATICS
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THEORY OF MATHEMATICS

by

John N. M. Jones

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Submitted to
the University of Chicago
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DEPARTMENT OF MATHEMATICS

Department of Zoology

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ACKNOWLEDGMENTS

I wish to give thanks first of all to God through Christ for the grace that has been given me to reach this point, and I ask his continued grace that I may use the knowledge that I have gained during this period of study in a wise and Christian manner.

To my wife, Rebecca, I give a very special "Thank you" for the efforts she has made in helping me through the joys and trials of this period of work and study.

Larry York and Prafulla Pani are sincerely thanked for the assistance that they gave me during this study.

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D. R. L.

TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
II. LITERATURE REVIEW.	3
A. Muscle Classification.	3
B. General Composition of Muscle.	4
C. Structure of Muscle.	4
D. Muscle Proteins.	8
1. Sarcoplasmic Proteins.	8
2. Myofibrillar Proteins.	10
a. Myosin	10
b. Actin.	11
c. Tropomyosin.	12
3. Stroma Proteins.	13
E. Muscle Contraction	13
F. Rigor Mortis	15
1. Physical Changes	15
2. Chemical Changes	16
G. Post-mortem Physicochemical Changes in Poultry Muscle	17
1. Rigor Mortis	17
2. Cold Shortening.	18
3. Thaw Rigor	19
4. Glycogen Degradation and pH Decline.	19
5. Phosphocreatine and ATP Degradation.	20
6. Inosinic Acid Formation and Degradation.	21
H. Effects of Processing on Tenderness of Poultry Muscle	22
1. Slaughter.	22
2. Scalding	23
3. Feather Removal.	24
4. Aging.	25
I. Extractability and Fractionation of Poultry Muscle Proteins.	29
1. Chicken Studies.	29
2. Turkey Studies	30
III. EXPERIMENTAL METHODS	33
A. Reagents	33
B. Centrifugation	33
C. pH Measurements.	33
D. Experimental Animals	34
E. Processing Procedure	34

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1947-1948

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1947-1948

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1947-1948

1947-1948

1947-1948

	Page
F. Sampling Procedure	35
G. Muscle pH Determination.	35
H. Protein Extraction and Fractionation	36
1. Primary Extractions.	36
2. Secondary Extractions.	38
3. Fractionation of C-1	39
I. Nitrogen Determination	40
J. Estimation of Protein Fractions.	40
K. Cooking and Tenderness Evaluation of the Birds . .	41
L. Statistical Analyses	42
IV. RESULTS AND DISCUSSION	43
A. Post-mortem pH Changes	43
B. Post-mortem Changes in Protein Extractability. . .	46
1. Total Extractable Nitrogen	49
2. Non-protein Nitrogen	49
3. Sarcoplasmic Protein Nitrogen.	49
4. Total Fibrillar Protein Nitrogen	52
5. Actomyosin Nitrogen.	55
6. Residual Myosin Nitrogen	55
7. Residual Actin Nitrogen.	58
8. Unextracted Soluble Protein Nitrogen	58
C. Implications of the Post-mortem Changes in Protein Extractability	60
1. Non-protein Nitrogen	60
2. Sarcoplasmic Protein Nitrogen.	62
3. Total Extractable Nitrogen, Total Fibrillar Protein Nitrogen and Actomyosin Nitrogen . . .	65
4. Residual Myosin Nitrogen, Residual Actin Nitrogen and Unextracted Soluble Protein Nitrogen	70
D. Tenderness Evaluation.	71
E. Correlation of Tenderness with Post-mortem Changes in pH and Protein Extractability	73
V. SUMMARY.	78
VI. LITERATURE CITED	81
VII. APPENDICES	92

LIST OF TABLES

Table	Page
1. Effect of ante-mortem injection of turkeys with pentobarbital on pH of the breast muscle, 0 to 72 hours post-mortem	44
2. pH decline of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital.	44
3. Effect of ante-mortem injection of turkeys with pentobarbital on total extractable nitrogen of the breast muscle, 0 to 72 hours post-mortem	50
4. Total extractable nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital.	50
5. Effect of ante-mortem injection of turkeys with pentobarbital on non-protein nitrogen of the breast muscle, 0 to 72 hours post-mortem.	51
6. Non-protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital.	51
7. Effect of ante-mortem injection of turkeys with pentobarbital on sarcoplasmic protein nitrogen of the breast muscle, 0 to 72 hours post-mortem.	53
8. Sarcoplasmic protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital.	53
9. Effect of ante-mortem injection of turkeys with pentobarbital on total fibrillar protein nitrogen of the breast muscle, 0 to 72 hours post-mortem.	54
10. Total fibrillar protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital.	54

Table		Page
11.	Effect of ante-mortem injection of turkeys with pentobarbital on actomyosin nitrogen of the breast muscle, 0 to 72 hours post-mortem	56
12.	Actomyosin nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital. . . .	56
13.	Effect of ante-mortem injection of turkeys with pentobarbital on residual myosin nitrogen of the breast muscle, 0 to 72 hours post-mortem	57
14.	Residual myosin nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital. . . .	57
15.	Effect of ante-mortem injection of turkeys with pentobarbital on residual actin nitrogen of the breast muscle, 0 to 72 hours post-mortem	59
16.	Residual actin nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital. . . .	59
17.	Effect of ante-mortem injection of turkeys with pentobarbital on unextracted soluble protein nitrogen of the breast muscle, 0 to 72 hours post-mortem.	61
18.	Unextracted soluble protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital	61
19.	Shear values of cooked turkey breast muscle from birds aged for 72 hours with and without ante-mortem injection of pentobarbital.	72
20.	Correlation coefficients of shear values with pH and protein extractability of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injection of pentobarbital.	74, 75

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LIST OF FIGURES

Figure		Page
1.	Diagram of the organization of skeletal muscle from the gross to the molecular level	5
2.	Flowsheet for extraction and fractionation of muscle proteins	37
3.	Extractability of protein fractions from turkey breast muscle, 0 to 72 hours post-mortem, of birds given ante-mortem injections of pentobarbital	47
4.	Extractability of protein fractions from turkey breast muscle, 0 to 72 hours post-mortem, of birds with no ante-mortem treatment	48

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- The Earth is a sphere, and its surface is covered by water.
- The Earth is divided into four main parts: North America, South America, Europe, and Asia.
- The Earth is divided into five main parts: North America, South America, Europe, Asia, and Africa.
- The Earth is divided into six main parts: North America, South America, Europe, Asia, Africa, and Australia.
- The Earth is divided into seven main parts: North America, South America, Europe, Asia, Africa, Australia, and Antarctica.

INTRODUCTION

Tenderness may be considered to be one of the most critical attributes of meat for consumer acceptance. However, tenderness of poultry muscle is a variable factor that is often unpredictable. It does not seem to be controlled by any one single factor. Marion (1967) has reviewed the effects of breed, strain, nutrition, grade, enzymes and processing procedures on tenderness in a concise and understandable manner.

The study of meat tenderness covers the transition of muscle from the living state to the dead state, a period which includes rigor mortis. During this period the levels of many biochemical compounds change extensively. Scientists at the U. S. Western Regional Research Laboratory (deFremery and associates) have studied extensively the biochemical changes in chickens subjected to various processing conditions. During conversion of muscle to meat the physical properties of muscle tissue also change profoundly. Probably the most outstanding change is a rapid increase in the modulus of elasticity of the muscle tissue during onset of rigor. The group of researchers in the Division of Biosciences of the National Research Council of Canada (Khan and associates) has studied extensively the changes in extractability of proteins from chicken muscles during the aging process. From the results of these two

groups, and results of various other individuals and groups, it appears that post-mortem biochemical and physical changes that occur are closely related to tenderness of chickens.

Since most studies of these changes have been done with chickens this present study was initiated to determine some of the biochemical and physical changes in breast muscle of turkeys treated to produce slow and fast rates of post-mortem glycolysis. The relationship of these changes to ultimate tenderness was also investigated. This study was approached in the following manner.

1. Different rates of post-mortem glycolysis were produced by injecting one group of birds with pentobarbital before slaughter to prevent the death struggle while a second group of birds was allowed to struggle freely during slaughter.
2. Quantitative changes in sarcoplasmic and fibrillar proteins were observed at 0, 1/4, 1/2, 1, 3, 6, 12, 24, 48 and 72 hours post-mortem.
3. Rate of post-mortem glycolysis was monitored by measuring pH at the various time intervals.
4. The aged turkeys were cooked and the level of tenderness was determined.
5. The relationship of tenderness to the post-mortem biochemical and physical changes was determined.

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author to the reader, in which he explains the purpose of the study and the methods used.

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LITERATURE REVIEW

Muscle Classification

Muscle is defined as a contractile tissue composed of bundles of elongated cells (muscle fibers) that function to produce bodily movements (Funk and Wagnalls, 1963). It can be divided into the general categories of smooth and striated muscle. Smooth muscle is innervated by the autonomic nervous system and its contraction is not subject to voluntary control. Striated muscle is subdivided into two distinct types, skeletal and cardiac. The fibers of skeletal muscle are syncytial and are innervated by the cerebrospinal system of nerves. Their contraction is under voluntary control. The fibers of cardiac muscle are made up of separate cellular units, and their rhythmical contraction is involuntary. In general, visceral musculature is composed of smooth muscle. The somatic musculature, comprising the flesh of the body wall and of the extremities, is striated skeletal muscle. Cardiac muscle makes up the wall of the heart and may extend into the proximal portions of the pulmonary veins (Bloom and Fawcett, 1968).

The major constituent of meat is striated skeletal muscle, thus, for convenience, the term muscle as used herein will refer to striated skeletal muscle.

General Composition of Muscle

Lawrie (1966) stated that the chemical composition of typical adult mammalian muscle after rigor mortis but before degradative changes post-mortem can be approximated to 75 percent of water, 18 percent of protein, 3.5 percent of soluble non-protein substances and 3 percent of fat. In contrast to this Watt and Merrill (1963) stated that the average composition of all classes of turkey light meat can be approximated to 73 percent of water, 24.6 percent of protein, 1.2 percent of fat and 1.2 percent of ash. The dark meat can be approximated to 73.6 percent of water, 20.9 percent of protein, 4.3 percent of fat and 1.1 percent of ash. However, an understanding of the nature and behavior of muscle and of its variability cannot be based on such a simple approach.

Structure of Muscle

Figure 1 is a diagram of the organization of muscle from the gross to the molecular level and can be referred to as the various structures are discussed.

The unit of organization of muscle is the fiber, a long cylindrical multinucleated cell whose diameter is usually in the range of 10-100 μ . Individual cells may extend along the entire length of the muscle (Huxley, 1960), however, this is not the rule. Large numbers of parallel muscle fibers are grouped into fascicles, which are visible in fresh muscle. The fascicles are associated in

SKELETAL MUSCLE

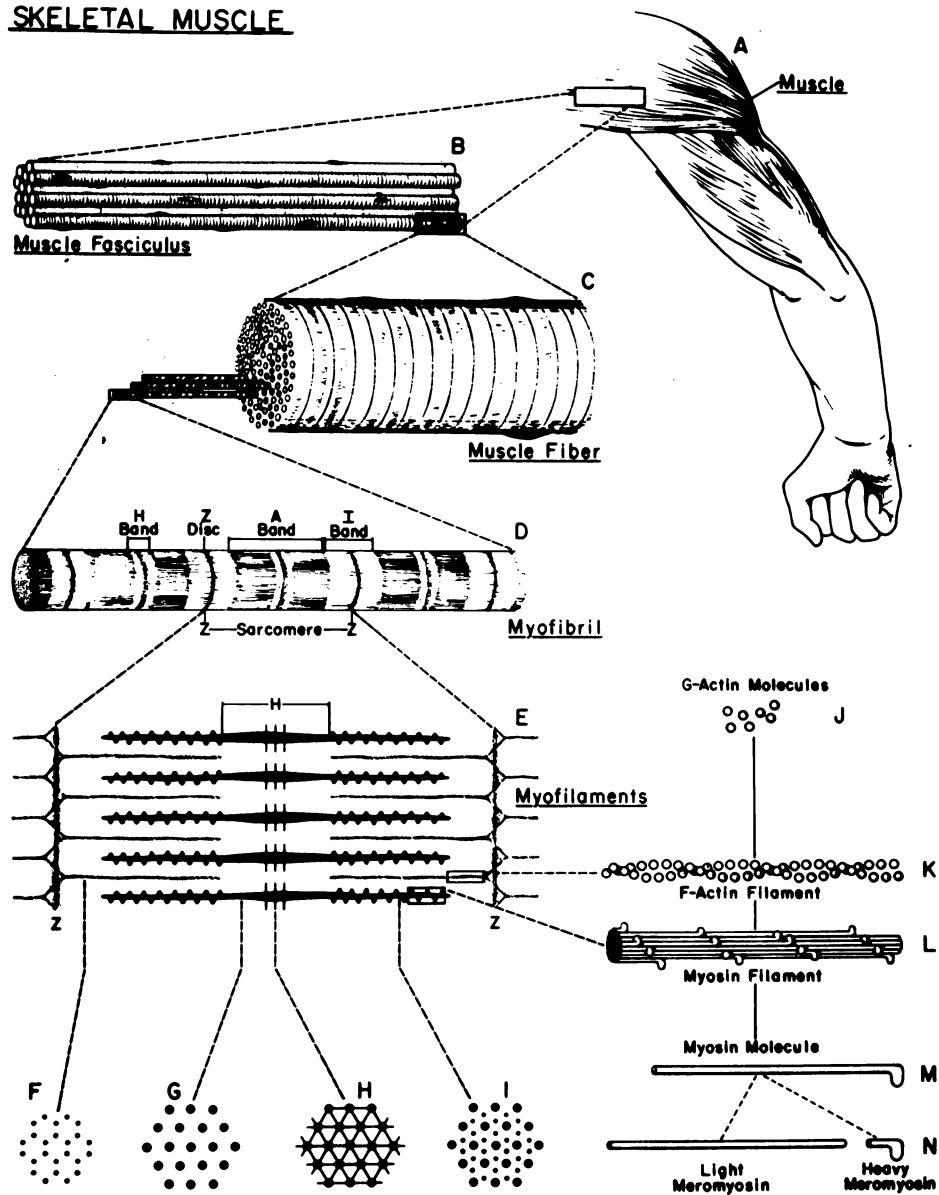
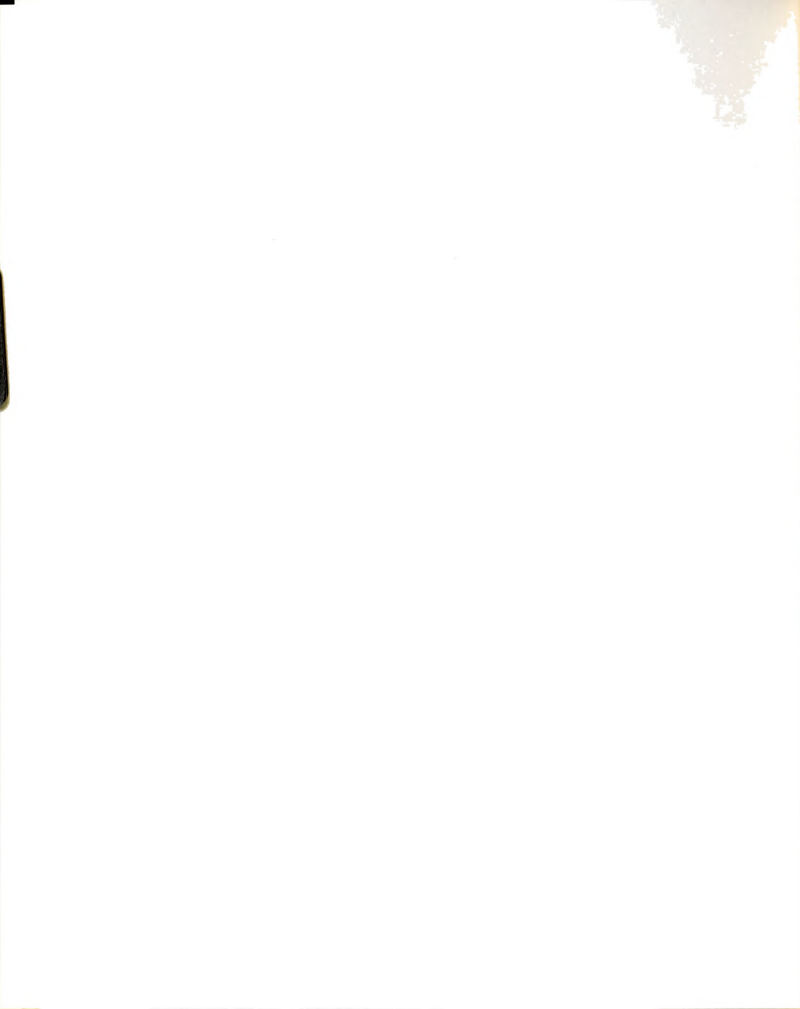


Figure 1. Diagram of the organization of skeletal muscle from the gross to the molecular level. F, G, H and I are cross sections at the levels indicated. (Drawing by Sylvia Colard Keene, Printed with permission from A Textbook of Histology, Bloom, W. and D. W. Fawcett, W. B. Saunders Co. 1968.)



various patterns to form the several types of muscles recognized by the anatomist - unipennate, bipennate, and so on.

Surrounding the muscle as a whole is a sheath of connective tissue known as the epimysium. Thin collagenous septa extend inward from the epimysium surrounding all of the fascicles or bundles: these separating septa constitute the perimysium. From the perimysium a fine connective tissue reticulum invests the individual muscle fibers and constitutes the endomysium. This total network of connective tissue binds together individual units and groups of units and integrates their action, and gives a certain degree of freedom of motion between them. Thus, although muscle fibers are closely packed together, each is somewhat independent of adjacent fibers and each bundle can move independently of neighboring bundles (Lawrie, 1966; Bloom and Fawcett, 1968).

The muscle fiber can be divided into three main constituents, the sarcolemma, sarcoplasm and myofibrils.

The sarcolemma is located just beneath the endomysium and is the limiting sheath of the fiber. It was once thought to be structureless but has now been shown to be a double membrane with the components about 50-60 Å apart (Robertson, 1958). Bloom and Fawcett (1968) and Venable (1963) indicate that this structure may have three components - the plasmalemma, its protein-polysaccharide external coating and a delicate network of associated reticular fibers. They indicate that the term sarcolemma should be reserved for the plasmalemma only.

Sarcoplasm is the cytoplasmic matrix of the cell which contains the usual cell organelles and inclusions. Some of these are

mitochondria, Golgi apparatus, sarcoplasmic reticulum, lipid bodies, the protein myoglobin and various other dissolved or suspended substances. Nuclei of the muscle fiber are also in the sarcoplasm and are usually located just beneath the sarcolemma (Bloom and Fawcett, 1968; Bennett, 1960; Walls, 1960).

According to Bloom and Fawcett (1968) the rest of the cell interior is occupied by myofibrils $1-2\ \mu$ in diameter which are highly organized contractile structures peculiar to muscle. In longitudinal sections of muscle the feature of greatest interest is the identification of the bands observed in cross striated myofibrils. The main bands observed are the A-band, I-band, Z-line and M-line which are briefly described below.

1. The A-band consists mainly of myosin filaments $100\ \text{\AA}$ in diameter and $1.5\ \mu$ long. This band stains darkly with iron-hematoxylin but appears bright when viewed with a polarizing microscope.
2. The I-band consists mainly of actin filaments $50\ \text{\AA}$ in diameter extending about $1\ \mu$ in either direction from the Z-line. Tropomyosin may also be associated with actin in the filaments making up this band. The I-band is not stained with iron-hematoxylin and appears dark when viewed with a polarizing microscope.
3. The Z-line bisects each I-band and contains tropomyosin. This line marks the bounds of the sarcomere or the repeating structural unit to which all morphological events of the contractile cycle are referred.

4. The M-line bisects each A-band and is thought to hold the myosin filaments together at their mid-point. The clarity of this line varies with the degree of contraction and method of preparation of the histological section.

Transverse sections of the myofibril in the A-band where overlap of actin and myosin occurs shows each myosin filament surrounded by six actin filaments in a hexagonal array. Also each of the thick filaments has short lateral projections along its length that are postulated to react with the thin filaments during muscle contraction (Huxley, 1960; Huxley and Hanson, 1960).

Muscle Proteins

Lawrie (1966) broadly divided the proteins of muscle tissue into those which are soluble in water or dilute salt solutions (sarco-plasmic proteins), those which are soluble in concentrated salt solutions (myofibrillar proteins) and those which are insoluble in the latter, at least at low temperature (proteins of connective tissue and other formed structure).

Sarcoplasmic Proteins

According to Mommaerts (1950) the sarcoplasmic protein is a fairly constant unit in preparative work but is far from homogenous. Now it is known to be a complex mixture of about 50 components, many of which are enzymes of the glycolytic pathway (Lawrie, 1966). Bendall (1964) stated that many of these components are easily

denatured under mild acid conditions (pH 4-5), which may play a role in the denaturation which sometimes occurs during processing and aging of meat.

Also present in the sarcoplasm is the sarcoplasmic reticulum. Ebashi and Lipmann (1962) demonstrated that vesicles of the sarcoplasmic reticulum show properties of the relaxing factor, which was first discovered by Marsh (1952). It has been shown that the relaxing factor is involved in changing the calcium level in the cell, and in the presence of adenosine triphosphate (ATP) may reduce the calcium concentration in the region of the myofibrils to about $0.02 \mu\text{M}$ or less (Weber et al., 1963). Ebashi and Lipmann (1962) showed that a constant supply of ATP was necessary to hold the calcium ions in the membrane fraction. Gergely (1968) indicated that sarcoplasmic reticulum particles he isolated had a globular head with a diameter of $0.1 - 0.2 \mu$ to which one or more tails were attached. These particles appeared to accumulate calcium only in the head portion. Also the adenosine triphosphatase (ATPase) activity that is characteristic of these particles (Hasselbach, 1964) was found to be concentrated at the junction of the tail and globular part. Lee (1965) was able to show that electrical stimulation causes the release of calcium from the sarcoplasmic reticulum which will allow muscle contraction to occur (Bendall, 1964; Davies, 1963), and when the electrical stimulation ceases there is reuptake of calcium ions by this system in the presence of ATP.

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Myofibrillar Proteins

The proteins of the myofibril make up the filamentous organization of the fiber and participate in contraction of the muscle. Major proteins of the myofibril (80-90 percent) are myosin, actin and tropomyosin (Poglazov, 1966; Perry, 1967). Ebashi (1968) discussed characteristics of three additional proteins he found in the myofibril: α -actinin, β -actinin and troponin. Perry (1967) and Poglazov (1966) also indicated the presence of the proteins - inhibitory factor, fibrillin, ribonucleoprotein, contractin, meta-myosin, α' -myosin, A-protein and Y protein. However, some of these may be complexes of other known proteins. No review of properties and characteristics of these minor protein components will be made. See Rampton (1969) for a detailed discussion of these proteins.

Myosin

Myosin is the chief protein of the thick myofilaments of the myofibril (Bloom and Fawcett, 1968). It is composed of light (L-) meromyosin and heavy (H-) meromyosin (Szent-Györgyi, 1953). Dreizen et al. (1967) stated that the molecular weight of myosin is about 500,000, and it is a highly asymmetric molecule with the ratio of length to diameter being about 100:1 (Lawrie, 1966). L-meromyosin is about 100 percent α -helical whereas H-meromyosin is only about 45 percent α -helical (Bendall, 1964). He also stated that the features of overwhelming importance in the myosin molecule are its enzymatic activity as an ATPase and its involvement in contraction.

1. The first step in the process of creating a new product is to identify a market need. This can be done through market research, which involves gathering information about the target market and its needs. Once a market need has been identified, the next step is to develop a concept for a new product that addresses this need. This concept should be based on the market research and should be unique and innovative. The next step is to develop a business plan for the new product. This plan should outline the costs of production, the pricing strategy, and the marketing strategy. Once the business plan has been developed, the next step is to secure funding for the new product. This can be done through a variety of sources, including venture capitalists, angel investors, and crowdfunding. Once funding has been secured, the next step is to develop a prototype of the new product. This prototype should be used to test the product and to gather feedback from potential customers. Finally, the next step is to launch the new product into the market. This can be done through a variety of channels, including retail stores, online marketplaces, and direct sales.

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The H-meromyosin portion of the molecule contains the enzymatic properties and the actin combining power of myosin while the L-meromyosin portion appears to be of purely structural importance.

Huxley (1963) was able to reconstitute filaments from purified myosin which are very similar in appearance to the thick filaments present in muscle. These filaments were formed in such a way that the L-meromyosin portion of the myosin molecules made up most of the backbone of the filaments and the H-meromyosin portion made up the crossbridges. This was accomplished when aggregates of myosin molecules were laid down with opposite polarity, the tail ends (L-meromyosin portion) lying next to each other towards the center, the H-meromyosin heads being oriented away from the center. Thus a completed myosin filament has a polarity and a center of symmetry with all the heads facing away from the center.

The ATPase of myosin is activated by calcium ions and inhibited by magnesium ions (Bendall, 1964). This enzymatic activity allows release of energy for contraction of the muscle.

Actin

Actin was first isolated by Straub in 1942. The actin isolated was shown to be a globular protein. This molecule has a bound nucleotide, ATP. It will polymerize under certain conditions such as the addition of neutral salts. The fibrous polymer contains bound adenosine diphosphate (ADP), therefore, an inorganic phosphate is released during the polymerization process. Fibrous actin can be depolymerized both in the presence and absence of ATP, however, if

ATP is absent the process is irreversible (Hayashi, 1967; Bendall, 1964). Mommaerts (1966) stated that globular actin has an affinity for ATP, but is not an ATPase. However, during polymerization it may undergo a conformational change. While in this transient state, the actin molecule temporarily acquires ATPase activity and splits ATP if it comes in contact. It is thought by Davies (1963) that the bound dinucleotide of fibrous actin is involved in the binding of actin to myosin during contraction of the muscle.

Globular actin has a molecular weight of about 60,000 whereas fibrous actin may have a weight of many millions. Fibrous actin consists of a double helix, each helix consisting of globular monomers of actin, about 55 \AA in diameter (Perry, 1965).

Tropomyosin

Tropomyosin is a highly charged protein discovered by Bailey (1948). It forms viscous solutions in water, but the viscosity falls sharply upon addition of only 0.01 M potassium chloride. It is high (approximately 100 percent) in α -helical content with a monomer molecular weight of about 60,000. The axial ratio is about 25:1. It resembles myosin in its chemical composition but differs markedly in most of its characteristics (Bendall, 1964; Lawrie, 1966). It is thought to be located at the Z-line and in association with the actin filaments (Hanson and Lowy, 1963). Also the work of Huxley (1963) tends to support the presence of tropomyosin at the Z-line. Ebashi (1968) working with fluorescein isothiocyanate labeled tropomyosin found that it binds with actin and the Z-line.

Stroma Proteins

The stroma proteins are those that remain as residue after repeated extraction of a well homogenized muscle sample with strong salt solutions. This material is of a collagenous nature and contributes to the structure of the sarcolemma and possibly the Z-line (Szent-Györgyi, 1960).

Muscle Contraction

A sliding filament theory of muscle contraction was proposed by Hanson and Huxley in 1955. Essential observations on which the sliding filament theory is based may be summarized as follows. There is a succession of ordered arrays of thick and thin filaments which overlap each other. In the A-band of the myofibril there are myosin containing filaments spaced about 450 Å^o apart in a hexagonal pattern. Thin actin containing filaments run from the Z-lines through the I-bands and overlap with the thick filaments of the A-band. At rest length the thin filaments do not reach to the center; thus, there is a less dense area in the center of the A-band known as the H-zone. When the muscle changes its length the A-bands remain constant in length while the I-bands either shorten or lengthen according to whether the sarcomere length is decreasing or increasing. Corresponding changes are observed in the width of the H-zone (Huxley, 1965).

Needham (1960) discussed the intimate involvement of ATP, calcium and magnesium in muscle contraction. Davies (1963) advanced a theory on the mechanism by which sliding of the filaments can occur. This

theory was based on the present knowledge of muscle microstructure, properties of muscle proteins and their interaction with ATP and calcium. It proposed that activation of muscle releases bound calcium from the sarcoplasmic reticulum and sarcolemma. This calcium diffuses and forms chelate links between bound ATP at the end of an asymmetrically extended, rapidly snaking polypeptide of the crossbridges of the H-meromyosins of the myosin filaments and bound ADP of the fibrous actin filaments. The calcium neutralizes the electric charge on the bound ATP of the polypeptide which spontaneously contracts to an α -helix by the energy of hydrogen-bond and hydrophobic-bond formation. This contraction drags the actin filament along the myosin filament. This brings the ATP into the range of action of the H-meromyosin ATPase, which cleaves off the terminal phosphate and breaks the link. On rephosphorylation of the ADP the helix is pulled out to a largely extended chain by the repulsion of the negative charge on the ATP and a fixed charge on the H-meromyosin. This cycle is repeated during the active state, which ends when the calcium is pumped back into the sarcoplasmic reticulum and sarcolemma.

In a different type of mechanism by which sliding of the filaments can occur, it is assumed that the overall length of one of the filaments undergoes a small amount of periodic change. The periodicity associated with actin is different from that associated with myosin. Thus, at any given time not all myosin sites can combine with actin sites. If at the site of interaction there is a lengthening or shortening of the actin filament the result will be that new sites on the actin will be brought into an interaction with myosin sites,

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and the cycle can repeat itself moving the actin filaments along the myosin filaments (Szent-Györgi, 1966).

Rigor Mortis

Physical Changes

At some period of time after death muscle becomes inextensible, and this is the state that has long been called rigor mortis. The loss of extensibility is due to actomyosin formation which proceeds slowly at first (the delay period) then with great rapidity (the fast phase): extensibility then remains constant at a low level (Lawrie, 1966). deFremery (1966a) stated that in pre-rigor poultry muscle, the modulus of elasticity is generally in the range of $0.5-2 \times 10^3$ g/cm² which remains more or less constant until the muscle begins to stiffen. The modulus of elasticity then increases rapidly to $8-10 \times 10^3$ g/cm². Pool (1963) indicated that shortening accompanied stiffening in poultry muscle. Bendall (1951) postulated that shortening of muscle fibers in rigor occurs essentially by the same mechanism as physiological contraction. Later he indicated that only a fraction of the muscle fibers are involved in shortening during rigor mortis and this shortening is irreversible (Bendall, 1960). Marsh (1954) also indicated that shortening during rigor may be considered a slow, irreversible physiological contraction. However, Nauss and Davies (1966) pointed out that the contractile components develop tension on stimulation during normal contraction, but in rigor mortis contraction and tension development occurs in the absence of external

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stimulation and can bear a load in the absence of ATP.

Poultry muscle is tender when cooked before onset of rigor mortis, but tenderness decreases as rigor proceeds. After a period of aging the muscle becomes tender again (deFremery, 1966b). This tenderization with aging has been called "resolution of rigor" by some researchers and people in the meat industry (Fischer, 1963; Goll, 1968). This "resolution of rigor" has been taken to mean dissociation of actomyosin and has been refuted by Bendall (1963). However, recently several different groups (Goll, 1968; Gothard et al., 1966; Stromer and Goll, 1967a, 1967b; Stromer et al., 1967; Scharpf et al., 1966; Takahashi et al., 1967) have indicated that there may be some dissociation of actomyosin during aging but not to the degree as before rigor. Also another contributor to this "resolution of rigor" is loss of the Z-line structure and weakening and eventual rupture of bonds between the I-band filaments and the Z-line material (Stromer and Goll, 1967b; Stromer et al., 1967; Fukazawa and Yasui, 1967; Sayre, 1968a).

Chemical Changes

In 1943 Erdős^u showed that disappearance of ATP was closely related to onset of stiffening in post-mortem muscle. Soon afterwards the central role of ATP in rigor mortis was established largely as a result of the work of Bate-Smith (1939, 1948), Bate-Smith and Bendall (1947, 1949, 1956), Bendall (1951, 1960), Marsh (1952, 1954), Marsh and Thompson (1958) and Lawrie (1953). These early studies elucidated many of the relationships among ante-mortem stress,

glycogen level, ultimate pH, ATP degradation and onset of rigor mortis that are generally accepted today. On the basis of these and other studies, the following chemical changes are now considered to be more or less characteristic of post-mortem muscle:

1. anaerobic breakdown of muscle glycogen to lactic acid, starting immediately after death;
2. a decrease in pH due primarily to formation of lactic acid;
3. a fall in phosphocreatine content of muscle, this fall occurring very rapidly after muscle glycogen reserves have been exhausted;
4. a decrease in ATP concentration, this decrease occurring very slowly until after disappearance of phosphocreatine after which time it proceeds rapidly to a level usually less than 20 percent of its initial level and
5. the appearance of ammonia and inosinic acid from deamination of adenylic acid.

Post-Mortem Physicochemical Changes in Poultry Muscle

Rigor Mortis

The processes associated with rigor mortis already described are also applicable to poultry, and deFremery (1966b) indicated that onset of rigor occurs normally in poultry at about 1 hour post-mortem.

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The processes associated with the various stages of development are also applicable to poetry, and especially the importance of rigor comes naturally in poetry as well as in science.

Cold Shortening

The phenomenon of cold shortening was first described by Locker et al., (1963) in freshly excised beef muscle. They found that minimal muscle shortening occurred at post-mortem temperatures between 14° and 19°C, with increased shortening at temperatures on either side of this range. deFremery and Pool (1960) reported that rate of ATP disappearance and toughness in excised chicken breast muscle follow the same general pattern and are minimal in the 10° to 20°C temperature range, however, at 0°C disappearance of ATP was more rapid than at 10°C. Also deFremery (1966a) indicated that ATP disappearance from chicken muscle was more rapid at 43°C than at 14°C. These results suggest that loss of muscle extensibility may also proceed more rapidly at temperatures on both sides of the range of 10° - 20°C. Reportedly, Dr. Cook of the University of Sidney (Briskey et al., 1966) has observed cold shortening in chicken breast muscle. Welbourn et al., (1968) found that sarcomere lengths of excised turkey muscle cooled at 0°C were shorter than those of muscle cooled at 16°C, but there was only slight shortening in sarcomere lengths of intact muscles as the cooling temperature decreased. Smith et al. (1969) also observed cold shortening in excised chicken and turkey muscle. They found that shortening in muscles stored at 0°C was significantly greater than that observed in muscles stored in a temperature range of 12° - 18°C, and shortening was greatest in muscles stored at 20°C.

Thaw Rigor

Thaw rigor, rapid development of rigor mortis that occurs when pre-rigor muscle is frozen and thawed, has been recognized for many years. deFremery (1966b) investigated the effect of thaw rigor on rate of onset of rigor mortis in chickens by monitoring the ATP and glycogen levels over time. He found that birds undergoing thaw rigor had an accelerated rate of glycogen and ATP degradation. He also reported that birds exhibiting accelerated rates of post-mortem glycolysis or thaw rigor were less tender than those exhibiting normal post-mortem glycolysis or normal rigor. Stumbo and Stadelman (1964) found that onset of rigor in freeze-dried pre-rigor poultry muscle is even faster than thaw rigor. They suggested that the increased calcium content in the soluble fraction of freeze-dried muscle may be the reason for this accelerated onset of rigor.

Glycogen Degradation and pH Decline

deFremery (1966a, 1966b) stated that accumulation of lactic acid as a result of anaerobic glycogen degradation lowers chicken muscle pH from above 7.0 to ultimate values of 5.7 to 5.9. He also indicated that onset of rigor mortis is delayed by ante-mortem injection of anesthetic, and this was thought to be due to a reduced rate of glycogen degradation. The anesthetized birds were also shown to be more tender than birds allowed to struggle freely at slaughter. Minimization of post-mortem glycolysis by (1) subcutaneous

1. *Journal of the American Medical Association*, 1997; 278: 1039-1044.

injections of adrenaline, which eliminates muscle glycogen ante-mortem; (2) intravenous injections of sodium iodoacetate, which inhibits phosphoglyceraldehyde dehydrogenase or (3) rapid cooking, has resulted in poultry meat that is tender without aging. Since these treatments accelerate rigor mortis, elimination of post-mortem glycolysis appeared to eliminate toughness associated with an acceleration of rigor mortis in normal birds (deFremery and Pool, 1963). In contrast to these results Price and Dawson (1967) found that turkeys exhibiting rapid post-mortem glycolysis are not necessarily tough, however, there appeared to be a possibility that tenderization was inhibited when glycolysis was essentially complete within 30 minutes after slaughter.

Phosphocreatine and ATP Degradation

Observations by Pool (1963) on changes in elastic properties of epinephrine-treated poultry muscle provided evidence that extensibility changes undergone by muscle during the course of rigor mortis and the development of tenderness in muscle are separate phenomena. Only the first is closely related to the level of ATP present and may involve some irreversible protein-protein interaction. In living muscle, creatine exists in two forms, free creatine and N-phosphoryl creatine (PC). Experiments with broilers showed that breast muscle contains about 45 μM total creatine/g of muscle tissue with approximately 45 percent as PC. When these birds were slaughtered with electrical stunning, PC concentration dropped to 13 percent of its resting concentration within 30 minutes. In birds which were

injections of curarines, which inhibit muscle contraction in
mammals; (2) intravenous injections of sodium bicarbonate, which
inhibits phosphocreatine breakdown; (3) and (4) and (5) and (6)
has resulted in results that are somewhat similar. Since
these treatments accelerate right action, stimulation of post-mortem
glycolysis appeared to eliminate the differences associated with an
acceleration of right action in normal birds (chickens and geese),
(1953). In contrast to these results, Price and Dawson (1953) found
that curarines exhibiting rapid post-mortem glycolysis are not
necessarily toxic; however, there appeared to be a possibility
that tenderness was inhibited when glycolysis was essentially
complete within 10 minutes after slaughter.

Phosphocreatine and ATP Degradation

Observations by Lee (1953) on changes in elastic properties of
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muscle, creatine exists in two forms, free creatine and phosphocreatine
(PCr). Experiments with poultry muscle showed that breast muscle
contains about 45 mg of creatine/g of muscle tissue with ATP levels
merely 45 percent of PCr. When these birds were slaughtered with
electric stunning, the concentration dropped to 15 percent of the
resting concentration within 10 minutes. Birds which were

anesthetized with pentobarbital prior to slaughter, PC concentration did not drop to this level until more than 4 hours had elapsed. As would be expected from the fact that PC is a phosphate donor for ATP, ATP levels were significantly elevated in anesthetized birds when compared to electrically stunned birds (deFremery, 1965). Price and Dawson (1967) found that practically all of the ATP had disappeared from light and dark turkey muscle at 6 hours post-mortem in birds that had been slaughtered (without stunning) and machine picked as well as birds slaughtered by brain sticking and hand picked without scalding. However, they did not observe extensive changes in the PC levels during post-mortem aging.

Inosinic Acid Formation and Degradation

The work of Davidek and Khan (1967, 1968) and Khan et al. (1968) has indicated that formation of inosinic acid (IMP) increased as breakdown of ATP increased during the period of time between slaughter and onset of rigor mortis in chickens. At onset of rigor the level of IMP remained constant, but during prolonged aging (over 24 hours) IMP content of muscle began to decrease. Their work suggested that IMP is produced in a sequence of successive changes of ATP to ADP, ADP to adenosine monophosphate (AMP) and AMP to IMP; and IMP is degraded to inosine and inosine to hypoxanthine. Loss of IMP also continues during frozen storage and appears to be caused by action of intrinsic enzymes. It was proposed by this group of scientists that IMP content of poultry muscle may be a good objective index of quality.

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Effects of Processing on Tenderness of Poultry Muscle

Slaughter

Effects of immobilization and slaughter methods have been investigated since passage of the Humane Slaughter Law in the United States in 1958.

May and Huston (1959) used oral administration of sodium pentobarbital to anesthetize birds in order to make handling and picking easier. Several attempts were made to incorporate this anesthetic into a practical ration for feeding birds prior to marketing. Variable results were obtained, but the product appeared to have practical implications. Kotula et al. (1961) demonstrated the possibility of in-line carbon dioxide immobilization of chickens under commercial processing conditions. Electrical stunning is also used in many poultry processing plants, especially those that process turkeys. These methods of immobilization are applied mainly to prevent struggle during slaughter, but they may affect post-mortem changes in other ways.

Dodge and Stadelman (1960) observed the extent of struggle in broilers and rated the movements from very slight to strong activity. They concluded that struggling and post-mortem tenderization were not related, when aging periods of 2 to 5 hours were used. They also fed an oral tranquilizer (Tyazine) in the diet before slaughter but it did not appear to affect either levels of tenderness and struggling or variation in these factors. Goodwin et al. (1961) studied the

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The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry, no matter how small, should be recorded to ensure the integrity of the financial data. This includes not only sales and purchases but also expenses and income. The document then moves on to discuss the importance of regular reconciliation. It states that accounts should be reconciled at least once a month to identify any discrepancies early on. This process involves comparing the company's records with the bank statements and ensuring that they match. If there are any differences, the document suggests investigating the cause and making necessary adjustments. The next section discusses the importance of proper documentation. It states that all transactions should be supported by valid receipts or invoices. This not only helps in proving the accuracy of the records but also provides a clear audit trail. The document then discusses the importance of maintaining a clear and organized system for storing these documents. It suggests using a filing system that allows for easy retrieval of documents when needed. Finally, the document discusses the importance of regular backups. It states that all financial data should be backed up regularly to prevent loss in case of a system failure or disaster. The document concludes by emphasizing that maintaining accurate and up-to-date financial records is essential for the success of any business.

influence of humane slaughter on tenderness of turkey meat. They compared humane slaughter methods--carbon dioxide immobilization, electrical stunning, oral administered nembutal immobilization, reserpine tranquilization and debraining by knife puncture of the anterior lobe of the brain--with a control or external cut method with no ante-mortem treatment using 4 and 24 hour aging periods. The humane methods of slaughter had no significant effect on shear values of the breast muscles. However, oral administration of nembutal resulted in a significantly increased shear value of the thigh muscle over the control. Stadelman and Wise (1961) concluded that oral administered nembutal significantly extended the period of maximum toughness as determined by shear value of cooked breast muscle of chicken.

deFremery (1965) found that onset of rigor was delayed in chickens which were anesthetized with pentobarbital prior to slaughter but contrary to the findings of Goodwin et al. (1961) these birds were significantly more tender, as measured by shear press values, than birds slaughtered by an external cut with no ante-mortem treatment.

Scalding

Under normal scalding conditions, it is doubtful that water temperatures between 53° and 61°C exert significant influences on poultry tenderness (Marion, 1967). However, the following reports in the literature indicate detrimental effects of extremes in scalding times and temperatures. Klose and Pool (1954) found that

tenderness of turkey muscle was not affected appreciably by scalding temperatures of 49° - 60°C, but increases in scalding temperature produced marked increases in toughness and wrinkling of the skin. These workers along with Pool et al. (1954) concluded that scalding temperatures of 60°C could be used if care was maintained to prevent moisture loss from the skin. Shannon et al. (1957) reported that increases in time of scald and temperature of scald as well as the interaction of time with temperature significantly reduced tenderness using a range of 49° - 91°C scalding temperature for 5 - 160 seconds. They also stated that effect of time was greater than that of temperature. Wise and Stadelman (1959, 1961) found that the toughening effect of high temperature-long time scalding of broilers is related to the depth to which scald heat penetrates the muscle tissue. They came to these same general conclusions when working with turkeys. It was also concluded that the presence of any substance such as skin or of an environment of lower temperature during the relatively critical early post-mortem period tends to result in a decrease of the scald effect.

Feather Removal

A fast, efficient method of removing feathers from poultry is essential in processing. This is generally accomplished by rubber fingered mechanical pickers. It has been shown by several groups (Klose et al., 1956, 1959; Wise and Stadelman, 1957; Pool et al., 1959; deFremery and Pool, 1959) that excessive beating by mechanical pickers causes toughening of poultry meat. The results of Klose et al.

(1956) indicated that toughness induced by excessive beating cannot be resolved completely by prolonged aging, and the effects of beating are cumulative and may be reduced by limiting the beating action to that barely essential for complete feather removal. Pool et al. (1959) found that the beating action during feather removal exerted its greatest toughening effect when the feathers were removed immediately after slaughter, but if the birds were not picked until 1-3 hours after slaughter the toughening effect was decreased.

Aging

It has been shown that chicken muscle is tender when cooked immediately post-mortem, but when it is allowed to age for 1 hour and is then cooked it is significantly toughened. However, when allowed to complete the aging process the muscle will be tender again upon cooking (deFremery, 1966b). This is no new conclusion because there are many reports in the literature concerning the effects of aging on tenderness and post-mortem changes in poultry meat.

Dawson et al. (1958) concluded that an aging time of 3 - 6 hours is sufficient for broilers although it appeared that tenderization continued after this point. It seems to be the general conclusion, however, that an aging period of 12 - 24 hours will give a more desirable product.

It is known that chilling poultry in water will result in some uptake of the chilling solution. Kahlenberg et al. (1960) found that birds were chilled by a continuous "spin-chill" method in 30 minutes, and water uptake was as high as 22.7 percent while unagitated slush

ice immersion cooling resulted in water uptake of as much as 13 percent. Much of this water was lost in further processing procedures, and there was no significant difference in shelf-life, flavor and tenderness in the cooked meat. Goodwin et al. (1962) and Froning and Swanson (1959) obtained similar results concerning the effect of liquid chilling methods on final tenderness and flavor.

It seems that tenderization during aging is a more prevalent problem with turkeys than with chickens because turkeys are generally frozen for marketing whereas chickens are generally marketed fresh. It is the general conclusion of several groups of scientists that aging of fryer-roaster turkeys is more critical than aging of larger birds (Dodge and Stadelman, 1959; Klose et al., 1961; Marion and Goodman, 1967; Brodine and Carlin, 1968), and these birds generally need more aging than larger birds. This may be due in part to the fact that longer freezing times required for larger birds will allow for adequate aging.

Mechanical chillers tumble the birds during the chilling process, and Goodwin et al. (1962) indicated that flexing of the wings and legs by tumbling during chilling retarded development of maximum tenderness. However, no significant effect was observed when the turkeys were aged for 32 hours at 2°C.

Studies dealing with cold shortening in poultry conducted by Welbourn et al. (1968) indicated that shortening which occurred in excised muscle was not significantly correlated with tenderness as measured by a shear press.

van den Berg et al. (1963) held chicken muscle at 0°C under aseptic conditions. Proteolysis in both breast and leg muscle was appreciable, resulting in the formation of free amino acids and other breakdown products. Ion-binding properties (as measured by loss of weight and minerals during cooking) changed markedly during storage. The water binding capacity of breast muscle decreased appreciably during the first week, whereas that of leg muscle did not change significantly. These same workers (van den Berg et al., 1964) indicated that water holding capacity is not related to juiciness, but it appears to be related to tenderness, at least for breast muscle. Changes in ion-binding properties and off odor and flavor development appeared to be caused by proteolysis. Khan (1968) indicated that differences in tenderness and rate of post-mortem tenderization between breast and leg muscle and between birds of different age groups appeared to be related to differences in stroma protein content of the muscle. In contrast to this deFremery and Streeter (1969) indicated that the stroma protein fraction had little effect on tenderness or tenderization.

Studies of several methods of fast, intermediate and slow freezing of poultry have been made by several groups of researchers (Marion and Stadelman, 1958; Miller and May, 1965; Pickett and Miller, 1967). The general conclusion of these studies was that there was no significant effect due to freezing time on tenderness. However, Marion and Stadelman (1958) did find that slow freezing methods resulted in a darker colored frozen turkey and Miller and May (1965) indicated that shorter storage times resulted in more tender meat.

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Stewart et al. (1945) indicated that broilers aged for 2 hours and then frozen at -67.8°C had vacuoles within the fibers of breast and thigh muscles. These vacuoles were considered as an indication of intrafibrillar freezing, ice crystals having formerly occupied the sites of the vacuoles. When birds were aged for 18 hours before freezing at this temperature, intrafibrillar freezing did not occur. Studies of quality and biochemical changes during frozen storage of chicken muscle conducted by Khan et al. (1963) and Khan and van den Berg (1967) produced results that indicated freezing caused small but detectable changes in eating quality. Changes in muscle proteins during freezing depended on the freezing rate. Slow freezing caused a larger loss of drip on thawing, a larger loss of nitrogenous constituents and nucleic acid derivatives to the drip and a larger loss of water-holding capacity of meat, than fast freezing. The results of Crigler and Dawson (1968) are in general agreement with these findings. Also the work of Khan et al. (1963) and Khan and van den Berg (1967) indicated that rapid freezing preserves the integrity of muscle proteins to a greater extent than slow freezing. However, there was a decrease in total sulfhydryl group content and myosin ATPase activity of muscle as a result of freezing. The rate of these changes depended directly on storage temperature and time.

Extractability and Fractionation of Poultry

Muscle Proteins

Chicken Studies

Swanson and Sloan (1953) observed changes in total nitrogen, water soluble nitrogen, non-precipitable nitrogen, soluble free alpha-amino nitrogen and non-precipitable alpha-amino nitrogen in New Hampshire fowl over a 40 week period at -21°C. Proteolysis was indicated during storage by increases in soluble nitrogen and non-protein nitrogen. Decreases in amino nitrogen over time suggested that amino acids released by proteolysis were being degraded by some mechanism.

Weinberg and Rose (1960) extracted chicken breast muscle with KCl-phosphate buffer ($\text{pH} = 7.5$, $I/2 = 0.55$). The extracts were diluted to lower ionic strengths so that actomyosin, myosin and sarcoplasmic proteins could be separated. The amount of nitrogen extracted increased when carcasses were held for 24 hours at 4°C, and this increase was entirely accounted for by an increase in the actomyosin fraction.

Different buffer systems were compared for efficiency of extraction of chicken muscle proteins, and a technique of dilution to specific ionic strengths was developed for routine fractionation and determination of major protein fractions in one operation. KCl-borate and KCl-phosphate buffers ($\text{pH} = 7.3-7.5$ and $I/2 = 1.0$) gave maximum extraction of protein. Protein fractionation in KCl-borate

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Figure 1. The effect of the number of trials on the number of correct responses. The number of correct responses was significantly higher than the number of incorrect responses in all conditions. Error bars represent the standard error of the mean.

buffer showed that in 1-year-old chicken muscle, stroma-, myofibrillar- and sarcoplasmic-protein nitrogen respectively contributed 13, 42 and 30 percent of total nitrogen in breast muscle and 24, 30 and 22 percent of total nitrogen in leg muscle (Khan, 1962). Khan and fellow workers (Khan, 1968; Khan and van den Berg, 1964a, 1964b; Khan and Lentz, 1965; Khan et al., 1963) using this procedure studied changes in protein extractability of chicken muscle during post-mortem aging under various aging and storage conditions. They have found that in birds held for aging at 0°C, the buffer extractable nitrogen rapidly decreased after death during onset of rigor and gradually increased to a maximum value during post-rigor aging. Changes in extractable nitrogen occurred mainly as a result of changes in extractability of myofibrillar proteins. Analysis of muscle proteins of 10-week-, 4-month- and 8-month-old birds, stored under aseptic conditions at 0°, 2° and 5°C, showed quantitative changes in the total extractable-, myofibrillar-, sarcoplasmic- and non-protein-nitrogen fractions during 7 weeks of storage. The myosin fraction increased during storage except in breast muscle of 10-week-old birds. The sarcoplasmic protein fraction decreased in the leg muscle of 10-week-old birds and the breast muscle of 4- and 8-month-old birds but not in the breast muscle of 10-week-old birds. The non-protein nitrogen and the amount of protein breakdown products increased in all samples. Proteolysis increased as storage time and temperature increased. When birds were subjected to frozen storage, total protein extractability in both breast and leg muscle decreased with storage time because of loss of stability of the actomyosin fraction. The

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sarcoplasmic protein fraction decreased only after long storage. The stroma protein fraction remained constant and the non-protein nitrogen fraction increased over time. The rate of these changes depended directly on time and temperature of storage. Birds were frozen before rigor, during rigor and after rigor, and it was found that protein extractability was lower in the birds frozen during rigor. The work of Sayre (1968b) and McIntosh (1967) is in general agreement with the conclusions of Khan and his fellow workers concerning the decrease in myofibrillar protein extractability with onset of rigor.

Turkey Studies

Marion and Forsythe (1962) estimated the sarcoplasmic protein fraction and non-protein amino nitrogen fraction in turkey light and dark muscle during pre-rigor, post-rigor, following rapid freezing and thawing and after 60 days storage at -29°C . There were no significant changes as the muscles underwent rigor and subsequent storage, however, a pronounced difference existed between the two muscles studied with results from the light muscle being consistently higher. Scharpf and Marion (1964) estimated total-, extractable-, coagulable-, actomyosin-, myosin- and sarcoplasmic-protein fractions in light and dark muscle before rigor and after 48 hours storage at $5^{\circ} - 10^{\circ}\text{C}$. Change over time was not significant, but the light muscle gave consistently higher nitrogen values than dark muscle.

Hoke et al. (1968) studied the effect of frozen storage on total-, extractable-, non-protein-, actomyosin-, myosin- and

sarcoplasmic-protein nitrogen fractions of light and dark muscle. Changes in these fractions were not marked. However, there was a decrease in the actomyosin fraction of light muscle and some indication of proteolytic changes.

Maxon and Marion (1969) followed sarcoplasmic and myofibrillar protein extractability from breast muscle from 0 - 72 hours post-mortem. The sarcoplasmic protein extracted remained relatively unchanged over the entire period whereas the myofibrillar fraction extractability increased steadily up to 48 hours post-mortem and then decreased slightly at 72 hours post-mortem. No protein solubility decrease was noticed during rigor onset.

Very little work dealing with post-mortem changes in protein extractability from turkey muscle has been done. There are no reports in the literature discussing the relationship between tenderness and protein extractability from either chicken or turkey muscle. With these two facts in mind the investigation reported in this thesis is of a timely and useful nature.

anticoagulant-protein ratio in relation to the antithrombotic effect. Changes in the fibrinolytic activity of the plasma and the effect of the decrease in the anticoagulant function of fibrinogen and plasminogen on the action of proteolytic enzymes.

Naxon and Hanson (1967) studied the effect of anticoagulant and metabolizing protein extractability from plasma, muscle, liver, and kidney in the rat. The anticoagulant protein was found to be increased during the unchanged over the entire period whereas the metabolizing function extractability increased during the 48 hours observation and then decreased slightly after 48 hours observation. The anticoagulant solubility decrease was not observed during the first 24 hours. Very little was found with most-soluble fibrin in protein extractability from kidney muscle and heart. The authors report in the literature a rising and falling between kidney mass and protein extractability from 48 hours and 144 hours. With these two data in mind, the investigation reported in this thesis is of a clinical and basic nature.

EXPERIMENTAL METHODS

Reagents

Reagents used in this study were reagent grade except the sodium hydroxide used in nitrogen determination. This chemical was purified grade for nitrogen determination. Deionized distilled water was used throughout the study. Details for preparation of solutions used in this study are given in Appendix A.

Centrifugation

A Model CS International Centrifuge equipped with Heads No. 240 and No. 242 was used throughout this study.

pH Measurements

All pH measurements were made using a Corning pH Meter Model 10 equipped with a Beckman Combination Electrode No. 39003. The expanded scale was used for pH determination, and the pH meter was calibrated using Beckman pH 7 buffer solution (No. 3501).

Reagents

Reagents used in this study were reagent grade. The sodium hydroxide used in nitrogen determination, after being dried, was used for nitrogen determination. Deionized distilled water was used throughout the study. Details for preparation of solutions used in this study are given in Appendix A.

Calibration

A Model 100 Instrument with a nitrogen electrode was used for calibration. The instrument was used to determine the nitrogen content of the samples.

Measurements

All measurements were made using a nitrogen electrode in a solution of known concentration. The electrode was calibrated with a solution of known concentration. The electrode was used for the determination of the pH of the water was calibrated using a solution of known pH.

Experimental Animals

Birds used in this study were mature, Broad-Breast White, female turkeys weighing approximately 17 pounds. They were obtained from the Michigan State University Poultry Farm where they were raised under identical conditions to reduce bird to bird variability as much as possible.

Processing Procedure

The turkeys were processed in five groups of four birds. Two birds in each group were selected at random and were given an injection of sodium pentobarbital (20 mg/kg body weight) in the brachial vein of the wing just before slaughter. The other two birds in each group received no pre-slaughter treatment. Hereafter the anesthetized treatment will be referred to as AN and the non-anesthetized treatment will be referred to as N-AN. The birds were sacrificed by severing the jugular vein and carotid arteries which will be referred to as bleeding. No attempt was made to restrain any of the birds after the throat was cut and the zero time sample taken. After bleeding the birds were scalded at 57°C for 55 seconds in a Rotomatic scalding machine. Feather removal was accomplished by a picking time of 45 seconds in an automatic rubber fingered picking machine. After picking, the birds were eviscerated, washed and placed in Cryovac bags. The birds were held in slush ice throughout the sampling period, and after 72 hours the bags were evacuated

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and the birds frozen at -23°C for storage until cooking and tenderness evaluation.

Sampling Procedure

Approximately 10 g samples were taken from the Pectoralis major muscle of each bird at 0, 1/4, 1/2, 1, 3, 6, 12, 24, 48 and 72 hours post-mortem. The samples were taken immediately to a 2°C cold room for extraction and fractionation. All samples except the zero time sample were taken from the right half of the birds. They were removed along the grain of the muscle in order to prevent excessive cutting across muscle fibers. Zero time samples were taken immediately after bleeding. Other samples were taken at the times given.

Muscle pH Determination

Within 3 minutes from the time each sample was taken approximately 3.0 g of the sample were placed in a micro-Waring blender jar containing about 75 ml of sodium iodoacetate solution (Appendix A) and blended for 30 seconds. The homogenates were transferred to glass containers and allowed to warm to room temperature. pH of each homogenate was determined and recorded.

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Protein Extraction and Fractionation

The procedure used for extraction and fractionation of muscle proteins was adapted from a procedure designed by Price et al. (1965).

Primary Extractions

Within 2.5 minutes from the time the samples were removed from the birds, 5 ± 0.2 g of muscle tissue were weighed and placed in a cold micro-Waring blender jar containing enough KCl-buffer solution (Appendix A) to fill the constricted portion of the jar. A 50 ml volumetric flask was held in place on top of the constricted part of the blender jar to prevent formation of a vortex while the sample was being blended for 30 seconds at high speed. The blended sample was poured into a 100 ml, graduated, conical centrifuge tube. The blender jar was washed with more KCl-buffer solution, and the washings were added to the centrifuge tube until a volume of 100 ml was reached. This was mixed well, and after standing 1 hour it was centrifuged for 20 minutes at 2760 X G.

The centrifugate was decanted off into a sample container. Another 100 ml of KCl-buffer solution were added to the residue and this was mixed well. After standing for 1 hour it was centrifuged for 20 minutes at 2760 X G. The centrifugate was added to the initial centrifugate and saved for fractionation and nitrogen analysis. This fraction was designated C-1 as is indicated in Figure 2. Volume

The procedure used for extraction and fractionation of muscle

proteins was adapted from a procedure described by (1963).

(1963).

Primary Extractions

Within 7-12 minutes after the time the animals were sacrificed, the birds, 3-4 g of muscle tissue were weighed and placed in a cold micro-Waring blender jar containing 10 ml of water. (Appendix 1) to find the concentration of water in the muscle. A volumetric flask was used to place on top of the concentrated water. The blender jar to provide a concentration of a volume of water was being a number of seconds after the water was poured into a cold jar, blended, cooled, and then poured into a cold jar was washed with more of concentrated water. The birds were added to the concentrated water and the water was reached. This was mixed well, and after standing about 1 hour it was centrifuged for 10 minutes at 1000 g.

The centrifuge was used to separate the water from the muscle. Another 10 ml of 0.1M sodium chloride solution were added to the residue and this was mixed well. After standing for 1 hour it was centrifuged for 10 minutes at 1000 g. The centrifuge was used to separate the initial centrifugate and saved for fractionation in subsequent steps. This fraction was designated as fraction 1, and is indicated in Figure 1.

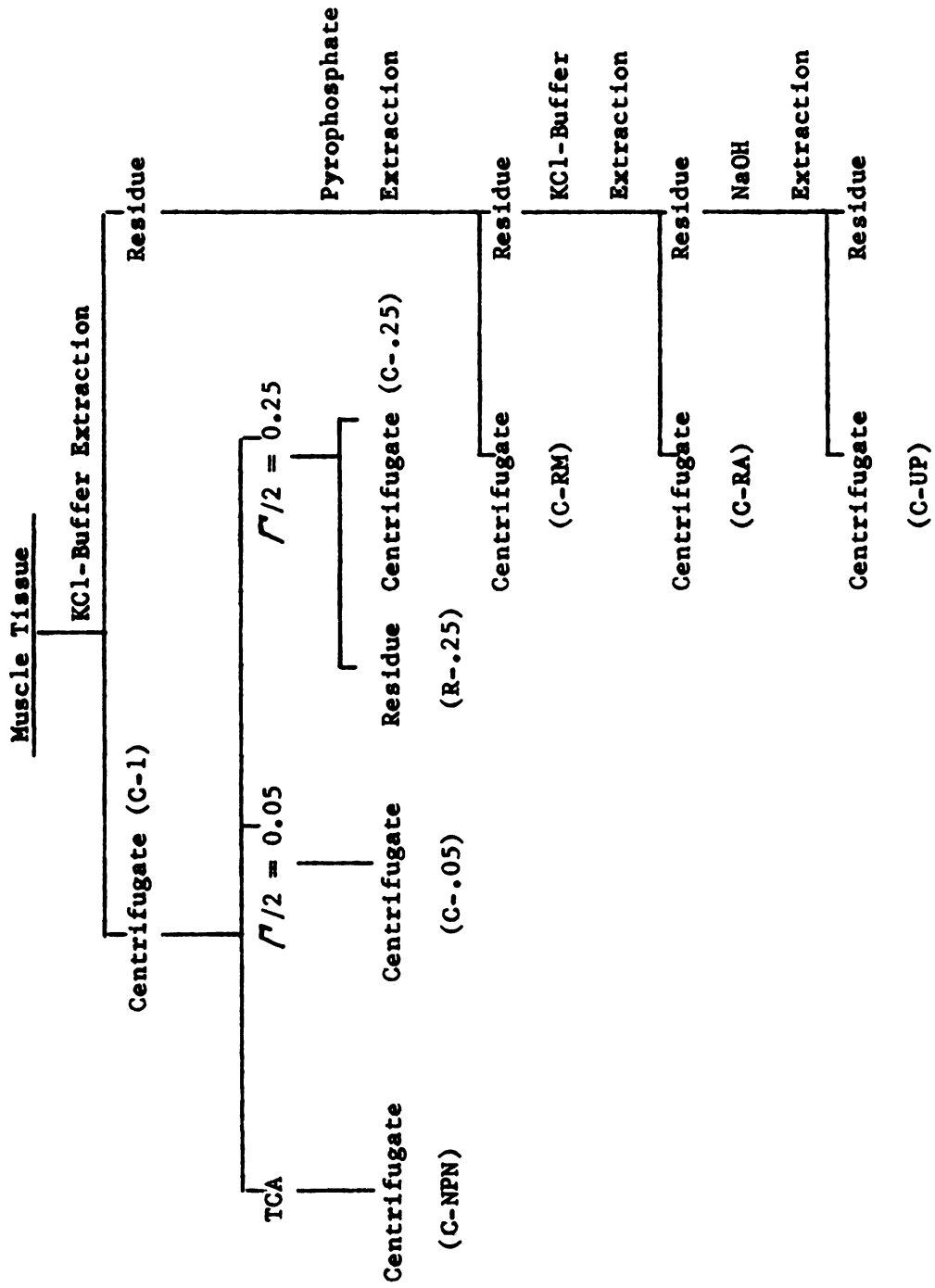
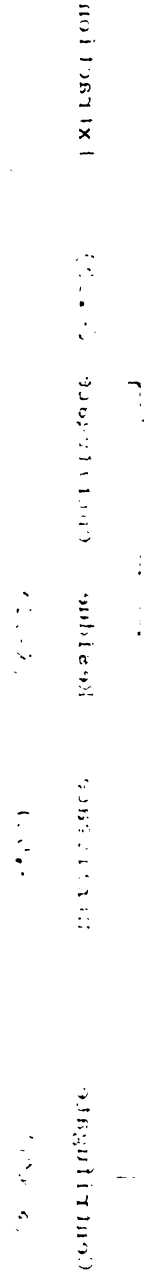
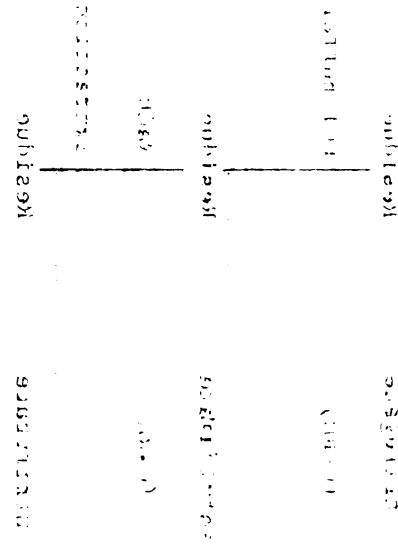


Figure 2. Flowsheet for extraction and fractionation of muscle proteins.

Fig. 2. Schematic diagram of the experimental setup.

(C-05)



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Centrifuge (C-1)

Residue

KCl-100% Extraction

100% 100%

of the residue remaining in the centrifuge tube was determined, and volume of the centrifugate was calculated. The residue was left in the centrifuge tube for further extraction.

Secondary Extractions

Fifty ml of sodium pyrophosphate solution (Appendix A) were added to the residue left after the primary extractions. After being mixed well and standing for 30 minutes the mixture was centrifuged for 20 minutes at 2760 X G. The centrifugate was decanted into a sample container and saved for nitrogen analysis. This fraction was designated C-RM as indicated in Figure 2. Volume of the residue was determined and volume of the centrifugate calculated.

To the residue of the pyrophosphate solution extraction, 50 ml of KCl-buffer solution were added. After mixing well this was allowed to stand for 30 minutes before centrifuging for 20 minutes at 2760 X G. The centrifugate was decanted into a sample container and saved for nitrogen analysis. This fraction was designated C-RA (Figure 2). Volume of the residue was determined and volume of the centrifugate calculated.

The remaining residue was subjected to a final extraction with 100 ml of sodium hydroxide solution (Appendix A). After mixing well this was allowed to stand for 30 minutes before centrifuging 20 minutes at 2760 X G. The centrifugate was decanted into a sample container and saved for nitrogen analysis. This fraction was designated C-UP (Figure 2). Volume of the residue was measured and discarded, and volume of the centrifugate was calculated.

of the residue remaining in the centrifuge tube was determined and the volume of the centrifugate was calculated. The residue was then placed in the centrifuge tube for further extraction.

Secondary Extractions

Fifty ml of sodium hydroxide solution (Appendix A) was added to the residue left after the primary extraction. After being mixed well and standing for 10 minutes the mixture was centrifuged for 10 minutes at 1500 X G. The supernatant was decanted into a sample container and saved for nitrogen analysis. This fraction was designated C-2H as indicated in Figure 1. Volume of the residue was determined and volume of the centrifugate calculated.

To the residue of the hydroxide solution extraction, 100 ml of KCl-bulter solution were added. After mixing well this was allowed to stand for 10 minutes before centrifuging for 10 minutes at 1500 X G. The centrifugate was decanted into a sample container and saved for nitrogen analysis. This fraction was designated C-2H (Figure 1). Volume of the residue was determined and volume of the centrifugate calculated.

The remaining residue was subjected to a final extraction with 100 ml of sodium hydroxide solution (Appendix A). After mixing well this was allowed to stand for 10 minutes before centrifuging for 10 minutes at 1500 X G. The supernatant was decanted into a sample container and saved for nitrogen analysis. This fraction was designated C-2H (Figure 1). Volume of the residue was determined and volume of the centrifugate was calculated.

Fractionation of C-1

A 10 ml portion of the centrifugate C-1 (Figure 2) was placed in a 100 ml, graduated, conical centrifuge tube and diluted to $f/2 = 0.25$ by adding 30 ml of water. This was mixed well and allowed to stand overnight. The mixture was centrifuged for 20 minutes at 2760 X G, and the centrifugate was transferred to a sample container using an aspirator. This fraction was saved for nitrogen analysis and designated C-.25 (Figure 2). Residue volume was determined and centrifugate volume calculated. The residue was diluted to 25 ml with a 0.1 N sodium hydroxide solution. This was mixed until the residue was dissolved and was decanted into a sample container and saved for nitrogen analysis. This fraction was labeled R-.25 (Figure 2).

Another 10 ml portion of centrifugate C-1 (Figure 2) was placed in a 250 ml flask and diluted to $f/2 = 0.05$ by adding 190 ml of water. This was mixed well and allowed to stand overnight. After standing the mixture was mixed again and a 100 ml portion was placed in a 100 ml, graduated, conical centrifuge tube. After centrifuging for 20 minutes at 2760 X G, volume of the residue was determined and volume of the centrifugate calculated. A portion of the centrifugate was decanted into a sample container and saved for nitrogen analysis. This fraction was labeled C-.05 (Figure 2). The remainder of the centrifugate and residue was discarded.

A 20 ml portion of the centrifugate C-1 (Figure 2) was placed in a 50 ml centrifuge tube, and 20 ml of TCA solution (Appendix A) was added. This was mixed well and centrifuged at 2410 X G for 20

minutes. The centrifugate was decanted into a sample container and saved for nitrogen analysis. This fraction was designated C-NPN. Volume of the residue was determined and volume of the centrifugate calculated. The residue was discarded.

Nitrogen Determination

All nitrogen determinations were conducted using the micro-Kjeldahl method as outlined by the American Instrument Company (1961). Titration of nitrogen liberated was accomplished using a Sargent Spectro-Electro Model SE automatic titrator. All nitrogen determinations were run in duplicate. Nitrogen content was reported as mg of nitrogen per 100 g of wet tissue.

Estimation of Protein Fractions

The following nitrogen containing fractions were determined from the data collected using the extraction, fractionation, analysis and calculation procedures described above and in Appendix B:

1. total extractable nitrogen - nitrogen of C-1;
2. non-protein nitrogen - nitrogen of C-NPN;
3. sarcoplasmic protein nitrogen - nitrogen remaining when C-NPN was subtracted from C-.05;
4. Total fibrillar protein nitrogen - nitrogen remaining when C-.05 was subtracted from C-1;
5. actomyosin nitrogen - nitrogen of R-.25;

6. residual myosin nitrogen - nitrogen of C-RM;
7. residual actin nitrogen - nitrogen of C-RA;
8. unextracted soluble protein nitrogen - nitrogen of C-UP.

Cooking and Tenderness Evaluation of the Birds

The turkeys were removed from the -23°C freezer, halved and allowed to thaw overnight at 2°C. They were placed in stainless steel pans and covered with aluminum foil. Thermocouples were inserted through the foil into the center of the thickest part of the breast muscle. The birds were cooked in groups of five to an internal temperature of 85°C in a circulating air oven set at 149°C. They were cooled overnight at 2°C before tenderness evaluation. After cooling, wings were removed along with the skin and four slices 0.5 cm thick were taken from the Pectoralis major muscle of each bird using a meat slicer. Samples approximately 3.8 X 4.0 cm were cut from these slices along the grain of the muscle. The samples were trimmed to weigh 8 ± 0.2 g. A Kramer shear press was used to shear the samples. The shear press was equipped with a 1360.8 kg ring and a 15 second downstroke was used. Amount of force required to shear the samples was obtained by measuring the height of peaks recorded by an electronic recorder as the samples were sheared. Shear values were recorded as kg of force per g of sample.

6. residual nitrogen - nitrogen of 0.1;
7. residual acid nitrogen - nitrogen of 0.1;
8. unextracted soluble protein nitrogen - nitrogen of 0.1.

Cooking and temperature evaluation of the birds

The turkeys were removed from the 45°C freezer, bled and allowed to thaw overnight at 10°C. They were placed in stainless steel pans and covered with aluminum foil. Thermocouples were inserted through the foil into the center of the thickest part of the breast muscle. The birds were cooked in groups of five to an internal temperature of 62°C in a circulating air oven set at 180°C. They were cooled overnight at 4°C before temperature evaluation. After cooling, wings were removed along with the skin and joint slices 0.5 cm thick were taken from the ventral wing muscles of each bird using a meat slicer. Samples approximately 3.0 x 4.0 cm were cut from these slices along the grain of the muscle. The samples were trimmed to weight 6 ± 0.2 g. A Warner shear press was used to shear the samples. The shear press was calibrated with a 1300.8 kg ring and a 12 sec on downstroke was used. Means of three replicates to shear the samples was obtained by averaging the first three of peaks recorded by an electronic recorder as the samples were sheared. Shear values were recorded as 1/3 of force per g of sample.

Statistical Analyses

Tenderness, pH and protein extractability data collected were subjected to analysis of variance (Snedecor and Cochran, 1967). Variance due to treatment was determined on the tenderness data, and variance due to treatment, time and treatment X time interaction was determined on the pH and protein extractability data. Duncan's multiple range test was used to compare means where significant differences (probability of differences due to sampling alone - 0.05 or less) were established.

Correlation of tenderness with pH and protein extractability at various times post-mortem was determined using STAT routines available from the Michigan State University Agricultural Experiment Station. Analyses were carried out on a CDC 3600 computer.

Statistical analyses

Tenderness, pH and protein extractability were subjected to analysis of variance (ANOVA) and Duncan's multiple range test was used to compare means where significant differences (probability of differences due to sampling alone = 0.05 or less) were established.

Correlation of responses with pH and protein extractability at various times post-mortem was determined using TAT routines available from the Michigan State University Agricultural Experiment Station. Analyses were carried out on a CDC 3600 computer.

RESULTS AND DISCUSSION

Post-Mortem pH Changes

Changes in pH of turkey breast muscle were followed for a 72 hour period post-mortem in birds that were subjected to ante-mortem injections of pentobarbital and in birds without ante-mortem treatment. Results of statistical analyses of the pH data collected are summarized in Tables 1 and 2 and Appendix C. It is obvious that rate of pH decline in breast muscle during the first 6 hours post-mortem was greater in the N-AN birds than in the AN birds. Statistical analyses also indicated that pH decline reached a statistical minimum level^{1/} at 6 hours in muscle of the N-AN birds whereas pH decline in muscle of the AN birds did not reach a statistical minimum level until 12 hours post-mortem. It is interesting to note that pH of the muscle of N-AN birds at 1/4 hour (6.02) was lower than the values at 1/2 (6.22) and 1 hour (6.10) post-mortem. Although this difference was not significant, it was quite noticeable in some birds.

^{1/} The terms statistical minimum level and statistical maximum level used in this report in reference to changes in a variable refer to the level at which further increases or decreases in that variable were not significantly different at the 1 percent level of probability.

Changes in pH of turkey breast muscle were followed for a 12 hour period post-mortem in birds that were subjected to stress-infection of hemolymph and in birds without any previous treatment. Results of statistical analyses of the pH data collected are summarized in Tables I and II and Appendix I. It is obvious that rate of pH decline in breast muscle during the first 6 hours post-mortem was greater in the 1-4% birds than in the 4-6% birds. Statistical analyses also indicated that the decline reached a statistical minimum level at 6 hours in muscle of the 1-4% birds whereas the decline in muscle of the 4-6% birds did not reach a statistical minimum level until 12 hours post-mortem. It is interesting to note that pH of the muscle of 1-4% birds at 12 hours (6.02) was lower than the values at 12 (6.12) and 1 hour (6.12) post-mortem. Although this difference was not significant, it was quite noticeable in some cases.

The above statistical minimum level was statistically significant level used in this report in reference to changes in a certain variable to the level at which further increases or decreases in that variable were not significantly different at the 1 percent level of probability.

Table 1. Effect of ante-mortem injection of turkeys with pentobarbital on pH of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment ^{2/} | |
|--------------|-------------------------|------------------------|
| | Anesthetized
pH | Non-anesthetized
pH |
| 0 | 6.86 ^a | 6.78 ^a |
| 1/4 | 6.70 ^a | 6.02 ^b |
| 1/2 | 6.71 ^a | 6.22 ^b |
| 1 | 6.69 ^a | 6.10 ^b |
| 3 | 6.55 ^a | 5.99 ^b |
| 6 | 6.32 ^a | 5.85 ^b |
| 12 | 5.87 ^a | 5.74 ^a |
| 24 | 5.77 ^a | 5.74 ^a |
| 48 | 5.78 ^a | 5.68 ^a |
| 72 | 5.79 ^a | 5.66 ^a |

^{1/} All values are means of ten birds.

^{2/} Values in a row marked by the same letter are not different at the 1 percent level of probability.

Table 2. pH decline of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized ^{2/} | | Non-anesthetized ^{2/} | |
|----------------------------|--------------------|--------------------------------|---------------------|
| Time (hr) | pH | Time (hr) | pH |
| 0 | 6.86 ^a | 0 | 6.78 ^a |
| 1/2 | 6.71 ^{ab} | 1/2 | 6.22 ^b |
| 1/4 | 6.70 ^{ab} | 1 | 6.10 ^b |
| 1 | 6.69 ^{ab} | 1/4 | 6.02 ^{bc} |
| 3 | 6.55 ^b | 3 | 5.99 ^{bcd} |
| 6 | 6.32 ^c | 6 | 5.85 ^{cde} |
| 12 | 5.87 ^d | 12 | 5.74 ^{de} |
| 72 | 5.79 ^d | 24 | 5.74 ^{de} |
| 48 | 5.78 ^d | 48 | 5.68 ^e |
| 24 | 5.77 ^d | 72 | 5.66 ^e |

^{1/} All values are means of ten birds.

^{2/} Values in a column marked by the same letter are not different at the 1 percent level of probability.

pH values for breast muscle of the N-AN birds at comparable times post-mortem are similar to values obtained from turkeys by Price and Dawson (1967). deFremery (1963) reported that pH of chicken breast muscle was about 6.8 at 1 hour and declined to about 5.95 at 6 hours post-mortem. The initial value (6.8) reported by deFremery is higher than those found in this study, particularly the value at 1 hour post-mortem for muscle of the N-AN birds. However, pH (5.95) at 6 hours post-mortem is similar to that of the N-AN birds. No reports were found in the literature dealing with rate of pH decline in birds anesthetized before slaughter. However, deFremery (1966b) stated that breakdown of N-phosphorylcreatine and ATP was delayed and initial glycogen levels were higher in birds anesthetized before slaughter than in control birds which are allowed to struggle freely during slaughter. He further stated that onset of rigor mortis was delayed in the anesthetized birds. These findings would lead one to assume that rate of pH decline was also decreased when birds were anesthetized before slaughter. The decreased rate of pH decline observed in breast muscle from AN birds of this study affirms this assumption.

The low pH value at 1/4 hour post-mortem for muscle of the N-AN birds may possibly be explained in the following manner. During the first few minutes after the birds were slaughtered anaerobic glycolysis proceeded rapidly producing lactic acid which caused pH to decline rapidly as was observed at 1/4 hour. After this initial surge of glycolysis, N-phosphorylcreatine began to be dephosphorylated at a significant rate releasing creatine. This caused an increase in muscle pH as was observed at 1/2 hour. Then as time passed glycogen continued

ph values for breast muscle of the 10 birds in the control group
post-mortem are similar to values obtained for chicken breast muscle
Dawson (1967). DeLong (1967) reported the pH of chicken breast
muscle was about 6.3 at 1 hour and 6.2 at 2 hours after death. In a 6 hours
post-mortem, the initial pH is reported to be 6.2 and 6.1 at 6 hours
than those found in this study. However, the pH of chicken breast
post-mortem for muscle of the 10 birds, however, was 6.1 at 1 hour
hours post-mortem is similar to that of the 10 birds, the reported
were found in the literature most of which were at 1 hour and 2 hours
anesthetized before slaughter. However, DeLong (1967) also stated
that breakdown of 1-phosphorylation and ATP was delayed and reduced
glycogen levels were higher in birds anesthetized before slaughter
than in control birds which were allowed to bleed to death before
slaughter. He further stated that most of the birds were delayed
in the anesthetized birds. These findings would mean that the
that rate of breakdown of ATP was also decreased in the birds anesthetized
before slaughter. The decrease rate of ATP breakdown observed in birds
muscle from 10 birds of the same age and sex was observed at
The low pH value at 1 hour post-mortem for muscle of the 10 birds
birds may possibly be explained by the following reasons. During the
first few minutes after the birds were slaughtered a period of rigor
proceeded rapidly during which time the pH of the muscle was
rapidly as was observed at 1 hour. After this initial period
glycolysis, phosphorylation, and ATP breakdown were
significant rate of ATP breakdown. These results are in accord with
pH as was observed at 1 hour. Then as the rate of glycolysis continued

to be metabolized to lactic acid causing pH to decline again. Changes in lactic acid, glycogen and N-phosphorylcreatine concentrations during the first hour after slaughter reported by deFremery (1966b) and Price and Dawson (1967) indicate that the above proposal could explain pH changes in breast muscle of the N-AN birds during the first hour post-mortem.

Post-Mortem Changes in Protein Extractability

Changes in extractability of several different protein fractions from breast muscle were investigated over a 72 hour time period post-mortem in birds that received ante-mortem injections of pentobarbital and birds that received no ante-mortem treatment. Fractions investigated in this study were: total extractable nitrogen, non-protein nitrogen, sarcoplasmic protein nitrogen, total fibrillar protein nitrogen, actomyosin nitrogen, residual myosin nitrogen, residual actin nitrogen and unextracted soluble protein nitrogen. Figures 3 and 4 demonstrate graphically the changes in extractability of each fraction. Results of this investigation will be described for each individual protein fraction but implications of these results will be discussed collectively because of interrelationships that exist between fractions.

Total Extractable Nitrogen

This fraction contained non-protein nitrogen as well as sarcoplasmic and fibrillar protein nitrogen. Data collected dealing with

to be metabolized to lactic acid (vanadium) in the liver. The lactic acid, glycogen and -phosphorylase concentrations were the first hour after slaughter (100%) and (100%) and (100%) respectively. It is indicated that the above information could be used in changes in breast muscle of the bird during the first hour post-mortem.

Post-mortem changes in protein metabolism

Changes in extractability of protein during post-mortem from breast muscle were investigated over a 24 hour time period. In birds that received and were in the process of (autolytic) and birds that received no post-mortem treatment, protein extractability in this study were: total extractable nitrogen, non-protein nitrogen, sarcoplasmic protein nitrogen, total fibrillar protein nitrogen, actomyosin nitrogen, residual protein nitrogen, and actin nitrogen and unextracted soluble protein nitrogen. Figures 1 and 2 demonstrate graphically the changes in extractability of each fraction. Results of this investigation will be described in a future individual protein fraction but implications of the results of the discussed data are very obvious of the protein levels that exist between fractions.

Total Extractable Nitrogen

This fraction contains non-protein nitrogen as well as soluble and fibrillar protein nitrogen. The level of soluble with

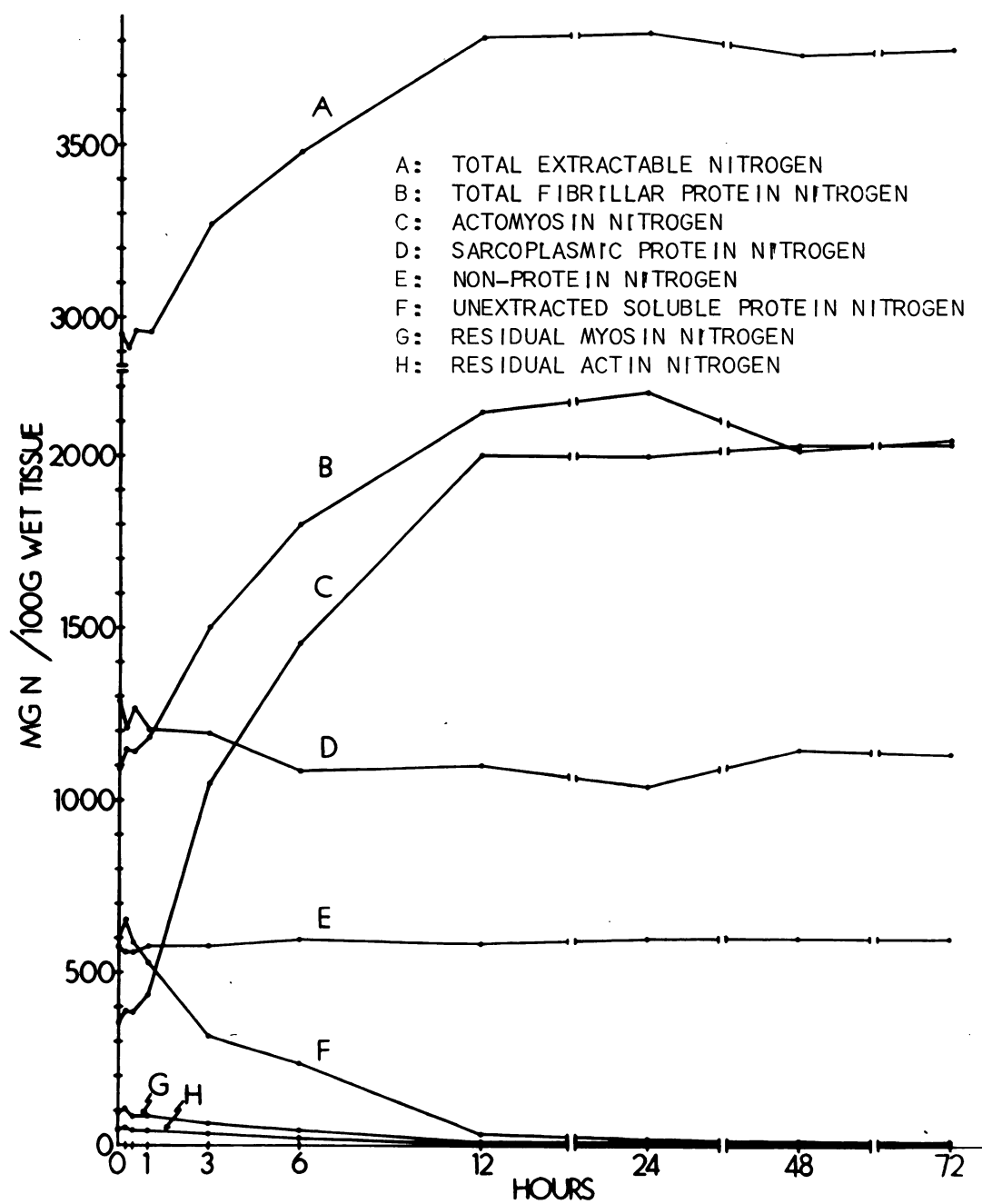


Figure 3. Extractability of protein fractions from turkey breast muscle, 0 to 72 hours post-mortem, of birds given ante-mortem injections of pentobarbital.

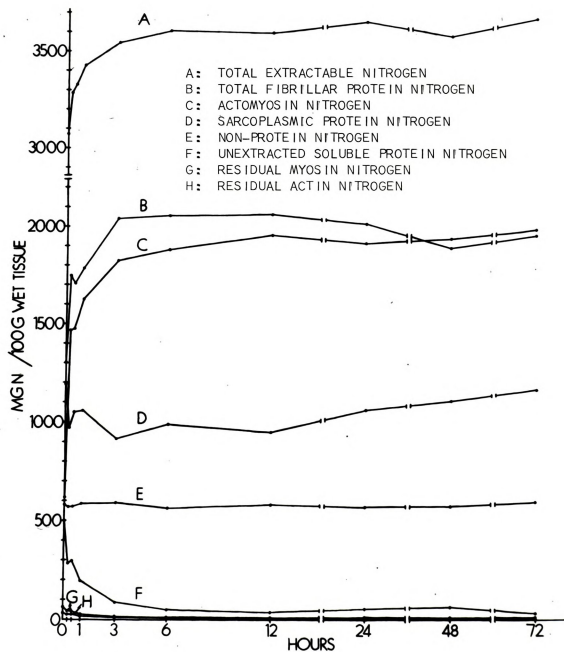
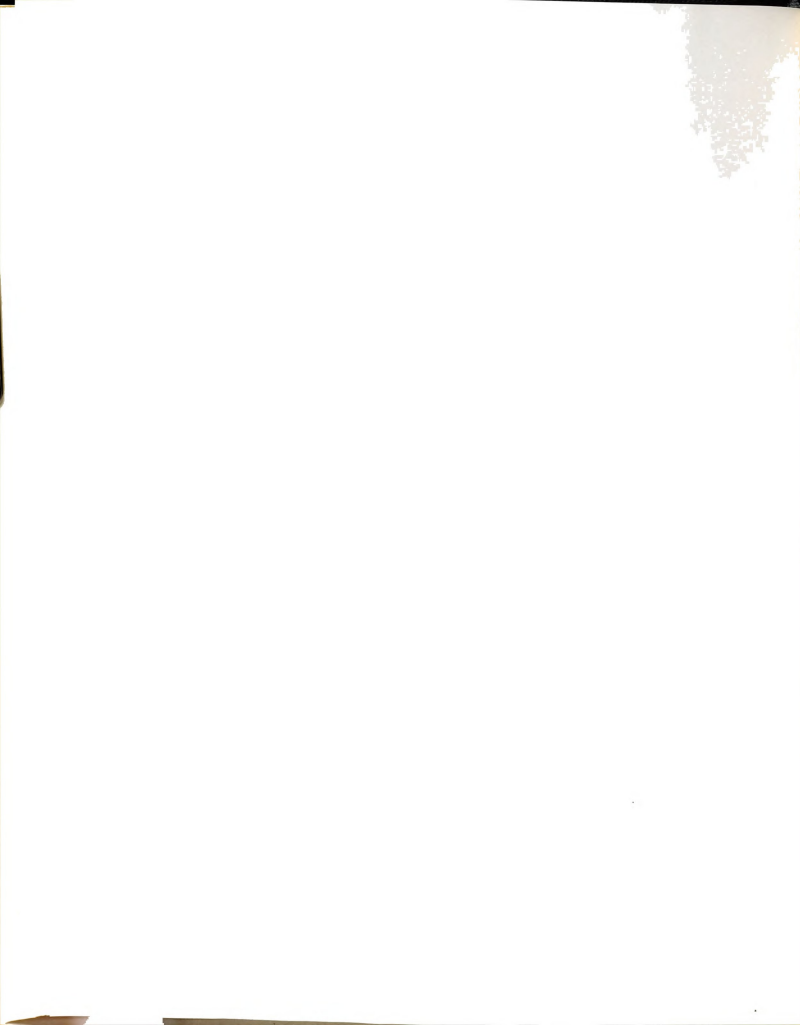


Figure 4. Extractability of protein fractions from turkey breast muscle, 0 to 72 hours post-mortem, of birds with no ante-mortem treatment.



this fraction are summarized in Tables 3 and 4 and Appendix C. Total extractable nitrogen was extracted in significantly greater amounts from breast muscle of the N-AN birds from 1/4 - 1 hour post-mortem than from muscle of the AN birds. Extractability of this fraction from muscle of the AN birds was relatively constant at a low level over the first hour, but after 1 hour it began to increase to a statistical maximum level at 12 hours post-mortem. This increase was similar to that from muscle of the N-AN birds which increased steadily from the zero time sampling to its statistical maximum level at 1 hour post-mortem. Although extractability of this fraction from breast muscle of the AN birds was lower than from muscle of the N-AN birds initially, maximum extractability was higher.

Non-protein Nitrogen

This fraction was composed of nitrogen containing compounds such as free amino acids that were not precipitated by trichloroacetic acid. Tables 5 and 6 and Appendix C include a summary of the data collected dealing with non-protein nitrogen. There were no significant differences either between treatments or over time. However, non-protein nitrogen appeared to increase during aging in muscle from the AN birds, but no similar trend was observed in muscle of the N-AN birds.

Sarcoplasmic Protein Nitrogen

Sarcoplasmic protein was defined in this study as that protein which remained in solution at an ionic strength of 0.05. Variance due to treatment was significant, and overall extractability of the

Table 3. Effect of ante-mortem injection of turkeys with pentobarbital on total extractable nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment ^{2/} | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 2949 ^a | 3074 ^a |
| 1/4 | 2911 ^a | 3285 ^b |
| 1/2 | 2961 ^a | 3328 ^b |
| 1 | 2957 ^a | 3426 ^b |
| 3 | 3269 ^a | 3545 ^a |
| 6 | 3477 ^a | 3607 ^a |
| 12 | 3805 ^a | 3596 ^a |
| 24 | 3816 ^a | 3655 ^a |
| 48 | 3751 ^a | 3583 ^a |
| 72 | 3766 ^a | 3680 ^a |

^{1/} All values are means of ten birds.

^{2/} Values in a row marked by the same letter are not different at the 1 percent level of probability.

Table 4. Total extractable nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized ^{2/} | | Non-anesthetized ^{2/} | |
|----------------------------|-----------------------|--------------------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 1/4 | 2911 ^a | 0 | 3074 ^a |
| 0 | 2949 ^a | 1/4 | 3285 ^{ab} |
| 1 | 2957 ^a | 1/2 | 3328 ^{abc} |
| 1/2 | 2961 ^a | 1 | 3426 ^{bcd} |
| 3 | 3269 ^b | 3 | 3545 ^{bcd} |
| 6 | 3477 ^{bc} | 48 | 3583 ^{cd} |
| 48 | 3751 ^{cd} | 12 | 3596 ^{cd} |
| 72 | 3766 ^{cd} | 6 | 3607 ^{cd} |
| 12 | 3805 ^d | 24 | 3655 ^d |
| 24 | 3816 ^d | 72 | 3680 ^d |

^{1/} All values are means of ten birds.

^{2/} Values in a column marked by the same letter are not different at the 1 percent level of probability.

Table 5. Effect of ante-mortem injection of turkeys with pentobarbital on non-protein nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 575 | 580 |
| 1/4 | 560 | 569 |
| 1/2 | 559 | 571 |
| 1 | 575 | 585 |
| 3 | 577 | 590 |
| 6 | 596 | 563 |
| 12 | 583 | 582 |
| 24 | 597 | 572 |
| 48 | 599 | 575 |
| 72 | 598 | 599 |

^{1/} All values are means of ten birds. Statistical analyses indicated that the variance due to treatment was not significant at the 1 percent level of probability.

Table 6. Non-protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized | | Non-anesthetized | |
|--------------|-----------------------|------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 1/2 | 559 | 6 | 563 |
| 1/4 | 560 | 1/4 | 569 |
| 0 | 575 | 1/2 | 571 |
| 1 | 575 | 24 | 572 |
| 3 | 577 | 48 | 575 |
| 12 | 583 | 0 | 580 |
| 6 | 596 | 12 | 582 |
| 24 | 597 | 1 | 585 |
| 72 | 598 | 3 | 590 |
| 48 | 599 | 72 | 599 |

^{1/} All values are means of ten birds. Statistical analyses indicated that the variance due to time was not significant at the 1 percent level of probability.

Table 1. The results of the analysis of variance for the effect of the concentration of the solution on the rate of the reaction.

| Concentration of the solution, g/l | Rate of the reaction, g/h |
|------------------------------------|---------------------------|
| 0.1 | 0.1 |
| 0.2 | 0.2 |
| 0.3 | 0.3 |
| 0.4 | 0.4 |
| 0.5 | 0.5 |
| 0.6 | 0.6 |
| 0.7 | 0.7 |
| 0.8 | 0.8 |
| 0.9 | 0.9 |
| 1.0 | 1.0 |

It is seen from the results of the analysis of variance that the rate of the reaction increases with an increase in the concentration of the solution. The rate of the reaction is directly proportional to the concentration of the solution.

Table 2. The results of the analysis of variance for the effect of the concentration of the solution on the rate of the reaction.

| Concentration of the solution, g/l | Rate of the reaction, g/h |
|------------------------------------|---------------------------|
| 0.1 | 0.1 |
| 0.2 | 0.2 |
| 0.3 | 0.3 |
| 0.4 | 0.4 |
| 0.5 | 0.5 |
| 0.6 | 0.6 |
| 0.7 | 0.7 |
| 0.8 | 0.8 |
| 0.9 | 0.9 |
| 1.0 | 1.0 |

It is seen from the results of the analysis of variance that the rate of the reaction increases with an increase in the concentration of the solution. The rate of the reaction is directly proportional to the concentration of the solution.

sarcoplasmic protein from breast muscle was greater in the AN than in the N-AN birds (Table 7 and Appendix C). Decline in extractability of this fraction from the muscle of both groups of birds appeared to follow changes in muscle pH fairly closely until the minimum pH level was reached, then it appeared to increase (Table 8 and Appendix C).

Total Fibrillar Protein Nitrogen

This fraction was composed of proteins of the myofibril: primarily actin, myosin and the actin-myosin complex -- actomyosin. Tables 9 and 10 and Appendix C include a summary of the data for this fraction. From 1/4 - 3 hours post-mortem extractability of this fraction from breast muscle was significantly greater in the N-AN birds than in the AN birds. Extractability remained relatively constant at a low level over the first hour post-mortem from muscle of the AN birds. After this time it began to rise in a manner similar to that of the N-AN birds in which extractability of this fraction from breast muscle increased from zero time to a statistical maximum level at 1/4 hour post-mortem. The statistical maximum level of extractability of this fraction from muscle of the AN birds was not reached until 12 hours post-mortem. Again extractability of this fraction began at a lower level from muscle of the AN birds but increased to a higher final extractability than from muscle of the N-AN birds.

Table 7. Effect of ante-mortem injection of turkeys with pentobarbital on sarcoplasmic protein nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 1285 | 1192 |
| 1/4 | 1207 | 971 |
| 1/2 | 1264 | 1050 |
| 1 | 1203 | 1057 |
| 3 | 1192 | 915 |
| 6 | 1083 | 990 |
| 12 | 1099 | 950 |
| 24 | 1039 | 1065 |
| 48 | 1142 | 1112 |
| 72 | 1131 | 1174 |

^{1/} All values are means of ten birds. Statistical analysis indicated that the variance due to treatment was significant at the 1 percent level of probability.

Table 8. Sarcoplasmic protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized | | Non-anesthetized | |
|--------------|-----------------------|------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 0 | 1285 | 0 | 1192 |
| 1/2 | 1264 | 72 | 1174 |
| 1/4 | 1207 | 48 | 1112 |
| 1 | 1203 | 24 | 1065 |
| 3 | 1192 | 1 | 1057 |
| 48 | 1142 | 1/2 | 1050 |
| 72 | 1131 | 6 | 990 |
| 12 | 1099 | 1/4 | 971 |
| 6 | 1083 | 12 | 950 |
| 24 | 1039 | 3 | 915 |

^{1/} All values are means of ten birds. Statistical analysis indicated that the variance due to time was not significant at the 1 percent level of probability.

Table 1. The results of the analysis of variance for the effect of the concentration of the active substance on the growth of the plants.

| Concentration of active substance, mg/l | Height of plants, cm | Weight of plants, g | Number of roots |
|---|----------------------|---------------------|-----------------|
| 0 | 10.5 | 1.2 | 1.5 |
| 0.1 | 11.2 | 1.3 | 1.6 |
| 0.2 | 11.8 | 1.4 | 1.7 |
| 0.3 | 12.5 | 1.5 | 1.8 |
| 0.4 | 13.2 | 1.6 | 1.9 |
| 0.5 | 14.0 | 1.7 | 2.0 |
| 0.6 | 14.8 | 1.8 | 2.1 |
| 0.7 | 15.5 | 1.9 | 2.2 |
| 0.8 | 16.2 | 2.0 | 2.3 |
| 0.9 | 17.0 | 2.1 | 2.4 |
| 1.0 | 17.8 | 2.2 | 2.5 |

The results of the analysis of variance for the effect of the concentration of the active substance on the growth of the plants are presented in Table 1. It is seen from the table that the growth of the plants increases with increasing concentration of the active substance. The maximum growth is observed at a concentration of 1.0 mg/l.

Table 2. The results of the analysis of variance for the effect of the concentration of the active substance on the growth of the plants.

| Concentration of active substance, mg/l | Height of plants, cm | Weight of plants, g | Number of roots |
|---|----------------------|---------------------|-----------------|
| 0 | 10.5 | 1.2 | 1.5 |
| 0.1 | 11.2 | 1.3 | 1.6 |
| 0.2 | 11.8 | 1.4 | 1.7 |
| 0.3 | 12.5 | 1.5 | 1.8 |
| 0.4 | 13.2 | 1.6 | 1.9 |
| 0.5 | 14.0 | 1.7 | 2.0 |
| 0.6 | 14.8 | 1.8 | 2.1 |
| 0.7 | 15.5 | 1.9 | 2.2 |
| 0.8 | 16.2 | 2.0 | 2.3 |
| 0.9 | 17.0 | 2.1 | 2.4 |
| 1.0 | 17.8 | 2.2 | 2.5 |

The results of the analysis of variance for the effect of the concentration of the active substance on the growth of the plants are presented in Table 2. It is seen from the table that the growth of the plants increases with increasing concentration of the active substance. The maximum growth is observed at a concentration of 1.0 mg/l.

Table 9. Effect of ante-mortem injection of turkeys with pentobarbital on total fibrillar protein nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment ^{2/} | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 1089 ^a | 1302 ^a |
| 1/4 | 1144 ^a | 1744 ^b |
| 1/2 | 1138 ^a | 1707 ^b |
| 1 | 1179 ^a | 1783 ^b |
| 3 | 1500 ^a | 2039 ^b |
| 6 | 1799 ^a | 2054 ^a |
| 12 | 2123 ^a | 2063 ^a |
| 24 | 2179 ^a | 2018 ^a |
| 48 | 2009 ^a | 1896 ^a |
| 72 | 2038 ^a | 1962 ^a |

^{1/} All values are means of ten birds.

^{2/} Values in a row marked by the same letter are not different at the 1 percent level of probability.

Table 10. Total fibrillar protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized ^{2/} | | Non-anesthetized ^{2/} | |
|----------------------------|-----------------------|--------------------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 0 | 1089 ^a | 0 | 1302 ^a |
| 1/2 | 1138 ^a | 1/2 | 1707 ^b |
| 1/4 | 1144 ^a | 1/4 | 1744 ^b |
| 1 | 1179 ^{ab} | 1 | 1783 ^b |
| 3 | 1500 ^{bc} | 48 | 1896 ^b |
| 6 | 1799 ^{cd} | 72 | 1962 ^b |
| 48 | 2009 ^{de} | 24 | 2018 ^b |
| 72 | 2038 ^{de} | 3 | 2039 ^b |
| 12 | 2123 ^{de} | 6 | 2054 ^b |
| 24 | 2179 ^e | 12 | 2063 ^b |

^{1/} All values are means of ten birds.

^{2/} Values in a column marked by the same letter are not different at the 1 percent level of probability.

Table 9. Effect of anaesthesia on injection of drugs in wild ponies. Total fibrinogen, serum albumin and protein mean, 0 to 12 hours post-mortem.

| Time (hr) | mg N/100 g wet tissue | mg N/100 g wet tissue | mg N/100 g wet tissue |
|-----------|-----------------------|-----------------------|-----------------------|
| 0 | 1.089 ^a | 1.089 ^a | 1.089 ^a |
| 1/2 | 1.134 ^a | 1.134 ^a | 1.134 ^a |
| 1 | 1.144 ^a | 1.144 ^a | 1.144 ^a |
| 2 | 1.158 ^a | 1.158 ^a | 1.158 ^a |
| 3 | 1.172 ^a | 1.172 ^a | 1.172 ^a |
| 4 | 1.186 ^a | 1.186 ^a | 1.186 ^a |
| 5 | 1.200 ^a | 1.200 ^a | 1.200 ^a |
| 6 | 1.214 ^a | 1.214 ^a | 1.214 ^a |
| 7 | 1.228 ^a | 1.228 ^a | 1.228 ^a |
| 8 | 1.242 ^a | 1.242 ^a | 1.242 ^a |
| 9 | 1.256 ^a | 1.256 ^a | 1.256 ^a |
| 10 | 1.270 ^a | 1.270 ^a | 1.270 ^a |
| 11 | 1.284 ^a | 1.284 ^a | 1.284 ^a |
| 12 | 1.298 ^a | 1.298 ^a | 1.298 ^a |
| 13 | 1.312 ^a | 1.312 ^a | 1.312 ^a |
| 14 | 1.326 ^a | 1.326 ^a | 1.326 ^a |
| 15 | 1.340 ^a | 1.340 ^a | 1.340 ^a |
| 16 | 1.354 ^a | 1.354 ^a | 1.354 ^a |
| 17 | 1.368 ^a | 1.368 ^a | 1.368 ^a |
| 18 | 1.382 ^a | 1.382 ^a | 1.382 ^a |
| 19 | 1.396 ^a | 1.396 ^a | 1.396 ^a |
| 20 | 1.410 ^a | 1.410 ^a | 1.410 ^a |
| 21 | 1.424 ^a | 1.424 ^a | 1.424 ^a |
| 22 | 1.438 ^a | 1.438 ^a | 1.438 ^a |
| 23 | 1.452 ^a | 1.452 ^a | 1.452 ^a |
| 24 | 1.466 ^a | 1.466 ^a | 1.466 ^a |
| 25 | 1.480 ^a | 1.480 ^a | 1.480 ^a |
| 26 | 1.494 ^a | 1.494 ^a | 1.494 ^a |
| 27 | 1.508 ^a | 1.508 ^a | 1.508 ^a |
| 28 | 1.522 ^a | 1.522 ^a | 1.522 ^a |
| 29 | 1.536 ^a | 1.536 ^a | 1.536 ^a |
| 30 | 1.550 ^a | 1.550 ^a | 1.550 ^a |
| 31 | 1.564 ^a | 1.564 ^a | 1.564 ^a |
| 32 | 1.578 ^a | 1.578 ^a | 1.578 ^a |
| 33 | 1.592 ^a | 1.592 ^a | 1.592 ^a |
| 34 | 1.606 ^a | 1.606 ^a | 1.606 ^a |
| 35 | 1.620 ^a | 1.620 ^a | 1.620 ^a |
| 36 | 1.634 ^a | 1.634 ^a | 1.634 ^a |
| 37 | 1.648 ^a | 1.648 ^a | 1.648 ^a |
| 38 | 1.662 ^a | 1.662 ^a | 1.662 ^a |
| 39 | 1.676 ^a | 1.676 ^a | 1.676 ^a |
| 40 | 1.690 ^a | 1.690 ^a | 1.690 ^a |
| 41 | 1.704 ^a | 1.704 ^a | 1.704 ^a |
| 42 | 1.718 ^a | 1.718 ^a | 1.718 ^a |
| 43 | 1.732 ^a | 1.732 ^a | 1.732 ^a |
| 44 | 1.746 ^a | 1.746 ^a | 1.746 ^a |
| 45 | 1.760 ^a | 1.760 ^a | 1.760 ^a |
| 46 | 1.774 ^a | 1.774 ^a | 1.774 ^a |
| 47 | 1.788 ^a | 1.788 ^a | 1.788 ^a |
| 48 | 1.802 ^a | 1.802 ^a | 1.802 ^a |
| 49 | 1.816 ^a | 1.816 ^a | 1.816 ^a |
| 50 | 1.830 ^a | 1.830 ^a | 1.830 ^a |
| 51 | 1.844 ^a | 1.844 ^a | 1.844 ^a |
| 52 | 1.858 ^a | 1.858 ^a | 1.858 ^a |
| 53 | 1.872 ^a | 1.872 ^a | 1.872 ^a |
| 54 | 1.886 ^a | 1.886 ^a | 1.886 ^a |
| 55 | 1.900 ^a | 1.900 ^a | 1.900 ^a |
| 56 | 1.914 ^a | 1.914 ^a | 1.914 ^a |
| 57 | 1.928 ^a | 1.928 ^a | 1.928 ^a |
| 58 | 1.942 ^a | 1.942 ^a | 1.942 ^a |
| 59 | 1.956 ^a | 1.956 ^a | 1.956 ^a |
| 60 | 1.970 ^a | 1.970 ^a | 1.970 ^a |
| 61 | 1.984 ^a | 1.984 ^a | 1.984 ^a |
| 62 | 1.998 ^a | 1.998 ^a | 1.998 ^a |
| 63 | 2.012 ^a | 2.012 ^a | 2.012 ^a |
| 64 | 2.026 ^a | 2.026 ^a | 2.026 ^a |
| 65 | 2.040 ^a | 2.040 ^a | 2.040 ^a |
| 66 | 2.054 ^a | 2.054 ^a | 2.054 ^a |
| 67 | 2.068 ^a | 2.068 ^a | 2.068 ^a |
| 68 | 2.082 ^a | 2.082 ^a | 2.082 ^a |
| 69 | 2.096 ^a | 2.096 ^a | 2.096 ^a |
| 70 | 2.110 ^a | 2.110 ^a | 2.110 ^a |
| 71 | 2.124 ^a | 2.124 ^a | 2.124 ^a |
| 72 | 2.138 ^a | 2.138 ^a | 2.138 ^a |
| 73 | 2.152 ^a | 2.152 ^a | 2.152 ^a |
| 74 | 2.166 ^a | 2.166 ^a | 2.166 ^a |
| 75 | 2.180 ^a | 2.180 ^a | 2.180 ^a |
| 76 | 2.194 ^a | 2.194 ^a | 2.194 ^a |
| 77 | 2.208 ^a | 2.208 ^a | 2.208 ^a |
| 78 | 2.222 ^a | 2.222 ^a | 2.222 ^a |
| 79 | 2.236 ^a | 2.236 ^a | 2.236 ^a |
| 80 | 2.250 ^a | 2.250 ^a | 2.250 ^a |
| 81 | 2.264 ^a | 2.264 ^a | 2.264 ^a |
| 82 | 2.278 ^a | 2.278 ^a | 2.278 ^a |
| 83 | 2.292 ^a | 2.292 ^a | 2.292 ^a |
| 84 | 2.306 ^a | 2.306 ^a | 2.306 ^a |
| 85 | 2.320 ^a | 2.320 ^a | 2.320 ^a |
| 86 | 2.334 ^a | 2.334 ^a | 2.334 ^a |
| 87 | 2.348 ^a | 2.348 ^a | 2.348 ^a |
| 88 | 2.362 ^a | 2.362 ^a | 2.362 ^a |
| 89 | 2.376 ^a | 2.376 ^a | 2.376 ^a |
| 90 | 2.390 ^a | 2.390 ^a | 2.390 ^a |
| 91 | 2.404 ^a | 2.404 ^a | 2.404 ^a |
| 92 | 2.418 ^a | 2.418 ^a | 2.418 ^a |
| 93 | 2.432 ^a | 2.432 ^a | 2.432 ^a |
| 94 | 2.446 ^a | 2.446 ^a | 2.446 ^a |
| 95 | 2.460 ^a | 2.460 ^a | 2.460 ^a |
| 96 | 2.474 ^a | 2.474 ^a | 2.474 ^a |
| 97 | 2.488 ^a | 2.488 ^a | 2.488 ^a |
| 98 | 2.502 ^a | 2.502 ^a | 2.502 ^a |
| 99 | 2.516 ^a | 2.516 ^a | 2.516 ^a |
| 100 | 2.530 ^a | 2.530 ^a | 2.530 ^a |

1/ All values are means of ten birds.
 2/ Values in a column marked by the same letter are not different at the 1 percent level of probability.

Table 10. Total fibrinogen, serum albumin and protein mean, 0 to 12 hours post-mortem, from birds which had been anaesthetized prior to injection of probenidol.

| Time (hr) | mg N/100 g wet tissue | mg N/100 g wet tissue | mg N/100 g wet tissue |
|-----------|-----------------------|-----------------------|-----------------------|
| 0 | 1.089 ^a | 1.089 ^a | 1.089 ^a |
| 1/2 | 1.134 ^a | 1.134 ^a | 1.134 ^a |
| 1 | 1.144 ^a | 1.144 ^a | 1.144 ^a |
| 2 | 1.158 ^a | 1.158 ^a | 1.158 ^a |
| 3 | 1.172 ^a | 1.172 ^a | 1.172 ^a |
| 4 | 1.186 ^a | 1.186 ^a | 1.186 ^a |
| 5 | 1.200 ^a | 1.200 ^a | 1.200 ^a |
| 6 | 1.214 ^a | 1.214 ^a | 1.214 ^a |
| 7 | 1.228 ^a | 1.228 ^a | 1.228 ^a |
| 8 | 1.242 ^a | 1.242 ^a | 1.242 ^a |
| 9 | 1.256 ^a | 1.256 ^a | 1.256 ^a |
| 10 | 1.270 ^a | 1.270 ^a | 1.270 ^a |
| 11 | 1.284 ^a | 1.284 ^a | 1.284 ^a |
| 12 | 1.298 ^a | 1.298 ^a | 1.298 ^a |
| 13 | 1.312 ^a | 1.312 ^a | 1.312 ^a |
| 14 | 1.326 ^a | 1.326 ^a | 1.326 ^a |
| 15 | 1.340 ^a | 1.340 ^a | 1.340 ^a |
| 16 | 1.354 ^a | 1.354 ^a | 1.354 ^a |
| 17 | 1.368 ^a | 1.368 ^a | 1.368 ^a |
| 18 | 1.382 ^a | 1.382 ^a | 1.382 ^a |
| 19 | 1.396 ^a | 1.396 ^a | 1.396 ^a |
| 20 | 1.410 ^a | 1.410 ^a | 1.410 ^a |
| 21 | 1.424 ^a | 1.424 ^a | 1.424 ^a |
| 22 | 1.438 ^a | 1.438 ^a | 1.438 ^a |
| 23 | 1.452 ^a | 1.452 ^a | 1.452 ^a |
| 24 | 1.466 ^a | 1.466 ^a | 1.466 ^a |
| 25 | 1.480 ^a | 1.480 ^a | 1.480 ^a |
| 26 | 1.494 ^a | 1.494 ^a | 1.494 ^a |
| 27 | 1.508 ^a | 1.508 ^a | 1.508 ^a |
| 28 | 1.522 ^a | 1.522 ^a | 1.522 ^a |
| 29 | 1.536 ^a | 1.536 ^a | 1.536 ^a |
| 30 | 1.550 ^a | 1.550 ^a | 1.550 ^a |
| 31 | 1.564 ^a | 1.564 ^a | 1.564 ^a |
| 32 | 1.578 ^a | 1.578 ^a | 1.578 ^a |
| 33 | 1.592 ^a | 1.592 ^a | 1.592 ^a |
| 34 | 1.606 ^a | 1.606 ^a | 1.606 ^a |
| 35 | 1.620 ^a | 1.620 ^a | 1.620 ^a |
| 36 | 1.634 ^a | 1.634 ^a | 1.634 ^a |
| 37 | 1.648 ^a | 1.648 ^a | 1.648 ^a |
| 38 | 1.662 ^a | 1.662 ^a | 1.662 ^a |
| 39 | 1.676 ^a | 1.676 ^a | 1.676 ^a |
| 40 | 1.690 ^a | 1.690 ^a | 1.690 ^a |
| 41 | 1.704 ^a | 1.704 ^a | 1.704 ^a |
| 42 | 1.718 ^a | 1.718 ^a | 1.718 ^a |
| 43 | 1.732 ^a | 1.732 ^a | 1.732 ^a |
| 44 | 1.746 ^a | 1.746 ^a | 1.746 ^a |
| 45 | 1.760 ^a | 1.760 ^a | 1.760 ^a |
| 46 | 1.774 ^a | 1.774 ^a | 1.774 ^a |
| 47 | 1.788 ^a | 1.788 ^a | 1.788 ^a |
| 48 | 1.802 ^a | 1.802 ^a | 1.802 ^a |
| 49 | 1.816 ^a | 1.816 ^a | 1.816 ^a |
| 50 | 1.830 ^a | 1.830 ^a | 1.830 ^a |
| 51 | 1.844 ^a | 1.844 ^a | 1.844 ^a |
| 52 | 1.858 ^a | 1.858 ^a | 1.858 ^a |
| 53 | 1.872 ^a | 1.872 ^a | 1.872 ^a |
| 54 | 1.886 ^a | 1.886 ^a | 1.886 ^a |
| 55 | 1.900 ^a | 1.900 ^a | 1.900 ^a |
| 56 | 1.914 ^a | 1.914 ^a | 1.914 ^a |
| 57 | 1.928 ^a | 1.928 ^a | 1.928 ^a |
| 58 | 1.942 ^a | 1.942 ^a | 1.942 ^a |
| 59 | 1.956 ^a | 1.956 ^a | 1.956 ^a |
| 60 | 1.970 ^a | 1.970 ^a | 1.970 ^a |
| 61 | 1.984 ^a | 1.984 ^a | 1.984 ^a |
| 62 | 1.998 ^a | 1.998 ^a | 1.998 ^a |
| 63 | 2.012 ^a | 2.012 ^a | 2.012 ^a |
| 64 | 2.026 ^a | 2.026 ^a | 2.026 ^a |
| 65 | 2.040 ^a | 2.040 ^a | 2.040 ^a |
| 66 | 2.054 ^a | 2.054 ^a | 2.054 ^a |
| 67 | 2.068 ^a | 2.068 ^a | 2.068 ^a |
| 68 | 2.082 ^a | 2.082 ^a | 2.082 ^a |
| 69 | 2.096 ^a | 2.096 ^a | 2.096 ^a |
| 70 | 2.110 ^a | 2.110 ^a | 2.110 ^a |
| 71 | 2.124 ^a | 2.124 ^a | 2.124 ^a |
| 72 | 2.138 ^a | 2.138 ^a | 2.138 ^a |
| 73 | 2.152 ^a | 2.152 ^a | 2.152 ^a |
| 74 | 2.166 ^a | 2.166 ^a | 2.166 ^a |
| 75 | 2.180 ^a | 2.180 ^a | 2.180 ^a |
| 76 | 2.194 ^a | 2.194 ^a | 2.194 ^a |
| 77 | 2.208 ^a | 2.208 ^a | 2.208 ^a |
| 78 | 2.222 ^a | 2.222 ^a | 2.222 ^a |
| 79 | 2.236 ^a | 2.236 ^a | 2.236 ^a |
| 80 | 2.250 ^a | 2.250 ^a | 2.250 ^a |
| 81 | 2.264 ^a | 2.264 ^a | 2.264 ^a |
| 82 | 2.278 ^a | 2.278 ^a | 2.278 ^a |
| 83 | 2.292 ^a | 2.292 ^a | 2.292 ^a |
| 84 | 2.306 ^a | 2.306 ^a | 2.306 ^a |
| 85 | 2.320 ^a | 2.320 ^a | 2.320 ^a |
| 86 | 2.334 ^a | 2.334 ^a | 2.334 ^a |
| 87 | 2.348 ^a | 2.348 ^a | 2.348 ^a |
| 88 | 2.362 ^a | 2.362 ^a | 2.362 ^a |
| 89 | 2.376 ^a | 2.376 ^a | 2.376 ^a |
| 90 | 2.390 ^a | 2.390 ^a | 2.390 ^a |
| 91 | 2.404 ^a | 2.404 ^a | 2.404 ^a |
| 92 | 2.418 ^a | 2.418 ^a | 2.418 ^a |
| 93 | 2.432 ^a | 2.432 ^a | 2.432 ^a |
| 94 | 2.446 ^a | 2.446 ^a | 2.446 ^a |
| 95 | 2.460 ^a | 2.460 ^a | 2.460 ^a |
| 96 | 2.474 ^a | 2.474 ^a | 2.474 ^a |
| 97 | 2.488 ^a | 2.488 ^a | 2.488 ^a |
| 98 | 2.502 ^a | 2.502 ^a | 2.502 ^a |
| 99 | 2.516 ^a | 2.516 ^a | 2.516 ^a |
| 100 | 2.530 ^a | 2.530 ^a | 2.530 ^a |

1/ All values are means of ten birds.
 2/ Values in a column marked by the same letter are not different at the 1 percent level of probability.

Actomyosin Nitrogen

Actomyosin is defined as the protein that was soluble at an ionic strength of 1.0 but insoluble at an ionic strength of 0.25. Again statistical analyses indicated that extractability of this fraction was significantly greater from muscle of the N-AN birds from 1/4 - 6 hours post-mortem than from muscle of the AN birds (Table 11 and Appendix C). As with some of the other fractions, extractability of actomyosin from breast muscle remained at a low level during the first hour in the AN birds before increasing to a statistical maximum at 12 hours post-mortem (Table 12). Actomyosin extractability from breast muscle of the N-AN birds increased from the zero time sample to a statistical maximum level at 1 hour (Table 12). As with total fibrillar protein, extractability of actomyosin from muscle began at a lower level in the AN birds, but final extractability was greater than that from muscle of the N-AN birds.

Residual Myosin Nitrogen

This fraction was defined as that protein extracted by a pyrophosphate containing solution from the residue of the KCl-buffer extraction of muscle (Hasselbach and Schneider, 1951; Baliga et al., 1962). Data collected dealing with this fraction are summarized in Tables 13 and 14 and Appendix C. Amount of residual myosin extracted was significantly less from muscle of the N-AN birds from 1/4 - 3 hours post-mortem than from muscle of the AN birds. The statistical minimum level of extractability of this fraction from breast muscle was reached

Actomyosin is defined as the protein that was released by an active strength of 1.0 but inactive at an active strength of 1.0. Statistical analyses indicated that extracellularly released actomyosin was significantly greater from muscle of the 10-day-old birds than from the 1-hour post-mortem than from muscle of the 10-day-old birds (Appendix C). As with most of the other treatments, extracellular actomyosin from breast muscle remained at a low level until the first hour in the 10-day-old birds, increasing to a maximum of 1.0 hour post-mortem (Table 1). Actomyosin extracellularly from breast muscle of the 10-day-old birds increased from the 10-day-old birds to a statistical maximum level at 1 hour post-mortem, as well as total fibrillar protein, extracellularly of actomyosin, and muscle protein at a lower level in the 10-day-old birds, but that extracellularly was greater than that from muscle of the 10-day-old birds.

Residual Myosin Nitrogen

This fraction was defined as that protein extracted by a phosphate containing solution from the residue of the 10-day-old birds. Extraction of muscle (residual and nonresidual) from the 10-day-old birds (1953). Data collected during this fraction was summarized in Tables 13 and 14 and Appendix C. Amount of residual myosin extracted was significantly less from muscle of the 10-day-old birds than from post-mortem than from muscle of the 10-day-old birds. The nonresidual myosin level of extracellularly released from breast muscle was reduced

Table 11. Effect of ante-mortem injection of turkeys with pentobarbital on actomyosin nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment ^{2/} | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 355 ^a | 620 ^a |
| 1/4 | 389 ^a | 1468 ^b |
| 1/2 | 383 ^a | 1476 ^b |
| 1 | 436 ^a | 1624 ^b |
| 3 | 1047 ^a | 1823 ^b |
| 6 | 1453 ^a | 1881 ^b |
| 12 | 1994 ^a | 1957 ^a |
| 24 | 1992 ^a | 1918 ^a |
| 48 | 2023 ^a | 1944 ^a |
| 72 | 2026 ^a | 1994 ^a |

^{1/} All values are means of ten birds.

^{2/} Values in a row marked by the same letter are not different at the 1 percent level of probability.

Table 12. Actomyosin nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized ^{2/} | | Non-anesthetized ^{2/} | |
|----------------------------|-----------------------|--------------------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 0 | 355 ^a | 0 | 620 ^a |
| 1/2 | 383 ^a | 1/4 | 1468 ^b |
| 1/4 | 389 ^a | 1/2 | 1476 ^b |
| 1 | 436 ^a | 1 | 1624 ^{bc} |
| 3 | 1047 ^b | 3 | 1823 ^c |
| 6 | 1453 ^c | 6 | 1881 ^c |
| 24 | 1992 ^d | 24 | 1918 ^c |
| 12 | 1994 ^d | 48 | 1944 ^c |
| 48 | 2023 ^d | 12 | 1957 ^c |
| 72 | 2026 ^d | 72 | 1994 ^c |

^{1/} All values are means of ten birds.

^{2/} Values in a column marked by the same letter are not different at the 1 percent level of probability.

Table 13. Effect of ante-mortem injection of turkeys with pentobarbital on residual myosin nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment ^{2/} | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 92 ^a | 64 ^{*a} |
| 1/4 | 105 ^{*a} | 43 ^b |
| 1/2 | 84 ^{*a} | 42 ^b |
| 1 | 86 ^a | 27 ^b |
| 3 | 64 ^{*a} | 15 ^b |
| 6 | 44 ^a | 13 ^a |
| 12 | 11 ^a | 12 ^a |
| 24 | 11 ^a | 14 ^a |
| 48 | 9 ^a | 11 ^a |
| 72 | 10 ^a | 12 ^a |

^{1/} All values are means of ten birds except those marked by an asterick which are means of nine birds.

^{2/} Values in a row marked by the same letter are not different at the 1 percent level of probability.

Table 14. Residual myosin nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized ^{2/} | | Non-anesthetized ^{2/} | |
|----------------------------|-----------------------|--------------------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 1/4 | 105 ^{*a} | 0 | 64 ^{*a} |
| 0 | 92 ^{ab} | 1/4 | 43 ^{ab} |
| 1 | 86 ^{ab} | 1/2 | 42 ^{ab} |
| 1/2 | 84 ^{*ab} | 1 | 27 ^b |
| 3 | 64 ^{*bc} | 3 | 15 ^b |
| 6 | 44 ^{cd} | 24 | 14 ^b |
| 12 | 11 ^d | 6 | 13 ^b |
| 24 | 11 ^d | 72 | 12 ^b |
| 72 | 10 ^d | 12 | 12 ^b |
| 48 | 9 ^d | 48 | 11 ^b |

^{1/} All values are means of ten birds except those marked by an asterick which are means of nine birds.

^{2/} Values in a column marked by the same letter are not different at the 1 percent level of probability.

1. The first part of the report is a general introduction to the subject of the study. It is followed by a description of the methods used in the study.

| TABLE I | | RESULTS OF THE STUDY | |
|---------|-----------------|----------------------|---------------------|
| Year | Number of cases | Percentage of cases | Percentage of total |
| 1950 | 100 | 100 | 100 |
| 1951 | 120 | 120 | 120 |
| 1952 | 150 | 150 | 150 |
| 1953 | 180 | 180 | 180 |
| 1954 | 200 | 200 | 200 |
| 1955 | 220 | 220 | 220 |
| 1956 | 250 | 250 | 250 |
| 1957 | 280 | 280 | 280 |
| 1958 | 300 | 300 | 300 |
| 1959 | 320 | 320 | 320 |
| 1960 | 350 | 350 | 350 |
| 1961 | 380 | 380 | 380 |
| 1962 | 400 | 400 | 400 |
| 1963 | 420 | 420 | 420 |
| 1964 | 450 | 450 | 450 |
| 1965 | 480 | 480 | 480 |
| 1966 | 500 | 500 | 500 |
| 1967 | 520 | 520 | 520 |
| 1968 | 550 | 550 | 550 |
| 1969 | 580 | 580 | 580 |
| 1970 | 600 | 600 | 600 |
| 1971 | 620 | 620 | 620 |
| 1972 | 650 | 650 | 650 |
| 1973 | 680 | 680 | 680 |
| 1974 | 700 | 700 | 700 |
| 1975 | 720 | 720 | 720 |
| 1976 | 750 | 750 | 750 |
| 1977 | 780 | 780 | 780 |
| 1978 | 800 | 800 | 800 |
| 1979 | 820 | 820 | 820 |
| 1980 | 850 | 850 | 850 |
| 1981 | 880 | 880 | 880 |
| 1982 | 900 | 900 | 900 |
| 1983 | 920 | 920 | 920 |
| 1984 | 950 | 950 | 950 |
| 1985 | 980 | 980 | 980 |
| 1986 | 1000 | 1000 | 1000 |
| 1987 | 1020 | 1020 | 1020 |
| 1988 | 1050 | 1050 | 1050 |
| 1989 | 1080 | 1080 | 1080 |
| 1990 | 1100 | 1100 | 1100 |
| 1991 | 1120 | 1120 | 1120 |
| 1992 | 1150 | 1150 | 1150 |
| 1993 | 1180 | 1180 | 1180 |
| 1994 | 1200 | 1200 | 1200 |
| 1995 | 1220 | 1220 | 1220 |
| 1996 | 1250 | 1250 | 1250 |
| 1997 | 1280 | 1280 | 1280 |
| 1998 | 1300 | 1300 | 1300 |
| 1999 | 1320 | 1320 | 1320 |
| 2000 | 1350 | 1350 | 1350 |
| 2001 | 1380 | 1380 | 1380 |
| 2002 | 1400 | 1400 | 1400 |
| 2003 | 1420 | 1420 | 1420 |
| 2004 | 1450 | 1450 | 1450 |
| 2005 | 1480 | 1480 | 1480 |
| 2006 | 1500 | 1500 | 1500 |
| 2007 | 1520 | 1520 | 1520 |
| 2008 | 1550 | 1550 | 1550 |
| 2009 | 1580 | 1580 | 1580 |
| 2010 | 1600 | 1600 | 1600 |
| 2011 | 1620 | 1620 | 1620 |
| 2012 | 1650 | 1650 | 1650 |
| 2013 | 1680 | 1680 | 1680 |
| 2014 | 1700 | 1700 | 1700 |
| 2015 | 1720 | 1720 | 1720 |
| 2016 | 1750 | 1750 | 1750 |
| 2017 | 1780 | 1780 | 1780 |
| 2018 | 1800 | 1800 | 1800 |
| 2019 | 1820 | 1820 | 1820 |
| 2020 | 1850 | 1850 | 1850 |
| 2021 | 1880 | 1880 | 1880 |
| 2022 | 1900 | 1900 | 1900 |
| 2023 | 1920 | 1920 | 1920 |
| 2024 | 1950 | 1950 | 1950 |
| 2025 | 1980 | 1980 | 1980 |
| 2026 | 2000 | 2000 | 2000 |
| 2027 | 2020 | 2020 | 2020 |
| 2028 | 2050 | 2050 | 2050 |
| 2029 | 2080 | 2080 | 2080 |
| 2030 | 2100 | 2100 | 2100 |
| 2031 | 2120 | 2120 | 2120 |
| 2032 | 2150 | 2150 | 2150 |
| 2033 | 2180 | 2180 | 2180 |
| 2034 | 2200 | 2200 | 2200 |
| 2035 | 2220 | 2220 | 2220 |
| 2036 | 2250 | 2250 | 2250 |
| 2037 | 2280 | 2280 | 2280 |
| 2038 | 2300 | 2300 | 2300 |
| 2039 | 2320 | 2320 | 2320 |
| 2040 | 2350 | 2350 | 2350 |
| 2041 | 2380 | 2380 | 2380 |
| 2042 | 2400 | 2400 | 2400 |
| 2043 | 2420 | 2420 | 2420 |
| 2044 | 2450 | 2450 | 2450 |
| 2045 | 2480 | 2480 | 2480 |
| 2046 | 2500 | 2500 | 2500 |
| 2047 | 2520 | 2520 | 2520 |
| 2048 | 2550 | 2550 | 2550 |
| 2049 | 2580 | 2580 | 2580 |
| 2050 | 2600 | 2600 | 2600 |
| 2051 | 2620 | 2620 | 2620 |
| 2052 | 2650 | 2650 | 2650 |
| 2053 | 2680 | 2680 | 2680 |
| 2054 | 2700 | 2700 | 2700 |
| 2055 | 2720 | 2720 | 2720 |
| 2056 | 2750 | 2750 | 2750 |
| 2057 | 2780 | 2780 | 2780 |
| 2058 | 2800 | 2800 | 2800 |
| 2059 | 2820 | 2820 | 2820 |
| 2060 | 2850 | 2850 | 2850 |
| 2061 | 2880 | 2880 | 2880 |
| 2062 | 2900 | 2900 | 2900 |
| 2063 | 2920 | 2920 | 2920 |
| 2064 | 2950 | 2950 | 2950 |
| 2065 | 2980 | 2980 | 2980 |
| 2066 | 3000 | 3000 | 3000 |
| 2067 | 3020 | 3020 | 3020 |
| 2068 | 3050 | 3050 | 3050 |
| 2069 | 3080 | 3080 | 3080 |
| 2070 | 3100 | 3100 | 3100 |
| 2071 | 3120 | 3120 | 3120 |
| 2072 | 3150 | 3150 | 3150 |
| 2073 | 3180 | 3180 | 3180 |
| 2074 | 3200 | 3200 | 3200 |
| 2075 | 3220 | 3220 | 3220 |
| 2076 | 3250 | 3250 | 3250 |
| 2077 | 3280 | 3280 | 3280 |
| 2078 | 3300 | 3300 | 3300 |
| 2079 | 3320 | 3320 | 3320 |
| 2080 | 3350 | 3350 | 3350 |
| 2081 | 3380 | 3380 | 3380 |
| 2082 | 3400 | 3400 | 3400 |
| 2083 | 3420 | 3420 | 3420 |
| 2084 | 3450 | 3450 | 3450 |
| 2085 | 3480 | 3480 | 3480 |
| 2086 | 3500 | 3500 | 3500 |
| 2087 | 3520 | 3520 | 3520 |
| 2088 | 3550 | 3550 | 3550 |
| 2089 | 3580 | 3580 | 3580 |
| 2090 | 3600 | 3600 | 3600 |
| 2091 | 3620 | 3620 | 3620 |
| 2092 | 3650 | 3650 | 3650 |
| 2093 | 3680 | 3680 | 3680 |
| 2094 | 3700 | 3700 | 3700 |
| 2095 | 3720 | 3720 | 3720 |
| 2096 | 3750 | 3750 | 3750 |
| 2097 | 3780 | 3780 | 3780 |
| 2098 | 3800 | 3800 | 3800 |
| 2099 | 3820 | 3820 | 3820 |
| 2100 | 3850 | 3850 | 3850 |
| 2101 | 3880 | 3880 | 3880 |
| 2102 | 3900 | 3900 | 3900 |
| 2103 | 3920 | 3920 | 3920 |
| 2104 | 3950 | 3950 | 3950 |
| 2105 | 3980 | 3980 | 3980 |
| 2106 | 4000 | 4000 | 4000 |
| 2107 | 4020 | 4020 | 4020 |
| 2108 | 4050 | 4050 | 4050 |
| 2109 | 4080 | 4080 | 4080 |
| 2110 | 4100 | 4100 | 4100 |
| 2111 | 4120 | 4120 | 4120 |
| 2112 | 4150 | 4150 | 4150 |
| 2113 | 4180 | 4180 | 4180 |
| 2114 | 4200 | 4200 | 4200 |
| 2115 | 4220 | 4220 | 4220 |
| 2116 | 4250 | 4250 | 4250 |
| 2117 | 4280 | 4280 | 4280 |
| 2118 | 4300 | 4300 | 4300 |
| 2119 | 4320 | 4320 | 4320 |
| 2120 | 4350 | 4350 | 4350 |
| 2121 | 4380 | 4380 | 4380 |
| 2122 | 4400 | 4400 | 4400 |
| 2123 | 4420 | 4420 | 4420 |
| 2124 | 4450 | 4450 | 4450 |
| 2125 | 4480 | 4480 | 4480 |
| 2126 | 4500 | 4500 | 4500 |
| 2127 | 4520 | 4520 | 4520 |
| 2128 | 4550 | 4550 | 4550 |
| 2129 | 4580 | 4580 | 4580 |
| 2130 | 4600 | 4600 | 4600 |
| 2131 | 4620 | 4620 | 4620 |
| 2132 | 4650 | 4650 | 4650 |
| 2133 | 4680 | 4680 | 4680 |
| 2134 | 4700 | 4700 | 4700 |
| 2135 | 4720 | 4720 | 4720 |
| 2136 | 4750 | 4750 | 4750 |
| 2137 | 4780 | 4780 | 4780 |
| 2138 | 4800 | 4800 | 4800 |
| 2139 | 4820 | 4820 | 4820 |
| 2140 | 4850 | 4850 | 4850 |
| 2141 | 4880 | 4880 | 4880 |
| 2142 | 4900 | 4900 | 4900 |
| 2143 | 4920 | 4920 | 4920 |
| 2144 | 4950 | 4950 | 4950 |
| 2145 | 4980 | 4980 | 4980 |
| 2146 | 5000 | 5000 | 5000 |
| 2147 | 5020 | 5020 | 5020 |
| 2148 | 5050 | 5050 | 5050 |
| 2149 | 5080 | 5080 | 5080 |
| 2150 | 5100 | 5100 | 5100 |
| 2151 | 5120 | 5120 | 5120 |
| 2152 | 5150 | 5150 | 5150 |
| 2153 | 5180 | 5180 | 5180 |
| 2154 | 5200 | 5200 | 5200 |
| 2155 | 5220 | 5220 | 5220 |
| 2156 | 5250 | 5250 | 5250 |
| 2157 | 5280 | 5280 | 5280 |
| 2158 | 5300 | 5300 | 5300 |
| 2159 | 5320 | 5320 | 5320 |
| 2160 | 5350 | 5350 | 5350 |
| 2161 | 5380 | 5380 | 5380 |
| 2162 | 5400 | 5400 | 5400 |
| 2163 | 5420 | 5420 | 5420 |
| 2164 | 5450 | 5450 | 5450 |
| 2165 | 5480 | 5480 | 5480 |
| 2166 | 5500 | 5500 | 5500 |
| 2167 | 5520 | 5520 | 5520 |
| 2168 | 5550 | 5550 | 5550 |
| 2169 | 5580 | 5580 | 5580 |
| 2170 | 5600 | 5600 | 5600 |
| 2171 | 5620 | 5620 | 5620 |
| 2172 | 5650 | 5650 | 5650 |
| 2173 | 5680 | 5680 | 5680 |
| 2174 | 5700 | 5700 | 5700 |
| 2175 | 5720 | 5720 | 5720 |
| 2176 | 5750 | 5750 | 5750 |
| 2177 | 5780 | 5780 | 5780 |
| 2178 | 5800 | 5800 | 5800 |
| 2179 | 5820 | 5820 | 5820 |
| 2180 | 5850 | 5850 | 5850 |
| 2181 | 5880 | 5880 | 5880 |
| 2182 | 5900 | 5900 | 5900 |
| 2183 | 5920 | 5920 | 5920 |
| 2184 | 5950 | 5950 | 5950 |
| 2185 | 5980 | 5980 | 5980 |
| 2186 | 6000 | 6000 | 6000 |
| 2187 | 6020 | 6020 | 6020 |
| 2188 | 6050 | 6050 | 6050 |
| 2189 | 6080 | 6080 | 6080 |
| 2190 | 6100 | 6100 | 6100 |
| 2191 | 6120 | 6120 | 6120 |
| 2192 | 6150 | 6150 | 6150 |
| 2193 | 6180 | 6180 | 6180 |
| 2194 | 6200 | 6200 | 6200 |
| 2195 | 6220 | 6220 | 6220 |
| 2196 | 6250 | 6250 | 6250 |
| 2197 | 6280 | 6280 | 6280 |
| 2198 | 6300 | 6300 | 6300 |
| 2199 | 6320 | 6320 | 6320 |
| 2200 | 6350 | 6350 | 6350 |
| 2201 | 6380 | 6380 | 6380 |
| 2202 | 6400 | 6400 | 6400 |
| 2203 | 6420 | 6420 | 6420 |
| 2204 | 6450 | 6450 | 6450 |
| 2205 | 6480 | 6480 | 6480 |
| 2206 | 6500 | 6500 | 6500 |
| 2207 | 6520 | 6520 | 6520 |
| 2208 | 6550 | 6550 | 6550 |
| 2209 | 6580 | 6580 | 6580 |
| 2210 | 6600 | 6600 | 6600 |
| 2211 | 6620 | 6620 | 6620 |
| 2212 | 6650 | 6650 | 6650 |
| 2213 | 6680 | 6680 | 6680 |
| 2214 | 6700 | 6700 | 6700 |
| 2215 | 6720 | 6720 | 6720 |
| 2216 | 6750 | 6750 | 6750 |
| 2217 | 6780 | 6780 | 6780 |
| 2218 | 6800 | 6800 | 6800 |
| 2219 | 6820 | 6820 | 6820 |
| 2220 | 6850 | 6850 | 6850 |
| 2221 | 6880 | 6880 | 6880 |
| 2222 | 6900 | 6900 | 6900 |
| 2223 | 6920 | 6920 | 6920 |
| 2224 | 6950 | 6950 | 6950 |
| 2225 | 6980 | 6980 | 6980 |
| 2226 | 7000 | 7000 | 7000 |
| 2227 | 7020 | 7020 | 7020 |
| 2228 | 7050 | 7050 | 7050 |
| 2229 | 7080 | 7080 | 7080 |
| 2230 | 7100 | 7100 | 7100 |
| 2231 | 7120 | 7120 | 7120 |
| 2232 | 7150 | 7150 | 7150 |
| 2233 | 7180 | 7180 | 7180 |
| 2234 | 7200 | 7200 | 7200 |
| 2235 | 7220 | 7220 | 7220 |
| 2236 | 7250 | 7250 | 7250 |
| 2237 | 7280 | 7280 | 7280 |
| 2238 | 7300 | 7300 | 7300 |
| 2239 | 7320 | 7320 | 7320 |
| 2240 | 7350 | 7350 | 7350 |
| 2241 | 7380 | 7380 | 7380 |
| 2242 | 7400 | 7400 | 740 |

at 1/4 hour in the N-AN birds but was not reached until 6 hours post-mortem in the AN birds (Table 14). It was noticed that extractability of residual myosin was greater initially from muscle of the AN birds and remained fairly constant for the first hour but then it began to decrease similar to extractability from muscle of the N-AN birds which decreased from the zero time sample.

Residual Actin Nitrogen

Residual actin was defined as the protein extracted by KCl-buffer from the residue of the pyrophosphate extraction (Baliga et al., 1962). Tables 15 and 16 and Appendix C include a summary of data collected for this fraction. This summary indicated that extractability of residual actin from breast muscle was significantly lower in the N-AN birds at 1/4, 1 and 3 hours post-mortem than in the AN birds. The statistical minimum extractability level of this fraction was reached at 1 hour in the N-AN birds and at 6 hours post-mortem in the AN birds. Extractability of this fraction from muscle was greater in the AN birds initially and remained fairly constant over the first hour before beginning to decrease similar to that of the N-AN birds.

Unextracted Soluble Protein Nitrogen

This fraction was defined as alkali soluble protein extracted from the residue of the residual actin extraction and was made up mostly of denatured proteins. These data are summarized in

[illegible]

Residual Actin Nitrogen

[illegible]

Unextracted Soluble Protein Nitrogen

Mostly of denatured proteins. Some of the substances in the residue of the residual acid solution are as follows: This reaction was an acid-soluble, protein-free.

Table 15. Effect of ante-mortem injection of turkeys with pentobarbital on residual actin nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment ^{2/} | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 45 ^a | 34 ^{*a} |
| 1/4 | 50 ^{*a} | 26 ^b |
| 1/2 | 43 ^{*a} | 28 ^a |
| 1 | 43 ^a | 19 ^b |
| 3 | 34 ^{*a} | 12 ^b |
| 6 | 22 ^a | 8 ^a |
| 12 | 6 ^a | 7 ^a |
| 24 | 7 ^a | 9 ^a |
| 48 | 5 ^a | 7 ^a |
| 72 | 5 ^a | 7 ^a |

^{1/} All values are means of ten birds except those marked by an asterick which are means of nine birds.

^{2/} Values in a row marked by the same letter are not different at the 1 percent level of probability.

Table 16. Residual actin nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized ^{2/} | | Non-anesthetized ^{2/} | |
|----------------------------|-----------------------|--------------------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 1/4 | 50 ^{*a} | 0 | 34 ^{*a} |
| 0 | 45 ^{ab} | 1/2 | 28 ^a |
| 1/2 | 43 ^{*ab} | 1/4 | 26 ^{ab} |
| 1 | 43 ^{ab} | 1 | 19 ^{abc} |
| 3 | 34 ^{*bc} | 3 | 12 ^{bc} |
| 6 | 22 ^{cd} | 24 | 9 ^c |
| 24 | 7 ^d | 6 | 8 ^c |
| 12 | 6 ^d | 12 | 7 ^c |
| 72 | 5 ^d | 72 | 7 ^c |
| 48 | 5 ^d | 48 | 7 ^c |

^{1/} All values are means of ten birds except those marked by an asterick which are means of nine birds.

^{2/} Values in a column marked by the same letter are not different at the 1 percent level of probability.

Table 17. Effect of temperature on the rate of growth of *Escherichia coli* in a nutrient broth medium.

| Time (hr) | Temperature (°C) | Optical density at 540 mμ |
|-----------|------------------|---------------------------|
| 0 | 20 | 0.00 |
| 1 | 20 | 0.05 |
| 2 | 20 | 0.10 |
| 3 | 20 | 0.15 |
| 4 | 20 | 0.20 |
| 5 | 20 | 0.25 |
| 6 | 20 | 0.30 |
| 7 | 20 | 0.35 |
| 8 | 20 | 0.40 |
| 9 | 20 | 0.45 |
| 10 | 20 | 0.50 |
| 11 | 20 | 0.55 |
| 12 | 20 | 0.60 |
| 13 | 20 | 0.65 |
| 14 | 20 | 0.70 |
| 15 | 20 | 0.75 |
| 16 | 20 | 0.80 |
| 17 | 20 | 0.85 |
| 18 | 20 | 0.90 |
| 19 | 20 | 0.95 |
| 20 | 20 | 1.00 |

1. All values are means of two or more determinations. 2. Asterisk indicates that the value is significantly different from the control value at the 1 percent level of probability.

Table 18. Effect of temperature on the rate of growth of *Escherichia coli* in a nutrient broth medium.

| Time (hr) | Temperature (°C) | Optical density at 540 mμ |
|-----------|------------------|---------------------------|
| 0 | 20 | 0.00 |
| 1 | 20 | 0.05 |
| 2 | 20 | 0.10 |
| 3 | 20 | 0.15 |
| 4 | 20 | 0.20 |
| 5 | 20 | 0.25 |
| 6 | 20 | 0.30 |
| 7 | 20 | 0.35 |
| 8 | 20 | 0.40 |
| 9 | 20 | 0.45 |
| 10 | 20 | 0.50 |
| 11 | 20 | 0.55 |
| 12 | 20 | 0.60 |
| 13 | 20 | 0.65 |
| 14 | 20 | 0.70 |
| 15 | 20 | 0.75 |
| 16 | 20 | 0.80 |
| 17 | 20 | 0.85 |
| 18 | 20 | 0.90 |
| 19 | 20 | 0.95 |
| 20 | 20 | 1.00 |

1. All values are means of two or more determinations. 2. Asterisk indicates that the value is significantly different from the control value at the 1 percent level of probability.

Tables 17 and 18 and Appendix C. Extractability of this fraction from breast muscle remained at a relatively constant, high level in the AN birds for the first hour post-mortem. Then it began to decrease to a statistical minimum level at 12 hours. This fraction was extracted in significantly smaller amounts from muscle of the N-AN birds from 1/4 - 6 hours than from muscle of the AN birds, with a statistical minimum extractability level being reached at 3 hours post-mortem. This fraction was extracted from breast muscle at a fairly constant, high level the first hour post-mortem in the AN birds before extractability began to decline similar to that of the N-AN birds.

Implications of the Post-Mortem Changes in Protein Extractability

Non-Protein Nitrogen

Small changes in non-protein nitrogen that occurred in this study are in general agreement with findings reported by several other researchers. Khan and van den Berg (1964b) found little change in non-protein nitrogen in chicken breast muscle over a 3 day storage period or in chicken leg muscle over a 6 day storage period. Similar results were found by Miller et al. (1965) working with chickens. In contrast to this, Aberle and Merkel (1966) found that soluble non-protein nitrogen increased significantly during post-mortem aging of Longissimus dorsi and Semitendinosus muscles of the bovine. Most of the increase occurred during the first 168 hours post-mortem. Similar

Tables 11 and 12 and Figures 11 and 12. Extrapolation of this fraction from breast muscle remained at a relatively constant, high level in the AN birds for the first four post-mortem hours. There was a decrease to a statistical minimum level at 6 hours. This fraction was extracted in significantly smaller amounts from muscle of the N-AN birds from 1 1/2 - 6 hours than from muscle of the AN birds, with a statistical minimum extrapolative level being reached at 1 1/2 hours post-mortem. This fraction was extracted from breast muscle at a fairly constant, high level the first four post-mortem hours in birds before extracorporeality began to exerting stimulus to loss of the N-AN birds.

Implications of the post-mortem response

Protein Extracorporeality

Non-Protein Nitrogen

Small changes in non-protein nitrogen level occurred in this study are in general agreement with results reported by several other researchers. Chan and van den Berg (1960) found little change in non-protein nitrogen in chicken serum must be over a 24 hour period or in chicken leg muscle over a 7 day storage period. In results were found by Miller et al. (1961) working with chickens. In contrast to this, Morris and Taylor (1962) found that extracorporeality protein nitrogen increased significantly during 24 hours post-mortem. Longissimus dorsi and semitendinosus muscles of the turkey, and of the increase occurred during the first 12 hours post-mortem. In this

Table 17. Effect of ante-mortem injection of turkeys with pentobarbital on unextracted soluble protein nitrogen of the breast muscle, 0 to 72 hours post-mortem^{1/}.

| Time
(hr) | Treatment ^{2/} | |
|--------------|---------------------------------------|---|
| | Anesthetized
mg N/100 g wet tissue | Non-anesthetized
mg N/100 g wet tissue |
| 0 | 599 ^a | 499 ^{*a} |
| 1/4 | 651 ^{*a} | 283 ^b |
| 1/2 | 588 ^{*a} | 297 ^b |
| 1 | 529 ^a | 192 ^b |
| 3 | 316 ^{*a} | 89 ^b |
| 6 | 239 ^a | 50 ^b |
| 12 | 33 ^a | 39 ^a |
| 24 | 20 ^a | 56 ^a |
| 48 | 16 ^a | 66 ^a |
| 72 | 13 ^a | 33 ^a |

^{1/} All values are means of ten birds except those marked by an asterisk which are means of nine birds.

^{2/} Values in a row marked by the same letter are not different at the 1 percent level of probability.

Table 18. Unextracted soluble protein nitrogen of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injections of pentobarbital^{1/}.

| Anesthetized ^{2/} | | Non-anesthetized ^{2/} | |
|----------------------------|-----------------------|--------------------------------|-----------------------|
| Time (hr) | mg N/100 g wet tissue | Time (hr) | mg N/100 g wet tissue |
| 1/4 | 651 ^{*a} | 0 | 499 ^{*a} |
| 0 | 599 ^a | 1/2 | 297 ^b |
| 1/2 | 588 ^{*a} | 1/4 | 283 ^b |
| 1 | 529 ^a | 1 | 192 ^{bc} |
| 3 | 316 ^{*b} | 3 | 89 ^{cd} |
| 6 | 239 ^b | 48 | 66 ^{cd} |
| 12 | 33 ^c | 24 | 56 ^{cd} |
| 24 | 20 ^c | 6 | 50 ^{cd} |
| 48 | 16 ^c | 12 | 39 ^{cd} |
| 72 | 13 ^c | 72 | 33 ^d |

^{1/} All values are means of ten birds except those marked by an asterisk which are means of nine birds.

^{2/} Values in a column marked by the same letter are not different at the 1 percent level of probability.

results were shown by Parrish et al. (1969) over a 312 hour aging period for beef, however, there was little change over the first 72 hours post-mortem.

There seemed to be no apparent explanation for the trend of increasing non-protein nitrogen during aging observed in the AN birds but not in the N-AN birds. Changes in the non-protein nitrogen fraction of muscle may result from both intrinsic enzymatic proteolysis and bacterial proteolysis. However, one would not expect the anesthetic to have an effect on either intrinsic proteolytic enzymes or microbial flora.

It was noted that the sarcoplasmic protein fraction tended to decrease during aging in the AN birds, therefore, this may possibly account for the increasing trend in the non-protein nitrogen in the AN birds.

Sarcoplasmic Protein Nitrogen

Changes that occurred in extractability of sarcoplasmic protein from breast muscle of the AN and N-AN birds used in this study are similar to changes reported in the literature dealing with other species as well as with turkeys.

Water soluble protein extractability was less at 24 hours than at 30 minutes post-mortem in breast muscle of broiler chickens aged in ice water (Maier and Fischer, 1966). This is similar to the trends observed in this study. However, Khan and van den Berg (1964b) found no change in the sarcoplasmic fraction of chicken breast muscle over 2 days of aging or of the leg muscle over 6 days of aging in crushed, drained ice.

results were shown by bacteria counts. In a control group, the period for bacterial growth was 10 to 12 hours. In the birds, however, there was little or no bacterial growth in the first 24 hours post-mortem.

There seemed to be no important explanation for the trend of increasing non-protein nitrogen during aging in the birds. It was not in the 2-4 birds. Changes in the non-protein nitrogen fraction of muscle may result from both nitrogenous and non-nitrogenous lysate and bacterial products. However, one would not expect an anesthetic to have an effect on either nitrogenous or non-nitrogenous or microbial flora.

It was noted that the sarcolemmal membrane of the muscle decreased during aging in the birds. Therefore, this decrease may account for the increasing trend in the non-protein nitrogen in the birds.

Sarcolemmal Protein Content

Changes that occurred in the sarcolemmal protein content of the breast muscle of the birds during aging in this study are similar to changes in other tissues and organs during aging in other species as well as with turnover.

Water soluble protein in the sarcolemma was less in 2 weeks than in 30 minutes post-mortem in breast muscle of the birds. This was also in ice water (24 hr and 48 hr). This is similar to the results observed in this study. However, there was no significant change in the sarcolemmal fraction of chicken breast muscle over 2 days of aging or in the muscle over 2 days of aging in crushed, drained ice.

Price et al. (1965) found that sarcoplasmic protein extractability changed only slightly in turkey breast and leg muscle over 14 days of storage at 2°C. Maxon and Marion (1969) reported similar results using turkey breast muscle stored for 72 hours at 2°C.

Thompson et al. (1968) held beef muscle at 30°C for 24 hours and then stored it at 3°C. Control samples were stored at 3°C throughout the 10 day aging period. They indicated that extractability of water soluble protein was greatest from muscles held at the elevated temperature for the first 3 days. After the third day, however, extractability was greater for muscles held at 3°C. Aberle and Merkel (1966) found that the sarcoplasmic protein fraction remained constant in beef Longissimus dorsi muscle over a two week aging period at 4°C, but this fraction decreased in the Semitendinosus muscle during the second week of aging. In contrast, Kronman and Winterbottom (1960) and Goll et al. (1964) respectively studied the Longissimus dorsi and Semitendinosus muscle, and both groups found that sarcoplasmic protein extractability was highest immediately after death and that its extractability decreased during post-mortem aging.

The effect of treatment observed in this study appears to be somewhat similar to reported observations of changes in pork. pH decline and overall extractability of the sarcoplasmic protein fraction observed in the AN and N-AN birds are somewhat analogous to the observations in normal and pale, soft and exudative (PSE) pigs respectively.

Sarcoplasmic protein is a heterogenous mixture of individual proteins, many of which are easily denatured under mild acid conditions (Bendall, 1964). Bendall and Wismer-Pedersen (1962)

Price et al. (1963) found that sarcoplasmic protein extractability changed only slightly in turkey breast and leg muscles over 14 days of storage at 2°C. Nakom and Nakom (1963) reported similar results using turkey breast muscle stored for 14 hours at 2°C. Thompson et al. (1966) held beef muscle at 20°C for 12 hours and then stored it at 2°C. Control samples were stored at 2°C throughout the 10 day aging period. They indicated that extractability of water soluble protein was greatest from muscle stored at the elevated temperature for the first 3 days. After the third day, however, extractability was greater for muscle held at 2°C. Adler and Metkei (1965) found that the sarcoplasmic protein fraction remained constant in beef longissimus dorsi muscle over a two week aging period at 4°C, but this fraction decreased in the semitendinosus muscle during the second week of aging. In contrast, Crawford and Winterbottom (1967) and Goll et al. (1969) respectively studied the longissimus dorsi and semitendinosus muscles and both groups found that sarcoplasmic protein extractability was highest immediately after death and that its extractability decreased during post-mortem aging. The effect of treatment appeared in this study appears to be somewhat similar to reported observations of changes in water, pH decline and overall extractability of the sarcoplasmic protein fraction observed in the AA and AA-0 birds are somewhat analogous to the observations in normal and hairy, gold and emperor (1967) pigs respectively.

Sarcoplasmic protein is a heterogeneous mixture of individual proteins, many of which are easily released and of mild acid conditions (Bendall, 1969). Bendall and Schmitt-Behnen (1969)

postulated that sarcoplasmic proteins may be adsorbed on the myofibrillar proteins during post-mortem changes in pork. In affirmation of this Sayre and Briskey (1963) and Briskey and Sayre (1964) found a marked reduction in extractability of sarcoplasmic protein at low ionic strength and myofibrillar protein at high ionic strength in pig skeletal muscle that went into rigor under acidic conditions soon after death. Conditions such as these are characteristic of pork muscle that is PSE. Scopes and Lawrie (1963) and Scopes (1964) through use of starch gel electrophoresis, concluded that the electrophoretically detectable band of creatine kinase was absent in PSE pork muscle. These workers postulated that creatine kinase had probably been denatured and precipitated onto the myofibrils and that this precipitated sarcoplasmic protein was responsible for the decrease in extractability of the myofibrillar proteins. However, Borchert et al. (1969) using starch gel electrophoresis found that diminished sarcoplasmic protein extractability in PSE pork muscle did not manifest itself in preferential denaturation of a specific component of this fraction.

The pH decline was more rapid (approximately 3 hours) and ultimate pH lower (approximately 5.3 - 5.4) in the PSE pigs (Sayre and Briskey, 1963) than was observed in the N-AN birds of this study. They also found that extractability of sarcoplasmic protein of PSE pork muscle was comparable with normal muscle before onset of rigor mortis. However, after onset of rigor, extractability of this fraction from PSE muscle was greatly reduced, whereas there was little change in extractability in normal muscle. A significant

postulated that sarcoplasmic proteins may be released in the muscle
liberated proteins could be released in part, a stimulation
of this type and Briskley (1967) and Briskley and Briskley (1967) found
a marked reduction in the concentration of sarcoplasmic protein in a low
ionic strength and myofibrillar protein a high ionic strength in
pig skeletal muscle that went into rigor after a short time
soon after death. Conditions such as these are characteristic of
pork muscle that is low in pH and low in ionic strength and
through use of starch gel electrophoresis, concluded that the electro-
phoretically detectable band of creatine kinase was absent in the
pork muscle. These workers postulated that creatine kinase may
probably been denatured and, hydrolyzed into the creatine and
that this precipitated sarcoplasmic protein was responsible for the
decrease in extractability of the myofibrillar proteins. However,
Hochberg et al. (1967) using starch gel electrophoresis found that
diminished sarcoplasmic protein extractability in the pork muscle
did not manifest itself in protein denaturation or a marked
component of this reaction.

The pH decline was more rapid (approximately 4 hours) and
ultimate pH lower (approximately 5.5 - 5.7) in the first 12 hours
and Briskley (1967) and was observed in the - 100°C and - 150°C studies.
They also found that extractability of sarcoplasmic protein of pork
pork muscle was comparable with normal muscle before rigor of 12
months. However, a low level of rigor extractability of this
fraction from the muscle was greatly reduced, whereas there was
little change in extractability of sarcoplasmic protein of a 24-hour

decrease in extractability did not occur in either group of birds during onset of rigor, but there were small decreases as pH declined in both groups which is somewhat similar to the findings in pork.

Total Extractable Nitrogen, Total Fibrillar Protein Nitrogen and Actomyosin Nitrogen

As indicated in the discussion above the non-protein nitrogen and sarcoplasmic protein fractions generally do not change much during the first 24 hours post-mortem in the various species except in abnormal cases such as PSE pork muscle. If this is the case, then changes in total extractable nitrogen must be due mainly to changes in extractability of the myofibrillar proteins.

The continuous increase in total extractable nitrogen, total fibrillar protein nitrogen and actomyosin nitrogen to maximum extractabilities at 1, 1/2 and 1 hour respectively in muscle of the N-AN birds appeared to be in general disagreement with previous findings in other species as well as the findings of Price et al. (1965) who were working with turkeys. They found that extractability of myofibrillar protein from light and dark turkey muscle in KCl-phosphate buffer, pH = 7.5, $\sqrt{t}/2 = 0.55$, changed only slightly during onset of rigor and post-rigor aging. However, the actomyosin fraction decreased during rigor and increased during post-rigor aging in light muscle, but in dark muscle this fraction increased up to 24 hours post-mortem but later decreased during post-rigor aging. In contrast, Maxon and Marion (1969) indicated that extractability of the myofibrillar fraction of turkey breast muscle with KCl-phosphate buffer solution increased steadily up to 48 hours and possibly decreased at

decrease in extractability of his not only in skeletal muscle but also during onset of rigor, but there were no differences in the decrease in both groups which is somewhat surprising in the light of the fact.

Total Extractable Nitrogen, Total for the Protein Nitrogen and Actomyosin Nitrogen

As indicated in the discussion above, the actomyosin nitrogen and sarcoplasmic, total extractable nitrogen is not constant during the first 4 hours post-mortem in the various species examined in previous cases and is not constant in the present case. Then changes in total extractable nitrogen could be due mainly to changes in extractability of the extractable proteins.

The conditions involved in total extractable nitrogen, total extractable protein nitrogen and actomyosin nitrogen to maximum extractability at 14 hours post-mortem and the decrease in extractability in other species as well as the decrease in the present case (1937) who were reported with subsequent studies and the extractability of myofibrillar protein was studied in the present study. The phosphate buffer, pH 7.4, was used and only 0.1% of the onset of rigor and post-rigor stages. However, the subsequent decrease during rigor and increased during post-rigor stage, in fact muscle, but in other studies the extractable nitrogen was not constant post-mortem and the decrease during onset of rigor and post-rigor stage. The present study has shown the action of the phosphate buffer and the decrease in extractability of myofibrillar protein and actomyosin protein during onset of rigor and post-rigor stage. The present study has shown the action of the phosphate buffer and the decrease in extractability of myofibrillar protein and actomyosin protein during onset of rigor and post-rigor stage.

72 hours post-mortem. This is in agreement with the changes in extractability of myofibrillar proteins from the breast muscle of the N-AN birds. An explanation for this finding was not given by Maxon and Marion nor was one evident from the results of this study.

The pattern of extractability of total extractable nitrogen, total fibrillar protein nitrogen and actomyosin nitrogen in the AN birds was similar to published results for other species. Extractabilities of these fractions remained constant over the first hour and then began to increase to maximum levels at 12 hours post-mortem for all three fractions.

Results of this investigation suggested that a relationship existed between rate of post-mortem glycolysis and myofibrillar protein extractability. These results indicated that extractability of myofibrillar proteins increased during rapid pH decline as was observed in the first hour post-mortem in the N-AN birds. A somewhat different type of interrelationship appeared to exist between rate of post-mortem glycolysis, onset of rigor mortis and myofibrillar protein extractability in the various species, especially in pork.

In studying properties of fibrillar proteins of normal and watery pork muscle, Bendall and Wismer-Pedersen (1962) determined the amount of protein extracted from fibrils washed free of soluble sarcoplasmic protein. Their results showed that normal fibrils were almost completely extracted, giving a highly viscous solution containing 88 percent of the fibrillar proteins. With "watery" fibrils, however, only 11 percent of the fibrillar proteins were extracted. From additional work they concluded that in watery meat, the main fibrillar protein, actomyosin, was in the native state but had



become covered with a layer of denatured sarcoplasmic protein. They suggested that this layer of denatured protein covering the fibrillar protein made it resistant to extraction at high ionic strength. Extractability of pork muscle under various conditions has also been reported by Sayre and Briskey (1963) and Briskey and Sayre (1964). These workers showed that myofibrillar protein extractability was grossly altered by the temperature and pH existing during onset of rigor or during the first few hours after death. They found no loss in myofibrillar protein extractability under conditions of slow pH decline (6.0+) regardless of the temperature at the onset of rigor. Likewise there was no loss in extractability with a medium pH (5.7 - 5.9) at onset of rigor as long as the temperature was low. However, under conditions of high temperature and medium or low pH (5.3 - 5.9) loss of myofibrillar protein extractability was severe. McIntosh (1967) followed changes in the actomyosin fraction of pork muscle through 14 days of storage at 4°C. This fraction made up 27 percent of the total nitrogen before onset of rigor and decreased to 2 percent of the total nitrogen at 4 days. It then increased to 50 percent of the total nitrogen at 14 days post-mortem.

Davey and Gilbert (1968a) investigated changes in extractability of myofibrillar proteins of beef and rabbit muscle during aging. Approximately 52 percent of the myofibrillar proteins of unaged muscle was extracted at 2°C whereas from aged muscle as much as 78 percent was extracted. They (Davey and Gilbert, 1968b) made another study of myofibrillar proteins extracted from beef and rabbit muscle by a buffer that dissociates the actomyosin complex of the muscle cell. Myosin which constituted 50 - 52 percent of the myofibrillar



protein was wholly extracted throughout aging, whereas actin was extracted in increasing amounts as aging proceeded. In contrast tropomyosin was not extracted and remained firmly held within the myofibrillar structures throughout aging. Effects of temperature and post-mortem storage on myofibrillar protein extractability of beef and rabbit muscle were reported by Chaudhry et al. (1969). The most noticeable result was that extractability of these proteins with 0.5 M potassium chloride, 0.1 M potassium phosphate, pH = 7.4, increased with increasing time of post-mortem storage at temperatures up to 25°C. Increased extractability began to appear about 16 - 24 hours post-mortem for both rabbit and beef muscle at 2°C, about 12 hours post-mortem for beef muscle at 16°C, and about 3 - 6 hours post-mortem for rabbit muscle at 25°C. However, in rabbit muscle at 37°C extractability of these proteins increased at 6 hours post-mortem but decreased to below the initial level of extractability at 24 hours post-mortem.

Weinberg and Rose (1960) found that nitrogen extracted from chicken breast muscle by a KCl-phosphate buffer, pH = 7.5, $\sqrt{I}/2 = 0.55$, increased when the carcasses were held for 24 hours at 4°C. This increase was entirely accounted for by an increase in extractability of the actomyosin fraction. Khan and van den Berg (1964b) found that KCl-buffer extractable nitrogen of chicken leg and breast muscle rapidly decreased after death during onset of rigor and gradually increased to a maximum value during post-rigor aging. Changes in the extractable nitrogen occurred mainly as a result of changes in extractability of myofibrillar proteins. Khan (1968) found that extractability of myosin remained relatively constant from 15 minutes

protein was whole... extracted in the presence of a... myofibrillar structure... and post-mortem... beef and rabbit... most noticeable... with 0.5%... increased with... up to 150C... hours post-mortem... hours post-mortem... at 150C... motion but... 10 hours post-mortem... Weinberg... chicken breast... increased when... increase was... of the... 20-l... rapidly... increased... extractable... extractability... extractability...

to 72 hours post-mortem in chicken leg and breast muscle, however, extractability of actomyosin decreased as the birds went into rigor and then increased again during post-rigor aging. Myofibrillar protein extractability from chicken breast muscle was measured after the muscle had aged in ice for various periods from 30 minutes to 24 hours post-mortem by Sayre (1968b). The extraction solution was KCl-phosphate buffer, pH = 7.0, $I/2 = 1.0$. Residue from the salt extraction was treated with 0.1 N sodium hydroxide to remove additional myofibrillar protein. Myosin extractability decreased rapidly during the first 3 - 4 hours of aging while the alkali soluble protein increased and actomyosin was extracted at a low constant level. Following 4 - 6 hours of aging the alkali soluble protein became constant, and actomyosin appeared in the extract in increasing quantities as myosin continued to decline.

Maximum levels of extractability for total extractable nitrogen, total fibrillar protein nitrogen and actomyosin nitrogen were greater in the AN birds although initial levels were lower than the extractability levels in the N-AN birds. This may be related to the suggestion made by Bendall and Wismer-Pedersen (1962) that sarcoplasmic proteins may be denatured and precipitated on the myofibrillar protein thus decreasing their extractability. This could have occurred in the birds used in this study to a small extent with less decrease in protein extractability occurring in the AN birds because of a slower pH decline.

When values for total fibrillar protein nitrogen were compared with actomyosin nitrogen at corresponding times in both groups of

birds, it was noted that at 48 hours in the AN birds and 48 and 72 hours post-mortem in the N-AN birds values for actomyosin nitrogen were greater than values for total fibrillar protein nitrogen. Theoretically this is impossible, however, variability in protein extractability from muscle from bird to bird and fraction to fraction is a possible explanation for this finding.

Residual Myosin Nitrogen, Residual Actin Nitrogen and Unextracted Soluble Protein Nitrogen

Changes observed in this study dealing with residual myosin, residual actin and unextracted soluble protein closely paralleled each other in both groups of birds. Changes in these fractions also appeared to be inversely proportional to changes in extractability of total fibrillar protein and actomyosin in both groups of birds. The small values for the residual myosin and actin indicated that there was no extensive dissociation of the actomyosin during the first hour post-mortem in either group of birds, although the amount of myofibrillar protein in the residue was greatest during this time. In a preliminary study, Price et al. (1965) also found that there was only a small amount of dissociation of buffered-salt insoluble actomyosin of turkey muscle when it was treated with a pyrophosphate buffer. This lack of dissociation may be an indication of protein denaturation as indicated by Baliga et al. (1962). These researchers found that extractability of myofibrillar protein decreased in fresh water fish after 5 and 15 days of storage. The residue from the buffered salt extraction was extracted with a pyrophosphate containing buffer and the results indicated that actomyosin was dissociated

1. The first part of the report is a general introduction to the subject.

2. The second part is a detailed description of the methods used.

3. The third part is a discussion of the results obtained.

4. The fourth part is a conclusion and a list of references.

5. The fifth part is a list of the names of the authors.

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30. The thirtieth part is a list of the names of the exporters.

31. The thirty-first part is a list of the names of the importers.

at 5 days but was not dissociated at 15 days of storage. This was interpreted to indicate that the first fall in extractability was related to rigor mortis whereas the decrease in extractability at 15 days was a result of protein denaturation. However, it was found in this present study that during the first hour the pH was still fairly high in both groups of birds, and rigor had not developed to a great extent. Thus one would not expect that denaturation of the protein was occurring to a great extent during this period, especially after observing increases in the myofibrillar protein extractability as time passed.

Tenderness Evaluation

Tenderness of the turkeys used in this study was determined by shearing cooked samples with a Kramer shear press. Data collected during tenderness evaluation of the birds are summarized in Table 19. As indicated the AN birds were significantly more tender than the N-AN birds.

Goodwin et al. (1961) studied the influence of humane slaughter on tenderness of turkeys. They found that birds subjected to humane slaughter treatments resulted in an creased shear value for the thigh muscles, with nembutal immobilized birds being significantly less tender than control birds that received no ante-mortem treatment. In contrast to this, deFremery (1965) stated that chickens anesthetized with pentobarbital prior to slaughter were significantly more tender (measured by shear value) than birds that were allowed



to struggle freely during slaughter. Other work by deFremery (1966b) indicated that increases in rate of post-mortem glycolysis tended to cause toughening in chickens. As the pH data indicated the N-AN birds of this present study had a more rapid post-mortem glycolytic rate than the AN birds. This tends to compare favorably with the results of deFremery as well as several other groups that investigated the effects of processing procedures on tenderness and post-mortem chemical changes.

Table 19. Shear values of cooked turkey breast muscle from birds aged for 72 hours with and without ante-mortem injection of pentobarbital^{1/}.

| Treatment | | | |
|--------------|---------------------|------------------|-------|
| Anesthetized | | Non-anesthetized | |
| Bird | Force ^{2/} | Bird | Force |
| 1 | 7.60 | 2 | 13.99 |
| 3 | 6.29 | 4 | 11.52 |
| 5 | 5.35 | 6 | 14.86 |
| 7 | 5.98 | 8 | 20.68 |
| 9 | 8.28 | 10 | 16.41 |
| 11 | 5.89 | 12 | 18.45 |
| 13 | 7.43 | 14 | 19.51 |
| 15 | 7.45 | 16 | 9.11 |
| 17 | 7.43 | 18 | 16.14 |
| 19 | 7.54 | 20 | 11.69 |
| Ave. | 6.93 | Ave. | 15.34 |

^{1/} The variance in shear values due to treatment was significant at the 1 percent level of probability.

^{2/} The force required to shear the samples is expressed in kg of force/g cooked muscle and the values given are averages of three or four samples.

the first of the two main groups of the
the second of the two main groups of the
the third of the two main groups of the
the fourth of the two main groups of the
the fifth of the two main groups of the
the sixth of the two main groups of the
the seventh of the two main groups of the
the eighth of the two main groups of the
the ninth of the two main groups of the
the tenth of the two main groups of the

the eleventh of the two main groups of the

the twelfth of the two main groups of the
the thirteenth of the two main groups of the

the fourteenth of the two main groups of the

the fifteenth of the two main groups of the
the sixteenth of the two main groups of the
the seventeenth of the two main groups of the
the eighteenth of the two main groups of the
the nineteenth of the two main groups of the
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the twenty-sixth of the two main groups of the
the twenty-seventh of the two main groups of the
the twenty-eighth of the two main groups of the
the twenty-ninth of the two main groups of the
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the thirty-sixth of the two main groups of the
the thirty-seventh of the two main groups of the
the thirty-eighth of the two main groups of the
the thirty-ninth of the two main groups of the
the fortieth of the two main groups of the

the forty-first of the two main groups of the
the forty-second of the two main groups of the
the forty-third of the two main groups of the
the forty-fourth of the two main groups of the
the forty-fifth of the two main groups of the
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the fifty-sixth of the two main groups of the
the fifty-seventh of the two main groups of the
the fifty-eighth of the two main groups of the
the fifty-ninth of the two main groups of the
the sixtieth of the two main groups of the

**Correlation of Tenderness with Post-Mortem Changes
in pH and Protein Extractability**

Snedecor and Cochran (1967) stated that correlation coefficients are measurements of mutual relationship between two variables, or degree of closeness of the linear relationships between these variables. Correlations of bird tenderness with post-mortem changes in pH and protein extractability are summarized in Table 20.

A positive correlation occurred between tenderness and pH decline in the AN birds from zero time to 6 hours post-mortem with r values at 1/4 - 3 hours being significant at the 5 percent level of probability. However, from 12 - 72 hours post-mortem there was a negative correlation between tenderness and pH decline with r values at 48 and 72 hours also being significant at the 5 percent level of probability. No significant correlation between tenderness and pH decline was observed in the N-AN birds.

Significant correlations between tenderness and changes in protein extractability were observed in only two isolated instances. One was the 24 hour post-mortem actomyosin fraction in the N-AN birds and the other was the 24 hour post-mortem residual actin fraction in the AN birds. These r values were significant at the 5 and 0.1 percent levels of probability respectively.

The relationship of change in pH or post-mortem glycolytic rate to tenderness has been studied extensively by deFremery (1966b). He found that accelerating of post-mortem glycolysis resulted in increases in toughness of chicken muscle. In contrast Price and



Table 20. Correlation coefficients of shear values with pH and protein extractability of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injection of pentobarbital.

| Time
(hr) | pH | | TEN ^{1/} | |
|--------------|---------------------|-----------|-------------------|-----------|
| | Anes. | Non-Anes. | Anes. | Non-Anes. |
| 0 | .406 | -.050 | -.181 | .287 |
| 1/4 | .680 ^{2/} | -.266 | -.224 | -.051 |
| 1/2 | .704 ^{2/} | .515 | -.116 | .097 |
| 1 | .630 ^{2/} | -.130 | -.208 | .092 |
| 3 | .673 ^{2/} | .150 | -.193 | .264 |
| 6 | .534 | -.269 | -.492 | -.016 |
| 12 | -.395 | .169 | -.247 | .078 |
| 24 | -.562 | .214 | -.274 | -.024 |
| 48 | -.700 ^{2/} | .221 | -.256 | .099 |
| 72 | -.733 ^{2/} | .303 | -.090 | .358 |

| Time
(hr) | NPN | | SPN | |
|--------------|-------|-----------|-------|-----------|
| | Anes. | Non-Anes. | Anes. | Non-Anes. |
| 0 | .437 | -.022 | -.570 | .430 |
| 1/4 | -.335 | .219 | -.255 | .443 |
| 1/2 | -.236 | -.208 | -.481 | -.160 |
| 1 | -.031 | .245 | -.306 | .087 |
| 3 | -.617 | -.252 | -.031 | .037 |
| 6 | .000 | .462 | -.428 | .318 |
| 12 | -.395 | .326 | -.393 | -.238 |
| 24 | .194 | -.227 | -.491 | .046 |
| 48 | .179 | -.014 | -.061 | -.029 |
| 72 | -.302 | -.386 | -.167 | .157 |

| Time
(hr) | TFPN | | AN | |
|--------------|-------|-----------|-------|--------------------|
| | Anes. | Non-Anes. | Anes. | Non-Anes. |
| 0 | .591 | -.108 | .236 | .232 |
| 1/4 | .167 | -.243 | -.455 | -.054 |
| 1/2 | .368 | .171 | .082 | .181 |
| 1 | .183 | -.169 | -.281 | .040 |
| 3 | -.074 | .227 | -.319 | .308 |
| 6 | -.282 | -.338 | -.502 | .086 |
| 12 | .399 | .371 | -.012 | .351 |
| 24 | .277 | -.066 | .385 | .656 ^{2/} |
| 48 | -.244 | .192 | .499 | -.223 |
| 72 | .133 | .278 | .121 | .034 |

Table 10. Correlation of the rate of change of the concentration of the protein in the plasma and the rate of change of the concentration of the protein in the urine.

| Time (hr) | Protein in plasma (g/l) | Protein in urine (g/l) | Time (hr) | Protein in plasma (g/l) | Protein in urine (g/l) |
|-----------|-------------------------|------------------------|-----------|-------------------------|------------------------|
| 0 | 1.0 | 1.0 | 0 | 1.0 | 1.0 |
| 1 | 1.1 | 1.1 | 1 | 1.1 | 1.1 |
| 2 | 1.2 | 1.2 | 2 | 1.2 | 1.2 |
| 3 | 1.3 | 1.3 | 3 | 1.3 | 1.3 |
| 4 | 1.4 | 1.4 | 4 | 1.4 | 1.4 |
| 5 | 1.5 | 1.5 | 5 | 1.5 | 1.5 |
| 6 | 1.6 | 1.6 | 6 | 1.6 | 1.6 |
| 7 | 1.7 | 1.7 | 7 | 1.7 | 1.7 |
| 8 | 1.8 | 1.8 | 8 | 1.8 | 1.8 |
| 9 | 1.9 | 1.9 | 9 | 1.9 | 1.9 |
| 10 | 2.0 | 2.0 | 10 | 2.0 | 2.0 |
| 11 | 2.1 | 2.1 | 11 | 2.1 | 2.1 |
| 12 | 2.2 | 2.2 | 12 | 2.2 | 2.2 |
| 13 | 2.3 | 2.3 | 13 | 2.3 | 2.3 |
| 14 | 2.4 | 2.4 | 14 | 2.4 | 2.4 |
| 15 | 2.5 | 2.5 | 15 | 2.5 | 2.5 |
| 16 | 2.6 | 2.6 | 16 | 2.6 | 2.6 |
| 17 | 2.7 | 2.7 | 17 | 2.7 | 2.7 |
| 18 | 2.8 | 2.8 | 18 | 2.8 | 2.8 |
| 19 | 2.9 | 2.9 | 19 | 2.9 | 2.9 |
| 20 | 3.0 | 3.0 | 20 | 3.0 | 3.0 |
| 21 | 3.1 | 3.1 | 21 | 3.1 | 3.1 |
| 22 | 3.2 | 3.2 | 22 | 3.2 | 3.2 |
| 23 | 3.3 | 3.3 | 23 | 3.3 | 3.3 |
| 24 | 3.4 | 3.4 | 24 | 3.4 | 3.4 |
| 25 | 3.5 | 3.5 | 25 | 3.5 | 3.5 |
| 26 | 3.6 | 3.6 | 26 | 3.6 | 3.6 |
| 27 | 3.7 | 3.7 | 27 | 3.7 | 3.7 |
| 28 | 3.8 | 3.8 | 28 | 3.8 | 3.8 |
| 29 | 3.9 | 3.9 | 29 | 3.9 | 3.9 |
| 30 | 4.0 | 4.0 | 30 | 4.0 | 4.0 |
| 31 | 4.1 | 4.1 | 31 | 4.1 | 4.1 |
| 32 | 4.2 | 4.2 | 32 | 4.2 | 4.2 |
| 33 | 4.3 | 4.3 | 33 | 4.3 | 4.3 |
| 34 | 4.4 | 4.4 | 34 | 4.4 | 4.4 |
| 35 | 4.5 | 4.5 | 35 | 4.5 | 4.5 |
| 36 | 4.6 | 4.6 | 36 | 4.6 | 4.6 |
| 37 | 4.7 | 4.7 | 37 | 4.7 | 4.7 |
| 38 | 4.8 | 4.8 | 38 | 4.8 | 4.8 |
| 39 | 4.9 | 4.9 | 39 | 4.9 | 4.9 |
| 40 | 5.0 | 5.0 | 40 | 5.0 | 5.0 |
| 41 | 5.1 | 5.1 | 41 | 5.1 | 5.1 |
| 42 | 5.2 | 5.2 | 42 | 5.2 | 5.2 |
| 43 | 5.3 | 5.3 | 43 | 5.3 | 5.3 |
| 44 | 5.4 | 5.4 | 44 | 5.4 | 5.4 |
| 45 | 5.5 | 5.5 | 45 | 5.5 | 5.5 |
| 46 | 5.6 | 5.6 | 46 | 5.6 | 5.6 |
| 47 | 5.7 | 5.7 | 47 | 5.7 | 5.7 |
| 48 | 5.8 | 5.8 | 48 | 5.8 | 5.8 |
| 49 | 5.9 | 5.9 | 49 | 5.9 | 5.9 |
| 50 | 6.0 | 6.0 | 50 | 6.0 | 6.0 |
| 51 | 6.1 | 6.1 | 51 | 6.1 | 6.1 |
| 52 | 6.2 | 6.2 | 52 | 6.2 | 6.2 |
| 53 | 6.3 | 6.3 | 53 | 6.3 | 6.3 |
| 54 | 6.4 | 6.4 | 54 | 6.4 | 6.4 |
| 55 | 6.5 | 6.5 | 55 | 6.5 | 6.5 |
| 56 | 6.6 | 6.6 | 56 | 6.6 | 6.6 |
| 57 | 6.7 | 6.7 | 57 | 6.7 | 6.7 |
| 58 | 6.8 | 6.8 | 58 | 6.8 | 6.8 |
| 59 | 6.9 | 6.9 | 59 | 6.9 | 6.9 |
| 60 | 7.0 | 7.0 | 60 | 7.0 | 7.0 |
| 61 | 7.1 | 7.1 | 61 | 7.1 | 7.1 |
| 62 | 7.2 | 7.2 | 62 | 7.2 | 7.2 |
| 63 | 7.3 | 7.3 | 63 | 7.3 | 7.3 |
| 64 | 7.4 | 7.4 | 64 | 7.4 | 7.4 |
| 65 | 7.5 | 7.5 | 65 | 7.5 | 7.5 |
| 66 | 7.6 | 7.6 | 66 | 7.6 | 7.6 |
| 67 | 7.7 | 7.7 | 67 | 7.7 | 7.7 |
| 68 | 7.8 | 7.8 | 68 | 7.8 | 7.8 |
| 69 | 7.9 | 7.9 | 69 | 7.9 | 7.9 |
| 70 | 8.0 | 8.0 | 70 | 8.0 | 8.0 |
| 71 | 8.1 | 8.1 | 71 | 8.1 | 8.1 |
| 72 | 8.2 | 8.2 | 72 | 8.2 | 8.2 |
| 73 | 8.3 | 8.3 | 73 | 8.3 | 8.3 |
| 74 | 8.4 | 8.4 | 74 | 8.4 | 8.4 |
| 75 | 8.5 | 8.5 | 75 | 8.5 | 8.5 |
| 76 | 8.6 | 8.6 | 76 | 8.6 | 8.6 |
| 77 | 8.7 | 8.7 | 77 | 8.7 | 8.7 |
| 78 | 8.8 | 8.8 | 78 | 8.8 | 8.8 |
| 79 | 8.9 | 8.9 | 79 | 8.9 | 8.9 |
| 80 | 9.0 | 9.0 | 80 | 9.0 | 9.0 |
| 81 | 9.1 | 9.1 | 81 | 9.1 | 9.1 |
| 82 | 9.2 | 9.2 | 82 | 9.2 | 9.2 |
| 83 | 9.3 | 9.3 | 83 | 9.3 | 9.3 |
| 84 | 9.4 | 9.4 | 84 | 9.4 | 9.4 |
| 85 | 9.5 | 9.5 | 85 | 9.5 | 9.5 |
| 86 | 9.6 | 9.6 | 86 | 9.6 | 9.6 |
| 87 | 9.7 | 9.7 | 87 | 9.7 | 9.7 |
| 88 | 9.8 | 9.8 | 88 | 9.8 | 9.8 |
| 89 | 9.9 | 9.9 | 89 | 9.9 | 9.9 |
| 90 | 10.0 | 10.0 | 90 | 10.0 | 10.0 |
| 91 | 10.1 | 10.1 | 91 | 10.1 | 10.1 |
| 92 | 10.2 | 10.2 | 92 | 10.2 | 10.2 |
| 93 | 10.3 | 10.3 | 93 | 10.3 | 10.3 |
| 94 | 10.4 | 10.4 | 94 | 10.4 | 10.4 |
| 95 | 10.5 | 10.5 | 95 | 10.5 | 10.5 |
| 96 | 10.6 | 10.6 | 96 | 10.6 | 10.6 |
| 97 | 10.7 | 10.7 | 97 | 10.7 | 10.7 |
| 98 | 10.8 | 10.8 | 98 | 10.8 | 10.8 |
| 99 | 10.9 | 10.9 | 99 | 10.9 | 10.9 |
| 100 | 11.0 | 11.0 | 100 | 11.0 | 11.0 |

Table 20. Contd.

| Time
(hr) | RMN | | RAN | |
|--------------|-------|-----------|---------------------|-----------|
| | Anes. | Non-Anes. | Anes. | Non-Anes. |
| 0 | .424 | -.436 | .189 | -.227 |
| 1/4 | -.020 | -.012 | -.051 | .036 |
| 1/2 | .163 | -.119 | -.197 | .044 |
| 1 | .371 | -.153 | .268 | -.230 |
| 3 | .362 | .505 | .073 | .376 |
| 6 | .312 | .268 | .503 | .513 |
| 12 | -.263 | .041 | -.139 | .195 |
| 24 | -.483 | .381 | -.893 ^{3/} | .459 |
| 48 | .458 | .180 | .426 | .312 |
| 72 | -.250 | -.287 | -.300 | -.214 |

| Time
(hr) | USPN | |
|--------------|-------|-----------|
| | Anes. | Non-Anes. |
| 0 | -.028 | -.168 |
| 1/4 | .275 | .146 |
| 1/2 | .136 | -.122 |
| 1 | .449 | -.165 |
| 3 | .255 | .429 |
| 6 | .488 | .593 |
| 12 | .055 | .484 |
| 24 | .266 | .415 |
| 48 | .405 | .244 |
| 72 | -.302 | .382 |

1/ The various protein fractions are abbreviated in the following manner: total extractable nitrogen, TEN; non-protein nitrogen, NPN; sarcoplasmic protein nitrogen, SPN; total fibrillar protein nitrogen, TFPN; actomyosin nitrogen, AN; residual myosin nitrogen, RMN, residual actin nitrogen, RAN and unextracted soluble protein nitrogen, USPN.

2/ Significant at the 5 percent level of probability.

3/ Significant at the 0.1 percent level of probability.

Table 10. Contd.

| Time
(hr) | Area | Time
(hr) | Area |
|--------------|------|--------------|------|
| 0 | 1.0 | 12 | 1.0 |
| 1/4 | 1.0 | 13 | 1.0 |
| 1/2 | 1.0 | 14 | 1.0 |
| 1 | 1.0 | 15 | 1.0 |
| 2 | 1.0 | 16 | 1.0 |
| 3 | 1.0 | 17 | 1.0 |
| 4 | 1.0 | 18 | 1.0 |
| 5 | 1.0 | 19 | 1.0 |
| 6 | 1.0 | 20 | 1.0 |
| 7 | 1.0 | 21 | 1.0 |
| 8 | 1.0 | 22 | 1.0 |
| 9 | 1.0 | 23 | 1.0 |
| 10 | 1.0 | 24 | 1.0 |
| 11 | 1.0 | 25 | 1.0 |
| 12 | 1.0 | 26 | 1.0 |
| 13 | 1.0 | 27 | 1.0 |
| 14 | 1.0 | 28 | 1.0 |
| 15 | 1.0 | 29 | 1.0 |
| 16 | 1.0 | 30 | 1.0 |
| 17 | 1.0 | 31 | 1.0 |
| 18 | 1.0 | 32 | 1.0 |
| 19 | 1.0 | 33 | 1.0 |
| 20 | 1.0 | 34 | 1.0 |
| 21 | 1.0 | 35 | 1.0 |
| 22 | 1.0 | 36 | 1.0 |
| 23 | 1.0 | 37 | 1.0 |
| 24 | 1.0 | 38 | 1.0 |
| 25 | 1.0 | 39 | 1.0 |
| 26 | 1.0 | 40 | 1.0 |
| 27 | 1.0 | 41 | 1.0 |
| 28 | 1.0 | 42 | 1.0 |
| 29 | 1.0 | 43 | 1.0 |
| 30 | 1.0 | 44 | 1.0 |
| 31 | 1.0 | 45 | 1.0 |
| 32 | 1.0 | 46 | 1.0 |
| 33 | 1.0 | 47 | 1.0 |
| 34 | 1.0 | 48 | 1.0 |
| 35 | 1.0 | 49 | 1.0 |
| 36 | 1.0 | 50 | 1.0 |
| 37 | 1.0 | 51 | 1.0 |
| 38 | 1.0 | 52 | 1.0 |
| 39 | 1.0 | 53 | 1.0 |
| 40 | 1.0 | 54 | 1.0 |
| 41 | 1.0 | 55 | 1.0 |
| 42 | 1.0 | 56 | 1.0 |
| 43 | 1.0 | 57 | 1.0 |
| 44 | 1.0 | 58 | 1.0 |
| 45 | 1.0 | 59 | 1.0 |
| 46 | 1.0 | 60 | 1.0 |
| 47 | 1.0 | 61 | 1.0 |
| 48 | 1.0 | 62 | 1.0 |
| 49 | 1.0 | 63 | 1.0 |
| 50 | 1.0 | 64 | 1.0 |
| 51 | 1.0 | 65 | 1.0 |
| 52 | 1.0 | 66 | 1.0 |
| 53 | 1.0 | 67 | 1.0 |
| 54 | 1.0 | 68 | 1.0 |
| 55 | 1.0 | 69 | 1.0 |
| 56 | 1.0 | 70 | 1.0 |
| 57 | 1.0 | 71 | 1.0 |
| 58 | 1.0 | 72 | 1.0 |
| 59 | 1.0 | 73 | 1.0 |
| 60 | 1.0 | 74 | 1.0 |
| 61 | 1.0 | 75 | 1.0 |
| 62 | 1.0 | 76 | 1.0 |
| 63 | 1.0 | 77 | 1.0 |
| 64 | 1.0 | 78 | 1.0 |
| 65 | 1.0 | 79 | 1.0 |
| 66 | 1.0 | 80 | 1.0 |
| 67 | 1.0 | 81 | 1.0 |
| 68 | 1.0 | 82 | 1.0 |
| 69 | 1.0 | 83 | 1.0 |
| 70 | 1.0 | 84 | 1.0 |
| 71 | 1.0 | 85 | 1.0 |
| 72 | 1.0 | 86 | 1.0 |
| 73 | 1.0 | 87 | 1.0 |
| 74 | 1.0 | 88 | 1.0 |
| 75 | 1.0 | 89 | 1.0 |
| 76 | 1.0 | 90 | 1.0 |
| 77 | 1.0 | 91 | 1.0 |
| 78 | 1.0 | 92 | 1.0 |
| 79 | 1.0 | 93 | 1.0 |
| 80 | 1.0 | 94 | 1.0 |
| 81 | 1.0 | 95 | 1.0 |
| 82 | 1.0 | 96 | 1.0 |
| 83 | 1.0 | 97 | 1.0 |
| 84 | 1.0 | 98 | 1.0 |
| 85 | 1.0 | 99 | 1.0 |
| 86 | 1.0 | 100 | 1.0 |
| 87 | 1.0 | 101 | 1.0 |
| 88 | 1.0 | 102 | 1.0 |
| 89 | 1.0 | 103 | 1.0 |
| 90 | 1.0 | 104 | 1.0 |
| 91 | 1.0 | 105 | 1.0 |
| 92 | 1.0 | 106 | 1.0 |
| 93 | 1.0 | 107 | 1.0 |
| 94 | 1.0 | 108 | 1.0 |
| 95 | 1.0 | 109 | 1.0 |
| 96 | 1.0 | 110 | 1.0 |
| 97 | 1.0 | 111 | 1.0 |
| 98 | 1.0 | 112 | 1.0 |
| 99 | 1.0 | 113 | 1.0 |
| 100 | 1.0 | 114 | 1.0 |
| 101 | 1.0 | 115 | 1.0 |
| 102 | 1.0 | 116 | 1.0 |
| 103 | 1.0 | 117 | 1.0 |
| 104 | 1.0 | 118 | 1.0 |
| 105 | 1.0 | 119 | 1.0 |
| 106 | 1.0 | 120 | 1.0 |
| 107 | 1.0 | 121 | 1.0 |
| 108 | 1.0 | 122 | 1.0 |
| 109 | 1.0 | 123 | 1.0 |
| 110 | 1.0 | 124 | 1.0 |
| 111 | 1.0 | 125 | 1.0 |
| 112 | 1.0 | 126 | 1.0 |
| 113 | 1.0 | 127 | 1.0 |
| 114 | 1.0 | 128 | 1.0 |
| 115 | 1.0 | 129 | 1.0 |
| 116 | 1.0 | 130 | 1.0 |
| 117 | 1.0 | 131 | 1.0 |
| 118 | 1.0 | 132 | 1.0 |
| 119 | 1.0 | 133 | 1.0 |
| 120 | 1.0 | 134 | 1.0 |
| 121 | 1.0 | 135 | 1.0 |
| 122 | 1.0 | 136 | 1.0 |
| 123 | 1.0 | 137 | 1.0 |
| 124 | 1.0 | 138 | 1.0 |
| 125 | 1.0 | 139 | 1.0 |
| 126 | 1.0 | 140 | 1.0 |
| 127 | 1.0 | 141 | 1.0 |
| 128 | 1.0 | 142 | 1.0 |
| 129 | 1.0 | 143 | 1.0 |
| 130 | 1.0 | 144 | 1.0 |
| 131 | 1.0 | 145 | 1.0 |
| 132 | 1.0 | 146 | 1.0 |
| 133 | 1.0 | 147 | 1.0 |
| 134 | 1.0 | 148 | 1.0 |
| 135 | 1.0 | 149 | 1.0 |
| 136 | 1.0 | 150 | 1.0 |
| 137 | 1.0 | 151 | 1.0 |
| 138 | 1.0 | 152 | 1.0 |
| 139 | 1.0 | 153 | 1.0 |
| 140 | 1.0 | 154 | 1.0 |
| 141 | 1.0 | 155 | 1.0 |
| 142 | 1.0 | 156 | 1.0 |
| 143 | 1.0 | 157 | 1.0 |
| 144 | 1.0 | 158 | 1.0 |
| 145 | 1.0 | 159 | 1.0 |
| 146 | 1.0 | 160 | 1.0 |
| 147 | 1.0 | 161 | 1.0 |
| 148 | 1.0 | 162 | 1.0 |
| 149 | 1.0 | 163 | 1.0 |
| 150 | 1.0 | 164 | 1.0 |
| 151 | 1.0 | 165 | 1.0 |
| 152 | 1.0 | 166 | 1.0 |
| 153 | 1.0 | 167 | 1.0 |
| 154 | 1.0 | 168 | 1.0 |
| 155 | 1.0 | 169 | 1.0 |
| 156 | 1.0 | 170 | 1.0 |
| 157 | 1.0 | 171 | 1.0 |
| 158 | 1.0 | 172 | 1.0 |
| 159 | 1.0 | 173 | 1.0 |
| 160 | 1.0 | 174 | 1.0 |
| 161 | 1.0 | 175 | 1.0 |
| 162 | 1.0 | 176 | 1.0 |
| 163 | 1.0 | 177 | 1.0 |
| 164 | 1.0 | 178 | 1.0 |
| 165 | 1.0 | 179 | 1.0 |
| 166 | 1.0 | 180 | 1.0 |
| 167 | 1.0 | 181 | 1.0 |
| 168 | 1.0 | 182 | 1.0 |
| 169 | 1.0 | 183 | 1.0 |
| 170 | 1.0 | 184 | 1.0 |
| 171 | 1.0 | 185 | 1.0 |
| 172 | 1.0 | 186 | 1.0 |
| 173 | 1.0 | 187 | 1.0 |
| 174 | 1.0 | 188 | 1.0 |
| 175 | 1.0 | 189 | 1.0 |
| 176 | 1.0 | 190 | 1.0 |
| 177 | 1.0 | 191 | 1.0 |
| 178 | 1.0 | 192 | 1.0 |
| 179 | 1.0 | 193 | 1.0 |
| 180 | 1.0 | 194 | 1.0 |
| 181 | 1.0 | 195 | 1.0 |
| 182 | 1.0 | 196 | 1.0 |
| 183 | 1.0 | 197 | 1.0 |
| 184 | 1.0 | 198 | 1.0 |
| 185 | 1.0 | 199 | 1.0 |
| 186 | 1.0 | 200 | 1.0 |
| 187 | 1.0 | 201 | 1.0 |
| 188 | 1.0 | 202 | 1.0 |
| 189 | 1.0 | 203 | 1.0 |
| 190 | 1.0 | 204 | 1.0 |
| 191 | 1.0 | 205 | 1.0 |
| 192 | 1.0 | 206 | 1.0 |
| 193 | 1.0 | 207 | 1.0 |
| 194 | 1.0 | 208 | 1.0 |
| 195 | 1.0 | 209 | 1.0 |
| 196 | 1.0 | 210 | 1.0 |
| 197 | 1.0 | 211 | 1.0 |
| 198 | 1.0 | 212 | 1.0 |
| 199 | 1.0 | 213 | 1.0 |
| 200 | 1.0 | 214 | 1.0 |
| 201 | 1.0 | 215 | 1.0 |
| 202 | 1.0 | 216 | 1.0 |
| 203 | 1.0 | 217 | 1.0 |
| 204 | 1.0 | 218 | 1.0 |
| 205 | 1.0 | 219 | 1.0 |
| 206 | 1.0 | 220 | 1.0 |
| 207 | 1.0 | 221 | 1.0 |
| 208 | 1.0 | 222 | 1.0 |
| 209 | 1.0 | 223 | 1.0 |
| 210 | 1.0 | 224 | 1.0 |
| 211 | 1.0 | 225 | 1.0 |
| 212 | 1.0 | 226 | 1.0 |
| 213 | 1.0 | 227 | 1.0 |
| 214 | 1.0 | 228 | 1.0 |
| 215 | 1.0 | 229 | 1.0 |
| 216 | 1.0 | 230 | 1.0 |
| 217 | 1.0 | 231 | 1.0 |
| 218 | 1.0 | 232 | 1.0 |
| 219 | 1.0 | 233 | 1.0 |
| 220 | 1.0 | 234 | 1.0 |
| 221 | 1.0 | 235 | 1.0 |
| 222 | 1.0 | 236 | 1.0 |
| 223 | 1.0 | 237 | 1.0 |
| 224 | 1.0 | 238 | 1.0 |
| 225 | 1.0 | 239 | 1.0 |
| 226 | 1.0 | 240 | 1.0 |
| 227 | 1.0 | 241 | 1.0 |
| 228 | 1.0 | 242 | 1.0 |
| 229 | 1.0 | 243 | 1.0 |
| 230 | 1.0 | 244 | 1.0 |
| 231 | 1.0 | 245 | 1.0 |
| 232 | 1.0 | 246 | 1.0 |
| 233 | 1.0 | 247 | 1.0 |
| 234 | 1.0 | 248 | 1.0 |
| 235 | 1.0 | 249 | 1.0 |
| 236 | 1.0 | 250 | 1.0 |
| 237 | 1.0 | 251 | 1.0 |
| 238 | 1.0 | 252 | 1.0 |
| 239 | 1.0 | 253 | 1.0 |
| 240 | 1.0 | 254 | 1.0 |
| 241 | 1.0 | 255 | 1.0 |
| 242 | 1.0 | 256 | 1.0 |
| 243 | 1.0 | 257 | 1.0 |
| 244 | 1.0 | 258 | 1.0 |
| 245 | 1.0 | 259 | 1.0 |
| 246 | 1.0 | 260 | 1.0 |
| 247 | 1.0 | 261 | 1.0 |
| 248 | 1.0 | 262 | 1.0 |
| 249 | 1.0 | 263 | 1.0 |
| 250 | 1.0 | 264 | 1.0 |
| 251 | 1.0 | 265 | 1.0 |
| 252 | 1.0 | 266 | 1.0 |
| 253 | 1.0 | 267 | 1.0 |
| 254 | 1.0 | 268 | 1.0 |
| 255 | 1.0 | 269 | 1.0 |
| 256 | 1.0 | 270 | 1.0 |
| 257 | 1.0 | 271 | 1.0 |
| 258 | 1.0 | 272 | 1.0 |
| 259 | 1.0 | 273 | 1.0 |
| 260 | 1.0 | 274 | 1.0 |
| 261 | 1.0 | 275 | 1.0 |
| 262 | 1.0 | 276 | 1.0 |
| 263 | 1.0 | 277 | 1.0 |
| 264 | 1.0 | 278 | 1.0 |
| 265 | 1.0 | 279 | 1.0 |
| 266 | 1.0 | 280 | 1.0 |
| 267 | 1.0 | 281 | 1.0 |
| 268 | 1.0 | 282 | 1.0 |
| 269 | 1.0 | 283 | 1.0 |
| 270 | 1.0 | 284 | 1.0 |
| 271 | 1.0 | 285 | 1.0 |
| 272 | 1.0 | 286 | 1.0 |
| 273 | 1.0 | 287 | 1.0 |
| 274 | 1.0 | 288 | 1.0 |
| 275 | 1.0 | 289 | 1.0 |
| 276 | 1.0 | 290 | 1.0 |
| 277 | 1.0 | 291 | 1.0 |
| 278 | 1.0 | 292 | 1.0 |
| 279 | 1.0 | 293 | 1.0 |
| 280 | 1.0 | 294 | 1.0 |
| 281 | 1.0 | 295 | 1.0 |
| 282 | 1.0 | 296 | 1.0 |
| 283 | 1.0 | 297 | 1.0 |
| 284 | 1.0 | 298 | 1.0 |
| 285 | 1.0 | 299 | 1.0 |
| 286 | 1.0 | 300 | 1.0 |
| 287 | 1.0 | 301 | 1.0 |
| 288 | 1.0 | 302 | 1.0 |
| 289 | 1.0 | 303 | 1.0 |
| 290 | 1.0 | 304 | 1.0 |
| 291 | 1.0 | 305 | 1.0 |
| 292 | 1.0 | 306 | 1.0 |
| 293 | 1.0 | 307 | 1.0 |
| 294 | 1.0 | 308 | 1.0 |
| 295 | 1.0 | 309 | 1.0 |
| 296 | 1.0 | 310 | 1.0 |
| 297 | 1.0 | 311 | 1.0 |
| 298 | 1.0 | 312 | 1.0 |
| 299 | 1.0 | 313 | 1.0 |
| 300 | 1.0 | 314 | 1.0 |
| 301 | 1.0 | 315 | 1.0 |
| 302 | 1.0 | 316 | 1.0 |
| 303 | 1.0 | 317 | 1.0 |
| 304 | 1.0 | 318 | 1.0 |
| 305 | 1.0 | 319 | 1.0 |
| 306 | 1.0 | 320 | 1.0 |
| 307 | 1.0 | 321 | 1.0 |
| 308 | 1.0 | 322 | 1.0 |
| 309 | 1.0 | 323 | 1.0 |
| 310 | 1.0 | 324 | 1.0 |
| 311 | 1.0 | 325 | 1.0 |
| 312 | 1.0 | 326 | 1.0 |
| 313 | 1.0 | 327 | 1.0 |
| 314 | 1.0 | 328 | 1.0 |
| 315 | 1.0 | 329 | 1.0 |
| 316 | 1.0 | 330 | 1.0 |
| 317 | 1.0 | 331 | 1.0 |
| 318 | 1.0 | 332 | 1.0 |
| 319 | 1.0 | 333 | 1.0 |
| 320 | 1.0 | 334 | 1.0 |
| 321 | 1.0 | 335 | 1.0 |
| 322 | 1.0 | 336 | 1.0 |
| 323 | 1.0 | 337 | 1.0 |
| 324 | 1.0 | 338 | 1.0 |
| 325 | 1.0 | 339 | 1.0 |
| 326 | 1. | | |

Dawson (1967) made a study of post-mortem biochemical changes in turkey muscle and their relationship to tenderness. They found that rate of glycolysis in all birds studied could be classed as rapid in relation to the data presented by deFremery (1966b). However, tenderness of breast muscle from all of the birds after 72 hours aging was quite acceptable. They concluded that rapid post-mortem glycolysis per se may not be the direct cause of toughness, but perhaps there is a critical period or muscle condition after slaughter where rapid glycolysis is certainly associated with toughness in aged turkey muscle.

The positive correlation between tenderness and pH decline in the AN birds during the first 6 hours post-mortem appeared to contradict the findings of deFremery (1966b) because this indicated that acceleration of post-mortem glycolysis resulted in tenderness development. The negative correlation observed after 6 hours post-mortem was in agreement with deFremery's findings, but by this time the statistical minimum pH had been reached. However, the N-AN birds as discussed earlier had a significantly faster rate of pH decline during the first 3 hours post-mortem and were also significantly less tender than the AN birds after aging for 72 hours. Thus the conclusion of Price and Dawson (1967) that rapid post-mortem glycolysis per se may not be the direct cause of toughness, but perhaps there is a critical period or muscle condition after slaughter where rapid glycolysis is certainly associated with toughness in aged turkey muscle has been affirmed by this present study.

Hegarty et al. (1963) studied the relationship of some intracellular protein characteristics of beef to muscle tenderness. They

found that the fibrillar protein extractability was highly correlated with tenderness. A similar study on beef was conducted later by Goll et al. (1964), but they concluded that protein extractability did not appear to be related to tenderness.

Results of this present study also indicated that tenderness did not appear to be related to the extractability of the various protein fractions from turkey muscle. Significant positive correlation between tenderness and actomyosin extractability at 24 hours post-mortem in the N-AN birds was probably due to chance because of its low level of significance (probability = 0.05) and the fact that there appeared to be no related trend when other r values for this fraction were considered. Significant negative correlation between tenderness and residual actin at 24 hours post-mortem in the AN birds was also isolated and was not part of an increasing or decreasing trend when compared with other r values for this fraction. However, the level of significance was high (probability = 0.001) indicating that something was occurring in this fraction that is related to tenderness development, although an explanation of this possible phenomenon was not apparent.

found that the fibrillation
laced with tenderness.
by Goll et al.
did not appear
technique of the
did not appear
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lation between
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fraction were
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SUMMARY

Different rates of post-mortem glycolysis in turkeys were achieved by anesthetizing one group of birds before slaughter to prevent the death struggle and allowing a second group of birds to struggle freely during slaughter. At 0, 1/4, 1/2, 1, 3, 6, 12, 24, 48 and 72 hours after slaughter rate of post-mortem glycolysis was monitored by muscle pH determination. A protein extraction and fractionation procedure was used to determine changes in extractability of sarcoplasmic and myofibrillar proteins during the 72 hour sampling period.

Muscle pH declined more rapidly during the first 6 hours post-mortem in the N-AN birds than in the AN birds. Statistical minimum pH levels of 5.85 - 5.66 and 5.87 - 5.77 were reached at 6 and 12 hours post-mortem in breast muscle from the N-AN and AN birds respectively.

Non-protein nitrogen of the muscle of both groups of birds was not significantly different. It did not change significantly in either group of birds during the 72 hour aging period. However, there appeared to be a trend for this fraction to increase with aging in muscle of the AN birds.

Although there were no significant differences due to treatment at any specific time, the overall extractability of sarcoplasmic

Different... achieved by... prevented... struggle... 48 and 72 hours... monitored by... fractionation... ability of... hour sampling...

Muscle... normal... by... hours post-mortem... respectively.

Non-protein... not significantly... other... there appears... using in...

Although... ment at...

protein was significantly greater in the AN than in the N-AN birds. A decline in extractability of this fraction from muscle was observed in both groups of birds, and it appeared to follow the decline in pH until a minimum pH level was reached. After this minimum pH level was reached it tended to increase.

Extractability of total extractable nitrogen, total fibrillar protein nitrogen and actomyosin nitrogen fractions from muscle closely paralleled each other in both groups of birds. Extractability of each of these fractions began to increase steadily from zero time to statistical maximum levels at 1, 1/2 and 1 hour respectively in the N-AN birds. However, extractability of these three fractions remained fairly constant at a low level from muscle of the AN birds during the first hour post-mortem, then it began to increase to statistical maximum levels at 12 hours for all three fractions.

Buffered-salt insoluble protein was extracted with a pyrophosphate containing buffer in an attempt to dissociate the unextracted actomyosin, and the residual myosin nitrogen, residual actin nitrogen and unextracted soluble protein nitrogen were determined. Very little dissociation of actomyosin occurred in muscle from either group of birds. The level of extractability of these three fractions from breast muscle was fairly constant during the first hour post-mortem in the AN birds. This was followed by a decline similar to the declining level of extractability from muscle of the N-AN birds which began at zero time. Statistical minimum levels of extractability for these fractions were reached at 1/4, 1 and 3 hours and 6, 6 and 12 hours post-mortem in muscle of the N-AN and AN birds respectively.

Tenderness level of all birds was measured by shear values and it was found that breast muscles from the AN birds were significantly more tender than those from the N-AN birds. The correlation of tenderness with changes in pH and protein extractability was determined. As pH declined in the AN birds during the first 6 hours post-mortem the birds became more tender, however, pH decline after 6 hours resulted in toughening. No similar trends were observed in the muscles from the N-AN birds. There appeared to be no relationship between tenderness and protein extractability from muscle of either group of birds, although there were two instances where significant correlation coefficients were observed. These two instances were isolated with only one r value being significant in each case and it was not part of an increasing or decreasing trend in correlation.

Tenderness was not found in the group of 100. It was found that only 22 males and 10 females were tender than the control group. The tenderness with control was not found in the group of 100. As an illustration of the tenderness post-mortem, the following is a list of the 6 hours tenderness in the control group. The tenderness from the control group was not found in the group of 100. The tenderness between tenderness and the control group of 100 was not found in the group of 100. The correlation between tenderness and the control group of 100 was not found in the group of 100. The tenderness with only one group of 100 was not found in the group of 100. The tenderness was not found in the group of 100.

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pyrophosphate con
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for 24-48.

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undergoing

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muscle, with
Res. 12.

Bate-Smith,
triphosphate.

Bate-Smith,
time course

Bate-Smith,
heart.

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muscle
exercise during
11-22.

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APPENDIX A

Reagents and Solutions

Pentobarbital solution - 3% wt/v sodium pentobarbital in water.

Sodium iodoacetate solution - 0.001 M aqueous solution.

KCl - buffer solution - $\sqrt{I}/2 = 1.0$, pH = 7.5. 0.800 M potassium chloride, 0.010 M potassium fluoride, 0.061 M disodium phosphate, 0.0075 M mono-sodium phosphate aqueous solution.

TCA solution - 10% wt/v trichloroacetic acid in water.

Sodium hydroxide solution - 0.1 N aqueous solution.

Sodium pyrophosphate solution - $\sqrt{I}/2 = 0.7$, pH = 7.5.

0.0688 M aqueous solution of sodium pyrophosphate adjusted to pH 7.5 by adding 1.0 M aqueous mono-sodium phosphate solution.

Sulfuric acid - 93 - 98% pure, nitrogen free.

Sodium hydroxide - 50% wt/v sodium hydroxide in water.

Potassium sulfate - powder.

Cupric sulfate solution - 10% wt/v cupric sulfate in water.

Boric acid solution - 4% wt/v boric acid in water.

Hydrochloric acid solution - standardized aqueous hydrochloric acid solution of 0.01 N and 0.1 N.

Bromcresol green indicator solution - 1% wt/v aqueous bromcresol green (sodium salt) solution.

1. Sodium hydroxide solution (10%)
2. Sodium carbonate solution (10%)
3. Sodium bicarbonate solution (10%)
4. Sodium chloride solution (10%)
5. Sodium phosphate solution (10%)
6. Sodium sulfate solution (10%)
7. Sodium nitrate solution (10%)
8. Sodium acetate solution (10%)
9. Sodium formate solution (10%)
10. Sodium oxalate solution (10%)
11. Sodium citrate solution (10%)
12. Sodium tartrate solution (10%)
13. Sodium succinate solution (10%)
14. Sodium malate solution (10%)
15. Sodium lactate solution (10%)
16. Sodium pyruvate solution (10%)
17. Sodium ascorbate solution (10%)
18. Sodium glutamate solution (10%)
19. Sodium proline solution (10%)
20. Sodium glycine solution (10%)
21. Sodium alanine solution (10%)
22. Sodium valine solution (10%)
23. Sodium leucine solution (10%)
24. Sodium isoleucine solution (10%)
25. Sodium threonine solution (10%)
26. Sodium serine solution (10%)
27. Sodium methionine solution (10%)
28. Sodium cysteine solution (10%)
29. Sodium proline solution (10%)
30. Sodium glycine solution (10%)

APPENDIX B

Procedure for calculation of nitrogen containing fractions.

Total Extractable Nitrogen (TEN):

$$(\text{mg N/ml C-1}) (\text{ml C-1}) (100/\text{sample wt.}) = \text{nitrogen of C-1} = \text{TEN}$$

Non-protein Nitrogen (NPN):

$$(\text{mg N/ml C-NPN}) (\text{ml C-NPN}) (\text{ml C-1}/20) (100/\text{sample wt.}) = \\ \text{nitrogen of C-NPN} = \text{NPN}$$

Sarcoplasmic Protein Nitrogen (SPN):

$$(\text{mg N/ml C-.05}) (\text{ml C-.05}) (\text{ml C-1}/10) (100/\text{sample wt.}) = \\ \text{nitrogen of C-.05}$$

$$(\text{nitrogen of C-.05}) - (\text{nitrogen of C-NPN}) = \text{SPN}$$

Total Fibrillar Protein Nitrogen (TFPN):

$$(\text{nitrogen of C-1}) - (\text{nitrogen of C-.05}) = \text{TFPN}$$

Actomyosin Nitrogen (AN):

$$(\text{mg N/ml R-.25}) (\text{ml R-.25}) (\text{ml C-1}/10) (100/\text{sample wt.}) = \\ \text{nitrogen of R-.25} = \text{AN}$$

Residual Myosin Nitrogen (RMN):

$$(\text{mg N/ml C-RM}) (\text{ml C-RM}) (100/\text{sample wt.}) = \text{nitrogen of C-RM} = \\ \text{RMN}$$

Residual Actin Nitrogen (RAN):

$$(\text{mg N/ml C-RA}) (\text{ml C-RA}) (100/\text{sample wt.}) = \text{nitrogen of C-RA} = \text{RAN}$$

Unextracted Soluble Protein Nitrogen (USPN):

$$(\text{mg N/ml C-UP}) (\text{ml C-UP}) (100/\text{sample wt.}) = \text{nitrogen of C-UP} = \\ \text{USPN}$$

APPENDIX C

Combined ranking of the values for pH and protein extractability of turkey breast muscle, 0 to 72 hours post-mortem, from birds with and without ante-mortem injection of pentobarbital^{1/}.

| Time-Trt ^{2/} | pH ^{3/} | Time-Trt | TEN ^{4/} |
|------------------------|----------------------|----------|-----------------------|
| 0-A | 6.86 ^a | 1/4-A | 2911 ^a |
| 0-NA | 7.78 ^{ab} | 0-A | 2949 ^a |
| 1/2-A | 6.71 ^{ab} | 1-A | 2957 ^a |
| 1/4-A | 6.70 ^{ab} | 1/2-A | 2961 ^a |
| 1-A | 6.69 ^{ab} | 0-NA | 3074 ^{ab} |
| 3-A | 6.55 ^b | 3-A | 3269 ^{bc} |
| 6-A | 6.32 ^c | 1/4-NA | 3285 ^{bc} |
| 1/2-NA | 6.22 ^{cd} | 1/2-NA | 3328 ^{bcd} |
| 1-NA | 6.10 ^{cde} | 1-NA | 3426 ^{cde} |
| 1/4-NA | 6.02 ^{def} | 6-A | 3477 ^{cdef} |
| 3-NA | 5.99 ^{defg} | 3-NA | 3545 ^{cdefg} |
| 12-A | 5.87 ^{efgh} | 48-NA | 3583 ^{defg} |
| 6-NA | 5.85 ^{fgh} | 12-NA | 3596 ^{defg} |
| 72-A | 5.79 ^{fgh} | 6-NA | 3607 ^{defg} |
| 48-A | 5.78 ^{fgh} | 24-NA | 3655 ^{efg} |
| 24-A | 5.77 ^{fgh} | 72-NA | 3680 ^{efg} |
| 12-NA | 5.74 ^{gh} | 48-A | 3751 ^{fg} |
| 24-NA | 5.74 ^{gh} | 72-A | 3766 ^{fg} |
| 48-NA | 5.68 ^h | 12-A | 3805 ^g |
| 72-NA | 5.66 ^h | 24-A | 3816 ^g |

| Time-Trt | NPN | Time-Trt | SPN |
|----------|------------------|----------|-------------------|
| 1/2-A | 559 ^a | 3-NA | 915 ^a |
| 1/4-A | 560 ^a | 12-NA | 950 ^a |
| 6-NA | 563 ^a | 1/4-NA | 971 ^a |
| 1/4-NA | 569 ^a | 6-NA | 990 ^a |
| 1/2-NA | 571 ^a | 24-A | 1039 ^a |
| 24-NA | 572 ^a | 1/2-NA | 1050 ^a |
| 0-A | 575 ^a | 1-NA | 1057 ^a |
| 48-NA | 575 ^a | 24-NA | 1065 ^a |
| 1-A | 575 ^a | 6-A | 1083 ^a |
| 3-A | 577 ^a | 12-A | 1099 ^a |
| 0-NA | 580 ^a | 48-NA | 1112 ^a |
| 12-NA | 582 ^a | 72-A | 1131 ^a |
| 12-A | 583 ^a | 48-A | 1142 ^a |
| 1-NA | 585 ^a | 72-NA | 1174 ^a |
| 3-NA | 590 ^a | 0-NA | 1192 ^a |
| 6-A | 596 ^a | 3-A | 1192 ^a |
| 24-A | 597 ^a | 1-A | 1203 ^a |
| 72-A | 598 ^a | 1/4-A | 1207 ^a |
| 72-NA | 599 ^a | 1/2-A | 1264 ^a |
| 48-A | 599 ^a | 0-A | 1285 ^a |

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| Time-Track | | Time-Track | |
|------------|--|------------|--|
| 0-A | | 11-A | |
| 0-NA | | 11-NA | |
| 1-1-A | | 12-A | |
| 1-1-NA | | 12-NA | |
| 1-A | | 13-A | |
| 2-A | | 13-NA | |
| 2-NA | | 14-A | |
| 3-A | | 14-NA | |
| 3-NA | | 15-A | |
| 4-A | | 15-NA | |
| 4-NA | | 16-A | |
| 5-A | | 16-NA | |
| 5-NA | | 17-A | |
| 6-A | | 17-NA | |
| 6-NA | | 18-A | |
| 7-A | | 18-NA | |
| 7-NA | | 19-A | |
| 8-A | | 19-NA | |
| 8-NA | | 20-A | |
| 9-A | | 20-NA | |
| 9-NA | | 21-A | |
| 10-A | | 21-NA | |
| 10-NA | | 22-A | |
| 11-A | | 22-NA | |
| 11-NA | | 23-A | |
| 12-A | | 23-NA | |
| 12-NA | | 24-A | |
| 13-A | | 24-NA | |
| 13-NA | | 25-A | |
| 14-A | | 25-NA | |
| 14-NA | | 26-A | |
| 15-A | | 26-NA | |
| 15-NA | | 27-A | |
| 16-A | | 27-NA | |
| 16-NA | | 28-A | |
| 17-A | | 28-NA | |
| 17-NA | | 29-A | |
| 18-A | | 29-NA | |
| 18-NA | | 30-A | |
| 19-A | | 30-NA | |
| 19-NA | | 31-A | |
| 20-A | | 31-NA | |
| 20-NA | | | |

APPENDIX C continued

| Time-Trt | TFPN | Time-Trt | AN |
|----------|---------------------|----------|--------------------|
| 0-A | 1089a | 0-A | 355a |
| 1/2-A | 1138a | 1/2-A | 383a |
| 1/4-A | 1144a | 1/4-A | 389a |
| 1-A | 1179ab | 1-A | 436a |
| 0-NA | 1302ab | 0-NA | 620a |
| 3-A | 1500bc | 3-A | 1047b |
| 1/2-NA | 1707cd | 6-A | 1453c |
| 1/4-NA | 1744cde | 1/4-NA | 1468c |
| 1-NA | 1783cde | 1/2-NA | 1476 ^c |
| 6-A | 1799cde | 1-NA | 1624 ^{cd} |
| 48-NA | 1896 ^{def} | 3-NA | 1823 ^{cd} |
| 72-NA | 1962 ^{def} | 6-NA | 1881 ^d |
| 48-A | 2009 ^{def} | 24-NA | 1918 ^d |
| 24-NA | 2018 ^{def} | 48-NA | 1944 ^d |
| 72-A | 2038 ^{def} | 12-NA | 1957 ^d |
| 3-NA | 2039 ^{def} | 24-A | 1992 ^d |
| 6-NA | 2054 ^{def} | 72-NA | 1994 ^d |
| 12-NA | 2063 ^{def} | 12-A | 1999 ^d |
| 12-A | 2123 ^{ef} | 48-A | 2023 ^d |
| 24-A | 2179 ^f | 72-A | 2026 ^d |

| Time-Trt | RMN | Time-Trt | RAN |
|----------|-------------------|----------|-------------------|
| 1/4-A | 105 ^{*a} | 1/4-A | 50 ^{*a} |
| 0-A | 92 ^{ab} | 0-A | 45 ^{ab} |
| 1-A | 86 ^{ab} | 1/2-A | 43 ^{*ab} |
| 1/2-A | 84 ^{*ab} | 1-A | 43 ^{ab} |
| 0-NA | 64 ^{*bc} | 3-A | 34 ^{*bc} |
| 3-A | 64 ^{*bc} | 0-NA | 34 ^{*bc} |
| 6-A | 44 ^{cd} | 1/2-NA | 28 ^{bc} |
| 1/4-NA | 43 ^{cd} | 1/4-NA | 26 ^{cd} |
| 1/2-NA | 42 ^{cd} | 6-A | 22 ^{cde} |
| 1-NA | 27 ^d | 1-NA | 19 ^{cde} |
| 3-NA | 15 ^d | 3-NA | 12 ^{de} |
| 24-NA | 14 ^d | 24-NA | 9 ^e |
| 6-NA | 13 ^d | 6-NA | 8 ^e |
| 72-NA | 12 ^d | 12-NA | 7 ^e |
| 12-NA | 12 ^d | 72-NA | 7 ^e |
| 48-NA | 11 ^d | 24-A | 7 ^e |
| 12-A | 11 ^d | 48-NA | 7 ^e |
| 24-A | 11 ^d | 12-A | 6 ^e |
| 72-A | 10 ^d | 72-A | 5 ^e |
| 48-A | 9 ^d | 48-A | 5 ^e |

APPENDIX C continued

| Time-Trt | USPN |
|----------|-------------------|
| 1/4-A | 651 ^{*a} |
| 0-A | 599 ^a |
| 1/2-A | 588 ^{*a} |
| 1-A | 529 ^a |
| 0-NA | 499 ^{*a} |
| 3-A | 316 ^{*b} |
| 1/2-NA | 297 ^b |
| 1/4-NA | 283 ^b |
| 6-A | 239 ^b |
| 1-NA | 192 ^{bc} |
| 3-NA | 89 ^{cd} |
| 48-NA | 66 ^{cd} |
| 24-NA | 56 ^{cd} |
| 6-NA | 50 ^{cd} |
| 12-NA | 39 ^{cd} |
| 12-A | 33 ^d |
| 72-NA | 33 ^d |
| 24-A | 20 ^d |
| 48-A | 16 ^d |
| 72-A | 13 ^d |

- 1/ All values are means of ten birds except those marked by an asterisk which are means of nine birds. All values except those indicating pH are expressed as mg N/100 g of wet tissue.
- 2/ Time is expressed in hours and the anesthetized treatment is expressed as A and the non-anesthetized as NA.
- 3/ Values in a column marked by the same letter are not different at the 1 percent level of probability.
- 4/ The various protein fractions are abbreviated in the following manner: total extractable nitrogen, TEN; non-protein nitrogen, NPN; sarcoplasmic protein nitrogen, SPN; total fibrillar protein nitrogen, TFPN; actomyosin nitrogen, AN; residual myosin nitrogen, RMN; residual actin nitrogen, RAN and unextracted soluble protein nitrogen, USPN.

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