SOME POSTMORTEM ASPECTS OF BROILER BREAST MUSCLE

by

DARRELL FENWICK WOOD

B.Sc.(Agr.), McGill University, 1963
M.Sc., McGill University, 1965

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in the Department
of
Food Science

We accept this thesis as conforming to the
required standard.

THE UNIVERSITY OF BRITISH COLUMBIA

February, 1973
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of **Food Science**

The University of British Columbia
Vancouver 8, Canada

Date **March 30, 1973.**
ABSTRACT

The ability of broiler P. major muscle to develop postmortem isometric tension was studied under a variety of conditions. Muscle strips developed and released tension while suspended in phosphate buffer pH 7.2. The addition of calcium or magnesium to the buffer enhanced tension release, as did the presence of either of these two ions and EDTA.

Different rates of tension release were evident from bird to bird and the rate of release was fastest in birds which required the shortest time to reach maximum tension. The proportion of tension released within one hour of reaching maximum tension correlated significantly with subsequent proportions from 2 through 12 hours after maximum tension, making it possible to predict, with reasonable accuracy, tension release from 1 hour values. The one hour release values had a significant relationship with time to maximum tension but no relationship with tenderness suggesting that tension release, from bird to bird, is not indicative of tenderness.

Free struggle at slaughter, hot water scalding and mechanical plucking were shown to have an additive effect on tension parameters and tenderness of broiler P. major.

Pre-slaughter epinephrine injection was shown to deplete muscle glycogen levels within 8 - 12 hours post-injection. ATP levels remained high resulting in considerable tension development. It appears that such tension development is sufficient to account for the subsequent toughening observed
in the muscle.

Broilers were subjected to three different stress situations (commercial handling, cold and heat). No significant changes in postmortem muscle quality were observed as a result of these treatments. Broilers appeared to respond differently under the different conditions with heat stress shortening the time to reach maximum tension and commercial and cold stresses lengthening the time to maximum tension.

A cold shortening effect was observed during the study of the effect of temperature on tension pattern. The amount of cold shortening observed was increased as temperature was lowered from 5°C to 0°C. At 2°C, the initial cold shortening occurred and no further tension development was observed even after 36 hours at this temperature. Removal of strips from 2°C to room temperature after 12 and 24 hours resulted in some further tension development but essentially no tension was observed when 36 hour strips were brought to room temperature. The cold shortening did not significantly lower muscle ATP and creatine phosphate levels although a decrease was observed.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>iii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>viii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>x</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>General Muscle Tenderness</td>
<td>3</td>
</tr>
<tr>
<td>Antemortem Tenderness Related Factors</td>
<td>4</td>
</tr>
<tr>
<td>Breed and Strain</td>
<td>4</td>
</tr>
<tr>
<td>Sex and Age</td>
<td>5</td>
</tr>
<tr>
<td>Ration</td>
<td>6</td>
</tr>
<tr>
<td>Muscle Type</td>
<td>6</td>
</tr>
<tr>
<td>Slaughter and Processing Tenderness Related Factors</td>
<td>7</td>
</tr>
<tr>
<td>Humane Slaughter</td>
<td>7</td>
</tr>
<tr>
<td>Scalding</td>
<td>8</td>
</tr>
<tr>
<td>Beating-Picking</td>
<td>9</td>
</tr>
<tr>
<td>Hot Cutting</td>
<td>10</td>
</tr>
<tr>
<td>Pre-Rigor Tenderness Related Factors</td>
<td>10</td>
</tr>
<tr>
<td>Chemical Changes</td>
<td>11</td>
</tr>
<tr>
<td>Physical Changes</td>
<td>13</td>
</tr>
<tr>
<td>Post-Rigor Tenderness Related Factors</td>
<td>15</td>
</tr>
<tr>
<td>Resolution of Rigor</td>
<td>15</td>
</tr>
<tr>
<td>Aging and Frozen Storage</td>
<td>16</td>
</tr>
<tr>
<td>Methods of Measuring Muscle Tenderness</td>
<td>17</td>
</tr>
<tr>
<td>Isometric Tension Measurement</td>
<td>18</td>
</tr>
<tr>
<td>Antemortem Stress and Postmortem Muscle Quality</td>
<td>20</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

| Isometric Tension Experiments | 22 |
| Muscle Source | 22 |
| Isometric Tension Measurement | 22 |
| The Effect of Environment on Tension Pattern | 25 |
| The Effect of pH on Tension Pattern | 25 |
| The Effect of Calcium, Magnesium and EDTA on Tension Pattern | 25 |
| The Effect of Temperature on Tension Pattern | 26 |
| Effect of Processing Techniques on Tension Pattern and Tenderness | 26 |

Epinephrine Experiments

| Preliminary Experiment | 28 |
| Experiment 1 | 28 |
| Experiment 2 | 29 |

Sample Preparation for Metabolite Assay

| pH | 29 |
| Metabolite Analyses | 30 |

Stress Experiments

<p>| Commercial Stress | 30 |
| Heat Stress | 31 |</p>
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Stress</td>
<td>31</td>
</tr>
<tr>
<td>Cold Shortening Experiments</td>
<td>32</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>32</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>33</td>
</tr>
<tr>
<td><strong>RESULTS AND DISCUSSION</strong></td>
<td>34</td>
</tr>
<tr>
<td>Isometric Tension Experiments</td>
<td>34</td>
</tr>
<tr>
<td>The Effect of Environment on Tension Pattern</td>
<td>34</td>
</tr>
<tr>
<td>The Effect of pH on Tension Pattern</td>
<td>36</td>
</tr>
<tr>
<td>The Effect of Temperature on Tension Pattern</td>
<td>40</td>
</tr>
<tr>
<td>The Effect of Calcium, Magnesium and EDTA</td>
<td>44</td>
</tr>
<tr>
<td>on Tension Pattern</td>
<td></td>
</tr>
<tr>
<td>The Effect of Processing Techniques on Tension Pattern and Tenderness</td>
<td>55</td>
</tr>
<tr>
<td>Segregation of Broiler Controls on the Basis of Time to Reach Maximum Tension</td>
<td>58</td>
</tr>
<tr>
<td>The Relation Between One Hour Tension Release, Time to Maximum Tension and Shear Value</td>
<td>64</td>
</tr>
<tr>
<td>Epinephrine Experiments</td>
<td>66</td>
</tr>
<tr>
<td>Preliminary Experiment</td>
<td>66</td>
</tr>
<tr>
<td>Epinephrine Experiment 1</td>
<td>68</td>
</tr>
<tr>
<td>Epinephrine Experiment 2</td>
<td>71</td>
</tr>
<tr>
<td>Stress Experiments</td>
<td>81</td>
</tr>
<tr>
<td>Cold Shortening Studies</td>
<td>88</td>
</tr>
<tr>
<td><strong>SUMMARY AND CONCLUSIONS</strong></td>
<td>95</td>
</tr>
<tr>
<td><strong>LITERATURE CITED</strong></td>
<td>99</td>
</tr>
<tr>
<td>Table</td>
<td>Title</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>I</td>
<td>Means and Standard Errors of Time and Tension Development for Strips of Broiler P. major Muscle Run in Phosphate Buffer at Four Different pH Levels.</td>
</tr>
<tr>
<td>II</td>
<td>Means and Standard Errors of Time and Tension Development for Strips of Broiler P. major Muscle Run in Buffers at Various Temperatures.</td>
</tr>
<tr>
<td>III</td>
<td>Means and Standard Errors of Time and Tension Development for Strips of Broiler and Fowl P. major Muscle Run in Phosphate Buffer and Buffer Containing Three Levels of Calcium.</td>
</tr>
<tr>
<td>IV</td>
<td>Means and Standard Errors of Time and Tension Development for Strips of Broiler P. major Muscle in Phosphate Buffer and Buffer Containing Mg++, EDTA, Ca++ + EDTA and Mg++ + EDTA.</td>
</tr>
<tr>
<td>V</td>
<td>Means and Standard Errors of Tension Parameters for Inner and Outer Strips of Broiler P. major Muscle Subjected to Various Post-Slaughter Treatment.</td>
</tr>
<tr>
<td>VI</td>
<td>Means and Standard Errors of Pooled Tension Parameters and Shear Value Data for Inner and Outer Strips of Broiler P. major Subjected to Various Post-Slaughter Treatments.</td>
</tr>
<tr>
<td>VII</td>
<td>Time and Tension Means and Standard Deviations for Three Broiler Groups Segregated on the Basis of Time to Reach Maximum Tension.</td>
</tr>
<tr>
<td>VIII</td>
<td>Regression Line Parameters for Tension Release (Independent Variable) Versus Time (Dependent Variable) from Three Groups of Broiler Controls and for the Pooled Groups.</td>
</tr>
<tr>
<td>IX</td>
<td>Simple Correlations of One Hour Tension Release Values with Subsequent Hourly Values from Three Groups of Control Broilers and for the Pooled Data from the Three Groups.</td>
</tr>
<tr>
<td>X</td>
<td>Simple Correlations of Two Hour Tension Release Values with Subsequent Hourly Values from Three Groups of Control Broilers and for the Pooled Data from the Three Groups.</td>
</tr>
</tbody>
</table>
Table

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>XI Tension Parameters and Shear Values for P. major Muscle from Broilers Injected with Epinephrine at Various Times Pre-Slaughter.</td>
<td>67</td>
</tr>
<tr>
<td>XII Analysis of Variance for Parameters Studied in Epinephrine Experiment 1.</td>
<td>68</td>
</tr>
<tr>
<td>XIII Duncan's New Multiple Range Test on Significant Treatment Means from Epinephrine Experiment 1.</td>
<td>69</td>
</tr>
<tr>
<td>XIV Correlation Matrix for Parameters Studied in Epinephrine Experiment 1.</td>
<td>70</td>
</tr>
<tr>
<td>XV Analysis of Variance of Parameters Studied in Epinephrine Experiment 2.</td>
<td>72</td>
</tr>
<tr>
<td>XVI Duncan's New Multiple Range Test on Significant Treatment Means from Epinephrine Experiment 2.</td>
<td>73</td>
</tr>
<tr>
<td>XVII Correlation Matrix for Parameters Studied in Epinephrine Experiment 2.</td>
<td>78</td>
</tr>
<tr>
<td>XVIII Means and Standard Errors of Parameters of P. major Muscle from Broilers in the Commercial Stress Experiment.</td>
<td>82</td>
</tr>
<tr>
<td>XIX Means and Standard Errors of Parameters of P. major Muscle from Broilers in the Heat Stress Experiment.</td>
<td>84</td>
</tr>
<tr>
<td>XX Means and Standard Errors of Parameters of P. major Muscle from Broilers in the Cold Stress Experiment.</td>
<td>85</td>
</tr>
<tr>
<td>XXI Means and Standard Errors of Tension Parameters of Broiler P. major Muscle Subjected to Various Post-Slaughter Temperature Treatments.</td>
<td>89</td>
</tr>
<tr>
<td>XXII Means and Standard Errors of ATP, HMP and CP Content of Broiler P. major Muscle Subjected to Various Temperature Treatments.</td>
<td>92</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Effect of different extracellular incubation media on isometric tension pattern of broiler P. major muscle.</td>
</tr>
<tr>
<td>2</td>
<td>Effect of extracellular pK on isometric tension pattern of broiler P. major muscle.</td>
</tr>
<tr>
<td>3</td>
<td>Effect of $10^{-3}$ M calcium on tension decline in broiler P. major muscle.</td>
</tr>
<tr>
<td>4</td>
<td>Effect of $10^{-3}$ M calcium on tension decline in fowl P. major muscle.</td>
</tr>
<tr>
<td>5</td>
<td>Effect of $10^{-3}$ M magnesium, EDTA and calcium-EDTA on tension decline in broiler P. major muscle.</td>
</tr>
<tr>
<td>6</td>
<td>Isometric tension decline in three groups of broilers separated on the basis of time required to reach maximum tension.</td>
</tr>
<tr>
<td>7</td>
<td>Relation between tension, time to maximum tension and shear value in P. major muscle from epinephrine treated broilers.</td>
</tr>
<tr>
<td>8</td>
<td>Relation between muscle glycogen and ATP levels and shear value in P. major from epinephrine treated broilers.</td>
</tr>
<tr>
<td>9</td>
<td>Relation between pH, muscle lactate and shear value in P. major from epinephrine treated broilers.</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to his advisor, Dr. J.F. Richards, Associate Professor, Department of Food Science for his guidance and encouragement during the course of this study.

He is also thankful to the members of his graduate committee:

Dr. S. Nakai, Department of Food Science
Dr. W.D. Powrie, Department of Food Science
Dr. C.W. Roberts, Department of Poultry Science
Professor L.M. Staley, Department of Agricultural Engineering
Dr. M.A. Tung, Department of Food Science.

for their encouragement and continued interest in the research and for the review of this thesis and a special note of thanks to Dr. Tung for computer assistance.

The author is grateful to the Department of Poultry Science for the use of poultry farm facilities and for supplying some of the broilers used in this study. He is also grateful to Mr. Garth Sundeen for technical assistance, to Miss Lynne Robinson for computer assistance and preparation of the figures and to the late Mr. W. Gleave for construction of the chambers used in the isometric tension studies.

Financial support of the University of British Columbia through a post-graduate Fellowship is gratefully acknowledged.

A special note of gratitude is also expressed to my wife, Carol, for her encouragement and understanding during the course of this study and for the typing of this manuscript.
INTRODUCTION

Until recently, muscle biology has been divided into the study of muscle and the study of meat. The complementary nature of these two areas of study is obvious and was stressed by Dr. B.B. Marsh in the introduction to the Second Symposium on the Physiology and Biochemistry of Muscle as a Food (1970), in which he stated:

"In our study of postmortem muscle we must consider more and more the factors which determine energy production and utilization in the living tissue: the purpose which the muscle serves, the speed at which it moves, the duration of its periods of continuous use, the age, species, and degree of domestication of the animal, the stresses (both long- and short-term) to which it has been exposed. Only when the facts of life in muscle are known can we expect to influence its qualities in death."

Two closely related physical changes occur in pre-rigor, postmortem muscle. These are a shortening or contraction and a loss of extensibility. The shortening phase, on the animal carcass, is characterized by an isometric tension development which results in the so-called rigor stiffening. This phenomenon and associated chemical changes have been repeatedly shown to influence the ultimate acceptability of many muscle systems as food. However, only a meagre amount of information on these relationships is available for poultry muscle systems.

A study of simulated isometric tension development and its relation to tenderness in broiler Pectoralis major muscle is reported in this thesis. Broilers were subjected
to some stressors which may be encountered prior to slaughter under commercial conditions and the effect of these stressors on postmortem muscle quality is also reported.
LITERATURE REVIEW

General Muscle Tenderness

Tenderness, from the consumer viewpoint, is high on the list of factors determining the acceptability of muscle as a food. In view of this fact, food scientists have devoted considerable time and effort to the study of meat tenderness.

One of the greatest concerns in studying tenderness is its variability. Paul et al. (1959) compared cooking losses and tenderness of chickens hatched from eggs laid by the same hen with those of chickens from eggs of different hens in the same flock. The use of half-sibs did not reduce variation although cooking loss and tenderness were similar for both groups.

Tenderness varies from muscle to muscle within an animal and from animals of the same or different species and this variation may be influenced by both ante- and postmortem events.

A symposium in 1963 (Campbell Soup Company, 1963) dealt with many of the factors then thought to influence muscle tenderness. Antemortem factors such as breed, sex and management practices and postmortem factors such as aging and cooking method were discussed. This chronological sequence of events overlooked one relatively short, but highly significant, time period -- that from slaughter to rigor onset. This period
varies from 2 - 4.5 hours in poultry and up to 18 - 20 hours in beef and its importance had been demonstrated by several workers prior to the symposium (Ramsbottom and Strandine, 1949; Koonz et al., 1954; deFremery and Pool, 1960; Locker, 1960).

A review of the factors influencing avian tenderness was published by Marion (1967).

Two basic physical changes occur in pre-rigor post-mortem muscle, a shortening or tendency to contract and a loss of extensibility. Factors which affect these changes and their relation to tenderness have been reviewed by Marsh (1972) and Newbold and Harris (1972). Both are excellent reviews of pertinent literature in this area.

Since shortening or the tendency of the muscle to contract affects eventual muscle tenderness, it follows in the sequence of events that the post-rigor resolution of these parameters must also be important. Goll (1968) reviewed the present literature in this area dealing, in particular, with the evidence which clearly indicates that a resolution of rigor actually occurs in postmortem muscle. The author emphasized the need for further research on the effects of lysosomes and pH changes on postmortem myofibrillar proteins in order to better understand what causes the resolution of rigor.

**Antemortem Tenderness Related Factors**

**Breed and Strain**

The effect of breed and strain on tenderness has been
difficult to establish. Shrimpton and Miller (1960) compared two strains of chickens and found male Leghorns to be less tender than White Rock males and females. Goodwin (1966) compared six strains of Broad Breasted Bronze turkeys and reported that strain did not significantly influence shear values.

Larmond et al. (1968) found that the effect of breed on goose quality was negative for the genotypes studied. Moran et al. (1970) and Larmond et al. (1970) reported that a Cornish male - White Rock female cross gave better carcass quality than the pure strains or reciprocal crosses but no flavor or shear differences were evident between sexes or crosses.

Sex and Age

It has been generally established that the older the poultry at slaughter the less tender the meat. May et al. (1962) found that 72 - week-old chickens were less tender than 10 - week-old chickens regardless of the time analyzed postmortem or the aging temperature.

Goodwin et al. (1969) studied 12 strains of birds grown to 8 weeks of age before processing. No difference was found between commercial strains in the shear values of breast and thigh. There was, however, a slight difference in tenderness of the sexes with males having lower shear values for both breast and thigh muscle.
A sex related tenderness difference in broilers was also demonstrated by Larmond et al. (1969), but a study of Large White turkeys revealed no significant sex differences in eating quality (Larmond et al., 1971). These authors also found that tenderness decreased with age at slaughter.

**Ration**

The composition of the diet has very little or no effect on tenderness as long as the diet provides for optimum growth rate (Marsden et al., 1957 a, b; Goerty et al., 1961). Varying the energy level, however, has been shown to alter the body composition, basically through protein and fat changes (Donaldson et al., 1956; Summers et al., 1965; Marion et al., 1967; Goodwin et al., 1969).

Shrimpton and Miller (1960) showed that birds kept on full feed were more tender than birds kept on a restricted diet.

**Muscle Type**

In most reports shear values for breast muscle (light) are higher than those for thigh muscle (dark). Peterson et al. (1959) found that light muscle in young birds was significantly tougher than dark but in older birds the reverse was found. van den Berg et al. (1963, 1964) found that tenderization in breast muscle was essentially complete after 1 - 2 days post-
mortem, whereas in leg muscle a second tenderization period occurred 2 - 5 days postmortem.

**Slaughter and Processing Tenderness Related Factors**

**Humane Slaughter**

Normal slaughtering procedure for poultry consists of suspending birds by their feet, brief electrical stunning and exsanguination by an external throat cut. This procedure can result in a great deal of struggle before electrical stunning and a considerable lapse of time before the bird becomes unconscious. deFremery and Pool (1958) found that a relationship existed between struggle at slaughter and tenderness whereas Lineweaver (1959) and Dodge and Stadelman (1960) found no relationship between struggling and postmortem tenderization.

Anesthetization, by sodium pentobarbital (Nembutal) has been used in an attempt to find a more humane method of slaughter (May and Huston, 1959; Goodwin et al., 1961; Stadelman and Wise, 1961; deFremery, 1965). Goodwin et al. (1961) found that the method of slaughter had no significant effect on breast muscle tenderness but the use of Nembutal produced some detrimental effects on tenderness. Stadelman and Wise (1961) found that anesthetization with Nembutal lengthened the period of maximum toughness in chickens. This was later verified by deFremery (1965) but his tenderness findings were contrary to those of Goodwin et al. (1961). Landes et al.
(1971) also found that turkeys anesthetized with Nembutal were more tender than nonanesthetized controls.

Immobilization of turkeys and chickens using carbon dioxide has been demonstrated (Drewniak et al., 1955; Kotula et al., 1957, 1961). A 75 second exposure to 33 - 36 percent carbon dioxide concentration was shown to keep broilers unconscious, but alive, during shackling, sticking and bleeding (Kotula et al., 1961).

Scalding

The adverse effects of high scalding temperatures on poultry tenderness have long been known. Present scalding techniques vary but in general fall within the range of 125 - 140°F for times of from 30 - 150 seconds.

Shannon et al. (1957) studied 6 levels of scald temperatures for 6 different times at each level. The study showed that time of scald and temperature of scald significantly reduced tenderness and time had a greater effect than temperature. These findings were verified on turkeys by Klose et al. (1959) and chickens by Pool et al. (1959).

Wise and Stadelman (1959) investigated the effect of scald time and temperature on tenderness at various depths within chicken Pectoralis major muscle. The authors found that the toughening effect of high temperature-long time scalding was related to the depth to which the scald heat penetrated the muscle. These same authors (Wise and Stadelman,
1961) suggested that two aspects may be important in describing the scalding effect on tenderness. Firstly, protein denaturation at elevated temperatures and secondly, an undefined effect caused by holding the carcass at temperatures in excess of normal body temperature.

Klose et al. (1971a) and Kaufman et al. (1972) experimented with a technique using a chamber and subatmospheric steam in an effort to eliminate immersion scalding. The technique was effective in reducing pollution and minimizing water requirements but the end product had about the same shelf life and degree of tenderness as immersion scalded birds.

**Beating-Picking**

Picking machines, which have stout rubber fingers on a rapidly revolving drum, have been shown to produce muscle which is significantly tougher than hand picked controls (Wise and Stadelman, 1957; Pool et al., 1959; Klose et al., 1959).

deFremery and Pool (1960) showed that severe mechanical handling of fresh chicken muscle caused rapid loss of ATP and a rapid drop in muscle pH. Sayre (1969, 1970) also showed that mechanical picking led to a rapid drop in pH and subsequent toughness in the muscle. The author reported that a combination of scalding and beating produced postmortem changes similar to those produced by scalding and picking alone.
Hot Cutting

Lowe (1948) found induced toughness in breast muscle which was cut within 1 hour of slaughter. This toughness persisted even after 24 hours of aging and subsequent cooling. Toughening by pre-rigor cutting has also been reported by Koonz et al. (1954), Pool et al. (1959) and Nixon and Miller (1967).

The above reports did not give particular attention to the type of cutting. Klose et al. (1971b) have extensively studied the effect of cutting poultry carcasses at specific times post-slaughter, the types of cuts made and the specific muscles affected by the cut made. They found that knife cutting the wings at the shoulder joint and flattening the breast at 20, 60 and 120 minutes postmortem gave shear values twice that obtained following the same procedure at 22 hours postmortem. On the other hand, if the wing was sawed off at a point beyond the breast muscle insertion, thereby leaving the wing stub attached to the breast section, no pre-rigor toughening effect was obtained. The authors thus concluded that for optimum tenderness in cut-up poultry consideration should be given to time postmortem and location of cut in relation to breast muscle.

Pre-Rigor Tenderness Related Factors

The biophysical and biochemical changes which take place in postmortem mammalian muscle have been the subject of
extensive research. The changes which occur in postmortem avian muscle are, in general, the same as those in the mammalian species. The most obvious change is the stiffening of the muscle as it passes into rigor mortis. This phenomenon is accompanied by several basic chemical changes including the disappearance of glycogen, ATP and N-phosphorylcreatine; the appearance of ammonia and inosinic acid from the deamination of adenylic acid; and the accumulation of lactic acid through anaerobic glycolysis of muscle glycogen stores (deFremery, 1966a).

Reviews on pre-rigor changes (Newbold and Harris, 1972) and post-rigor resolution (Goll, 1968) have covered many of the pertinent references in these areas.

Chemical Changes

One of the most important factors in the relationship between postmortem chemical changes and tenderness is the rate and extent of the pH change in the muscle. Treatments which increase the rate of pH drop bring about residual toughness in the muscle (deFremery and Pool, 1960; Khan and Nakamura, 1970, 1971).

The effect of pH change on tenderness has been determined basically through acceleration, retardation and blockage or prevention of postmortem glycolysis. Acceleration of glycolysis is usually accomplished through free struggle at death, scalding or mechanical picking. These effects have been discussed in previous sections.
Retardation of glycolysis in poultry muscle has been effected by electrical stunning or injection of Nembutal prior to exsanguination (deFremery, 1965; Sayre, 1969, 1970). Both treatments result in high pH values post-slaughter and a subsequent slow decline of muscle pH.

The blockage of glycolysis may be accomplished by antemortem injection of sodium iodoacetate (deFremery and Pool, 1963; Sayre 1969, 1970) to inhibit the enzyme phosphoglyceraldehyde dehydrogenase. Antemortem epinephrine injections, to eliminate muscle glycogen, has been widely used as a means of eliminating glycolysis (deFremery and Pool, 1963; deFremery, 1965; Khan and Nakamura, 1970; Sayre, 1969, 1970). These authors found minimized postmortem glycolysis and increased tenderness through injection of epinephrine.

Klose et al. (1970) found that muscle depleted of glycogen by antemortem epinephrine injections did not have lower shear values than normal muscle. The authors were unable to explain reasons for this discrepancy but did question the overall effectiveness of epinephrine injections for lowering muscle glycogen.

The decrease of N-phosphorylcreatine (PC) and ATP in muscle are closely related since the immediate postmortem source of ATP occurs through dephosphorylation of PC and phosphorylation of adenosine diphosphate (ADP). The PC in chicken breast muscle is very labile (deFremery, 1965, 1966a)
and the transitory pH increase in poultry muscle immediately postmortem has been attributed to the free creatine liberated at slaughter (Dodge and Peters, 1960).

Under normal conditions the level of ATP in chicken breast muscle is approximately 10 μ moles/g fresh tissue. This level remains relatively high for the first 1-3 hours postmortem then drops rapidly and the muscle passes into rigor mortis when the ATP level has fallen to about 30 percent of its initial level (deFremery, 1966a).

**Physical Changes**

Muscle in the living animal is soft, plastic and extensible, but in rigor it becomes rigid and relatively inextensible. In addition to losing extensibility unrestrained muscle shortens during rigor development. Newbold and Harris (1972) have extensively reviewed the aspects of pre-rigor shortening.

This shortening is greatly dependent on temperature but not all muscles show the same extent of shortening. Locker and Hagyard (1963) defined a "cold shortening" phenomenon in ox neck muscle and since that report ovine (Cook and Langsworth, 1966), porcine (Galloway and Goll, 1967), Hendricks et al., 1971) and avian muscles (Smith et al., 1969) have been reported to "cold shorten". Jungk and Marion (1970) reported that no "cold shortening" was evident in turkey breast muscle but thigh
muscle did exhibit "cold shortening" (Marion, 1971).

Marsh and Thompson (1958), Locker and Hagyard (1963) and Marsh and Leet (1966) reported that the amount of "cold shortening" decreased as the period between slaughter and exposure to cold increased.

Interesting effects on tenderness of beef and lamb carcasses have been effected by changing postmortem hanging practices (Herring et al., 1965 a, b; Bouton and Harris, 1972b). Different points of attachment prevent different muscles from shortening leading to an improved tenderness in these muscles. No work of this nature has been done with avian species. However, Hegarty and Allen (1972) found that stretching pre-rigor turkey muscles, which had been excised from the carcass, did not significantly lower shear values and in some cases the stretched muscles were significantly tougher than unstretched controls.

Klose et al., (1970) studied the effect of pre-rigor contraction on the tenderness of postmortem chicken muscle. Electrical stimulation, beating, freeze-thawing or heating reduced muscle length, in most cases, to between 40 and 50 percent of the original rest length. Subsequent shear values for the contracted, cooked muscles were found to be about one-half those for uncontracted controls. The authors speculated that the extreme state of contraction effected changes at the sarcomere level which resulted in the myofibrils being more susceptible to a shearing stress.
Post-Rigor Tenderness Related Factors

Resolution of Rigor

Post-rigor tenderization in muscle involves a resolution of rigor mortis. The evidence relating to the actual resolution of rigor was reviewed by Goll (1968). Two lines of evidence were discussed. Firstly, muscle held isometrically begins to develop tension immediately after death and increases to a maximum at varying times postmortem. Regardless of species, the ability to maintain isometric tension slowly declines after the point of maximum tension has been reached. The second line of evidence suggesting that a resolution of rigor occurs in postmortem muscle is the observation that sarcomeres which have undergone extensive postmortem shortening will, after several days, lengthen again (Gothard et al., 1966; Stromer and Goll, 1967; Takahashi et al., 1967).

One possible explanation for the observed resolution of rigor is loss of Z-line structure and weakening and eventual rupture of the bonds between the I and Z filaments. Henderson et al. (1970) showed that the Z-line of bovine, porcine and rabbit muscle lost its integrity during postmortem storage. Similar findings have been reported in chicken muscle by Takahashi et al. (1967) but Sayre (1969, 1970) found I-Z weakening to be more prominent than Z-line degradation in postmortem chicken muscle.
A second possible cause of the resolution of rigor is the weakening of the actin-myosin interaction. Such weakening may be effected by a very specific and limited proteolysis of myosin, actin and/or one of the regulatory proteins (Goll, 1968). Evidence of such a weakening was first reported by Fujimaki et al. (1965).

Aging and Frozen Storage

The aging period is considered the time when a processor can most influence the tenderness of chicken and turkey muscle. The different aging requirements for red and white muscle (van den Berg et al., 1964) must be taken into consideration, when considering an adequate aging period.

Marion and Goodman (1967) and Welbourn et al. (1968) have studied turkey quality in relation to aging, time and chilling treatment prior to freezing. Larger turkeys were found to require a shorter aging period prior to freezing suggesting that additional tenderization occurs in large turkeys during freezing and thawing (Marion and Goodman, 1967).

Several chemical changes have been shown to occur in frozen poultry muscle that may relate to tenderness (Khan et al., 1963; Khan, 1964). The change in protein and nonprotein constituents of chicken breast muscle was followed during frozen storage. Nonprotein constituents increased with frozen storage indicating that some proteolysis had occurred. This proteolysis could affect the solubility and ion-binding properties of the
protein, and thus affect tenderness, loss of juiciness and subsequent development of dryness in the meat.

**Methods of Measuring Muscle Tenderness**

One of the greatest difficulties in comparing available data on avian tenderness is the tremendous variation in procedures used to determine tenderness. Marion (1967) emphasized the need to standardize mechanical and sensory methodology so that individual researchers may be able to determine how their data compares with researchers in other laboratories.

Pearson (1963) presented a review of objective and subjective methods for measuring meat tenderness and pointed out the advantages and limitations of each method.

Most of the objective methods of determining meat tenderness use some form of a shearing device and record the force required to shear or compress a sample of standard size.

The relationship between instrument tenderness values and sensory panels has been widely studied over the last two decades (Deatherage and Garnatz, 1952; Klose et al., 1961; White et al., 1964; Pangborn et al., 1965; Sharrah et al., 1965a, b; Pool and Klose, 1969, Szczesniak et al., 1970; Larmond and Petrasovits, 1972). A comparison of objective methods has been published by Bouton and Harris (1972a). Some of the more recent studies have raised objections on both the theoretical and practical aspects of the shear type method.
for determining meat tenderness (Sharrah et al., 1965 b; Pool and Klose, 1969; Szczesniak et al., 1970).

A different approach to the measurement of meat tenderness has been tried by some workers. Nakamura (1972) measured tensile strength of muscle fibers in order to study postmortem aging of chicken breast muscle. Stanley et al. (1972) performed two basic types of objective measurements on raw porcine muscle -- shearing and break strength tests; and sarcomere length, elasticity, stress relaxation and break elongation. The results of these tests were compared with taste panel evaluations of tenderness, elasticity and chew count on cooked meat. Texturized vegetable protein was used as a reference by the panel and proved useful in reducing variations between chew count and objective evaluation. The authors concluded that there are two major structural contributions of raw muscle to cooked meat tenderness, a connective tissue factor and a contraction factor, and different objective methods should be used for their evaluation.

Zachariah et al. (1971) have also tested a method for predicting tenderness on raw muscle. Electrical impedance measurements made on poultry tended to indicate that high impedance was associated with tender birds and low impedance with tough birds.

Isometric Tension Measurement

The first studies on the use of isometric tension
measurements to follow the time-course of rigor mortis in bovine and rabbit muscle were reported by Busch et al. (1967) and Jungk et al. (1967). Prior to this time extensibility measurements were used in order to quantatively follow rigor mortis.

The measurement of isometric tension provides several unique advantages over extensibility measurements (Busch et al., 1972). The most important advantage is its ability to detect changes which correspond to not only onset of rigor but resolution of rigor as well.

Schmidt et al. (1968) describe the development of an isotonic and isometric rigorometer which allowed measurement of isometric tension (shortening) and isotonic tension (loss of extensibility) simultaneously. These same authors (Schmidt et al., 1970 a, b) have used the rigorometer to study some factors affecting the time-course of rigor mortis in porcine muscle. Jungk and Marion (1970) have demonstrated isometric tension development and decline in turkey muscle and have established a linear relationship between temperature and tension development in breast muscle.

Busch et al. (1972a) published some improvements in the procedure for measuring postmortem isometric tension and have reported extensive data on tension development from three mammalian species (porcine, bovine, rabbit).
Antemortem Stress and Postmortem Muscle Quality

Animals are exposed to many forms of stress during growth and in particular during shipment to market and slaughter. These stresses increase the need for hormones which are produced, by the adrenal gland.

Selye (1956) defines stress as:

"the state manifested by a specific syndrome which consists of all the non-specifically induced changes within a biological system".

In his early work (Selye, 1950) noted that animals exposed to a number of stress-producing factors, such as emotional excitement, fatigue, cold and inanition, always reacted with an increased secretion of hormones from the adrenal medulla and adrenal cortex. One effect of these hormones is alteration of liver and muscle glycogen levels and changes in the latter are of particular importance in relation to postmortem muscle quality.

The effect of stress on postmortem muscle quality is exemplified by the Poland China breed of pigs. These animals have been shown to be extremely susceptible to antemortem stress and yield a high incidence of pale soft exudative (PSE) muscle (Sayre et al., 1963 a, b; Briskey, 1964; Kastenschmidt et al., 1966, 1968; Lister et al., 1970; Sair et al., 1970). In beef animals, stress has been implicated in the incidence of dark cutting beef (Lawrie, 1958, 1966 a,b). To date, no such stress effect has been demonstrated in poultry muscle although the
tremendous variation in tenderness may, in some way, be related to variations in stress susceptibility in the avian species.

The influence of stress on bovine, porcine and ovine meat quality has been reviewed by Hedrick (1965) and Judge (1969) and on growth and performance by Wilson (1971). Ringer (1971) and Siegel (1971) have reviewed the literature on poultry adaptation to confinement rearing systems and stress and environment respectively. However, a study of the effects of antemortem stress on postmortem muscle quality in the avian species is lacking.

Lack of information in the above mentioned area and the need for a comprehensive study of the relationship between isometric tension development and decline and broiler tenderness prompted the research which is presented in this thesis.
MATERIALS AND METHODS

Isometric Tension Experiments

Muscle Source

The laying hens used in this study were obtained from the Department of Poultry Science at U.B.C. The birds were approximately 2 year old New Hampshires. The broilers were obtained from a local processing plant, transported to the U.B.C. poultry farm and kept in a range house for 7 - 10 days prior to use to allow for adjustment to the new surroundings. The broilers were maintained on a 20 percent broiler grower ration and were slaughtered at 8 - 12 weeks of age.

For slaughter, birds were placed in a metal funnel, exsanguinated by an outside neck cut and allowed to bleed for about 2 minutes. The wings and legs were manually restrained during slaughter in addition to restriction provided by the funnel.

Isometric Tension Measurement

Isometric tension development and decline were measured using an E & M 6-channel physiograph fitted with isometric transducers. The physiograph was obtained from Narco-Bio-Systems Inc. formerly E & M Instrument Co., Houston, Texas.
Muscle for tension measurement was obtained immediately after exsanguination by cutting the breast skin and excising a 1 cm wide strip of muscle tissue from the anterior portion of the Pectoralis major. The strip was cut parallel to the direction of the muscle fibers and care was taken to prevent stretching of the muscle during excision and subsequent strip preparation. A regression line was prepared by carefully measuring the weight and cross-sectional area of several muscle strips 5 cm in length and 0.1 - 1.0 cm$^2$ in cross section. All strips used in subsequent studies were cut to 5 cm in length, weighed and the cross-sectional area determined from the regression line. Most strips ranged between 0.15 - 0.25 cm$^2$ since this size best fitted the clamping system used.

One end of a muscle strip was clamped in a battery cable clamp (Mueller No. 48B) and the other end was attached to a second clamp. This second clamp was fixed on a plexiglass rod within a plexiglass cylinder, 9.5 cm in diameter and 20 cm high. The rod was fixed about 4 cm above the chamber bottom in order to permit the use of magnetic stirring. The chamber was filled with enough buffer to cover the muscle strip and top clamp and the muscle strip was attached to an isometric transducer by means of 6 lb test monofilament fishing line tied to the free clamp. Approximately 5.0 g/cm$^2$ tension was applied to each strip in order to attain some measure of uniformity of starting conditions.
The physiograph was calibrated so that a 1 cm pen deflection was equivalent to 5 g tension. Up to 6 strips could be studied from each bird. The maximum time lapse from exsanguination to attachment of strips from one bird was 20 minutes. The entire system used in this and subsequent studies is shown in Plate 1.

Plate 1. Isometric tension measuring apparatus.
The Effect of Environment on Tension Pattern

Four different systems were studied in order to select the appropriate chamber media for the muscle strips. System 1 consisted of a humid chamber produced by lining the chambers with moistened chromatography paper, placing about 3 cm of salt solution in the bottom of the chamber and covering the top of the chamber with Saran wrap allowing a small space for the attachment of the strip to the transducer. The other 3 systems consisted of filling the chamber with one of distilled water, phosphate buffer pH 7.2, ionic strength 0.15 or Tris-acetate buffer pH 7.1, ionic strength 0.22 (Goll et al., 1970).

The Effect of pH on Tension Pattern

Phosphate buffers of pH 5.8, 6.3, 6.7 and 7.2 were prepared as described by Gomori (1955). Six broilers were used in this study and four strips were cut from each bird. One strip was run at each pH and tension measurements were made at room temperature (22 - 25°C). The length of measurements varied between 16 - 20 hours.

The Effect of Calcium, Magnesium and EDTA on Tension Pattern

Calcium chloride, magnesium chloride and EDTA were
added to the pH 7.2 phosphate buffer. Solutions were prepared to contain $10^{-7}$, $10^{-4}$, $10^{-3}$ M calcium, $10^{-3}$ M magnesium, $10^{-3}$ M EDTA, $10^{-3}$ M calcium + $10^{-3}$ M EDTA and $10^{-3}$ M magnesium + $10^{-3}$ M EDTA. Several strips from broilers were run in each solution and strips from 6 laying hens were run in the buffer plus $10^{-3}$ M calcium.

The Effect of Temperature on Tension Pattern

Muscle strips were run in phosphate buffer ranging from 0 - 60°C. The cold temperature strips were run in cold-rooms at about 2 and 5°C and the 0°C readings were obtained by pre-cooling the buffer to 0°C in a freezer. Temperatures above room temperature were attained by placing small, 50 watt aquarium heaters in the buffer chambers and stirring magnetically to maintain even heat distribution. The heaters were able to maintain preset temperatures within ± 1°C. Temperatures studied were 0, 2, 5, 23°C, 30, 37, 43, 50 and 60°C.

Effect of Processing Techniques on Tension Pattern and Tenderness

Five different treatments were studied within this experiment.

1. Exsanguination + restricted struggle - control

---

1 This Temperature was selected as representative of room temperature which varied between 22 - 25°C.
2. Exsanguination + restricted struggle + hot water scald
3. Exsanguination + free struggle + hot water scald
4. Exsanguination + restricted struggle + pluck
5. Exsanguination + free struggle + hot water scald + pluck

Exsanguination with restricted struggle was done as previously described. Simulation of processing plant conditions was done by suspending birds by their feet and allowing them to struggle freely both before and after exsanguination. Scalding consisted of 90 second submersion in water at 60°C and plucking was done on a rotary picker, fitted with pliable rubber fingers, for 60 seconds. Six muscle strips were taken immediately from one side of the breast for tension measurement. Three strips were taken from muscle close to the skin and three from muscle near the breast bone in order to study an "inner" and "outer" effect in the muscle. The exposed breast muscle was covered with Saran wrap, packed in drained crushed ice and aged for 24 hours at 2°C after which tenderness measurements were performed in a manner similar to that reported by deFremery and Pool (1960). The excised breast muscle was clamped between two 1/8 inch aluminum plates fitted with metal spacers so that cooked muscle approximately 0.7 cm in thickness was obtained. The muscle was cooked in boiling water for 30 minutes and
cooled in running tap water for 5 minutes. Strips of parallel fibres, 1.5 cm wide were prepared and sheared using an Allo-Kramer shear press. A single blade shear cell, 250 lb ring and 9 cm/min cross head speed were used for all shears. A minimum of 10 shears per bird was obtained and in most cases attenuation was set at the 5 percent level.

**Epinephrine Experiments**

**Preliminary Experiment**

The equivalent of 4 mg epinephrine/kg (Sigma Chemical Co., St. Louis, Missouri) body weight was injected into the breast muscle of 6 broilers which were killed at 3, 6, 9, 12, 15 and 18 hours post-injection. Tension and tenderness measurements were made as previously described. Two control birds were also run.

**Experiment 1**

A total of 18 male and 18 female broilers were used in this experiment. A 2 x 3 x 6 randomized complete block design (Cochran and Cox, 1964) was used. Treatments consisted of uninjected controls and exsanguination at 2, 4, 8, 12 and 16 hours post-injection. The experiment was run over a 6 day period and males and females were killed on separate days. Injection was done intramuscularly into the thigh and dosage was as used
in the preliminary experiment. Blood was collected at death for blood lactate analysis according to the method of Hadjwassiliou and Rieder (1968). Tension and tenderness were also measured.

**Experiment 2**

The procedure and design of experiment 1 were duplicated except that post-injection slaughter times of 4, 8, 10, 12 and 24 hours were used. Muscle samples were taken within 2 minutes after exsanguination, frozen in liquid nitrogen (LN$_2$) and stored in aluminum foil envelopes under LN$_2$ for subsequent metabolite analysis.

**Sample Preparation for Metabolite Assay**

The samples stored under LN$_2$ were powdered by the method of Borchert and Briskey (1965) as modified by Vanderstoep (1971). The frozen samples were pulverized in a macro-model Virtis homogenizer for 1.5 minutes at approximately 11,000 rpm. The powdered sample was replaced in aluminum foil envelopes and stored under LN$_2$.

**pH**

pH of the muscle samples was determined by modifying the method of Cassens and Newbold (1967). 1 - 2 g powdered sample was homogenized in 10 ml neutralized 0.005 M sodium
iodoacetate at 2°C. The slurry was allowed to warm to room temperature and pH was measured using a Corning Model 10 pH meter.

Metabolite Analyses

ATP was determined by the method of Lamprecht and Trautschold (1963) with two modifications. Two grams of previously powdered sample were added to 7.5 ml instead of 6.5 ml perchloric acid and 1 cm cuvettes were used instead of 2 cm cells. This latter change necessitated alteration of the quantities of intermediates, cofactors and enzymes used.

Lactate was determined by the method of Hohorst (1963) and glycogen by the method of Pfleiderer (1963).

Optical density measurements were made using a Unicam SP 800 recording spectrophotometer. All chemicals were of reagent grade, made up in glass distilled water. Enzymes, cofactors and intermediates used were obtained from Sigma Chemical Co., St. Louis, Missouri.

Stress Experiments

Commercial Stress

Twenty-eight broilers were used in this experiment. One male and one female was obtained from a local processing plant just prior to slaughter, transported to the Food Science
laboratory at U.B.C. and killed immediately. This procedure was repeated on seven different days. One control male and female, from a supply maintained at U.B.C., was killed on each of these days.

Immediately after exsanguination, 15 - 20 g breast muscle was frozen in LN$_2$ and subsequently analysed for pH and ATP as previously described. Tension and tenderness were also measured as previously described.

**Heat Stress**

Twenty female broilers were used in this study. Fourteen birds were placed in hot air at 110°F for 3 hours prior to exsanguination. Seven of these birds were killed with restricted struggle and seven were allowed to struggle freely. Six birds were killed as controls.

Samples of muscle were excised immediately post-slaughter and frozen in LN$_2$ for "0" hour pH and ATP analysis. Tension and tenderness were measured and 24 hour pH was also determined.

**Cold Stress**

Twenty-seven female broilers were studied in this experiment. Eighteen birds were placed in a 2°C coldroom. Nine were exsanguinated after 2 hours and 9 after 6 hours. The remaining nine birds were killed as controls.
Samples of muscle were excised immediately post-slaughter and frozen in LN₂ for subsequent ATP, glycogen and hexose monophosphate analysis and tension and tenderness were measured.

**Cold Shortening Experiments**

**Experiment 1**

Broilers, of mixed sex, were used in this experiment. The birds were exsanguinated with restricted struggle and 6 muscle strips were prepared from each bird for tension measurement. Two strips were run in buffer at room temperature, 4 strips were attached to transducers in a 2°C coldroom then pre-cooled buffer at 2°C was added to the chambers. These four strips were kept in the coldroom in order to observe tension development at this temperature. Two strips were removed from the coldroom at 12, 24 and 36 hours post-maximum tension. The time of maximum tension was determined from the two control strips run at room temperature. The 2°C buffer was exchanged for room temperature buffer and the strips were attached to isometric transducers at room temperature to observe the ability of the strips to develop tension. A similar series of tests was done in which strips were attached to transducers at room temperature, 2°C buffer was added and after the initial "cold shortening" occurred the buffer was replaced with room temperature buffer for further observation of tension pattern.
These strips were designated as "0" time samples. A total of 15 birds was used so that each treatment contained data from 6 different birds and 12 different muscle strips.

**Experiment 2**

Six birds were used in this experiment. Birds were exsanguinated with restricted struggle and a sample of muscle was immediately frozen in LN₂. Twelve strips of muscle, proportional in size to strips used for tension measurement, were then prepared. Eight strips were placed in phosphate buffer at 2°C and after 3 minutes, four were removed, dried rapidly on a paper towel and frozen in LN₂. The other four were removed after 7 minutes and similarly frozen. The remaining 4 strips were placed in buffer at room temperature at the same time as the eight strips were placed in the cold buffer. These strips were removed after 7 minutes and similarly frozen. ATP, creatine phosphate and hexose monophosphate analyses were subsequently performed on the muscle samples according to the method of Lamprecht and Stein (1963).
RESULTS AND DISCUSSION

Isometric Tension Experiments.

The Effect of Environment on Tension Pattern

Ideally, isometric tension pattern should be studied on strips suspended in air, thereby minimizing external effects. The isometric tension pattern of several strips was studied by suspending strips in chambers maintained at a high relative humidity. These conditions, however, did not prevent the surface of the muscle strips from drying and spurious tension patterns were obtained. These findings are similar to those of Busch et al. (1972 a) who found that occasionally, surface dehydration occurred even at 95-98 percent relative humidity. These same authors also found that the isometric tension pattern of rabbit psoas muscle was identical whether the strips were suspended in air or in a saline buffer. On the basis of this finding, two different buffer systems and distilled water were used as liquid media and their effect on broiler muscle tension pattern was studied.

The tension patterns obtained in the Tris-acetate buffer were almost identical to those obtained in the phosphate buffer (Figure 1). Tension maximum was reached in 4.3 hours in both buffers compared to 8.3 hours in the distilled water. The main difference between the two buffer systems was in the tension decline. After 16 hours postmortem, strips in the Tris-acetate buffer had declined to about 60 percent of the
Figure 1. Effect of different extracellular incubation media on isometric tension pattern of broiler \textit{P. major} muscle.
maximum tension and were declining slowly. Strips in phosphate buffer had declined to about 45 percent and were continuing to show a rapid rate of decline. The Tris-acetate buffer appeared cloudy after 16 hours, whether or not sodium azide was added to the buffer, suggesting that some form of exchange may have occurred between the buffer and the muscle. This may explain why the rate of tension decline was levelling off at this point in time. In contrast, the phosphate buffer remained clear.

The tension pattern obtained in distilled water, as expected, was quite different from the patterns obtained in the two buffers. The tension developed much slower and after peaking, tension declined slowly until about 85 percent maximum tension was reached. Very little decline was observed beyond this point. This is probably due to a flow of soluble materials, in particular salt ions, from inside the muscle into the lower ionic strength water.

On the basis of the above findings, phosphate buffer was selected for use in all subsequent studies. Other points considered in making this selection were its ease and simplicity of preparation and its previous use by Jungk and Marion (1970) and Marion (1971) for tension studies on turkey muscle.

The Effect of pH on Tension Pattern

Average tension patterns for strips maintained in buffers of varying pH are reported in Figure 2. The values
Figure 2. Effect of extracellular pH on isometric tension pattern of broiler *P. major* muscle.
were obtained by averaging the hourly values for tension and time to maximum tension of 6 broilers. The patterns do not differ substantially in rate of tension development or decline. This method of obtaining a tension pattern does, however, give misleading values for both time to maximum tension (referred to as time henceforth) and the actual maximum tension developed. This arises through the fact that all birds do not reach maximum tension at the same time. The true mean values for tension and time are shown in Table 1. These values were obtained by averaging the actual values for tension and for time for the strips from the broilers. Paired comparison t-tests were done by pairing the pH 5.8, 6.3 and 6.8 strips individually, with the control (pH 7.2) strips.

### Table 1. Means and Standard Errors of Time and Tension Development for Strips of Broiler P. Major Muscle Run in Phosphate Buffer at Four Different pH Levels.

<table>
<thead>
<tr>
<th>pH</th>
<th>Time, hr</th>
<th>Tension, g/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>4.50 ± 0.61</td>
<td>40.26 ± 2.80</td>
</tr>
<tr>
<td>6.7</td>
<td>3.90 ± 0.63*</td>
<td>34.75 ± 3.92</td>
</tr>
<tr>
<td>6.3</td>
<td>3.60 ± 0.39*</td>
<td>27.73 ± 1.51 **</td>
</tr>
<tr>
<td>5.8</td>
<td>3.33 ± 0.22*</td>
<td>28.15 ± 2.08 *</td>
</tr>
</tbody>
</table>

* Significantly different from pH 7.2 (p<0.05).
** p<0.01
Time values for pH 6.7 and 7.2 are both approximately 4.3 hours when taken from the Figure 2. This compares to true values of 3.9 and 4.5 for pH 6.7 and 7.2 respectively. Similarly, peak tension values for pH 6.7 and 7.2 are 31 and 34 g/cm² from Figure 2 versus 34.75 and 40.26 respectively from Table 1.

A definite trend is evident from the pH data. As pH decreased so did the time required to reach maximum tension and amount of tension. The times for pH 5.8, 6.3 and 6.7 are significantly lower (p<0.05) than for the control, pH 7.2. The average tension of strips at pH 5.8 or 6.3 was significantly lower than at pH 7.2.

The results obtained here differ somewhat from those obtained by Busch et al. (1972a) who reported no change in the amount of tension in rabbit psoas muscle between pH 5.5 - 7.0. However, at pH 5.0, tension appeared to decrease. They also found a decrease in time required to reach maximum tension as the pH decreased from 7.0 to 5.0. These data were obtained at 37°C. When the experiment was conducted at 2°C, the opposite result was obtained i.e. decreasing pH from 7.0 to 5.0 increased the time required for maximum tension to develop.

The fact that the above data were obtained using rabbit muscle and that different temperatures gave different results make it difficult to draw analogies to the present data on broiler muscle which were obtained at approximately 25°C.
One similarity does exist between the two sets of data. Both show that decreasing the extracellular pH does not hinder the ability of muscle to develop or release isometric tension.

The fact that time and tension are altered by decreasing extracellular pH is difficult to explain. April et al., (1968) and Rome (1968) have shown that extracellular pH does not alter intracellular pH to any great extent but does change the selective permeability of the sarcolemma. This may or may not be the case in broiler muscle as it appears that in the lower pH buffers, anaerobic glycolysis is halted earlier than in control buffers, thus resulting in less tension and shorter time to maximum tension. If the observed phenomena were due to a change in sarcolemmal permeability then a greater change in the overall tension pattern would be expected, due to loss of ions and/or metabolites and co-factors vital to the contraction and relaxation phases of the tension pattern.

The Effect of Temperature on Tension Pattern

During post-slaughter handling poultry muscle encounters temperatures ranging from 60°C in scald tanks down to near 0°C during ice-slush cooling and aging. Because of this a study was initiated to determine the effect of temperatures within this range on postmortem isometric tension pattern. Temperatures between 5°C and 20°C were not studied because broilers are not exposed to this range during the pre-rigor
period and pass through it quite rapidly on subsequent cooling. The results of these studies on temperature effects are presented in Table II. The time values are reported as minutes instead of hours because of the rapid tension development in some of the treatments.

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Time, min</th>
<th>Tension, g/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.1 ± 0.2 **</td>
<td>65.37 ± 6.5 **</td>
</tr>
<tr>
<td>2</td>
<td>3.3 ± 0.2 **</td>
<td>38.23 ± 3.6 **</td>
</tr>
<tr>
<td>5</td>
<td>2.2 ± 0.2</td>
<td>17.24 ± 2.3</td>
</tr>
</tbody>
</table>

** Significantly different from 5°C (p<0.01)

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Time, min</th>
<th>Tension, g/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>276.7 ± 22.1</td>
<td>46.40 ± 2.0</td>
</tr>
<tr>
<td>30</td>
<td>217.8 ± 82.0</td>
<td>49.73 ± 6.5</td>
</tr>
<tr>
<td>37</td>
<td>116.5 ± 44.4 **</td>
<td>65.46 ± 11.7</td>
</tr>
<tr>
<td>43</td>
<td>49.3 ± 15.6 **</td>
<td>73.75 ± 9.4 **</td>
</tr>
<tr>
<td>50</td>
<td>8.1 ± 1.2 **</td>
<td>316.89 ± 19.3 **</td>
</tr>
<tr>
<td>60</td>
<td>0.5 ± -- **</td>
<td>290.18 ± 29.2 **</td>
</tr>
</tbody>
</table>

** Significantly different from 23°C (p<0.01)
The data for 0°, 2° and 5°C show definitely, that cold shortening \(^1\) occurs in broiler *p. major* muscle. In fact, the increasing effect of cold shortening is demonstrated within the temperature range from 0 - 5°C. DeFremery and Pool (1960) found that the rate of ATP decline in postmortem broiler muscle was faster at 0°C than at 10°C and minimal between 10 - 20°C. Smith *et al.* (1969) first demonstrated a cold shortening effect in avian muscle. They found that shortening at 0°C was significantly greater than in the 12 - 18°C range. The above results are in close accord with the present findings. The present data, however, demonstrated a more dramatic cold shortening effect that was reported by Smith *et al.* (1969). These authors found that shortening was essentially complete after 3 hours in broilers whereas the present study shows that shortening is virtually instantaneous and the tension developed is essentially all abated within 15 - 30 minutes after maximum development. The difference between these data probably reflect the fact that isometric tension as determined in the present experiment, is a much more sensitive technique than the measurement of length determined by Smith *et al.* (1969).

The fact that the time and tension are smallest at 5°C suggests also that a point of minimal tension development

---

\(^1\) Cold shortening in the literature refers to the actual length change which occurs in excised muscles subjected to low temperatures. In this thesis, however, cold shortening is used to describe the rapid tension development at 0 , 2 and 5°C.
is being approached as suggested by the data of deFremery and Pool (1960) and Smith et al. (1969). This cold temperature effect will be discussed further in a subsequent section of this thesis.

The time and tension data for temperatures of 23°C and above is consistent with an increase in glycolysis at higher temperatures. Though time tends to shorten and tension increase, with increasing temperature, no significant changes are apparent until 37°C is reached. The most striking results were observed at 50 and 60°C. These temperatures were studied because the outer layers of breast muscle could easily reach temperatures in this range during scalding. In view of the previously discussed toughening which occurs due to the scalding procedure, these data provide an insight as to why the toughening occurs.

The association between muscle contraction and tenderness has been well established (Herring et al., 1965 a; Marsh and Leet, 1966; Howard and Judge, 1968). The great increase in tension developed at 50 and 60°C (ca. 300 g/cm²) versus 23°C (ca. 50 g/cm²) suggests that, in the outer layers of broiler P. major, contraction would be maximal within a very short period of time. The increased glycolytic rate at these temperatures would result in a low pH. A combination of the low pH and high temperature could lead to denaturation of the highly contracted myofibrils thus preventing the normal
tenderization which occurs in postmortem muscle. Hamm (1966) showed that actomyosin solubility is greatly decreased by heating in the range from 40 - 60°C. Khan (1971) has shown that dephosphorylation of ATP at high temperatures affects the mode or extent of stiffening of the muscular tissue thus preventing tenderization. The present data indicates that the altered mode of stiffening could be the result of increased tension (contraction) developed at 50 - 60°C. This increased contraction would mean an increase in actomyosin formation which upon denaturation would result in the toughening observed in scalded poultry.

The combination of low pH and high temperature may also affect tenderness through alterations in connective tissue. Schaller and Powrie (1972) showed slight changes in connective tissue of broiler P. major due to heating at 60°C.

The Effect of Calcium, Magnesium and EDTA on Tension Pattern

Three different calcium concentrations were prepared and used in this study on broiler and fowl muscle. The time and tension data for broilers and the time data for the fowl are presented in Table III.

The presence of the 3 levels of calcium did not significantly affect the time to maximum tension for the broilers or fowl. The 10^{-3} M level did, however, significantly affect the amount of tension developed in the broiler muscle (p<0.01).
TABLE III. MEANS AND STANDARD ERRORS OF TIME AND TENSION DEVELOPMENT FOR STRIPS OF BROILER AND FOWL P. MAJOR MUSCLE RUN IN PHOSPHATE BUFFER AND BUFFER CONTAINING THREE LEVELS OF CALCIUM

<table>
<thead>
<tr>
<th>Calcium Concentration</th>
<th>Control</th>
<th>$10^{-3}M$</th>
<th>$10^{-4}M$</th>
<th>$10^{-7}M$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Broiler</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>4.18 ± 0.62</td>
<td>4.06 ± 0.50</td>
<td>4.04 ± 0.86</td>
<td>3.70 ± 0.72</td>
</tr>
<tr>
<td>Tension</td>
<td>51.71 ± 4.32</td>
<td>36.43 ± 4.63*</td>
<td>45.61 ± 4.09</td>
<td>54.89 ± 7.10</td>
</tr>
<tr>
<td><strong>Fowl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>9.63 ± 0.53</td>
<td>10.50 ± 0.62</td>
<td>9.10 ± 0.71</td>
<td>10.17 ± 0.67</td>
</tr>
</tbody>
</table>

**Significantly different from control (p<0.01)**

The most noticeable difference, with respect to broilers versus fowl, is the difference between the time to maximum tension. The time required in fowl is twice that of broilers which would indicate a much slower rate of anaerobic glycolysis in the post-mortem fowl or considerably higher initial levels of glycogen and ATP.

Although it is not possible to accurately compare tension values for fowl and broilers, it is possible to obtain a rough estimate. The cross-sectional area of the first 30 strips cut after preparing the correlation curve, when averaged, give an indication of the average size of muscle strips being prepared at that time. If this value is applied to the average
tension value for the fowl then approximately 40 g/cm² tension is obtained. This amount is in line with broiler control values.

The tension release data for control and 10⁻³ M calcium strips are shown in Figure 3 for broilers and Figure 4 for fowl. In both cases, the presence of 10⁻³ M calcium in the extracellular buffer caused a significant increase in the rate of tension release. In broiler muscle, 10⁻⁴ and 10⁻⁷ M calcium caused a slight but non-significant increase in rate of tension decline. The 10⁻⁴ M level in fowl produced a significant effect but 10⁻⁷ M was similar to the control.

There is an obvious difference in the rate of tension decline between broiler and fowl control strips. At 12 hours post-maximum tension, the broiler controls had declined to 40 percent of the maximum tension, whereas the fowl controls had declined to only 60 percent. This difference in tension release may be related to the observation that the Z-line in older animals is less labile than the Z-line in younger animals (Goll, 1970). This may further relate to the established fact that older poultry are less tender than broiler age poultry (May et al., 1962; Larmond et al., 1971).

The effect of calcium on tension development is not as clear cut as its effect on the rate of tension release. None of the levels tested affected the time to reach maximum tension and only the 10⁻³ M level decreased the amount of tension developed in broiler muscle. Since calcium is responsible for
Figure 3. Effect of $10^{-3}$ M calcium on tension decline in broiler P. major muscle. Circles and bars are means ± 1 standard error.
Figure 4. Effect of $10^{-3}$ M calcium on tension decline in fowl P. major muscle. Circles and bars are means ± 1 standard error.
contraction in vivo (Hasselbach, 1964), one must assume that the sarcolemma remains impermeable to extracellular calcium during most of the pre-rigor period. The observed decrease in the amount of tension developed in the presence of $10^{-3}$ M calcium suggests that a critical concentration may have been reached or exceeded thus promoting a more rapid penetration of the calcium into the muscle. Busch et al. (1972b) found that $10^{-3}$ M calcium reduced tension development in rabbit psoas muscle and suggested that a calcium stimulated process caused the loss of isometric tension and that the maximum tension developed represented a balance between tension development and loss of ability to maintain tension at any particular time. These authors isolated a "calcium activated sarcoplasmic factor" from rabbit muscle and demonstrated its ability to effect complete Z-line removal from rabbit muscle myofibrils in the presence of at least $10^{-3}$ M calcium. This critical calcium concentration is in accord with the tension release findings which show that the presence of $10^{-3}$ M calcium causes a marked increase in tension release while $10^{-4}$ M calcium does not differ substantially from the controls. Further data on the role of calcium in postmortem muscle has been provided by Davey and Gilbert (1969) and Haga et al. (1966). Haga et al. (1966) found that calcium ions promoted the extraction of actin from muscle. In this case, structural weakening within muscle could occur after rigor mortis since the sarcoplasmic reticulum loses its ability to sequester calcium at this time.
Davey and Gilbert (1969) suggested that calcium was necessary for the weakening of muscle structure since EDTA was found to stabilize muscle fine structure during aging. These findings are consistent with the enhanced tension release in the presence of $10^{-3}$ M calcium.

The events which are involved in loss of isometric tension (resolution of rigor) have been categorized (Goll, 1968) as loss of Z-line structure which leads to eventual rupture of the bonds between the I - Z filaments, and weakening of the actin-myosin interaction. Loss of Z-line structure has been demonstrated by several workers using muscle from different sources (Stromer and Goll, 1967, beef; Henderson et al., 1970 rabbit and porcine; Takahaski, et al., 1967, chicken). In most cases, however, this loss was demonstrated by blendorizing aged muscle pieces and examining the fragmented myofibrils. It is extremely difficult, using this technique, to determine if the resolution of rigor is due to breaks at the I - Z junction or loss of Z-line structure. However, it should be noted that loss of Z-line structure has also been demonstrated in situ,(Henderson et al., 1970).

Sayre (1969) found that the Z-line in chicken muscle, aged for 24 hours, was still intact. Upon blendorizing, the muscle appeared to fragment in the I-band region and not the A-band or Z-line regions. These results are in agreement with the results of Fukazawa et al. (1963) who found that breaks in myofibrils always took place in the I-band region. These data combined with the data of Takahaski et al. (1967) suggest that
resolution of rigor and tension release, in poultry muscle, may be due to weakening of the muscle structure at or near I-Z junction. If this junction is structurally weakened, then fragmentation procedures for myofibrillar preparation would rupture these weakened areas allowing Z-line material to diffuse away. This would give the appearance of Z-line disintegration during postmortem storage.

Busch et al. (1972 a, b) have shown that the presence of $10^{-3} M$ EDTA or EGTA (calcium chelators) in the extracellular buffer did not interfere with tension development in rabbit or porcine muscle. They did, however, prevent the release of tension for up to 48 hours post-maximum tension indicating that calcium had been successfully removed from its role in stimulating tension release.

The above findings were not observed in broiler muscle. A study of the effects of magnesium (necessary in contraction process as well as calcium), EDTA and equimolar combinations of calcium-EDTA and magnesium-EDTA on tension development and release is presented in Table IV and Figure 5. The presence of the above materials in the extracellular buffer did not substantially alter the time to reach maximum tension, further suggesting that the sarcolemma remains virtually impermeable to extracellular materials in early postmortem muscle. EDTA and equimolar concentrations of EDTA and calcium or magnesium significantly lower the amount of
Figure 5. Effect of $10^{-3}$ M magnesium, EDTA and calcium-EDTA on tension decline in broiler P. major muscle.
TABLE IV. MEANS AND STANDARD ERRORS OF TIME AND TENSION DEVELOPMENT FOR STRIPS OF BROILER P. MAJOR MUSCLE IN PHOSPHATE BUFFER AND BUFFER CONTAINING Mg++, EDTA, Ca++ + EDTA AND Mg++ + EDTA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, hr</th>
<th>Tension, g/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=11)</td>
<td>5.74 ± 0.76</td>
<td>51.25 ± 2.36</td>
</tr>
<tr>
<td>$10^{-3}$M Mg++ (n=5)</td>
<td>6.48 ± 1.21</td>
<td>41.62 ± 4.81</td>
</tr>
<tr>
<td>(6.44 ± 1.13)$^a$</td>
<td>(53.12 ± 3.79)</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$M EDTA (n=5)</td>
<td>4.70 ± 0.96</td>
<td>38.38 ± 1.17*</td>
</tr>
<tr>
<td>(5.39 ± 1.27)</td>
<td>(50.12 ± 3.79)</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$M Ca++ + $10^{-3}$M EDTA (n=5)</td>
<td>4.65 ± 0.96</td>
<td>32.57 ± 3.73*</td>
</tr>
<tr>
<td>(6.18 ± 1.48)</td>
<td>(49.22 ± 3.97)</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$M Mg++ (n=3)</td>
<td>4.56 ± 0.95</td>
<td>32.90 ± 0.95*</td>
</tr>
<tr>
<td>$10^{-3}$M EDTA (n=3)</td>
<td>5.77 ± 0.89</td>
<td>56.14 ± 5.40*</td>
</tr>
</tbody>
</table>

* Significantly different from control (p<0.05)

$^a$ Values within parentheses are averages for control strips run simultaneously with the various treatment strips and used in the paired comparison analyses.

Tension developed. Magnesium tended to lower tension somewhat but not significantly from control values.

The most unexpected finding in this study was the effect of these materials on the rate of tension release. EDTA, which prevents tension release in porcine and rabbit muscles, stimulates tension release in broiler P. major muscle. When equimolar concentrations of calcium or magnesium were added to
buffer containing $10^{-3}$ M EDTA an additive effect was observed on tension release (Figure 5). Only the release data for calcium-EDTA is shown because the magnesium-EDTA data were virtually the same. The release data for strips run in magnesium containing buffer, if superimposed on the calcium data in Figure 3, are again identical to calcium. The role of magnesium in tension release, though not verified, is probably somewhat different than that of calcium. Magnesium ions act as a plasticizer in muscle allowing actin and myosin to slip passively past each other. It is therefore possible, that the magnesium stimulates the dissociation of actomyosin resulting in an increased rate of tension decline.

The nature of the additive effects of calcium-EDTA and magnesium-EDTA is not known. Calcium and magnesium are both effectively chelated by EDTA (Blaedel and Meloche, 1963) with calcium forming a slightly more stable complex than magnesium. One possible explanation for the additive effect on tension release may be that the complex of EDTA-Ca or EDTA-Mg, which in this form is uncharged, may pass through the sarcolemma with greater ease than either of the ions when present singularly. Once inside the cell, other ions such as zinc, lead, iron, which displace calcium and magnesium in an EDTA complex, could bring about a release of calcium and magnesium ions thereby stimulating a more rapid rate of tension release. This explanation is purely speculative and a thorough examination of muscle strips, treated in the above manner, is warranted at the
electron microscopic level. This study is of particular impor-
tance in view of the unique transverse tubular system which
has been demonstrated in chicken pectoral muscle (Mendell,
1971).

The Effect of Processing Techniques on Tension Pattern and
Tenderness

Processing techniques have been shown to adversely
affect poultry tenderness by several workers (Shannon et al.,
1957; Pool et al., 1959; Wise and Stadelman, 1959, 1961). In
view of these findings, various processing techniques, singu-
larly and in combination, were applied to broilers postmortem
in order to study tension parameters in relation to tenderness
(shear value). The effects of the various treatments on tension
parameters from the two different levels of P. major muscle
are shown in Table V. Only the combination treatment of pre-
slaughter struggle, scalding and plucking produced a signifi-
cant difference in tension parameters between outside and inside
breast muscle.

Wise and Stadelman (1959) found that shear was
significantly related to the depth at which samples were taken,
to the temperature of the scald water and to the duration of
scald. Shear value were not determined for different muscle
depths, but based on the significant decrease in time to maxi-
mum tension, it is possible that treatment 5 could result in
significant differences between inner and outer layers.
TABLE V. MEANS AND STANDARD ERRORS OF TENSION PARAMETERS FOR INNER AND OUTER STRIPS OF BROILER P. MAJOR MUSCLE SUBJECTED TO VARIOUS POST-SLAUGHTER TREATMENTS.

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time, hr</th>
<th>Tension, g/cm²</th>
<th>Tension release&lt;sup&gt;b&lt;/sup&gt; at one hour, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 outer</td>
<td>3.9 ± 0.6</td>
<td>33.1 ± 1.7</td>
<td>21.3 ± 3.5</td>
</tr>
<tr>
<td>inner</td>
<td>3.5 ± 0.6</td>
<td>35.6 ± 1.7</td>
<td>26.1 ± 3.7</td>
</tr>
<tr>
<td>2 outer</td>
<td>2.7 ± 0.5</td>
<td>67.7 ± 6.7</td>
<td>25.0 ± 1.7</td>
</tr>
<tr>
<td>inner</td>
<td>3.0 ± 0.4</td>
<td>56.1 ± 2.7</td>
<td>20.7 ± 1.7</td>
</tr>
<tr>
<td>3 outer</td>
<td>2.7 ± 0.5</td>
<td>43.9 ± 4.4</td>
<td>21.4 ± 5.1</td>
</tr>
<tr>
<td>inner</td>
<td>3.1 ± 0.4</td>
<td>53.4 ± 3.7</td>
<td>19.0 ± 3.7</td>
</tr>
<tr>
<td>4 outer</td>
<td>2.2 ± 0.4</td>
<td>41.8 ± 4.1</td>
<td>28.1 ± 3.2</td>
</tr>
<tr>
<td>inner</td>
<td>2.1 ± 0.3</td>
<td>47.0 ± 3.9</td>
<td>28.4 ± 3.2</td>
</tr>
<tr>
<td>5 outer</td>
<td>1.9 ± 0.4*</td>
<td>65.6 ±10.5</td>
<td>29.5 ± 5.1*</td>
</tr>
<tr>
<td>inner</td>
<td>3.0 ± 0.3</td>
<td>55.0 ± 9.6</td>
<td>16.3 ± 2.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatments No. 1: Control - restricted struggle (n=15)

2: Struggle, pluck (n=5)

3: Restricted struggle, scald (n=6)

4: Struggle, scald (n=7)

5: Struggle, scald, pluck (n=8)

* Significantly different from inner strips (p<0.05)

<sup>b</sup> Amount of tension released at one hour post maximum tension.
The data for inner and outer strips combined and the shear values are presented in Table VI. On an overall basis, only treatment 4 produced a significant change in time to maximum tension. Treatments 2, 4 and 5 significantly increased the amount of tension developed compared to the control. The shear values indicate an additive response to the various processing techniques. The procedures which seem to have the greatest affect on the tenderness are free struggle at slaughter and scalding.

**TABLE VI.** MEANS AND STANDARD ERRORS OF POOLED TENSION PARAMETERS AND SHEAR VALUE DATA FOR INNER AND OUTER STRIPS OF BROILER P. MAJOR MUSCLE SUBJECTED TO VARIOUS POST-SLAUGHTER TREATMENTS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, hr</th>
<th>Tension, g/cm²</th>
<th>Tension Release at one hour, %</th>
<th>Shear Value, lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.7 ± 0.6</td>
<td>36.8 ± 1.5</td>
<td>23.7 ± 3.3</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>2.8 ± 0.4</td>
<td>61.9 ± 4.6**</td>
<td>22.8 ± 2.3</td>
<td>5.6 ± 0.3*</td>
</tr>
<tr>
<td>3</td>
<td>2.9 ± 0.5</td>
<td>48.6 ± 3.7**</td>
<td>20.2 ± 4.3</td>
<td>6.3 ± 0.6*</td>
</tr>
<tr>
<td>4</td>
<td>2.2 ± 0.3*</td>
<td>44.4 ± 3.9</td>
<td>28.3 ± 2.9</td>
<td>9.0 ± 0.5**</td>
</tr>
<tr>
<td>5</td>
<td>2.5 ± 0.6</td>
<td>59.8 ± 6.2**</td>
<td>22.9 ± 3.5</td>
<td>10.5 ± 0.9**</td>
</tr>
</tbody>
</table>

\( a \) For treatment breakdown see Table V.

\( b \) Amount of tension released at one hour post maximum tension.

* Significantly different from control (\( p < 0.05 \))

** \( p < 0.01 \)

The tenderness data are in accord with results found by other workers (Shannon et al., 1957; Pool et al., 1959; Wise and
The data also show that tension release does not correlate with shear value. All treatments showed first hour tension release values in the range of 20 - 28 percent and there were no significant differences between treatments and controls.

The fact that maximum toughening was observed for treatments involving scalding relates to the findings of the temperature studies discussed earlier. The tension values did not approach those found at 50 and 60°C because the outer layers of breast muscle, which may reach these temperatures, had a partially cooked appearance and were discarded.

It was noted during shear measurement that a sharp break in shear peak occurred after the blade had passed through the outer layer of the breast muscle demonstrating the existence of a toughened shell around the outer areas of breast muscle probably caused by high temperature-low pH denaturation.

Segregation of Broiler Controls on the Basis of Time to Reach Maximum Tension

It was noted during the course of the tension experiments that muscle strips in control buffer varied considerably in rate of tension release. There was a definite pattern between time to reach maximum tension and the proportion of tension released within 12 hours post-maximum tension. The data for control strips from 35 birds were segregated into three groups, on the basis of time required to reach maximum tension. The
means and standard deviations for the tension parameters of these three groups and for the pooled birds are presented in Table VII.

### TABLE VII. TIME AND TENSION MEANS AND STANDARD DEVIATIONS FOR THREE BROILER GROUPS SEGREGATED ON THE BASIS OF TIME TO REACH MAXIMUM TENSION.

<table>
<thead>
<tr>
<th>Group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time, min</th>
<th>sd&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Tension, g/cm&lt;sup&gt;2&lt;/sup&gt;</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n=10)</td>
<td>147.2**</td>
<td>30.3</td>
<td>47.74</td>
<td>15.65</td>
</tr>
<tr>
<td>II (n=15)</td>
<td>234.7</td>
<td>40.3</td>
<td>46.09</td>
<td>8.94</td>
</tr>
<tr>
<td>III (n=10)</td>
<td>473.1**</td>
<td>74.1</td>
<td>51.88</td>
<td>12.32</td>
</tr>
<tr>
<td>Pooled (n=35)</td>
<td>299.3</td>
<td>134.7</td>
<td>48.50</td>
<td>12.60</td>
</tr>
</tbody>
</table>

<sup>a</sup>Tension maximum for the three groups was observed between:
- Group I  - 0 - 3 hours postmortem
- Group II  - 3 - 6 hours postmortem
- Group III  - > 6 hours postmortem

sd Standard Deviation

** Significantly different from Group II (p<0.01)

Segregation on the above time basis gave three groups of birds which did not differ with regard to amount of tension developed but differed significantly from each other on the basis of the time required to reach maximum tension. The tension release data for the three groups are presented in Figure 6 and the regression line parameters for each Group are presented
Figure 6. Isometric tension decline in three groups of broilers separated on the basis of time required to reach maximum tension.
in Table VIII. It can be seen that the Group I birds, which reached maximum tension in less than 3 hours, released tension much more rapidly than did Groups II or III birds. Statistical t-test analysis of difference of means showed that Group I birds released tension at a faster rate (p<0.01) than Groups II and III birds. Groups II and III differed significantly from each other (p<0.05) only during the first 5 hours post-maximum tension.

<table>
<thead>
<tr>
<th>Group</th>
<th>Intercept</th>
<th>Slope</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n=10)</td>
<td>80.10</td>
<td>-5.33</td>
<td>0.711</td>
</tr>
<tr>
<td>II (n=15)</td>
<td>92.60</td>
<td>-4.56</td>
<td>0.776</td>
</tr>
<tr>
<td>III (n=10)</td>
<td>97.66</td>
<td>-4.54</td>
<td>0.849</td>
</tr>
<tr>
<td>Pooled (n=35)</td>
<td>90.47</td>
<td>-4.77</td>
<td>0.642</td>
</tr>
</tbody>
</table>

*a See footnote for Table VII.*

The tension pattern for Group I birds is almost identical to the release pattern observed when 10^{-3} M calcium was added to the buffer. It is possible that the rapid onset of rigor, as demonstrated by the short time to maximum tension, stimulated a more rapid or greater release of calcium from the sarcoplasmic reticulum once rigor had occurred.

The difference in release of tension was obvious at 1 hour post-maximum tension and it appeared possible to predict,
with some degree of accuracy, the 12 hour release from the 1 hour value. In order to test this hypothesis, the 1 hour release values for each group were correlated with the values for subsequent hourly values. The groups were analyzed individually and then the data were pooled and a general relationship was established. The results of these analyses are presented in Table IX. The data show that, for Group I birds,

**TABLE IX.** SIMPLE CORRELATIONS OF ONE HOUR TENSION RELEASE VALUES WITH SUBSEQUENT HOURLY VALUES FROM THREE GROUPS OF CONTROL BROILERS AND FOR THE POOLED DATA FROM THE THREE GROUPS.

<table>
<thead>
<tr>
<th>Time, hr</th>
<th>Group I&lt;sup&gt;a&lt;/sup&gt; (n=10)</th>
<th>Group II (n=15)</th>
<th>Group III (n=10)</th>
<th>Pooled (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>.958**</td>
<td>.825**</td>
<td>.564*</td>
<td>.950**</td>
</tr>
<tr>
<td>4</td>
<td>.904**</td>
<td>.596*</td>
<td>.475</td>
<td>.850**</td>
</tr>
<tr>
<td>6</td>
<td>.838**</td>
<td>.456</td>
<td>.480</td>
<td>.789**</td>
</tr>
<tr>
<td>8</td>
<td>.859**</td>
<td>.474</td>
<td>.224</td>
<td>.775**</td>
</tr>
<tr>
<td>10</td>
<td>.859**</td>
<td>.367</td>
<td>-.006</td>
<td>.731**</td>
</tr>
<tr>
<td>12</td>
<td>.901**</td>
<td>.306</td>
<td>-.088</td>
<td>.699**</td>
</tr>
</tbody>
</table>

<sup>a</sup> See footnote for Table VII.

* p<0.05

** p<0.01

there is a significant correlation (p<0.01) between 1 hour tension release and subsequent hourly values up to 12 hours post-maximum tension. The relationship is less pronounced for Groups II and III. This is probably due to the larger standard deviations
in time to maximum tension as demonstrated in Table VII. The correlation between 1 hour release and subsequent hourly values is significant ($p<0.01$) when the data for the 35 birds is pooled. This shows that one could obtain a reasonably accurate pattern of tension release by measuring only the 1 hour tension release.

The data presented in Table X show that the relationships between 2 hour values and subsequent hourly values are generally stronger than for the 1 hour data. The $r$ values for Groups II and III, in particular, are improved considerably as are the $r$ values for the pooled data.

TABLE X. SIMPLE CORRELATIONS OF TWO HOUR TENSION RELEASE VALUES WITH SUBSEQUENT HOURLY VALUES FROM THREE GROUPS OF CONTROL BROILERS AND FOR THE POOLED DATA FROM THE THREE GROUPS.

<table>
<thead>
<tr>
<th>Time, hr</th>
<th>Group I $^a$ (n=10)</th>
<th>Group II (n=15)</th>
<th>Group III (n=10)</th>
<th>Pooled (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>.965$^{**}$</td>
<td>.914$^{**}$</td>
<td>.866$^{**}$</td>
<td>.951$^{**}$</td>
</tr>
<tr>
<td>6</td>
<td>.889$^{**}$</td>
<td>.818$^{**}$</td>
<td>.767$^{**}$</td>
<td>.899$^{**}$</td>
</tr>
<tr>
<td>8</td>
<td>.873$^{**}$</td>
<td>.828$^{**}$</td>
<td>.689$^{**}$</td>
<td>.883$^{**}$</td>
</tr>
<tr>
<td>10</td>
<td>.845$^{**}$</td>
<td>.749$^{**}$</td>
<td>.506</td>
<td>.841$^{**}$</td>
</tr>
<tr>
<td>12</td>
<td>.828$^{**}$</td>
<td>.695$^{**}$</td>
<td>.414</td>
<td>.798$^{**}$</td>
</tr>
</tbody>
</table>

$^a$ See footnote for Table VII.

* $p<0.05$

** $p<0.01$
The Relation Between One Hour Tension Release, Time to Maximum Tension and Shear Value

Analyses were performed to establish if rate of tension release was significantly correlated to the time to reach maximum tension and if so to establish the relationship. The data from 150 strips were fitted to linear, logarithmic and hyperbolic models in order to determine the most suitable relationship for the data. It was found that the time to maximum tension and 1 hour tension release were linearly related. With percent relative tension (% RT) as the dependent variable and time as the independent variable, the following equation for the regression line was obtained: % RT = 61.74 + .408 Time, n= 150, R^2 = .462 (p<0.01).

Since tension release was more rapid when time to maximum tension was shortest and since 1 hour tension release was significantly related to release for subsequent hours (up to 12 hours post-maximum tension), it was decided to determine the relation between 1 hour tension release and the eventual tenderness observed in broilers. Linear, logarithmic and hyperbolic models were applied to the data for 1 hour tension release and shear values from 100 broilers. One hour tension release values ranged from 0 - 48 percent and shear values ranged from 2.5 to 14.3 pounds. No significant relationship was observed for any of the models studied, suggesting that the tension release observed in individual birds is not indicative of tenderness.
deFremery and Pool (1963) established that treatments which accelerate rigor mortis result in increased toughness (shear value) in broiler breast muscle. The present data on the relationship between shear value, time to maximum tension and one hour tension release raises some interesting questions with regard to the relationship between tension release in broiler *P. major* muscle and tenderness of the muscle. Birds which exhibit the most rapid rate of tension development also show the most rapid rate of decline even though, in most cases, the amount of tension developed does not differ significantly. The rapid tension development indicates accelerated rigor mortis which should lead to toughness according to deFremery and Pool (1963). This however, is not the case since a positive relationship exists between tension development and release and not between release and shear value.

Three possible reasons for the lack of a significant relationship between shear value and tension release are: sample error, the suitability of shear measurement for assessing overall muscle tenderness and the determination of shear values was done only at 24 hours postmortem. This 24 hour postmortem time may be long enough to allow a greater release of tension in the Group II and III birds thus bringing them more in line with Group I birds. It would be of interest to study the tensile and break strength parameters of uncooked muscle in relation to tension parameters and to study these parameters at a fixed time post-maximum tension for each bird. Perhaps in
this way a better idea of the relationship between tension release, the resolution of rigor mortis and tenderization may be obtained.

On the basis of the present data, one can conclude that variations in tension release, from bird to bird, are not indicative of the tenderness of the individual birds at 24 hours postmortem. The overall tension pattern for a pooled group of broilers is, however, indicative of the observed tenderization process in broilers. Most tenderization in P. major muscle of broilers occurs within a few hours post-rigor and during this period 50 percent or more of the isometric tension is released. The tenderization phenomenon in broilers is much more rapid than in pork and beef muscle, which show a slower tension development and decline. It may be concluded that the tension pattern is indicative of the tenderization phenomenon of a species but tension patterns for individuals within a species may or may not relate to the actual tenderness of the individual animal or muscle.

**Epinephrine Experiments**

**Preliminary Experiment**

This experiment was conducted to determine the approximate time when the effect of epinephrine injection was maximal in the broilers being used. deFremery and Pool (1963) and Sayre (1969, 1970) used a pre-slaughter injection time of 16 hours to
effect glycogen depletion in broilers, whereas deFremery (1966b) used 18 hours and Klose et al. (1970) 15 hours. Khan and Nakamura (1970) found that the epinephrine effect was maximal at 12 hours post-injection. The data obtained in this preliminary study are presented in Table XI. The data, though limited to one bird per time, suggest that the maximum effect of epinephrine occurs between 9 and 12 hours post-injection.

TABLE XI. TENSION PARAMETERS AND SHEAR VALUES FOR P. MAJOR MUSCLE FROM BROILERS INJECTED WITH EPINEPHRINE AT VARIOUS TIMES PRE-SLAUGHTER.

<table>
<thead>
<tr>
<th>Pre-slaughter Injection Time, hr</th>
<th>Time, min</th>
<th>Tension, g/cm²</th>
<th>Shear Value, lbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>399</td>
<td>54.85</td>
<td>4.06</td>
</tr>
<tr>
<td>3</td>
<td>213</td>
<td>41.96</td>
<td>4.15</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>94.90</td>
<td>6.58</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>157.70</td>
<td>7.01</td>
</tr>
<tr>
<td>12</td>
<td>43</td>
<td>96.72</td>
<td>7.40</td>
</tr>
<tr>
<td>15</td>
<td>92</td>
<td>99.36</td>
<td>4.62</td>
</tr>
<tr>
<td>18</td>
<td>49</td>
<td>87.93</td>
<td>6.53</td>
</tr>
</tbody>
</table>

The injections in this preliminary experiment were done intramuscularly into the P. major muscle. It was noted during subsequent tenderness measurements that shear values for muscle near the site of injection were negligible while values for areas away from the site of injection were generally higher
than control birds. In view of this localized epinephrine effect, injections for the subsequent experiments were done intramuscularly into the thigh muscle.

**Epinephrine Experiment 1**

The analysis of variance showed that there was a significant treatment effect for all parameters studied (Table XII). Treatment means and the results of Duncan's new multiple range test are presented in Table XIII. The tension and time data show an inverse relationship with maximum tension being developed in the shortest time. These values also indicate the effect of the epinephrine injections was maximal at 8 hours post-injection. This is somewhat earlier than the 12 hour value found by Khan and Nakamura (1970).

**TABLE XII. ANALYSIS OF VARIANCE FOR PARAMETERS STUDIED IN EPINEPHRINE EXPERIMENT 1.**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Tension</th>
<th>Time</th>
<th>Blood Lactate</th>
<th>Shear Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>2505.7</td>
<td>180.0</td>
<td>0.085**</td>
<td>9.28</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>11918.4**</td>
<td>32077.0**</td>
<td>0.044**</td>
<td>9.96*</td>
</tr>
<tr>
<td>SxT</td>
<td>5</td>
<td>461.0</td>
<td>7149.4</td>
<td>0.011</td>
<td>1.18</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>682.9</td>
<td>7389.5</td>
<td>0.011</td>
<td>3.23</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05

** p<0.01
A slight increase was found in blood lactate concentration in birds killed 2 hours after injection. This is probably due to the rapid breakdown of glycogen to lactic acid which then diffuses out of the muscle into the bloodstream. The lowest level of blood lactate was found in the 8 hour group where tension was maximum and time minimum.

Results of shear value analysis were contrary to most findings in that significant toughening was found instead of a tenderization. Khan and Nakamura (1970) found that muscle from broilers, injected with epinephrine 2 or 6 hours before slaughter, was more tender than control muscle after 24 hours of postmortem
storage. The present data shows no significant difference between control and 2 hour samples and significant toughening in 4, 8, 12 and 16 hour samples.

DeFremery and Pool (1963) and DeFremery (1966b) found that elimination of postmortem glycolysis by epinephrine injections gave chicken meat that was tender immediately post-slaughter. These authors, however, did not compare the effect on tenderness after 24 hours of aging.

In order to determine the relationship between tension, time, blood lactate and shear value, a simple correlation analysis was run on the data. The correlation analysis is presented in Table XIV.

**TABLE XIV. CORRELATION MATRIX FOR PARAMETERS STUDIED IN EPINEPHRINE EXPERIMENT 1.**

<table>
<thead>
<tr>
<th></th>
<th>Tension</th>
<th>Time</th>
<th>Blood Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td>.711**</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood Lactate</strong></td>
<td>.315</td>
<td>.196</td>
<td></td>
</tr>
<tr>
<td><strong>Shear</strong></td>
<td>.611**</td>
<td>.454**</td>
<td>.331*</td>
</tr>
</tbody>
</table>

*p<0.05

**p<0.01

As expected, the regression analysis showed significant relationships between the tension developed, the time to maximum tension and shear values. Blood lactate level was significantly related to shear value (p<0.05).
The analysis of variance (Table XII) showed that all parameters, except blood lactate, failed to show a significant sex effect. The male broilers were significantly higher than females with control levels being 0.44 and 0.32 m Moles/100mls respectively. The effect was particularly noticeable in the 2 hour treatments where males averaged 0.63 m Moles/100mls versus 0.39 m Moles/100mls for females.

On the basis of experiment, it was decided to repeat the experiment and in addition, collect data for muscle ATP, glycogen, pH and lactate. The times of sampling were changed somewhat to try to better define the time of maximal epinephrine effect.

Epinephrine Experiment 2

The analysis of variance is presented in Table XV and treatment means and the result of Duncan's new multiple range test are presented in Table XVI. As in Experiment 1, the 8 hour pre-slaughter injection time gave the maximal effect. However, in all cases, there were no significant differences between 8, 10 and 12 hour pre-slaughter injections. It would appear from this that the time of maximum effect extends over at least a 4 hour range and that the number of birds used in the test was not sufficient to establish a more exact time.

Muscle glycogen level, which indicates the extent of the epinephrine effect, was minimum at 8 hours and slightly higher at
TABLE XV.  ANALYSIS OF VARIANCE OF PARAMETERS STUDIED IN EPINEPHRINE EXPERIMENT 2.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Tension</th>
<th>Time</th>
<th>ATP</th>
<th>Glycogen</th>
<th>Blood Lactate</th>
<th>Tissue Lactate</th>
<th>pH</th>
<th>Shear Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>953.3</td>
<td>9184.0</td>
<td>24.1*</td>
<td>13.3**</td>
<td>0.006</td>
<td>34.0</td>
<td>0.06</td>
<td>24.6**</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>7920.8**</td>
<td>43361.0**</td>
<td>12.8*</td>
<td>10.7**</td>
<td>0.015</td>
<td>539.9</td>
<td>0.71</td>
<td>9.7**</td>
</tr>
<tr>
<td>SxT</td>
<td>5</td>
<td>692.3</td>
<td>682.1</td>
<td>1.9</td>
<td>3.8</td>
<td>0.027</td>
<td>124.5</td>
<td>0.09</td>
<td>2.2</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1122.2</td>
<td>4070.6</td>
<td>4.7</td>
<td>1.1</td>
<td>0.033</td>
<td>66.2</td>
<td>0.07</td>
<td>2.2</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05
** p<0.01
TABLE XVI. DUNCAN'S NEW MULTIPLE RANGE TEST ON SIGNIFICANT TREATMENT MEANS FROM EPINEPHRINE EXPERIMENT 2.

<table>
<thead>
<tr>
<th>Post-Injection Slaughter Time, hours</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension, g/cm²</td>
<td>40.19a</td>
<td>78.47a</td>
<td>128.35b</td>
<td>123.15b</td>
<td>127.20b</td>
<td>76.44a</td>
</tr>
<tr>
<td>Time, min</td>
<td>265.2</td>
<td>87.5a</td>
<td>45.2a</td>
<td>46.1a</td>
<td>50.5a</td>
<td>119.0a</td>
</tr>
<tr>
<td>ATP μ Moles/g</td>
<td>5.79ab</td>
<td>5.56ab</td>
<td>3.67b</td>
<td>3.76b</td>
<td>3.10b</td>
<td>6.76a</td>
</tr>
<tr>
<td>Glycogen, mg/g</td>
<td>3.88</td>
<td>1.18a</td>
<td>0.27a</td>
<td>0.45a</td>
<td>0.67a</td>
<td>1.52a</td>
</tr>
<tr>
<td>Slaughter pH</td>
<td>6.04a</td>
<td>6.19a</td>
<td>6.77b</td>
<td>6.79b</td>
<td>6.62b</td>
<td>6.11a</td>
</tr>
<tr>
<td>Tissue Lactate, μ Moles/g</td>
<td>45.0a</td>
<td>45.2a</td>
<td>28.2b</td>
<td>24.6b</td>
<td>30.8b</td>
<td>44.2a</td>
</tr>
<tr>
<td>Shear Value, lbs</td>
<td>4.57a</td>
<td>4.69a</td>
<td>6.51b</td>
<td>7.27b</td>
<td>6.84b</td>
<td>4.53a</td>
</tr>
</tbody>
</table>

*Means in the same row with similar superscripts do not differ significantly (p<0.05)

10 and 12 hours and by 24 hours had recovered to one half of the control level. It is of interest to note that the ATP level remained quite high throughout the entire time period studied. Since ATP is needed for shortening, this observation is consistent with the development of considerable tension in the 8, 10 and 12 hour muscle strips. There was very little glycogen present in the muscles at these time periods, therefore, little
postmortem glycolysis. Consequently, the time to maximum tension was very short even though tension was high.

The pH and tissue lactate values follow a pattern similar to glycogen and time to maximum tension. The control, 4 and 24 hour levels were similar and lactate dropped while pH rose in the 8, 10 and 12 hour samples.

The relationship between shear values and the significant treatment parameters is shown in Figures 7, 8 and 9. Shear value has a positive relationship with tension and pH and negative with glycogen, time to maximum tension, tissue lactate and ATP. It is of interest to note that no significant blood lactate effect was obtained in this experiment.

A simple correlation analysis was performed on the data from the second experiment and the correlation matrix is presented in Table XVII. It can be seen that there are significant correlations between all parameters except blood lactate. It is difficult to explain the difference between blood lactate values from experiments 1 and 2.

The analysis of variance (Table XV) shows that, similar to experiment 1, there was no sex difference for tension and time. Sex differences were observed for glycogen, ATP and shear value. The significant sex effect for shear value was not observed in experiment 1, however, shear values for females were consistently higher than for males. The shear value average for control males was 4.4 pounds versus 4.8 pounds for females. In the 10 hour group where shear values were maximum, the males averaged 5.8 pounds to 8.8 pounds for the females.
Figure 7. Relation between tension, time to maximum tension and shear value in P. major muscle from epinephrine treated broilers.
Figure 8. Relation between muscle glycogen and ATP levels and shear value in *P. major* from epinephrine treated broilers.
Figure 9. Relation between pH, muscle lactate and shear value in *P. major* muscle from epinephrine treated broilers.
<table>
<thead>
<tr>
<th></th>
<th>Tension</th>
<th>Time</th>
<th>ATP</th>
<th>Glycogen</th>
<th>pH</th>
<th>Blood Lactate</th>
<th>Tissue Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>.781**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>.705**</td>
<td>.587**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>.542**</td>
<td>.689**</td>
<td>.437**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>.731**</td>
<td>.598**</td>
<td>.631**</td>
<td>.475*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Lactate</td>
<td>.068</td>
<td>.010</td>
<td>.100</td>
<td>.115</td>
<td>.049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue Lactate</td>
<td>.610**</td>
<td>.466**</td>
<td>.479**</td>
<td>.404*</td>
<td>.816**</td>
<td>.149</td>
<td></td>
</tr>
<tr>
<td>Shear Value</td>
<td>.762**</td>
<td>.570**</td>
<td>.826**</td>
<td>.454**</td>
<td>.770**</td>
<td>.020</td>
<td>.647**</td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01
The greatest sex difference in glycogen levels was found in the control birds where values of 6.07 and 1.70 mg/g tissue were found for males and females respectively. In spite of this great difference in control levels, both males and females showed minimum glycogen levels (0.32 and 0.23 mg/g tissue respectively) at 8 hours. The ATP levels for females remained lower than for males throughout all of the treatment times. Both levels did, however, approach equality at 8 hours where the males averaged 3.8 and females 3.5 μ Moles/g tissue. After 8 hours the ATP level in males began rising while the level in females continued to drop reaching a minimum of 1.6 μ Moles/g tissue at 12 hours.

In general the sex differences seem to be related to differences in levels present in control birds. It also appears as if females may react somewhat differently to epinephrine than do males. This is particularly noticeable in the ATP and tenderness data.

The data from all the epinephrine experiments, including the preliminary experiment, show that depletion of glycogen induces toughness in broilers examined at 24 hours postmortem. This is in contrast to the data of Khan and Nakamura (1970) but the results obtained for the various parameters show why toughness may be expected.

Sayre (1969) found that shear values from chickens, injected pre-slaughter with epinephrine, did not change appre-
ciably during the aging period. He suggested that this muscle becomes inextensible quickly due to lack of ATP, but due to the high pH and possible integrity of the endoplasmic reticulum, there may be no great stimulus for contraction or tension development. The present data shows that this is not so. There is ample ATP remaining in the muscle, even when glycogen has been essentially depleted, and considerable tension is developed. The fact that the muscle becomes inextensible quickly is verified by the present data which demonstrated that the time required to reach maximum tension decreased to 45 minutes when the epinephrine effect was maximal. The fact that the epinephrine injected birds develop a great deal of tension post-mortem may explain why the toughening was observed. This ability to develop tension may also explain the observed tenderness if epinephrine treated birds are cooked without aging. Marsh and Leet (1966) found in beef neck muscle that 20 percent contraction was associated with a "fair" degree of tenderness; between 20 - 40 percent contraction was associated with a rapid decrease in tenderness to a minimum and between 40 - 60 percent contraction gave increased tenderness approximating that observed at 20 percent contraction.

Although most workers have found an increase in tenderness in epinephrine treated birds, Klose et al. (1970) did not find the expected lower shear values. They questioned the ability of epinephrine to deplete muscle glycogen but Cori
and Cori (1928) have shown that under proper conditions, this
technique depletes muscle glycogen prior to slaughter. Further­
more, the present data and that of Khan and Nakamura (1970) show
clearly that glycogen levels are depleted by pre-slaughter
epinephrine injections.

The epinephrine experiments were carried out to study
the response of the broiler under a severe stress situation.
An apparent adverse reaction, in the form of tougher muscle,
resulted from these studies and because females appeared to
react more adversely than males all subsequent stress experi­
ments except for the "commercial stress", were applied to females
only.

_Stress Experiments_

The results from the study on broilers, exposed to
antemortem commercial handling,(Table XVIII) show that there
are no significant differences between "stressed" and control
birds for any of the parameters studied except time required to
reach maximum tension. The "stressed" females required signi­
ficantly longer (p<0.05) than their control counterparts to
reach maximum tension. Though males showed the same trend, the
difference was not significant. On a combined sex basis
"stressed" birds were significantly different from controls
(p<0.05). For the most part, the differences between control
and "stressed" males are negligible. Although only the time
difference is significant in the females, most other parameters
### TABLE XVIII. MEANS AND STANDARD ERRORS OF PARAMETERS OF P. MAJOR MUSCLE FROM BROILERS IN THE COMMERCIAL STRESS EXPERIMENT

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
<th>Combined Sexes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stress</td>
<td></td>
<td>Stress</td>
<td></td>
<td>Stress</td>
<td></td>
</tr>
<tr>
<td>Time, hr</td>
<td>4.81±0.7*</td>
<td>3.02±0.7</td>
<td>6.25±1.2</td>
<td>4.49±0.4</td>
<td>5.53±0.7*</td>
<td>3.76±0.4</td>
</tr>
<tr>
<td>Tension, g/cm²</td>
<td>44.3 ±3.4</td>
<td>35.3 ±2.8</td>
<td>39.8 ±1.3</td>
<td>40.7 ±5.3</td>
<td>42.0 ±1.8</td>
<td>38.0 ±3.0</td>
</tr>
<tr>
<td>Shear Value, lbs</td>
<td>4.60±0.4</td>
<td>4.05±0.3</td>
<td>4.05±0.3</td>
<td>4.88±0.3</td>
<td>4.33±0.3</td>
<td>3.97±0.2</td>
</tr>
<tr>
<td>ATP, μMoles/g</td>
<td>7.98±0.5</td>
<td>5.92±0.8</td>
<td>8.48±0.9</td>
<td>8.46±0.9</td>
<td>8.23±0.4</td>
<td>7.19±0.7</td>
</tr>
<tr>
<td>pH</td>
<td>5.99±0.05</td>
<td>5.77±0.09</td>
<td>5.92±0.07</td>
<td>5.93±0.05</td>
<td>5.95±0.04</td>
<td>5.85±0.06</td>
</tr>
</tbody>
</table>

* Significantly different from corresponding control (p<0.05)
approached significance at the p<0.05 level. The most striking of these differences is the difference in muscle ATP levels. These data suggest a possible sex related difference in resistance or adaptation to commercial handling conditions.

The results for female broilers subjected to a thermal stress prior to slaughter (Table XIX) suggest that the birds were affected by the heat treatment and that the effect was intensified by allowing the birds to struggle freely during slaughter. The time to maximum tension was not shortened significantly by the heat treatment alone but the free struggle at slaughter apparently accelerated glycolysis so that the time difference was significant (p<0.05). The amount of tension was increased significantly by both "stress" treatments. The significant differences between "stressed"-free struggle birds and control birds for time to maximum tension and 1 hour tension release are consistent with data reported earlier on the effects of commercial processing techniques on tension parameters. They also confirm the lack of relationship between tension release and tenderness within the range of parameters studied.

The results for the "cold stress" experiment are presented in Table XX. As in the commercial stress experiment, there was a lengthening of the time required to reach maximum tension. This effect was accompanied by a tendency for tension, shear value, ATP and glycogen concentration to be high and hexose monophosphate to be lower in "cold-stressed" birds.
TABLE XIX. MEANS AND STANDARD ERRORS OF PARAMETERS OF P. MAJOR MUSCLE FROM BROILERS IN THE HEAT STRESS EXPERIMENT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control, RS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Stress, RS</th>
<th>Stress, S&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, hr</td>
<td>4.77 ± 1.1</td>
<td>3.12 ± 0.6</td>
<td>2.14 ± 0.2&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tension, g/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>38.2 ± 1.7</td>
<td>48.1 ± 4.1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>52.6 ± 4.2&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shear Value, lbs</td>
<td>5.06 ± 0.4</td>
<td>5.50 ± 0.5</td>
<td>5.10 ± 0.4</td>
</tr>
<tr>
<td>ATP, μ Moles/g</td>
<td>7.26 ± 1.0</td>
<td>6.11 ± 0.6</td>
<td>5.69 ± 0.7</td>
</tr>
<tr>
<td>pH, &quot;0&quot; hr</td>
<td>6.14 ± 0.04</td>
<td>6.05 ± 0.05</td>
<td>5.96 ± 0.03&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH, &quot;24&quot; hr</td>
<td>5.64 ± 0.03</td>
<td>5.61 ± 0.02</td>
<td>5.68 ± 0.03</td>
</tr>
<tr>
<td>1 Hour b Tension Release, %</td>
<td>22.6 ± 2.3</td>
<td>33.4 ± 4.0</td>
<td>38.5 ± 2.3&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>RS - Restricted struggle at slaughter  
<sup>b</sup>S - Free struggle at slaughter  
<sup>*</sup> Significantly different from control (p<0.05)  
<sup>**</sup> p<0.01  
<sup>b</sup> Amount of tension released at one hour post maximum tension.

The birds subjected to 2°C for 2 hours were significantly tougher (p<0.05) than control birds. Although the values do not differ significantly in most cases, the cold stress effect appeared to be maximal at 2 hours.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Stress 2 hr at 2°C</th>
<th>Stress 6 hr at 2°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, hr</td>
<td>5.59 ± 0.5</td>
<td>7.73 ± 0.8*</td>
<td>8.29 ± 1.2</td>
</tr>
<tr>
<td>Tension, g/cm²</td>
<td>49.06 ± 3.3</td>
<td>51.99 ± 3.2</td>
<td>46.86 ± 4.4</td>
</tr>
<tr>
<td>Shear Value, lbs</td>
<td>5.91 ± 0.3</td>
<td>6.97 ± 0.4*</td>
<td>6.11 ± 0.3</td>
</tr>
<tr>
<td>ATP, μ Moles/g</td>
<td>6.08 ± 0.9</td>
<td>6.66 ± 0.4</td>
<td>6.34 ± 0.5</td>
</tr>
<tr>
<td>Glycogen, mg/g</td>
<td>4.53 ± 0.4</td>
<td>5.60 ± 0.6</td>
<td>5.04 ± 0.7</td>
</tr>
<tr>
<td>HMPa</td>
<td>6.96 ± 0.3</td>
<td>6.12 ± 0.5</td>
<td>6.42 ± 0.5</td>
</tr>
</tbody>
</table>

*a HMP - Hexose Monophosphate

* Significantly different from control (p<0.05)

The most surprising effect in the "cold stress" study was the effect on time to maximum tension. The 6 hour birds had the longest time to maximum tension but since the variation was large, the values did not differ significantly from the controls. Three of the nine birds in this group, however, took from 11 - 12 hours to reach maximum tension. This is a relatively high incidence when compared to the fact that only 1 broiler, in over 100 control broilers studied in this project,
required as long as 12 hours to reach maximum tension.

The most important agents which have been proven to be stressors to the fowl are temperature extremes, handling, shaking, food and/or water deprivation and debeaking, (Freeman, 1971). In the initial "alarm phase", which results after exposure to a stressor, adrenalin or epinephrine is released from the adrenal medulla causing the passage of potassium from the muscles to the blood and the breakdown of liver and muscle glycogens to glucose and lactic acid (Lawrie, 1966).

If the effect of the stressor were severe enough, one would expect alterations in postmortem muscle due to depletion of muscle glycogen. The results from the epinephrine experiments, reported earlier in the thesis, verify this effect. The results from the stress experiments, however, are somewhat ambiguous and varied. This may be due, in part, to the fact that none of the stressors studied was severe enough to elicit the response observed by the actual injection of epinephrine.

The data from the "commercial stress" and "cold stress" experiments show a different trend than the data from the "heat stress" experiment. These findings are in accord with Seigel (1971) who found that different environmental stimuli produced evidence of "stress response" in birds but often several criteria of response had to be evaluated since responses to a particular stimulus may contradict or mask a single response. Such masking or contradiction of responses
may be of particular importance in studying a so-called "commercial stress" which involves several potential stressors (heat, cold, handling, crowding).

The apparent lengthening of the time to reach maximum tension observed in the "commercial and cold stress" experiments suggests that glycogen levels have not been disturbed and that the period of postmortem glycolysis has, in some manner, been lengthened. This effect has not been previously observed in broilers and is difficult to explain. Lawrie (1966) suggested that the difference in bovine response to pre-slaughter fasting or exercise, as compared to other species, may relate to the greater capacity of the ruminant muscle to gain energy by direct catabolism of fatty acids, thereby conserving carbohydrate. The possibility that poultry may be able to elicit this response, under certain stress situations, cannot be overlooked.

The commercial implications of such a phenomenon is obvious. The study on commercial processing techniques, reported earlier, showed that postmortem muscle parameters were significantly altered by the various procedures. It is therefore evident, that any change which affected postmortem glycolysis would be magnified by the processing techniques. In the case of the "cold and commercial stressed" birds, the apparent increase in time to maximum tension should have a beneficial effect on the ultimate muscle quality. Conversely,
the shortened time to maximum tension, observed in "heat stressed" birds would be magnified by the processing procedures and the muscle quality should be adversely affected.

Bovine, ovine and porcine species when subjected to stressors prior to slaughter, usually have lower muscle glycogen, higher postmortem pH, increased water holding capacity and improved tenderness and juiciness (Hedrick, 1965). However, under certain conditions postmortem quality is adversely affected in bovine and porcine muscles. This is evidenced by the occurrence of "dark cutting" beef and "pale, soft, exudative" pork which have been studied by several workers (Lawrie, 1958, 1966a, b; Sayre et al., 1963a, b; Lister et al., 1970)

The only adverse quality attribute observed during the study of avian muscle was the slight increase in shear value in the 2 hour cold stressed birds. This may be related to the fixed 24 hour aging period given all birds post-slaughter rather than the actual cold treatment. Control birds reached maximum tension at 5.6 hours post-slaughter whereas the 2 hour birds peaked at 7.7 hours post-slaughter. This shows that the 2 hour birds would have, on the average, a shorter aging time than the control birds.

**Cold Shortening Studies**

During the study of the effects of temperature on isometric tension pattern, a cold shortening phenomenon was observed which appeared to increase in intensity at 5°C down
to 0°C. The effect of this shortening on the ability of muscle strips to develop further tension is demonstrated in Table XXI. The 15 broilers studied in this experiment developed an average tension of 30.9 g/cm$^2$ within 3 minutes at 2°C. When this tension was developed through cold shortening, it was released almost as rapidly as it developed so that in most cases the strips had returned to the original starting point within 15 minutes after attachment to the transducers.

**TABLE XXI. MEANS AND STANDARDS ERROR OF TENSION PARAMETERS OF BROILER P. MAJOR MUSCLE SUBJECTED TO VARIOUS POST-SLAUGHTER TEMPERATURE TREATMENTS.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tension, g/cm$^2$</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature, control</td>
<td>61.31 $\pm$ 2.7</td>
<td>331.67 $\pm$ 23.5</td>
</tr>
<tr>
<td>Cold Shortening, 2°C</td>
<td>30.94 $\pm$ 5.9</td>
<td>2.93 $\pm$ 0.3</td>
</tr>
<tr>
<td>&quot;0&quot; time$^a$</td>
<td>43.57 $\pm$ 8.1</td>
<td>261.83 $\pm$ 42.0</td>
</tr>
<tr>
<td>12 hours PMT$^b$</td>
<td>12.62 $\pm$ 0.03</td>
<td>63.16 $\pm$ 11.8</td>
</tr>
<tr>
<td>24 hours PMT</td>
<td>7.93 $\pm$ 0.03</td>
<td>102.67 $\pm$ 35.5</td>
</tr>
<tr>
<td>36 hours PMT</td>
<td>1.77 $\pm$ 0.8</td>
<td>40.5 $\pm$ 10.3</td>
</tr>
</tbody>
</table>

$^a$"0" time refers to strips placed in buffer at 2°C to observe the cold shortening effect then immediately subjected to buffer at room temperature to observe further tension development.

$^b$These three times refer to muscle strips held in buffer at 2°C for various lengths of time post-maximum tension (PMT), then placed in buffer at room temperature to observe tension development. The time to maximum tension for these strips was determined from corresponding control strips run at room temperature.
When strips were allowed to shorten at 2°C, then brought up to room temperature by changing the buffer, they still developed a considerable amount of tension during the course of a normal tension pattern. The time to reach maximum tension was about one hour shorter than for control strips and the amount of tension was only about two-thirds that of the controls. If the tension developed on cold shortening is added to the subsequent tension developed at room temperature, then the total exceeds that developed by controls.

Holding muscle strips in buffer at 2°C for periods of 12, 24 and 36 hours demonstrated a decreasing ability of the strips to develop isometric tension subsequently. At 36 hours post-maximum tension, the strips had essentially lost their ability to develop tension. This indicates that glycolysis had continued in the strips at 2°C but the rate was probably too slow to effect a tension development, other than the initial cold shortening tension, at this temperature.

The mechanism of the cold shortening phenomenon is somewhat obscure. Locker and Nagyad (1963) showed that cold shortening, in beef muscle, started within a few minutes of commencement of cooling and was almost complete within one hour. Smith et al. (1969) demonstrated a cold shortening effect in turkey and chicken P. major and found that shortening was maximal at 0°C. This shortening was essentially complete after 3 hours in chickens and 5 hours in turkeys. The variation between
these studies and the present studies on broiler P. major may be due to the use of small strips of muscle in a buffer at 2°C as opposed to whole muscles cooled in air at 0°C. The temperature equilibrium would be attained much faster in the strips of muscle thereby accounting for the more rapid shortening observed.

The toughening, as a result of cold shortening, described by Newbold and Harris (1972) and Marsh (1972) is not likely to occur in poultry muscle. This is evident through the ability of the strips to relax to initial levels immediately after the cold shortening has reached its peak.

In order to better understand the nature of the cold shortening, a second study was initiated to measure the effect of cold shortening on some of the energy rich phosphate compounds in muscle. The effect of cold shortening on the ATP concentration is of particular interest since shortening occurs only when ATP is present in the muscle. The results of this study are shown in Table XXII.

The ATP levels were lowered somewhat by subjecting strips of muscle to buffer at 2°C for 3 or 7 minutes before freezing in liquid nitrogen for analysis. A lower ATP level was also evident in muscle strips held in buffer at room temperature for 7 minutes. Significant differences between cold-treated and control strips were found but cold-treated strips did not differ from strips held for 7 minutes in buffer at room tempera-
ture. This lack of a significant decrease in ATP levels is in accord with Busch et al. (1967) who observed only a small change in ATP level during large tension development at 2°C. It is evident, however, that a significant amount of ATP need not be hydrolyzed to effect the cold shortening. This is more plausible when one considers that a normal tetanic contraction in muscle is capable of developing 2 - 3 kg tension/cm² muscle (Huxley, 1958). The 30.9 g/cm² obtained during cold shortening represents only about 1 percent of the potential tetanus, therefore ATP levels may not change significantly. The presence of adequate levels of ATP subsequent to shortening would explain why the muscle is able to relax immediately after cold shortening since ATP also acts as a plasticizer in muscle allowing actin and myosin to slide freely past each other.

**TABLE XXII.** MEANS AND STANDARD ERRORS OF THE ATP, HMP AND CP CONTENT OF BROILER P. MAJOR MUSCLE SUBJECTED TO VARIOUS TEMPERATURE TREATMENTS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HMP</th>
<th>ATP</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.95 ± 0.68</td>
<td>9.23 ± 0.64</td>
<td>2.02 ± 0.4</td>
</tr>
<tr>
<td>3 min at 2°C</td>
<td>4.48 ± 0.56**</td>
<td>8.40 ± 0.49</td>
<td>2.23 ± 0.24</td>
</tr>
<tr>
<td>7 min at 2°C</td>
<td>4.17 ± 0.52**</td>
<td>8.06 ± 0.51*</td>
<td>2.52 ± 0.25</td>
</tr>
<tr>
<td>7 min at 25°C</td>
<td>5.35 ± 0.36</td>
<td>8.56 ± 0.55</td>
<td>2.44 ± 0.21</td>
</tr>
</tbody>
</table>

Abbreviations used are: HMP - hexose monophosphate
ATP - adenosine triphosphate
CP - creatine phosphate

* Significantly different from control (p<0.05)

** p<0.01
Marsh (1966) postulated that cold shortening, like thaw rigor, resulted from inactivation of a relaxing factor by calcium release, this being the result of a salt "flux". Such a salt "flux" may be explained in terms of the relative rates of diffusive and chemical processes since the temperature coefficient of diffusion is considerably lower than that of chemical reaction.

By applying the above postulation to the cold shortening phenomenon observed in broiler muscle, it is possible to suggest a sequence of events occurring in the muscle when subjected to cold buffer. A salt "flux" of calcium ions, released from the sarcoplasmic reticulum (SR), would stimulate the myofibrillar adenosine triphosphatase (myosin) to split ATP, thereby effecting shortening or contraction. The amount of contraction would be proportional to the concentration of the salt "flux" which apparently varies substantially between 5°C and 0°C. The salt "flux" may be a temporary phenomenon resulting from the "cold shock" and it would appear that after having lost the ability to sequester calcium, the SR again becomes operable. The calcium pump reverses and calcium is removed from the myofibrils and sequestered by the SR. Since there is still a considerable amount of ATP present to act as a plasticizer, the muscle relaxes to its original rest length. If held at or near 0°C, the strips maintain a slow rate of glycolysis which is not rapid enough to effect a further tension development. After 36 hours, at or near 0°C, sufficient glycolysis has occurred within the muscle
strips to eliminate any further ability to develop tension even if the strips are brought to room temperature.

The decrease in hexose monophosphate levels may result from an increased phosphorylation of fructose-6-phosphate to fructose-1, 6-diphosphate which is catalyzed by phosphofructokinase. This however, is speculative and warrants further study as does the apparent increase in creatine phosphate levels.
SUMMARY AND CONCLUSIONS

The effects of buffer, pH, temperature and various ions were studied in relation to tension development and decline in broiler \textit{P. major} muscle. A tension pattern, similar to those reported for turkey, porcine, bovine and rabbit muscle, was observed using phosphate buffer as an incubation media. Tension maximum was attained in control broilers at approximately 4.5 hours postmortem and an average of 45 g tension/cm$^2$ muscle was obtained.

Changing the pH or temperature of the buffer did not affect the ability of the broiler muscle to develop or release tension but both factors in some instances, altered the amount of tension developed and the time required to reach maximum tension. The effect of temperature was found to vary considerably. Temperatures in the range of those used in commercial scalding were shown to cause a 6 - 7 fold increase in tension development within a very short time. Such tension development may be related to the toughening which results from scalding of broilers.

The presence of $10^{-3}$ M calcium or magnesium ions in the incubation buffer produced a marked change in the rate of tension release. Their effect on tension development was minimal indicating that the sarcolemma of postmortem muscle may be impermeable to extracellular materials for a short
period of time postmortem. Chelation of the calcium or magnesium with EDTA produced an additive effect on tension release suggesting that non-charged complex may readily enter the muscle and that a displacement of calcium or magnesium from the complex may then take place thereby releasing these ions to effect a more rapid tension decline.

A pattern was observed in control strips run in phosphate buffer. Three groups of broilers were categorized on the basis of time required to reach maximum tension. Separation on this basis revealed that birds reaching maximum tension in less than 3 hours postmortem had a significantly greater rate of tension release than birds in the 3 - 6 hour or greater than 6 hour categories. The rate of release for strips from the 0 - 3 hour group coincided with the rate of release for strips run in buffer containing $10^{-3}$ M calcium, suggesting a difference in calcium regulation in this group of birds.

It was found possible to predict the proportion of maximum tension released at 12 hour post-maximum tension from the proportion released at 1 or 2 hours. The one hour value was also significantly correlated to the time required to reach maximum tension. Tenderness and 1 hour tension release value were not significantly related indicating that the rate of tension release from bird to bird is not indicative of subsequent tenderness.

The effect of various processing treatments on tension
parameters and broiler tenderness was studied. An additive response was observed for the different combinations studied with the combined commercial process of free struggle at slaughter, hot water scalding and mechanical plucking yielding the greatest changes and significantly toughening broiler breast muscle.

The effect of pre-slaughter epinephrine injections on postmortem tension parameters, tenderness and various compounds related to postmortem glycolysis in muscle was studied. The maximum effect of epinephrine injection occurred between 8 - 12 hours post-injection. At this time, muscle glycogen levels were essentially depleted but muscle ATP was still sufficient to effect considerable tension development during the extremely short period of postmortem glycolysis. This ability to develop considerable tension appeared to be the cause of the toughening observed in the muscle from epinephrine injected birds.

The effect of several antemortem stressors on postmortem broiler muscle was studied. The stressors studied (commercial, cold and heat) did not significantly alter muscle quality. A difference in response to the heat stress, as compared to the commercial and cold stresses, was observed. The response of birds to a stressful situation is characterized by release of epinephrine. It is evident that the situations studied in this thesis were not severe enough to elicit the magnitude of response observed from the actual injection of this hormone.
A cold shortening phenomenon was observed in the range from 0 - 5°C and the magnitude of the shortening increased as the temperature decreased towards 0°C. Subsequent studies at 2°C showed that after the initial cold shortening further tension development did not occur at this temperature. Further tension development did occur if the 2°C buffer was exchanged for buffer at room temperature. A holding period of 36 hours at 2°C was necessary to eliminate further tension development when buffer temperature was raised to room temperature. The cold shortening which occurred at 2°C did not significantly alter muscle concentrations of high energy phosphate compounds.
LITERATURE CITED


Love, B. 1948. Factors affecting the palatability of poultry with emphasis on histological postmortem changes. Adv. in Food Research. 1:203.


Schmidt, G.R., Cassens, R.G. and Briskey, E.J. 1970b. Relationship of calcium uptake by the sarcoplasmic reticulum to tension development and rigor mortis in striated muscle. J. Food Sci. 35:574.


