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Robert Arthur Jungk
Iowa State University

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Food Technology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Dean of Graduate College

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Post-mortem physiochemical changes in turkey muscle

Robert Arthur Jungk

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The effects of chronological age, muscle type (color) and environmental temperature on the post-mortem physiochemical changes in turkey muscle were studied. Post-mortem turkey muscle when held isometrically exhibits a pattern of tension development and relaxation. Excised muscle generally shortens during rigor mortis. Both phenomena were studied simultaneously, thus allowing a direct comparison.

The average time to maximum rigor was 4-1/2 hours, after which the muscles gradually relaxed. Shortening in excised strips, a parallel phenomenon, generally does not occur after 4 hours.

Chronological age significantly influenced shortening in excised strips ($p < 0.01$), rate of tension development ($p < 0.05$), time to maximum rigor ($p < 0.01$) and the amount of tension at maximum ($p < 0.05$). Turkeys of 14 to 27 weeks of age were studied. The younger birds developed more tension and took longer to relax than their older counterparts. Similarly the excised strips from younger birds shortened more.

The two muscles used in this study were Pectoralis superficialis and Biceps flexor. They were chosen as representative of the white and red muscle types respectively. Environmental temperature had a predominant influence on the post-mortem physiochemical differences between the two muscle types. The P. superficialis generally reached maximum rigor sooner

than the B. flexor. Similarly red muscle strips shortened more than white muscle strips.

Environmental temperature had a significant ($p < 0.01$) effect on extent of shortening in excised strips, rate of tension development, time to maximum rigor, amount of tension at maximum and rate of relaxation. The isometric tension and shortening characteristics of post-mortem white muscle were linearly related ($b = 0.61$) to environmental temperature over the range 2°-37°C. The temperature-shortening relationship for red muscle is more difficult to define. A "cold-shortening" effect definitely occurred. The temperature extremes studied, 0°-10°C and 30°-37°C, are stimulatory. This stimulation produces rapid tension development and relaxation within one hour post-mortem followed by, and frequently superimposed upon a "regular" pattern of post-mortem tension development and relaxation.

Age and muscle color had no significant effect on "thaw rigor". However the effects of freezing time and delay before thawing were significant at the ($p < 0.01$) level. The extent of shortening during thawing decreased with both delay before freezing and increased storage time before thawing. For the temperatures studied (0°, 18°, 36°C) extent of shortening increases with thawing temperature and red muscle strips shortened more than white muscle.

Chronological age, muscle type (color) and sampling time significantly affected amino acid concentration. The concentration of extractable free amino acids was higher in the older group of turkeys (17 vs. 27 weeks of age). Red muscle had a significantly ($p < 0.01$) higher concentration of glutamine, glycine, alanine and proline.

Muscle samples were excised at 0, 4 and 24 hours post-mortem. Of the 19 amino acids studied, the concentration of 17 were significantly affected by sampling time. With the exceptions of glutamine and ornithine, there was a general increase in free amino acid concentration with post-mortem aging. The increased extractability of amino acids was especially noteworthy in the older group of birds.

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INTRODUCTION

For many years meat research has been focused on the post-mortem changes which occur in muscle. Many of the quality attributes of meat are established shortly after death when muscle undergoes a series of physical and chemical changes. The principle factor of concern is tenderness. More recently it has been demonstrated that muscle post-mortem goes through a period of tension development followed by a gradual relaxation. This pattern can be demonstrated while the muscle is held isometrically. The isometric tension pattern is one of the first properties of post-mortem muscle that has been shown to be related to post-mortem changes in tenderness. A strong correlation has been established between contractile state of muscle and tenderness.

While meat scientists and food technologists have been trying to process muscle tissue into an improved meat product, the other scientific disciplines have been making rapid strides. Geneticists have selectively bred into the animal the desirable characteristics of meatiness, faster growth and rapid maturation. Similarly nutritionists have strived to improve feed efficiency. The impact of the combined sciences on the poultry industry has been pronounced, partly because of the short generation time and the speed in which some of the traits can be affected.

Because of these advances, another challenge has arisen. The bird that the processing plant receives for slaughter and preparation into meat is considerably different than 20 years ago. Broilers are now processed at 8 weeks of age whereas 20 years ago it was 12 to 15 weeks of age for the same size bird. Turkeys are also being marketed at a younger age.

Many turkeys are processed at only 15 weeks of age, whereas 10 years ago the average age at slaughter was about 25 weeks. Although traditionally young animals have been considered more tender, experience in the poultry industry is dispelling that notion. Besides exhibiting a higher degree of variability in tenderness, the younger birds also require a longer aging period to insure adequate tenderness. Because of the trend to processing younger birds and the inherent problems, modifications in current processing techniques are mandatory.

This study was undertaken to elucidate the post-mortem physiochemical changes which occur in muscles of birds of different ages. Emphasis was placed on variables which could be controlled by the industry to produce an improved product. The post-mortem alterations in muscle which occur during its conversion to meat are relatively universal for all the species of meat producing animals which have been investigated. While this study utilized turkey as an experimental animal it was designed so that the information obtained could be utilized by all the meat industries.

REVIEW OF LITERATURE

The most widely accepted theory of muscle contraction is that of the "sliding filament" proposed by Hanson and Huxley (1953, 1955). According to this theory myofibrils are composed of two structurally distinct filaments set in an interdigitating array. The two filaments are distinguished by size. The thick filaments are cylindrical tubes which form a regular hexagonal array in the A band. They are held in the proper three dimensional array by protein cross-bridges joining them at their centers. When muscle is viewed in appropriate cross section, the presence of these protein cross-bridges gives the structural entity called the M-line.

The thin filaments are primarily composed of the protein actin. They are held in the proper three dimensional array by the Z-line. From the Z-line they extend toward the center of the sarcomere, terminating at the edge of the H-zone. The thin filaments are polarized and "point" in opposite directions on either side of the H-zone (Huxley and Hanson, 1960).

The basic premise of the sliding filament theory of muscle contraction is that the length of the thick and thin filaments remains constant during contraction and the shortening which occurs in the muscle is accomplished by the interdigitation of the filaments (Huxley and Hanson, 1960). The interaction between the cross-bridges and the actin filament cause a directional force which shortens the sarcomere. This interdigitation occurs in many sarcomeres simultaneously and brings about muscle contraction. X-ray diffraction, light and electron microscopy studies have shown that the banding pattern during contraction are consistent with

the sliding filament theory, i.e. the I-band shortens and the H-zone narrows or disappears.

The myofibrils consist of two major proteins, myosin and actin. It is the interaction of these proteins that is associated with contraction. The contraction of artificially prepared actomyosin threads upon the addition of ATP was demonstrated by Szent-Gyorgyi (1950). In addition to actin and myosin there are four regulatory proteins known to exist in muscle which facilitate the actin-myosin interaction. They are: tropomyosin, troponin, α-actinin and β-actinin. Although their role is not completely understood it is known that they influence molecular conformation and ion sensitivity. Tropomyosin serves to bond troponin to the actin filament, and troponin in turn binds Ca^{++} very strongly. Troponin undergoes a conformational change when it binds Ca^{++} (Ebashi et al., 1967, 1968).

The high energy phosphate nucleotides and Ca^{++}, and Mg^{++} play a very important role in muscle contraction. The nucleotides act as a source of energy to accumulate Ca^{++} against a concentration gradient and provide the energy for contraction. The concentration of Ca^{++} and Mg^{++} at the site of contraction is an essential factor in determining whether contraction or relaxtion will occur. When Ca^{++} and Mg^{++} are present the terminal phosphate is split off ATP to produce energy for contraction. When Ca^{++} is absent and Mg^{++} present with ATP the muscle relaxes. In this latter case, the nucleotide is firmly bound to myosin and the actin-myosin complex is dissociated.

The level of Ca^{++} at the myofibrillar site is carefully controlled by the sarcoplasmic reticulum system. This system and its action were

elucidated after the discovery by Marsh (1951, 1952a) and Bendall (1953) of a subcellular muscle fraction which in the presence of ATP could influence the volume of a muscle homogenate. Latter work showed that this "relaxing factor" in muscle was particulate in nature and it was localized in the sarcoplasmic reticulum (Ebashi, 1960 and 1961, Ebashi and Lipman, 1962).

The sarcoplasmic reticulum consists of a network of fine tubules which surround the myofibrils and also communicates directly with the extracellular space. It is composed primarily of two systems. The T (transverse) system consists of larger tubules which extend from the surface into the center of the sarcolemma and is closely aligned with the L (longitudinal) system. The L system is composed of cisternae and smaller tubules which envelop the myofibrils.

The T and the L systems provide for efficient and rapid transmission of impulses to the sarcomeres, or working elements of the muscle. When the impulse progresses through the T system to the L system there is a release of Ca^{++} from the membraneous tubules of the L system. When the free Ca^{++} is attracted and bound to troponin, a structural change in the molecule results. This structural change allows for interaction of the actin and myosin filaments and thereby, contraction. When the impulse which caused the initial release of Ca^{++} stops, the free Ca^{++} is rebound by the L system. Simultaneously the actin resumes its original molecular configuration and relaxation occurs.

In 1811 Nysten published a paper, "On the Stiffening Affecting the Bodies of Human Beings and of Animals Post Mortem," which began the scientific investigations of rigor mortis. The physical change of state

from the highly extensible plastic condition of freshly excised muscle to the inextensible and rigid condition of muscle in full rigor has been noted from the earliest times, particularly in forensic medicine, where empirical observations of the relation between the time of onset of stiffening and the physical condition of the corpse have been made with considerable precision (Bendall, 1960). These observations are helpful to the practice of medical jurisprudence where they are used in the determination of the time and often the cause of death.

Almost all of the early studies on rigor mortis concerned observations of the human species. Since the beginning of the twentieth century, rigor studies have expanded to include other species. Studies of muscle physiology and associated changes at death have utilized such diverse animals as frog (Perry, 1950), rat (Forster, 1963a,b; Bate-Smith, 1939), and rabbit (Bendall, 1951; and Szent-Gyorgyi, 1950). Rigor as it affects meat quality has been studied in bovine (Locker, 1960; Lawrie, 1953), porcine (Briskey et al., 1962), ovine (Marsh and Thompson, 1958), avian (deFremery and Pool, 1960), equine (Lawrie, 1953), whale (Marsh, 1952b) and fish muscle (Partmann, 1963).

Current studies of rigor mortis cover two overlapping areas: those of muscle physiology and of muscle as meat. Investigations of muscle physiology are directed at understanding the mechanism of contraction and relaxation with its many ramifications in the areas of physics and biochemistry. The interest in muscle as meat stems from the effect of rigor mortis on important meat characteristics, such as tenderness, color, water-binding and emulsifying capacity.

For convenience, I will discuss the rigor studies involving muscle physiology under two separate headings: physical and chemical changes. Muscle undergoes both physical and chemical changes as it passes into rigor. These changes are not separate entities but rather are manifestations of interrelated phenomena.

Physical Changes

The hardening or stiffness of muscle was the first of the physical changes associated with rigor to be intensely studied. Muscle stiffening has been considered a result of changes in muscle proteins and not of a setting or hardening of the fat (Cassens and Newbold, 1966). Marsh (1952b) used a device called a penetrometer to provide a rough quantitative measure of the changing hardness of whale muscle. The pressures required to pierce the tissue at six points were recorded at each time interval and the mean value taken as a measure of the hardness of the tissue. Marsh demonstrated that the rapid transition to high resistance to penetration coincided with the commencement of a rapid decomposition of ATP. After hardening and over a period of time, the penetrometer readings gradually decreased to about their original level. At the same time, the muscle became soft and noncontractile and a small amount of weep exuded. The results were duplicated by Partmann (1963) who studied the hardening changes that occur in muscle of various species of fish.

A second physical characteristic, muscle extensibility, was studied after the development of a special device consisting of weights, a series of levers and a kymograph. With this device physiologists were able to

measure the change in length of muscle for any given weight. The most characteristic difference between active and resting muscle is an alteration in extensibility. Resting muscle is much more elastic than active muscle. Bate-Smith (1939) found a profound alteration of muscle extensibility as it passed from the living state. He delineated the overall post-mortem extensibility change into distinct phases. The delay phase was defined as that period immediately after death when there is only slight change in the modulus of elasticity, whereas the rapid phase is characterized by a very rapid increase in the modulus of elasticity to a value which may be 10-40 times higher than the initial value.

Forster (1963a), while studying post-mortem extensibility as it relates to forensic medicine, carefully differentiated the elastic and plastic characteristics of muscle. Elasticity can be considered as the property of a muscle system component that undergoes reversible lengthening upon loading and unloading. Plasticity is the irreversible lengthening that occurs upon loading and unloading. He attributes these two properties to different elements of the muscle system as is evidenced by their alteration at different times in the rigor cycle. Elasticity constantly decreases to a minimum during rigor mortis, whereas plastic deformation first increases and then decreases.

A modulus of elasticity in the range of 0.5-2 x 10^3 g/cm^2 was observed in pre-rigor poultry muscle. After 2 to 3 hours it increases rapidly to 8-10 x 10^3 g/cm^2 in normal chicken muscle (deFremery and Pool, 1960). A maximum modulus of elasticity is reached in about 8 to 10 hours, after which it declines approximately 40 percent in the next 12 hours (deFremery, 1966a). The effect of environmental temperature on

extensibility of poultry muscle has not been studied extensively. It has been suggested that lower temperatures delay the onset of rigor mortis. Some deviation from this general assumption has been predicted in the 0-2°C range because of relative rates of ATP degradation (deFremery, 1966a).

Various other extensibility studies have been conducted which verify the major findings already mentioned. Briskey et al. (1962) developed a rigorometer which not only automatically loads and unloads the muscles while recording changes in elasticity and extensibility, but also measures the changing electrical resistance. Briskey's rigorometer included a muscle chamber with environmental control. The studies conducted using this device showed that the time course of rigor mortis was temperature dependent, being prolonged as temperature was decreased.

The force needed to tear a muscle in rigor mortis is less than that of fresh muscle. Fresh muscle has an average tear limit of about 8 kg/cm^2, whereas rigor muscle tears with a load of about 4-6 kg/cm^2 (Forster, 1963a). Summarizing this observation, he noted that although the muscle in rigor mortis is harder and more inelastic, it is more brittle and has less tensile strength than fresh muscle.

Shortening is another physical change which is frequently observed in muscles undergoing rigor mortis. Prior to 1939, shortening was known to occur frequently in heavily loaded muscles. Sometimes, however, it failed to occur. Shortening usually can be observed in muscles undergoing a very rapid onset of rigor (Bate-Smith, 1939), and also when rigor develops at a pH higher than 6.2 (Bate-Smith and Bendall, 1947). Likewise a greater amount of shortening is associated with a short-time course as compared to

a long-time course of rigor mortis (Bendall, 1960; Marsh, 1954). These observations have been confirmed with porcine muscle by Sink et al. (1965). They found a high positive association between duration of rigor mortis delay phase and sarcomere length in the same muscle measured 24 hours post-mortem. Environmental temperature has considerable effect on the extent of shortening which is observed during rigor. In rabbit muscle, post-mortem shortening increases with increasing temperature for 0°-37°C (Bendall, 1951 and Jungk, 1965). Locker and Hagyard (1963) demonstrated a "cold shortening" of beef muscles in which minimal shortening was observed at 14-19°C, with a greater degree of shortening occurring either side of that range. The "cold shortening" phenomenon has also been demonstrated in the porcine (Galloway and Goll, 1967) and avian (Smith et al., 1969) species.

Bendall (1951) suggested that the changes in elasticity and shortening are physiologically related as is evidenced by their simultaneous occurrence during the rigor period. Concurrent with stiffening, Pool et al. (1959) found that poultry muscle strips shorten slightly under light load to about 90 percent of initial length, followed by a recovery almost to the original length. Forster (1963b) also found that shortening coincides with decreasing extensibility. He extended the observations on shortening and its dependence on temperature to rat muscle. Forster even went so far as to suppose that whatever causes shortening of muscle simultaneously diminishes extensibility of the elastic elements. Because of his particular interest in forensic medicine and the possibility of homicide being feigned as suicide, Forster studied muscle shortening in

rigor. He was able to show a relation between the magnitude of shortening during rigor mortis and weight or muscle tension. No measurable shortening occurred during rigor mortis in unloaded muscles. The effect of post-mortem shortening on meat quality will be discussed in another section of this thesis.

It is important to emphasize that loss of extensibility and shortening are two distinct, although closely related, aspects of rigor. This distinction is apparent when these physical changes are considered on the molecular level and with respect to time of occurrence.

On a molecular level, shortening can be depicted as a sliding of the actin and myosin filaments in a sarcomere past one another. Such a sliding depends on the occurrence of a contractile-producing stimulus at some time before ATP content is depleted. Inextensibility may be visualized on a molecular level as an interaction and fusion of actin and myosin to form actomyosin. On a macroscopic scale inextensibility is evident as resistance to stretch under a load (Goll, 1968). Both shortening and inextensibility are closely related to the ATP concentration. This relationship will be discussed with the other chemical changes associated with rigor.

When considered from a temporal sequence, the shortening of muscle, if it occurs, must always precede the loss of extensibility. In other words, shortening - the sliding of actin and myosin filaments - can only occur before the two filaments fuse to form actomyosin. The extent of post-mortem shortening which occurs is the responsible factor for the stiffness of rigor mortis. If little shortening occurs, the muscle may not appear stiff or hard, even though it is quite inextensible (Cassens

and Newbold, 1966; Goll, 1968).

In recent years several studies have established the influence of contraction state on meat tenderness. Lowe (1948) and Koonz et al. (1954) observed that muscle which was cut or excised in a pre-rigor condition was tougher than one which had been chilled while attached to the carcass. Locker (1960) suggested that the toughening that occurred might have been due to the shortening of the muscle during the onset of rigor mortis. Relaxed muscles were more tender than partly contracted muscles and this effect was especially significant in muscles of low connective tissue content. Goll et al. (1964a) evaluated the post-mortem changes in tenderness of muscles which were excised versus those left attached to the carcass. They found that muscles left attached to the carcass were least tender immediately after death and gradually increased in tenderness during post-mortem aging. Excised muscles, however, were least tender immediately after death and gradually increased in tenderness during post-mortem aging. Several investigators have loaded or restrained muscles during aging and found that muscles which are prevented from shortening are more tender (Partmann, 1963; Herring et al., 1965; Gillis and Henrickson, 1969; Buck and Black, 1967, 1968).

The relationship between shortening and tenderness is complex. Small differences in contraction state can cause marked differences in tenderness (Howard and Judge, 1968) and, indeed, in one study, investigators found no significant correlation between shear value and sarcomere length (Welbourn et al., 1968). This disparity may be caused by the fact that a muscle which is absolutely fixed in overall length is still capable of

appreciable shortening in one area with compensating lengthening elsewhere if the application of cold is uneven along its surface (Marsh and Leet, 1966).

A post-mortem isometric tension pattern was demonstrated by Jungk et al. (1967) after the development of a special device called an "isometer." A muscle strip which had been excised immediately after death was held by clamps isometrically thereby duplicating the condition of the muscle on the carcass. Tension exerted by the muscle was recorded as it went through rigor mortis. A pattern of gradual tension development is illustrative of the muscle's tendency to shorten. Tension development is followed by a failing ability to maintain tension and a subsequent decline in tension.

Busch et al. (1967) studied the effect of temperature on the post-mortem isometric tension pattern of bovine muscle and followed the concurrent changes in ATP concentration and shear values. A similar study was conducted using porcine muscle by Galloway and Goll (1967). A major improvement in measurement of rigor was made when it was realized that muscle strips immersed in Ringer's solution exhibited the same pattern of tension development as strips in air (Goll, 1968). Immersion of the strip eliminated dehydration and allowed increased flexibility in environmental control. The sensitivity and capacity of this method of measuring rigor has been increased through the use of isometric myographs with an E and M Physiograph. The simultaneous measurement of extensibility and isometric tension is allowed with the use of a combined rigorometer and physiograph (Schmidt et al., 1968a, 1968b). More recently the subcellular events

associated with the development and release of isometric tension in post-mortem rabbit and porcine striated muscle was studied by Busch (1969). Busch demonstrated that the isometric tension development closely parallels the post-mortem changes in length and that the loss in extensibility does not occur until maximum isometric tension has been reached. He also found that the addition of Ca^{++} ions to the buffer caused a rapid decline in muscle tension and further that this decline in tension is associated with the disruption and disappearance of the Z-line.

The electrical properties of post-mortem muscle have also been the focal point of several studies. Callow (1937) used an electrical resistance method to identify porcine carcasses which would yield superior bacon. This same technique was used by Ranken and Shrimpton (1968) to predict tenderness in turkeys. A potential of 14 volts a.c. (50 cycles) was applied by means of a double probe in the turkey breast and the current was recorded in milliamperes. Milliamp readings of $\geq$ 80 occurred in only about 1 percent of the birds. This group contained almost all the tough birds encountered. Shortly after death, muscle loses its excitability. Although this loss occurs long before completion of rigor mortis, the electrical response properties of porcine muscle taken immediately after exsanguination are correlated with the time course of rigor mortis. Forrest et al. (1966, 1967) observed the strength of contraction to be highest in muscles which exhibited long rigor, slow glycolytic rate and normal color morphology. The duration of contraction was also noted to be longer in this type of muscle. Similar results have also been reported for turkey muscle by Ma et al. (1971).

Besides the gross physical changes which occur during rigor mortis which have already been discussed, there are several less obvious but equally significant changes in morphology which occur at the ultrastructural level. Muscle which undergoes post-mortem shortening or contraction will lengthen with aging. This lengthening was first observed independently and almost simultaneously by Gothard et al. (1966) and Stromer and Goll (1967a,b) working with bovine muscle and Takahashi et al. (1967) working with chicken muscle. Gothard et al. (1966), in studying post-mortem changes in sarcomere length using phase microscopy, found considerable lengthening during the aging period. Also using phase microscopy, Stromer and Goll (1967a) found some reappearance of H-zones in the banding pattern of myofibrils from aged muscle, denoting a possible relaxation. Further examination, using electron microscopy, revealed that although the banded pattern of resting muscle does not reappear, some lengthening of sarcomeres clearly occurred between 24 and 312 hours of storage at 2°C (Stromer and Goll, 1967b). The Japanese workers examined the susceptibility of chicken pectoral muscle to fragmentation and also studied concurrent changes in sarcomere length. Myofibrils isolated immediately post-mortem were relatively long and frequently exhibited side-by-side orientation. With aging, myofibrils fragmented into small 1-4 sarcomere segments (Takahashi et al., 1967). They also found that besides the generally accepted concept of irreversible post-mortem contraction of sarcomeres, a reversible contraction can take place under particular conditions. This reversible contraction compares favorably with the relaxation or sarcomere lengthening demonstrated in the bovine species.

Two other ultrastructural changes which occur in muscle during post-mortem aging have been elucidated by Davey and Gilbert (1969). They found that during the aging of fiber pieces prepared from bovine sternomandibularis muscles, a loss of adhesion occurs between adjacent myofibrils. This was evidenced by increased readiness of fiber pieces to disintegrate into individual myofibrils upon homogenization. This finding corroborated that of Takahashi et al. (1967) who investigated chicken muscle. Alterations also appear within the myofibrils themselves in the regions of the Z-lines, sometimes leading to the apparent dissolution of this structure (Davey and Gilbert, 1969).

The structure of muscle in rigor mortis has also been studied using X-ray analysis. Muscles in rigor show an altered pattern of reflections when compared with resting muscle at the same sarcomere length. The major differences involve the myosin cross-bridges and actin helices. Huxley and Brown (1967) have demonstrated that a longitudinal movement of the cross-bridges occurs and there may be an attachment of other structures onto the outside of the units along the actin helices. They also noted that sharp sampling of the layer-line pattern is not observed in the patterns from rigor muscles, indicating that the registration of the filaments is lost. Upon further analysis of the structure differences between living and rigor muscles, Huxley (1968) postulated that 30 percent of the original mass of myosin moves from the vicinity of the thick filaments to the vicinity of the actin-containing filaments. This shift involves the heavy meromyosin component of the myosin molecule in the thick filaments.

Chemical Changes

The changes that a muscle undergoes post-mortem are chemical as well as physical. As might be expected in the transformation of a highly active, excitable and rapidly metabolizing muscle to the stiff and inactive post-mortem state, the chemical changes are primarily degradative.

Nineteenth century biochemists attributed the stiffening in rigor to two factors: a coagulation of muscle protein, comparable to coagulation of blood; or precipitation of muscle protein by lactic acid. These theories have been credited by Hoet and Marks (1926) to Kunne and Schipiloff who, in 1864 and 1882, respectively, studied the effects of acid on the water-soluble proteins of the sarcoplasm. These theories were seriously questioned after a number of workers were able to demonstrate that rigor occurs under alkaline conditions, even in the absence of lactic acid. Hoet and Marks (1926) therefore suggested that the factor providing common ground between the acid and alkaline types of rigor was the disappearance of hexose phosphate from the muscle. It was not until 1939 that ATPase activity was found in a muscle fraction by Engelhardt and Lyubimova (1939). Then in 1943, Erdos showed that the disappearance of ATP was closely related to the onset of stiffening in post-mortem muscle. The central role of ATP in rigor mortis was established by the Low Temperature research group at Cambridge, Bate-Smith (1939), Bate-Smith and Bendall (1947 and 1949) and Bendall (1951 and 1960). These early Cambridge studies elucidated many of the relationships among ante-mortem stress, glycogen level, ultimate pH, ATP degradation and the onset of rigor mortis that are generally accepted today. The close relationship

between ATP degradation and loss in extensibility has been verified for rabbit (Bendall, 1951; Bendall and Davey, 1957), bovine (Marsh, 1954), porcine (Briskey et al., 1966), and avian (deFremery and Pool, 1960). The post-mortem isometric tension pattern is also closely related to ATP degradation (Jungk et al., 1967; Busch et al., 1967).

The role of ATP in living muscle and its function in normal contraction was being studied throughout this same period. Szent-Gyorgyi (1950) demonstrated contraction in artificially prepared actomyosin threads upon addition of ATP. Glycerol extracted muscle fibers were also shown to exhibit contraction upon addition of ATP. Sarkar et al. (1950) showed that ATP splitting parallels isometric tension development in rabbit psoas muscle.

Another easily followed chemical change is the decline in pH. This results from an accumulation of lactic acid, the end product of anaerobic glycolysis. Glycolysis normally continues for a short time after death until the glycogen supply is depleted or the muscle reaches a critical pH where glycolysis in inhibited (Bate-Smith and Bendall, 1949).

During glycolysis ATP is continually being synthesized at the rate of 1.5 moles for every mole of lactic acid produced. The high energy phosphate in the muscle is stored as CP (creatine phosphate). When the need for ATP arises, the high energy phosphate is transferred from CP to ADP to yield ATP. Bendall (1951) and Lawrie (1953) have shown that the interval between death and onset of rigor is dependent on the quantity of CP present at death as well as the amount of glycogen. The role of CP in bolstering the ATP supply has been verified by the use of FDNB which inhibits the creatine phospho-transferase (Nauss and Davies, 1966).

Besides the chemical changes involving high energy phosphate compounds and glycolytic intermediates during rigor, the role of calcium is also very important. Post-mortem shortening of muscle is triggered by an efflux of Ca^{++} (Nauss and Davies, 1966). Greaser et al. (1967, 1969) have shown that soon after death the vesicles of the sarcoplasmic tubules lose their ability to accumulate Ca^{++}. Rigor, as defined in the tension development sense, can be inhibited in frog muscles by soaking in a Ca^{++}-free Ringer's solution containing various levels of EDTA or EGTA (Feinstein, 1966). Weiner and Pearson (1966) also prevented rigor shortening in rabbit by administration of a lethal injection of EDTA. Busch (1969) conducted experiments on the effect of Ca^{++} and chelators on the isometric tension pattern. Apparent lack of ready permeability into the muscle fiber prevented any effect on tension development; however, the latter stage of the pattern was profoundly affected. Metal chelators, EDTA and EGTA, prevented the gradual post-mortem relaxation of the muscle, whereas Ca^{++} addition to the muscle buffer accelerated the relaxation phase of the tension pattern.

Proteins are the major muscle constituent and account for about 85 percent of its total dry weight. On the basis of solubility, they can be divided into three major fractions. The sarcoplasmic proteins are soluble in water and are composed mostly of the soluble enzymes of the muscle system. The post-mortem chemical changes of glycolysis, ATP degradation and Ca^{++} movement already discussed occur in close conjunction with the sarcoplasmic proteins.

The muscle structure is mainly composed of the myofibrillar and the stroma proteins. Both of these protein groups have been studied rather

extensively with respect to their roles in muscle contraction, muscle disease and meat texture. The stroma proteins, often referred to as connective tissue, are insoluble in dilute alkali solutions and are composed primarily of collagen and elastin. The connective tissue of muscle undergoes considerable modification with age (Goll et al., 1963, 1964b,c,d). These changes no doubt account for much of the age-tenderness relationship. Degradative changes in connective tissue during post-mortem aging likewise have been studied. In chicken muscle there is no significant change in the stroma protein content during the time period required for maximum tenderization (Khan and van den Berg, 1964; Sayre, 1968). In their study of tenderization of chicken muscle, deFremery and Streeter (1969) found alkali-insoluble connective tissue of raw and cooked muscle (as measured by alkali-insoluble hydroxyproline) did not change significantly as a function of post-mortem aging. Although studies on the alkali-insoluble fraction have not revealed any significant post-mortem alterations, there may be subtle changes in the mucopolysaccharide fractions which have gone undetected.

The myofibrillar proteins are soluble in strong neutral salt solutions and are composed primarily of the contractile proteins, myosin, actin and tropomyosin. The solubility of these proteins change during post-mortem aging. The amount of nitrogen and actin extracted from poultry breast muscle increases with aging (Weinberg and Rose, 1960; Landes et al., 1971). Myosin extractability decreases rapidly in the first 3 to 4 hours of aging followed by an increase in extractability of actomyosin. The initial loss of myofibrillar protein solubility and subsequent release of

actomyosin corresponds with the time course of toughening and post-rigor tenderization in chicken muscle (Sayre, 1968). Scharpf et al. (1964, 1966) and Scharpf (1965), while studying post-rigor changes in the myosin B fraction of turkey muscle found evidence suggesting a dissociation of myosin B to myosin A and actin with resolution of rigor. Maxon and Marion (1969) found a steady increase in protein solubility of turkey breast muscle throughout 48 hours of aging. In beef muscle, the myofibrillar proteins from aged meat are much more readily extracted than from pre-rigor muscle (Davey and Gilbert, 1966, 1967, 1968a). Bovine myosin is extracted at a relatively constant level, whereas the solubility of actin increases with aging (Davey and Gilbert, 1968b). It has been suggested that the protein solubility changes associated with the aging of meat is due to proteolysis (Weinberg and Rose, 1960; Penny, 1968; Khan, 1968).

Proteolysis and its relationship to post-mortem tenderness has received considerable attention. Recent studies have focused on protein breakdown components such as nonprotein nitrogen and free amino acids, which have been shown to increase during post-mortem storage of beef (Parrish et al., 1969), poultry (Miller and May, 1965) and fish muscle (Hodgkiss and Jones, 1955). The more tender muscles (Longissimus dorsi and Psoas major) have been shown to contain more leucine-isoleucine than the less tender semitendinosus (Ma et al., 1961). Gardner and Stewart (1966) found a decrease in glutamine while glutamic acid and tryptophan increased in stored beef muscles. The increase in nonprotein nitrogen and free amino acids of bovine muscles upon post-mortem storage occurred after the post-mortem improvement in tenderness in the study by Parrish et al.

(1969). They concluded that the increase in concentration of these constituents was not related to the mechanism of tenderization. Field and Chang (1969) found individual and total free amino acids increased slightly with increasing tenderness within beef muscles.

Processing of Poultry

The poultry industry has considerable interest in the physiochemical changes which occur post-mortem. Their interest is even greater than that of the other animal industries. The small size of the avian species allows for rapid processing which may proceed simultaneously with the post-mortem physiochemical changes. The processing of the animal rapidly post-mortem may alter the normal pattern of rigor mortis. Such effect may be beneficial or detrimental to ultimate product quality.

Earlier studies have demonstrated the effect on quality of the various processing techniques. The method of dispatching and prior treatment to tranquilize the birds have been studied by several workers. Immobilization by carbon dioxide asphyxiation, electrical stunning, and tranquilization was studied by Goodwin et al. (1961) and shown to have little significance on tenderness. The scalding process may adversely influence tenderness when extremes of temperature or time are used (Klose et al., 1959; Pool et al., 1959 and Shrimpton, 1960). The defeathering operation also can exert a negative influence on meat tenderness (Pool et al., 1959).

One area which has received considerable study is the chilling process. The method of chilling poultry has progressed from the early

technique of cold air blast to the mechanical chilling systems which incorporate rapid agitation and a low temperature liquid medium to bring about rapid heat removal. The mechanical chilling systems provide economies of time, labor and space. In addition the mechanically chilled product may be superior from a microbial standpoint (Barnes and Shrimpton, 1968; Casale et al., 1965; Kraft et al., 1963).

Prior to 1960, research indicated that the time needed to chill the carcass was less than that required to properly tenderize the bird by aging. A minimum aging time of 12 hours was felt necessary and some benefit was derived by 6-12 additional hours. Klose et al. (1961a,b) studied several chill methods and found that 20 lb young toms, and possibly 12 lb young hens were adequately tender after a 1-2 hour chilling period. However the 6 lb fryer-roasters required longer aging. Marion and Goodman (1967) also found continuous chilling to be inadequate for the turkey fryer while it is sufficient to insure tenderness for the older, larger birds. Other workers have also noted extreme variability and relative toughness of young turkeys chilled for short periods (Scholtyssek and Klose, 1967). The three previously mentioned papers have demonstrated that young turkeys tenderize slower than the older birds.

The post-mortem physiochemical changes which occur in the carcass are known to affect ultimate product tenderness. The extreme variability in tenderness of young turkeys and the extended aging required to produce a satisfactorily tender product suggests there may be notable differences in post-mortem events. This study was undertaken to elucidate some of the post-mortem physiochemical changes which occur in turkeys of different ages.

METHODS AND MATERIALS

Source of Muscle Tissue

The source of muscle tissue for this study was the male domestic turkey, Meleagris gallopavo. The Pectoralis superficialis which is the largest muscle of the turkey was used as representative of the "white" muscles. The Biceps flexor was used as representative of the "red" muscles.

The birds used in this study were from the Iowa State University research farm. They had similar genetic background, had been raised under similar conditions, and for a given age group did not vary appreciably in weight. Exsanguination was accomplished by severing the jugular vein and the carotid artery. The birds were inverted in a metal cone during this process to minimize struggling. No prior stunning or anesthetic was used in this study except in special experiments where indicated.

Tension Measurements

Tension development and release was measured with an E and M Physiograph (E and M Instrument Co., Inc., Houston, Texas) equipped with both isometric and isotonic myograph transducers. The environment of the muscle strips was controlled by immersing the strips in saline solutions near physiological ionic strength. An environmental control chamber (Labline Inc., Chicago, Illinois) was used to maintain a constant temperature for each experiment.

For all tension measurements, muscle strips 0.1 to 0.3 cm^2 in cross

section and approximately 7 cm long were removed from each muscle by carefully cutting parallel to the muscle fibers to minimize structural damage. The excised strips were then weighed, and one end was immediately attached to the transducer by surgical thread. The other end of the strip was securely fastened with surgical thread to a glass rod projecting from the side of an 800-ml beaker at a point approximately 15 mm above the bottom.

Isometric tension studies were done by using isometric transducers supplied by the E and M Instrument Company. In order to keep each strip taut and to assist in maintaining uniformity of initial conditions, approximately 4 gm/cm^2 of tension was placed on each strip when it was first attached to the isometric myograph. Five isometric transducers were used in each experiment, thus allowing up to five comparisons on any one animal. This facilitated studies on the effects of various treatments since animal variation was eliminated by this design. Considerable care was taken to prevent stretching of the muscle strips while they were being excised and mounted on the instrument. Tension is expressed as g/cm^2 of muscle cross-sectional area; this was calculated by the method of Helander and Thulin (1962).

Adenosinetriphosphate Measurements

The concentration of ATP (adenosinetriphosphate) in the muscles was measured using the bioluminescent enzymatic method of Bodwell et al. (1965). Five grams of tissue were homogenized with 50 ml of cold (4°C) TCA. The homogenate, with the rinsing solution, was filtered through Whatman No. 42

filter paper and brought up to 100 ml with glass distilled water. Following the recommendation of Busch (1969), the TCA was removed from a 5-ml aliquot of the filtered extract by using two 10-ml extractions with ether. Twenty ml of 0.1M phosphate buffer, pH 7.4, was added to the TCA-free aliquot and the volume was then made up to 100 ml with glass distilled water. Samples were stored at -28°C until luminescence could be measured with a Turner fluorometer. All luminescence readings were made exactly 45 seconds after the addition of 0.2 ml of firefly extract (Sigma Chemical Company) which had been buffered in 0.05M potassium arsenate, pH 7.4. ATP values were determined by running standards of known concentration and then comparing the readings from the unknown with a standard curve.

pH Measurements

A 10-gram muscle sample was homogenized with 100 ml of glass distilled water in a Waring blender for 30 seconds. The pH of the homogenate was measured immediately using a Beckman Zeromatic pH meter with a glass electrode.

Shortening Measurements

Muscle strips were measured and placed in a phosphate buffer, pH 7.2, that had been previously adjusted to the desired temperature. Usually five temperatures, with three strips per bird for each temperature, were studied. The maximum time lapse from start of exsanguination to placement of the muscle strips in the environmental temperature was 15 minutes. The tabulated data are expressed as percent shortening from resting length.

Thaw Rigor Samples

The same muscles were used for the thaw rigor studies as in the other areas of experimentation. Immediately after excision the muscles were measured, wrapped in aluminum foil, placed in a polyethylene bag and immersed for 2 minutes in a dry ice-acetone solution. The muscles did not shorten during the freezing process. Muscle strips were stored at -20°C or below until sampled. The muscles were removed from the freezer and allowed to thaw. The shortening which occurred during thawing is expressed as percent shortening from resting length.

Analysis of Free Amino Acids

Free amino acids were determined in the Pectoralis superficialis and Biceps flexor muscles from 24 turkeys. Twelve birds from each of two age groups, 17 and 27 weeks, were studied. At any of the three sampling intervals, 0, 4 or 24 hours post-mortem, a 10-gram sample was homogenized in a Waring blender for 2 minutes with 100 ml of glass distilled water. Following 2 hours of agitation at room temperature, the samples were centrifuged for 10 minutes at 1600 x G at 10°C. The precipitate was discarded and the supernatant was recentrifuged under the same conditions for 20 minutes. To 20 ml of the final supernatant was added 20 ml of a 20 percent sulfosalicylic acid with norleucine as an internal standard. This mixture was then centrifuged at 10,000 x G for 20 minutes at 10°C. The supernatant was adjusted to pH 2.0 and brought up to 50 ml volume with physiological buffer (pH 2.0). The sample was held in a frozen state

until analyzed.

For the determination of free amino acids, the samples were brought to room temperature and 1.0 ml of the resulting solution was applied to the chromatographic column for analysis of free amino acids using an amino acid analyzer (Technicon TSM).

Least-squares analyses were used to estimate the effect of muscle, age and sampling time on free amino acids, expressed in μM/g of fresh tissue. Correlation coefficients between the various free amino acids were calculated.

RESULTS AND DISCUSSION

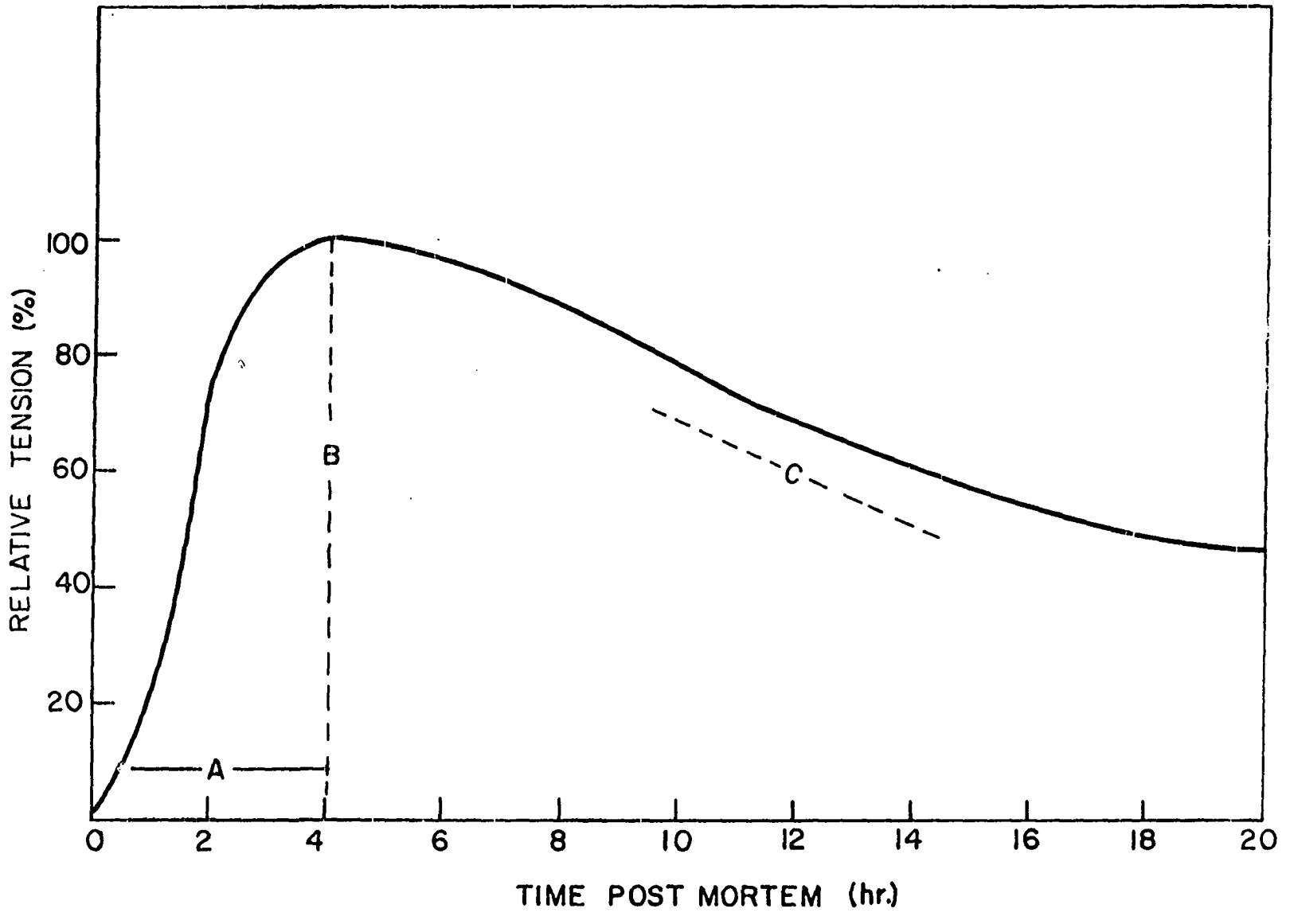
Isometric Tension

One of the methods available to study the overall physical changes which occur during rigor mortis is the isometric tension pattern. The pattern as it generally occurs in breast and thigh muscle of turkey at room temperature is shown in Figure 1. It includes a gradual development of tension followed by a slow loss of tension. This sequence is the physical manifestation of the combined physiochemical events which occur after death. The pattern can be divided into two general phases. The tension development phase includes the parameters of time to maximum tension development and the actual amount of tension at maximum. When these values are known, a rate of tension development can be calculated. The second phase of the pattern is associated with the release of tension. At any convenient reference point after the muscle begins to relax a rate of tension release can be calculated.

In this study the reference point of 20 hours post-mortem was used for the calculation of rate of tension release. For simplicity I will refer to the rates of developing and releasing tension as the rate up and rate down, respectively.

The variables of chronological age, muscle color and environmental temperature were studied. Turkeys of 13 to 27 weeks of age and both red and white muscle samples were utilized. The effect of the following environmental temperatures was investigated: 2, 10, 15, 20, 25, 30 and 35°C. Overall means and correlations involving red and white muscles from

Figure 1. General pattern of development and release of tension which occurs in post-mortem muscle strips held isometrically. A indicates time to maximum tension, B - tension at maximum and C - the release of tension


RELATIVE TENSION (%)
100
80
60
40
20
A
B
C
0
2
4
6
8
10
12
14
16
18
20
TIME POST MORTEM (hr.)

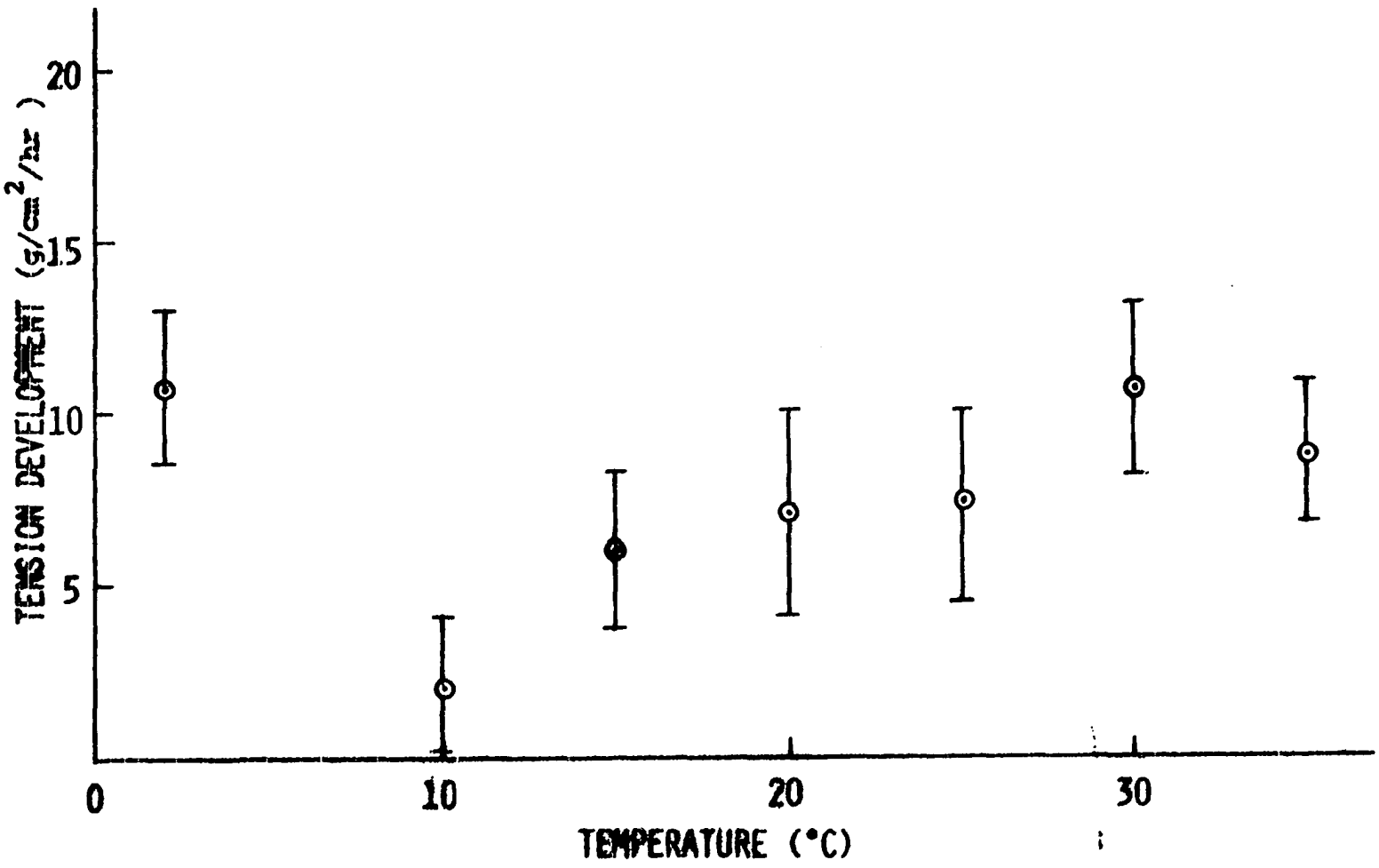


Figure 2. The effect of temperature on rate of tension development. Values shown are for red and white muscles combined

all ages of turkey studied at all temperatures, are presented in Tables 1 and 2.

Table 1. Tension development in turkey muscle

Factor	Average value
Rate up	8.54 ± 0.82[a] $g/cm^2/hr$
Time tc maximum	4.59 ± 0.35 hr
Maximum tension	25.10 ± 2.99 g/cm^2
Rate down	0.44 ± 0.09 $g/cm^2/hr$

[a]Each mean and standard error based on 172 observations.

Table 2. Relationship between isometric tension factors studied

Factors	Correlation
Rate up x Time of maximum	-0.28**
Rate up x Maximum tension	+0.86**
Rate up x Rate down	+0.80**
Rate down x Maximum tension	+0.93**

**Significant at 0.01 level.

A least-squares analysis of variance for the factors studied is given in Table 16 in the Appendix. Many of the factors indicated as significant will be further elucidated throughout this discussion section.

Rate of tension development

Rate up is significantly influenced by temperature ($p < 0.01$) and age ($p < 0.05$). Temperature and color moreover had a significant

($p < 0.05$) effect on rate up. Figure 2 illustrates this temperature effect. At the lowest temperature studied the greatest deviation from a regular pattern occurs. At 2°C red muscle developed tension at a rate of 13 $g/cm^2/hr$, whereas the rate for white muscle is about one-half that or 7 $g/cm^2/hr$. At the higher temperatures studied the rate up gradually increased with increasing temperature. The rate up for red muscle was always greater than that of white muscle. This result is consistent with the findings of Sexton and Gersten (1967) and Busch (1969). It should also be noted that the rate difference between the two muscle types averaged only about one or two units and only at 2°C was it greater.

Figure 3 shows the rate of tension development for the various age groups studied. The most noteworthy factor is the unusually rapid rate for young birds. Both red and white muscle strips at the young age exhibited a high rate. The rate difference between muscle type was consistent over the age groups.

Time to maximum tension

Age, color and temperature significantly ($p < .01$) influenced time to maximum tension (Table 16). The youngest group of birds studied took the longest time (Table 3).

Table 3. Time (hr) to maximum tension for each age category

Age (weeks)			
14	19	22	25
4.91±0.5	3.50±0.5	3.28±1.1	3.94±0.8

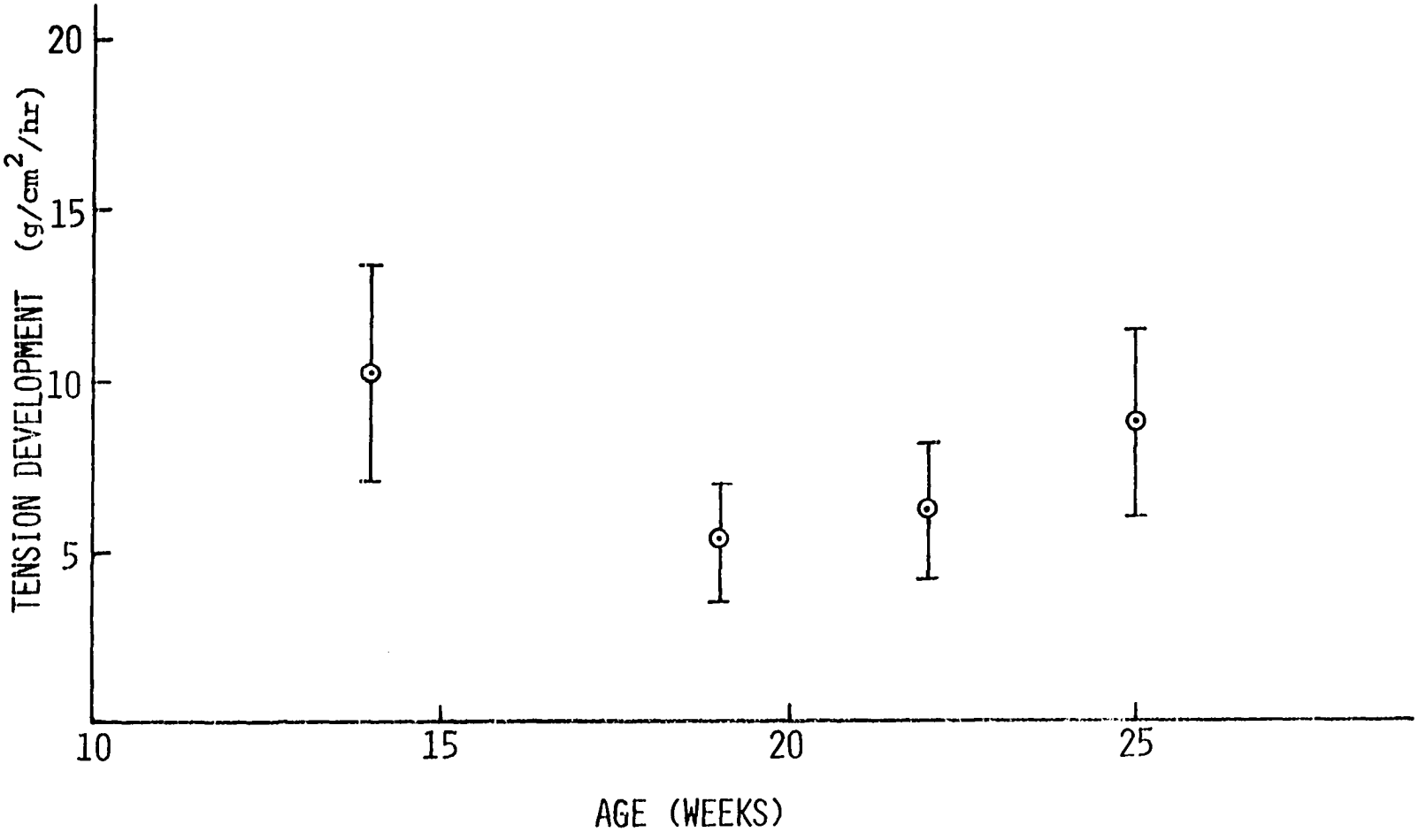


Figure 3. The effect of chronological age on rate of tension development post-mortem in red and white muscle strips

The red muscles required more time to reach maximum tension than white muscles, 5.55±0.5 and 3.58±0.4 hours respectively. This finding is consistent with the results of Schmidt et al. (1970). Muscle type also had an over-riding effect when the variable of temperature was introduced. The effect can be readily seen in Table 4. Especially noteworthy is the erratic influence of temperature on red muscle and the rather negligible effect of temperature on white muscle. The

Table 4. Effect of temperature on time to maximum tension

Temperature (°C)	Time (hours)	
	Red muscle	White muscle
2	3.80±1.2[a]	3.04±0.9
11	14.28±1.1	4.02±1.4
15	10.01±1.1	3.15±0.9
19	6.41±1.1	4.46±1.0
25	4.06±2.1	4.77±1.1
30	2.80±1.0	3.02±1.2
35	2.14±1.1	3.79±0.7

[a]Each mean and standard error based on 12 observations.

shortest times to maximum rigor for red muscle are at the temperature extremes studied, whereas the longest times are from 10°-20°C. This finding is consistent with other data reported in this thesis and lends credence to the occurrence of a thermal stimulus at the extreme temperatures.

The amount of tension which develops during rigor mortis was also studied (Table 5). The influence of temperature was significant ($p < 0.01$) and was similar to the temperature - time to maximum effect that was just discussed. The tension developed by red muscle strips at the temperature extremes was higher than that which develops at the intermediate temperatures.

Table 5. Maximum tension development in muscles

Temperature (°C)	Red muscle (g/cm^2)	White muscle (g/cm^2)
2	25.37±12.9[a]	15.93±10.3
11	22.11±12.7	10.67±15.1
15	22.14±12.0	20.32± 9.8
19	22.34±15.9	21.80±10.9
25	24.32±19.7	15.97±10.3
30	27.53±10.9	21.92±13.0
35	27.49±12.8	26.03± 8.0

[a]Each mean and standard error based on 12 observations.

Red muscle developed considerably more tension than white muscle (23.65 g/cm^2 and 19.45 g/cm^2, respectively). Sexton and Gersten (1967) also reported that red muscle undergoing isometric contraction developed more tension than white muscles.

Rate of tension release

This phase (Figure 1) is characterized by a gradual decline in tension and is expressed as the rate down. Rate down was determined by

taking the difference between tension at maximum and the tension at 20 hours post-mortem and dividing this difference by the number of hours which had elapsed between these reference points. Some error is introduced using this method in that the rate of relaxation is not uniform over the period. The muscles release tension slowly at first and then at a somewhat faster rate. In spite of this factor, the method used in determining rate down is considered the best available procedure.

Although a substantial numerical difference existed between the rates down for red and white muscle (0.32 and 0.59 $g/cm^2/hr$ respectively) this difference is not significant. Some of the variation is attributable to the fact that red muscle generally reached maximum tension at a later time and therefore its rate down was determined over a shorter period.

The only factor which had a significant effect on the rate down was temperature (Table 6). In addition there was a significant ($p < 0.05$) temperature-color interaction effect on rate down.

Table 6. Average rate of tension release (rate down) for red and white muscles

Temperature (°C)	Red muscle ($g/cm^2/hr$)	White muscle ($g/cm^2/hr$)	Average ($g/cm^2/hr$)
2	0.28[a]	0.30	0.29
11	0.23	0.24	0.23
15	0.13	0.75	0.44
19	0.13	0.48	0.30
25	0.53	0.99	0.76
30	0.77	0.68	0.73
35	0.16	0.75	0.45

[a]Each mean and standard error based on 12 observations.

Muscle Shortening

Another manifestation of rigor mortis is the shortening of excised muscle. Pre-rigor excision of muscle results in three types of shortening: 1) that associated with the excision, 2) that associated with temperature ("cold-shortening") (Locker and Hagyard, 1963), and 3) that associated with the onset of rigor mortis. Because of the current evidence relating post-mortem shortening to tenderness, which has been well summarized by Herring (1968), some of the factors that influence post-mortem shortening were investigated.

The factors studied include environmental temperature, muscle color and chronological age. All three of these factors proved to be significant as shown in Table 17 in the Appendix. The results shown in Table 17 are from averages of three muscle strips in every instance. The results are expressed as percentage of shortening from resting length. The muscle strips were excised, measured and then placed in the particular temperature environment. This technique discounts any shortening due to excision. The final percentage of shortening does, however, reflect the summation of shortening due to the effects of thermal stimulation or "cold shortening" and normal rigor shortening. Figure 4 graphically presents the data.

A distinct linear relationship ($b = 0.61^{**}$) existed between temperature and the percentage of shortening for white muscle strips. Contrary to the results which have been reported by Smith et al. (1969), this evidence suggests that "cold shortening" does not occur in breast muscle. The conflicting results may be caused by the method of determining the

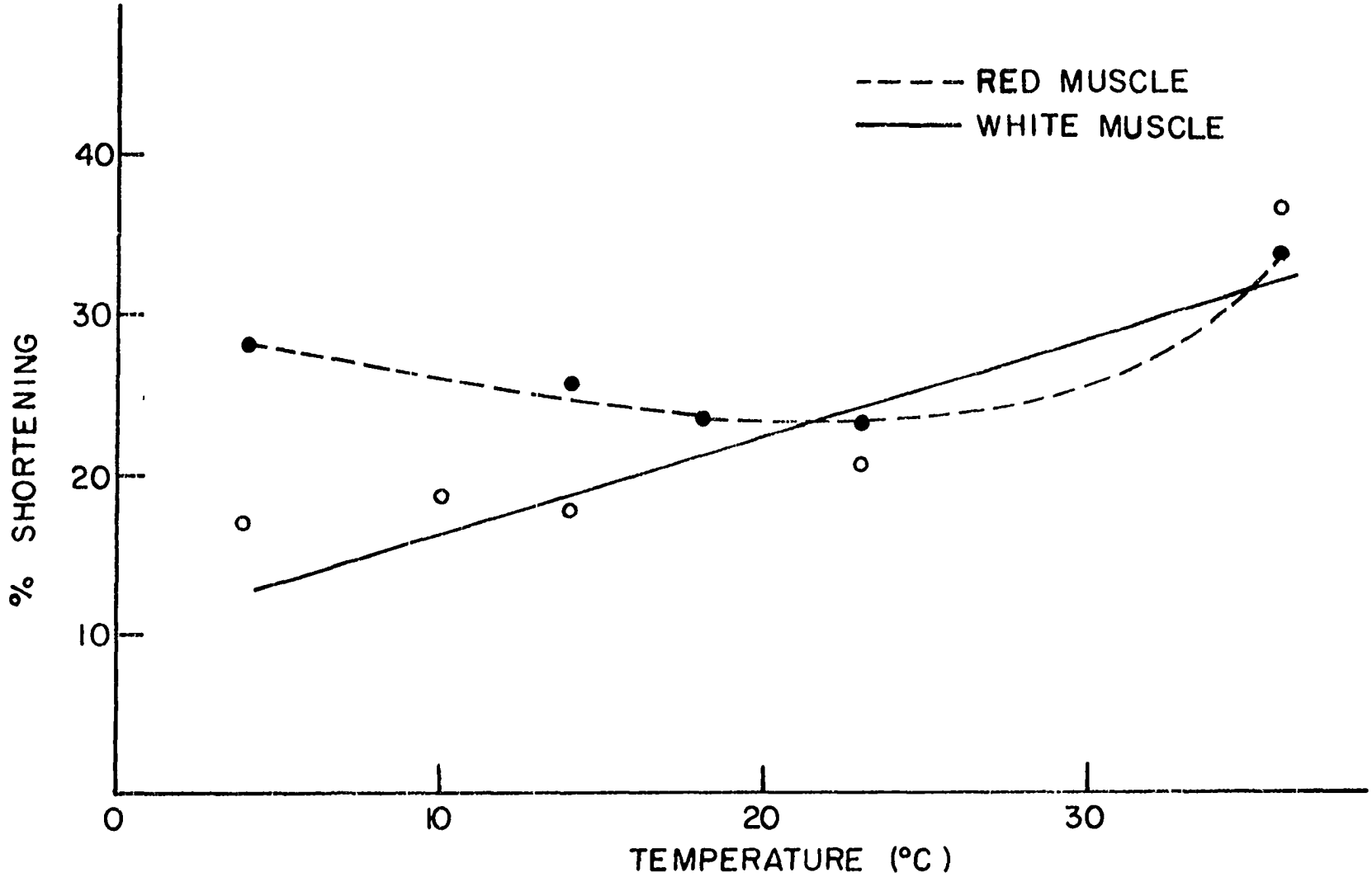


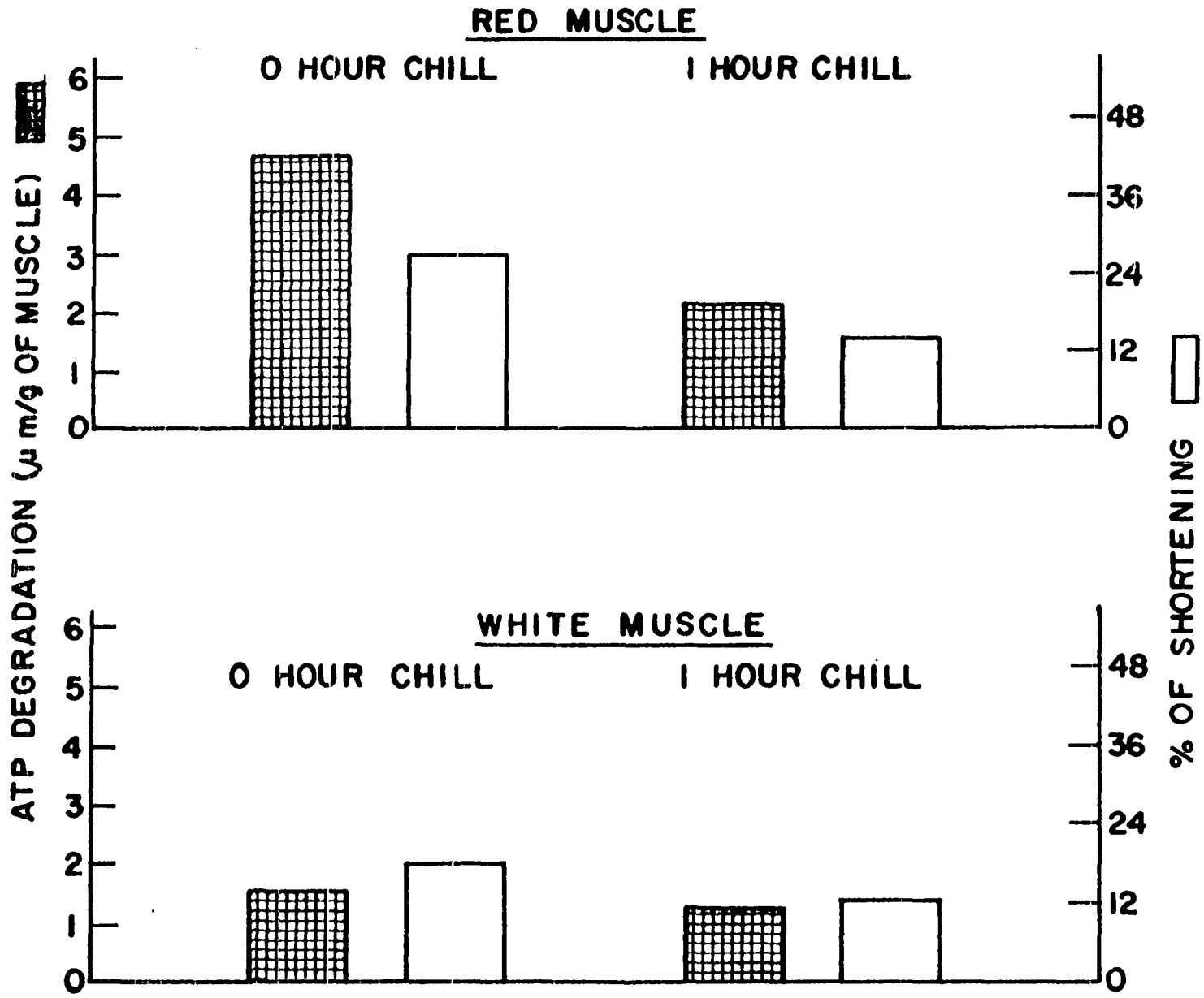
Figure 4. The effect of temperature on post-mortem shortening in excised turkey muscle

extent of shortening and the sufficiency of data. Smith et al. (1969) included the shortening due to excision in their data and made observations of shortening in excised turkey muscle strips at only two temperatures.

Red muscle does not undergo "cold shortening" either. Even though there is a significant curvilinear relationship of muscle shortening to temperature, a true "cold shortening" effect as defined by Locker and Hagyard (1963) apparently does not exist.

To further differentiate the muscle types, the extent of shortening specifically due to low temperature stimulation was measured. Muscle strips of each color type were excised. Initial ATP concentration was assessed while duplicate strips were immersed in an ice bath for 15 minutes. Upon removal from the ice bath, extent of shortening was measured and the strips were analyzed for ATP. This experiment was then repeated at one hour post-mortem. The results of analyses of three birds using three strips per color type for each time period are shown in Figure 5. When subjected to rapid chilling, immediately post-mortem, red muscles shorten considerably more than their white counterparts (27 percent versus 18 percent). There is likewise a greater amount of ATP utilized by the red muscle during such shortening (4.6 μM/g versus 1.5 μM/g). When the experiment was repeated with muscle strips which had been held at room temperature one hour, the differences between the two muscle types were not as apparent. The red muscle strips expended about 50 percent more ATP while shortening only 2 percent more in length than white muscle strips. From these results it is evident that immediately post-mortem

Figure 5. The effect of submersion in ice water on shortening and ATP degradation in red and white muscle strips


RED MUSCLE
0 HOUR CHILL
1 HOUR CHILL
WHITE MUSCLE
0 HOUR CHILL
1 HOUR CHILL
ATP DEGRADATION (μ m/g OF MUSCLE)
% OF SHORTENING
0
1
2
3
4
5
6
12
24
36
48

red muscle is more sensitive to thermal stimulation than white muscle. After one hour aging, the muscles react similarly to the chill treatment.

The results from Tables 7 and 17 indicate that chronological age of turkey significantly influenced the extent of muscle shortening. Overall

Table 7. Effect of chronological age, color and temperature on post-mortem shortening of excised muscle strip

Age	Color	5°C	14°C	18°C	23°C	36°C
(wks)		(%)	(%)	(%)	(%)	(%)
15	Red	33.7	29.3	27.8	25.5	36.2
	White	22.7	23.9	19.4	24.9	28.3
25	Red	21.2	18.2	20.2	20.1	25.5
	White	13.2	19.2	18.2	19.5	20.3
52	Red	29.7	26.7	21.9	23.9	39.3
	White	15.0	11.9	15.2	16.3	33.6

shortening was inversely related to age. The 15-week-old birds shortened 27.2 percent, whereas the 25- and 52-week-old birds shortened 19.5 percent and 23.4 percent, respectively. Muscle type (color) and temperature also had a significant influence on muscle shortening (Table 17).

Temperature Sensitivity

While conducting the preceding experiments there sometimes occurred erratic tension tracing over the initial 15 minutes of the testing period. The problem occurred in experiments at low temperatures. After a

preliminary check showed that the difficulty did not lie in the equipment, an experiment was designed to evaluate the stimulating effect of environmental temperature on muscle strips held isometrically. The conditions were the same for this experiment as those discussed earlier with one minor exception. The solution in which the muscle was immersed was replaced after 15 minutes and 30 minutes post-mortem. The replacement solutions differed only in temperature. The data reported will be the average results from at least three birds for each treatment. The results of this experiment are presented in Figures 6 and 7.

The muscle strips were subjected to various temperature regimes. When subjected to a 2°C temperature environment, red muscle was immediately stimulated to produce about 22 g/cm^2 isometric tension. This is three times the amount of tension exerted by white muscle strips under similar conditions. Both muscle types acclimate to this environmental temperature and within 15 minutes begin to relax. After a 15 minute interval the 2°C bathing solution was replaced by a 30°C solution. Both muscle types gradually developed tension in the normal manner. At 30 minutes post-mortem the solution was changed to 20°C and held for 20 hours. The last 18 hours depict an abnormal rigor pattern for muscle strips held at that temperature in that white muscle reached maximum tension at a time later than red muscle strips and red muscle released tension after 2 hours.

When the initial bathing solution is 30°C (Figure 6) white muscle strips develop tension at a faster rate than their red counterparts. When the temperature is lowered to 2°C the white muscle becomes stationary and the red muscle strips contract slightly followed by a loss of tension. At

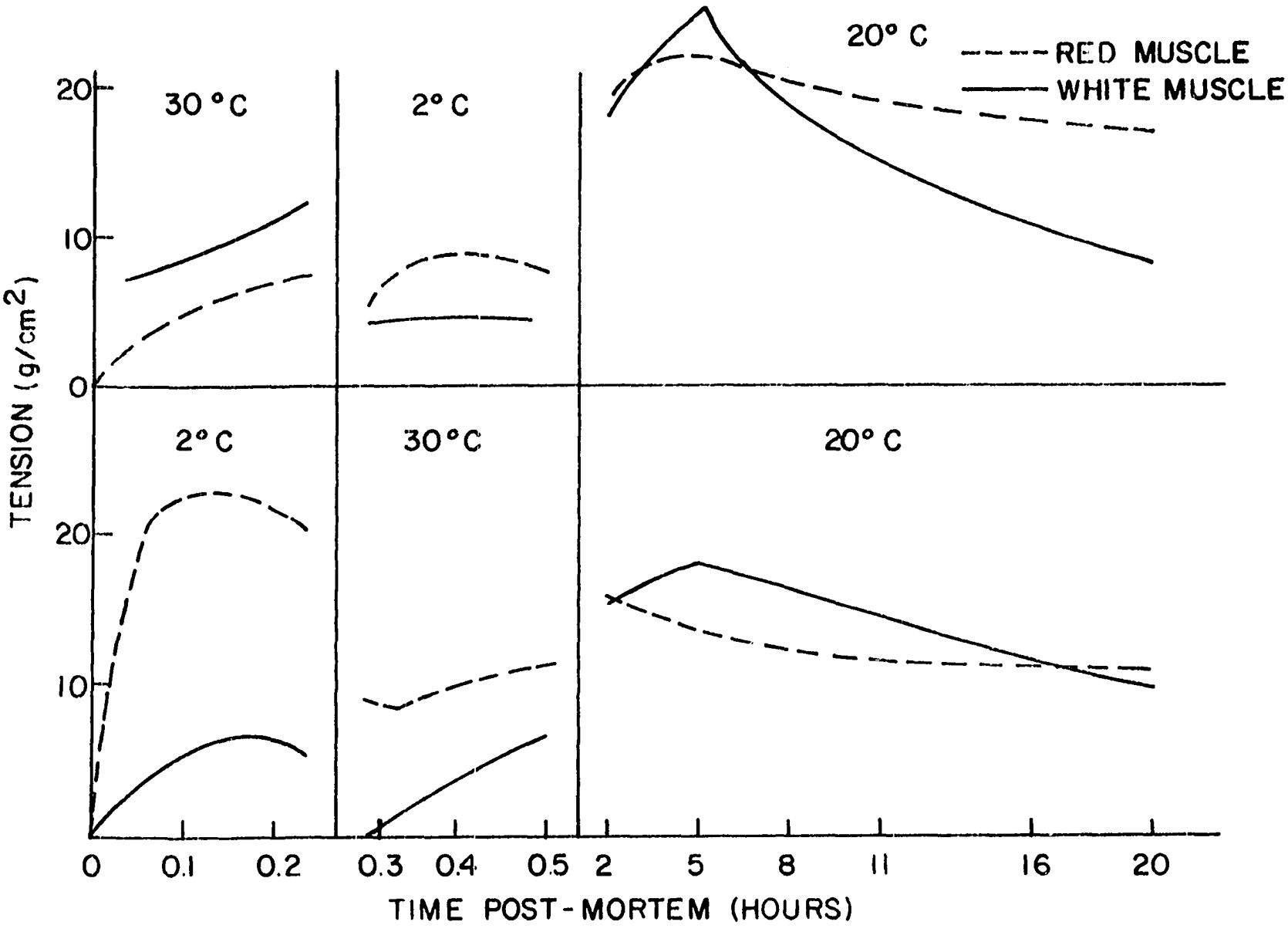


Figure 6. The influence of environmental temperature fluctuations on isometric tension

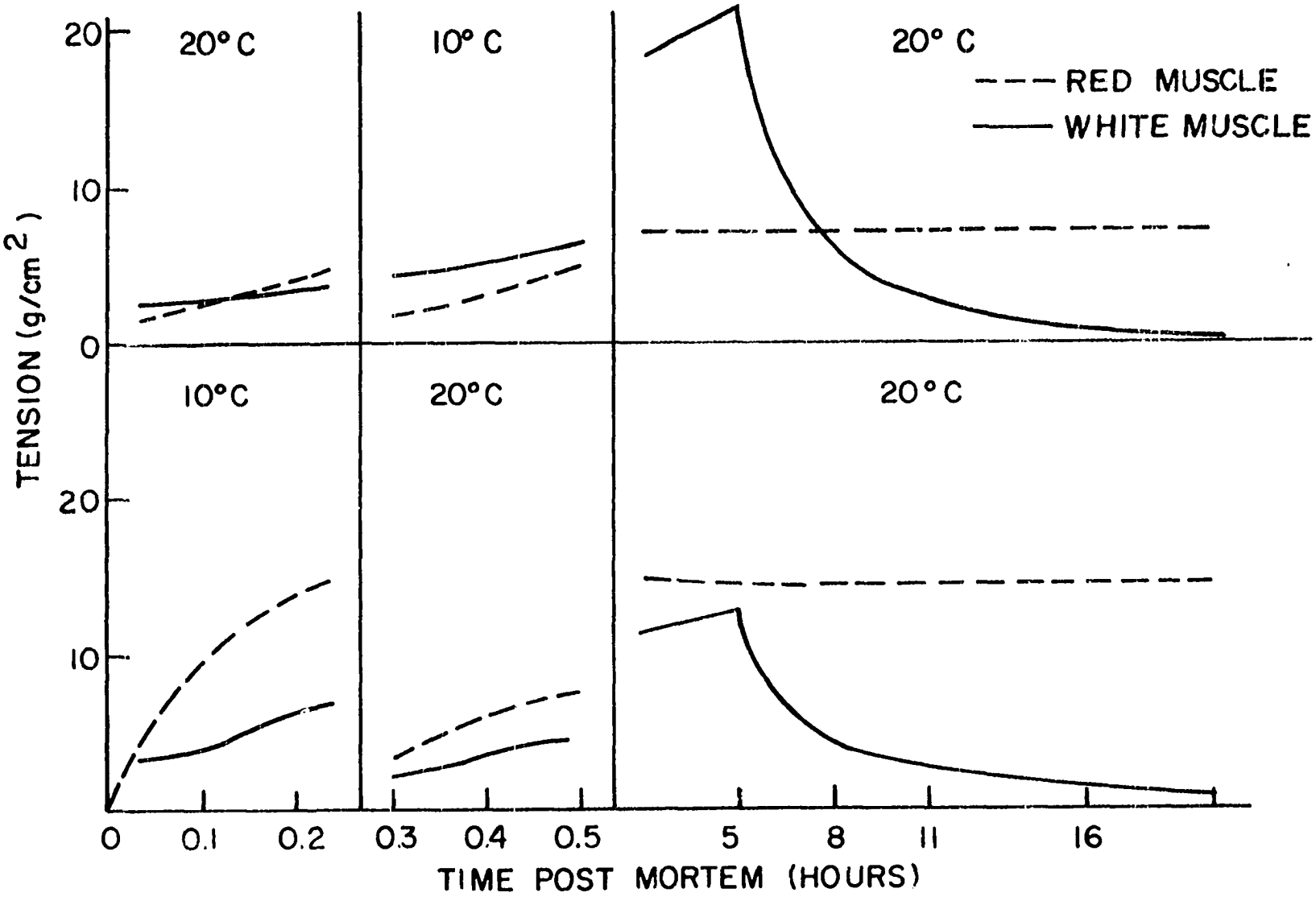


Figure 7. The influence of environmental temperature fluctuations on isometric tension

30 minutes post-mortem the environmental temperature was adjusted to 20°C and remained constant for 20 hours. During this time the red and white strips reached maximum tension at the same time, although the white muscle strips developed a greater amount of tension. The normal pattern of loss in tension ensued.

When the initial environmental temperature is 10°C, red muscle strips are stimulated to contract and produce twice the amount of tension as the white muscle strips (Figure 7). Contrary to that which occurred at 2°C, no loss of tension occurs within the initial 15 minute time interval at 10°C. The environmental temperature was raised to 20°C and held constant for the rest of the period. Both muscle types continued through a normal rigor pattern.

The isometric tension pattern which occurs when muscle strips are held for 15 minutes at 20°C, 15 minutes at 10°C and then for 20 hours at 20°C is very typical of the normal rigor pattern as first exemplified in Figure 1.

The most interesting thing to note about this experiment is that both red and white muscles are stimulated to produce tension by the 2°C environment and they will release the tension within 15 minutes. At 10°C red muscle is stimulated to contract, but no release phase was evident.

From this experiment it has been determined that post-mortem muscle strips when held isometrically can be stimulated by low temperature to develop and release tension. This stimulation cycle may be important for two reasons. First, if muscles attached to the carcass, even though stimulated to develop tension, release it; then this tension would have

no effect on product tenderness. Secondly, if the processor was looking for a method to rapidly deplete ATP stores in the muscles in order to minimize the adverse effects of rigor mortis on tenderness, he might use this technique to bring about such ATP depletion.

Shortening During Thawing

When muscle tissue is frozen very rapidly pre-rigor, the muscle upon thawing is subjected to severe shortening with massive exudation. This phenomenon commonly called "thaw rigor" was first demonstrated by Perry (1950). It has been investigated and shown to occur in many species (Perry, 1950; Marsh and Thompson, 1958; and Jungk, 1965).

The chemical and histological changes associated with "thaw rigor" as well as a theoretical discussion of this phenomenon are given by Bendall (1960).

With rapid processing and liquid nitrogen freezing, it is likely that turkeys might be frozen pre-rigor. For this reason, "thaw rigor" and some associated factors as they occur in turkey were studied. Eighteen birds were dispatched, muscles excised and frozen at various times. The factors studied were age, freezing time, muscle color and storage time. The samples were frozen immediately post-mortem and thawed at room temperature. Three strips of each color type were sampled per bird per thawing time. The average of the three strips for each bird was used in the analysis. Table 18 of the Appendix presents the analysis of the data. Significant effects are: freezing time, storage time and the interactions of age x freezing time, age x color, freezing time x storage time, and significant

at the 5 percent level is age x storage time.

Figure 8 depicts the shortening which occurs during thawing of red and white muscle strips held for various storage times. As can be seen, the extent of shortening decreases with increasing length of storage.

Figure 9 illustrates the effect of delay before freezing on "thaw rigor" shortening. The extent of shortening during thawing markedly decreases with delay before freezing. These samples were also thawed at room temperature.

In order to assess the effect of thawing temperature on extent of shortening, another study was conducted. Five birds were sacrificed and nine strips of each muscle color from each turkey were excised and frozen immediately. The following day three strips per bird of each color type were thawed at 4°, 18° and 36°C. The average shortening of the three strips were used in the statistical analysis. The data are presented in Table 19 of the Appendix and illustrated in Figure 10. Muscle color and thawing temperature were the significant factors. There was very little difference between birds.

Contrary to the results of the other thawing experiment, color was a significant factor. At two of the three temperatures studied, red muscle shortened more than white muscles. This result is consistent with the isometric tension data that showed red muscles usually developed greater tension than white muscles. Evidently with delay before freezing or extended storage time, changes occur in the red muscles which make them "weaker" and similar in shortening or tension development to white muscles.

Perhaps the most important thing to note about Figure 10 is that at

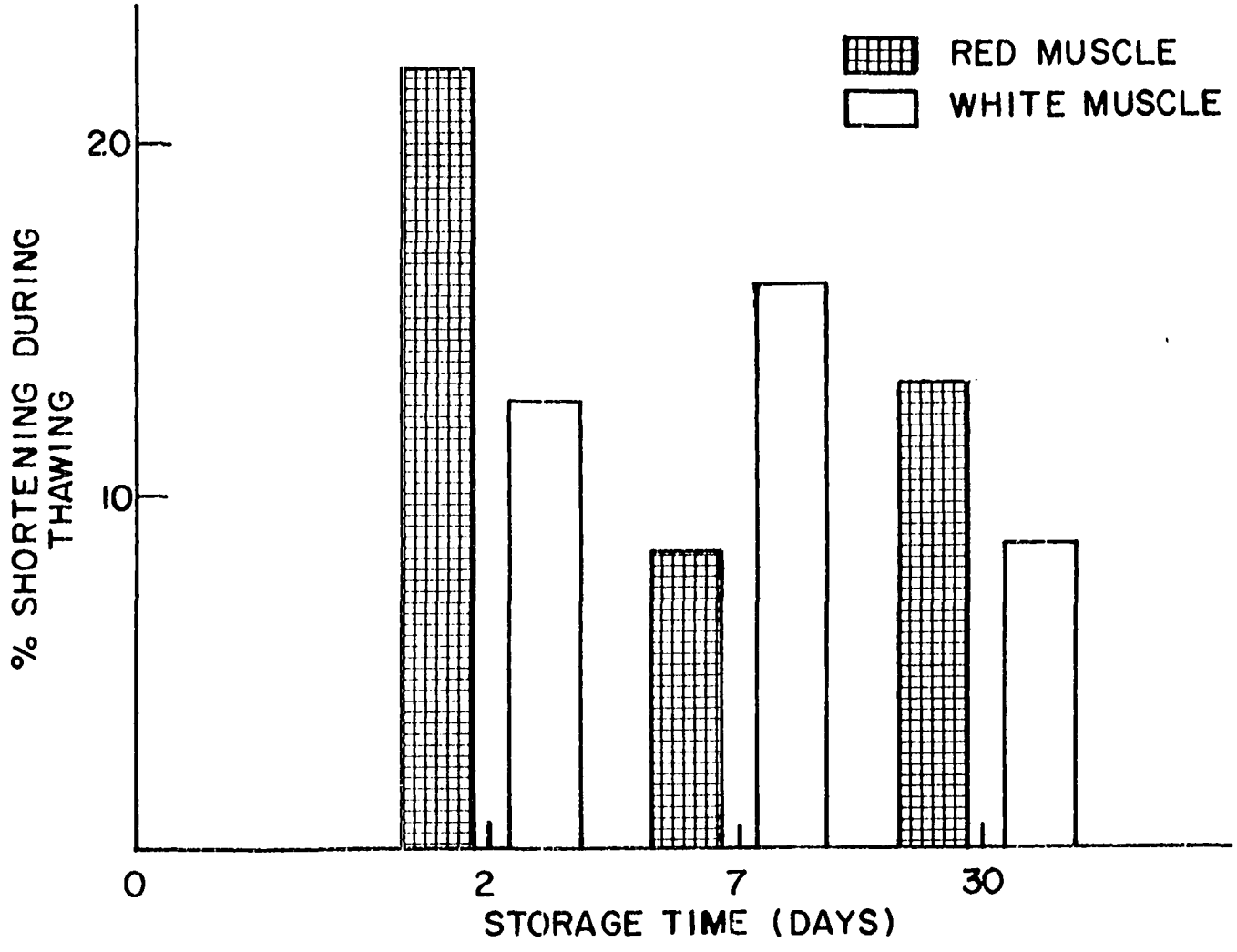


Figure 8. The effect of storage time on "thaw rigor" shortening. Storage temperature was -20°C

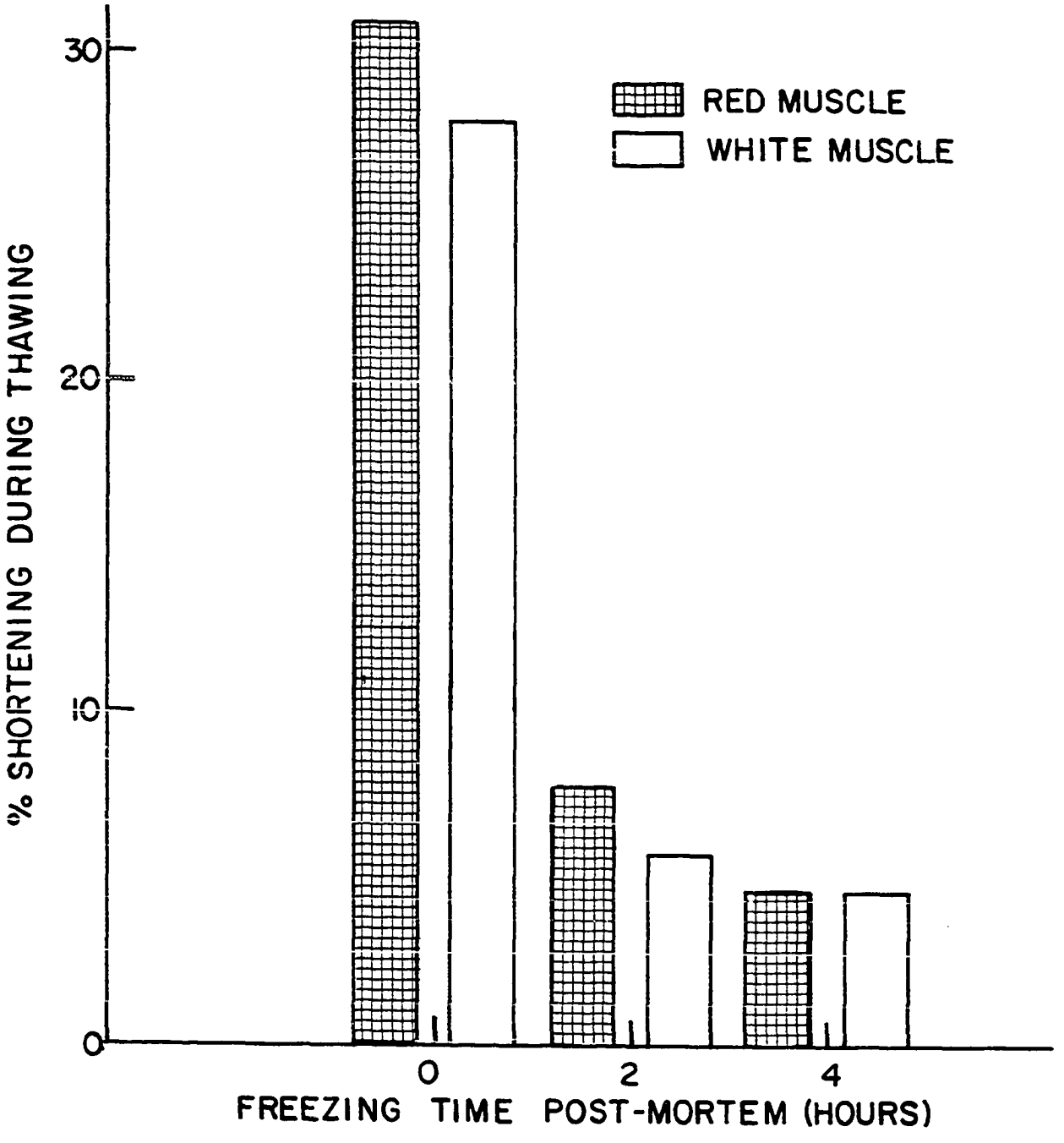
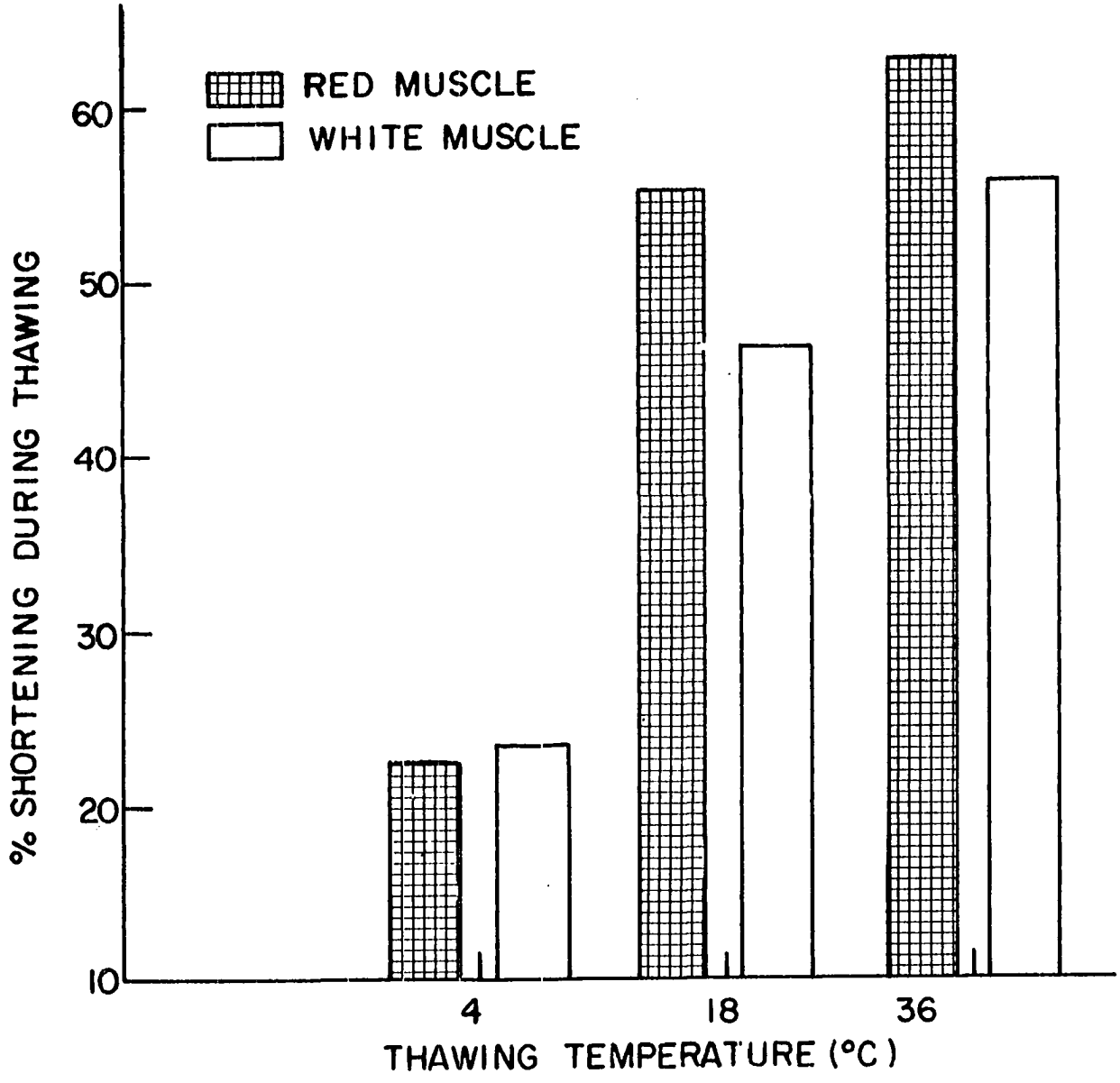


Figure 9. The effect of delay before freezing on the "thaw rigor" shortening. Muscles remained on carcass until freezing time

Figure 10. The effect of thawing temperature on "thaw rigor" shortening


RED MUSCLE
WHITE MUSCLE
% SHORTENING DURING THAWING
60
50
40
30
20
10
4
18
36
THAWING TEMPERATURE (°C)

lower thawing temperatures less shortening occurs. In light of the known relationship between shortening and tenderness, these results may explain the additional tenderization of muscle which may occur during thawing (Klose et al., 1959, 1961a,b). These workers reported that turkeys chilled for only a short period of time and then frozen may benefit from additional tenderization if thawed slowly. In other words, some tenderization occurs during slow thawing and this may substitute for aging before freezing. In actuality, what probably occurs is that the turkeys are being frozen pre-rigor and so the muscles do not undergo much shortening, and then with slow thawing, little additional shortening results. Such processing would minimize any toughness due to muscle shortening. This may be a desirable method to insure adequate tenderness in turkeys; however, the processor usually exerts little or no influence over thawing conditions. Thawing is done at the convenience of the cook.

In spite of the rapid freezing methods which are currently being utilized in the turkey industry there is no great concern about "thaw rigor" and the adverse tenderness associated with it. Two factors undoubtedly contribute to this lack of concern. First, even though present processing methods are rapid there is little pre-rigor freezing of muscle. This condition may change if liquid nitrogen or liquid carbon dioxide attains widespread use as a freezing method. Such methods are currently being used experimentally. Secondly, thaw rigor toughening, even though it could occur, may not appear because of slow marketing. The data from the first "thaw rigor" experiment showed that increased storage time diminishes shortening during thawing. With current

marketing conditions turkeys are often held in frozen storage for two to nine months before consumption. Such storage time allows for the gradual breakdown of muscle constituents (ATP) which otherwise, upon thawing, would cause rapid shortening with associated exudation and toughening. It is paradoxical that slow marketing prevents some of the problems associated with rapid processing. When both processing and marketing become rapid and efficient the problem of thaw rigor will have to be met.

Effect of Drugs on Muscle Characteristics

An experiment was conducted in which turkeys received various drugs. The effects of such drugs on the pattern of rigor mortis was assessed. The three drugs used were: Epinephrine, FDNB (Fluoro dinitro-benzene) and Nembutal. Epinephrine has been used in the study of rigor mortis in poultry by deFremery (1963, 1966a,b) and by deFremery et al. (1959, 1960 and 1963). Epinephrine stimulates the breakdown of glycogen and thereby accelerates the onset of rigor mortis. In the experiment reported here, the epinephrine was used at the same level as in deFremery's experiments. A 1.5 mg/kg dose was administered intramuscularly 16 to 18 hours before death. This dosage is sufficient to reduce muscle glycogen to 5 percent of the normal level.

FDNB is a compound which effectively blocks adenosine triphosphate - creatine phosphotransferase (Cain and Davies, 1962). It has been used as a tool in elucidating the role of the high energy phosphates in rigor mortis (Nauss and Davies, 1966 and Kushmerick and Davies, 1968). FDNB was administered intravenously at a level of 200 mg/kg approximately 15

minutes prior to sacrificing the bird.

Nembutal (sodium pentobarbital) has often been used as a general anesthetic in rigor experiments. Some of the physiological effects of this drug are the depression of vasomotor centers and ganglionic transmissions and uncoupling oxidative phosphorylation in brain mitochondria (Goth, 1968). In our experiments the drug was administered intravenously at a level of 40 mg/kg approximately three minutes before death.

The shortening and isometric tension measurements were conducted in a manner similar to the experiments reported earlier. The data represent the average results of at least three birds for each treatment. Table 8 presents the data.

Epinephrine causes a very rapid onset of rigor mortis. Both muscle types had reached maximum tension by 2.2 hours. The amount of tension which developed is less than would normally be expected during rigor but was similar to that observed in the nembutal treated birds. The nembutal birds took considerably longer to reach maximum rigor. The anesthetic nature of the drug allowed these birds to enter rigor without the severe stress and struggling that normally occurs and consequently the muscles of the nembutal treated birds retained a high energy reserve.

The FDNB birds demonstrated an intermediate time to maximum rigor and developed less tension at maximum rigor than did the controls. The theoretical reason for the epinephrine- and FDNB-treated birds developing less tension is that in each case a portion of the energy reserve that normally contributes to tension development in rigor mortis has been blocked or removed. The epinephrine-treated birds were deprived of any

Table 8. The effect of various drugs on post-mortem isometric tension characteristics. The results shown are average values of nine samples from three birds

Factor	Control		FDNB		Epinephrine		Nembutol	
Muscle type	Red	White	Red	White	Red	White	Red	White
Time to maximum tension (hr)	4	6	3.0	3.3	2.1	2.2	9.8	9.0
Maximum tension (g/cm^2)	38.9	25.8	22.1	9.6	10.3	17.4	17.3	14.6
Percent of maximum @ 40 hours	(59.3)[a]	(14.2)[a]	81	34	24	17	78	43

[a]Percent of maximum @ 20 hours.

energy that may have been produced through glycolysis and the FDNB-treated birds were prevented from utilizing the high energy stores of creatine phosphate.

The drug-treated birds were held under experimental conditions longer than the birds in the earlier experiments. This was done in order to better evaluate the effect of the drugs on the tension release phase of rigor mortis. The loss in tension is represented by the term entitled percent of maximum at 40 hr. Earlier in this section of the thesis a correlation value of +0.80** was reported between rate up and rate down for normal birds. In this experiment, the epinephrine-treated birds reached maximum tension quickly and also had released considerable tension,

($\bar{x}$ = 20.5%) by 40 hours post-mortem. The results of the nembutal are also consistent with the established correlation in that the muscle strips were slow to reach maximum tension and also slow to lose tension. The only apparent inconsistency to the correlation is the FDNB-treated birds. The muscle strips from these birds developed tension at a rapid pace but were slow to lose it. This was especially true with the samples of the Biceps flexor. In their work using FDNB-treated animals for studying "thaw" rigor, Kushmerick and Davies (1968) noted a double phase rigor. They noted that upon thawing the muscles contracted severely and then gradually lengthened. About thirty minutes after relaxation the muscles again went into rigor, but this time no relaxation followed. Based upon biochemical analysis they concluded that relaxation did not occur in this second phase because of the absence of ADP or any polyphosphate. Kushmerick and Davies (1968) maintained that only when both the ATP and ADP levels in the sarcoplasm are very low will rigor occur. If ADP is available the muscles would lengthen (relax).

Busch (1969) was able to prevent loss of tension by the addition of EDTA to the buffer in which the muscle was suspended. The EDTA also stabilized the Z-line of the sarcomere. He also found the addition of Ca++ to the bathing solution accelerated the decline of isometric tension. In relation to the work of Kushmerick and Davies (1968) and Busch (1969) the results obtained in turkeys treated with FDNB suggest that the loss of tension occurs only in the presence of ADP and the post-mortem release of Ca++ from the sarcoplasmic tubules.

A second experiment utilizing the drugged birds investigated the

effect of environmental temperature on post-mortem shortening of excised muscle strips. The data are presented in Figure 11. At the three temperatures studied, 2°, 18°, and 36°C, regardless of treatment, red muscles shorten more than the white muscle strips. The general pattern of shortening as influenced by temperature is consistent with that related in an earlier section of this thesis. Red muscle strips shorten more at 2° and 36° than at the intermediate temperature of 18°C, whereas the shortening of excised white muscle strips is directly related to temperature.

The muscles from the nembutal-treated birds shortened more than muscle strips from the birds with the other treatments. This was probably due to two factors: first, the nembutal-treated birds did not struggle when sacrificed so they probably had more total energy stores retained in the system, and secondly, the energy stores present were usable and not blocked as was the case in the FDNB-treated birds. The excised muscle strips from the epinephrine-treated birds shortened less than the strips from birds of the other treatments. This fact may have been predicted from the isometric tension data. The fact the epinephrine-treated birds reached maximum rigor faster would suggest that they had less energy available for shortening also. A direct comparison between shortening and isometric tension should be made cautiously, however. Busch (1969) has shown that isometric tension development and decline closely parallel post-mortem change in length of isotonically suspended strips. He noted however, that changes of 50 percent in isometric tension are paralleled by changes of only 4-5 percent in length.

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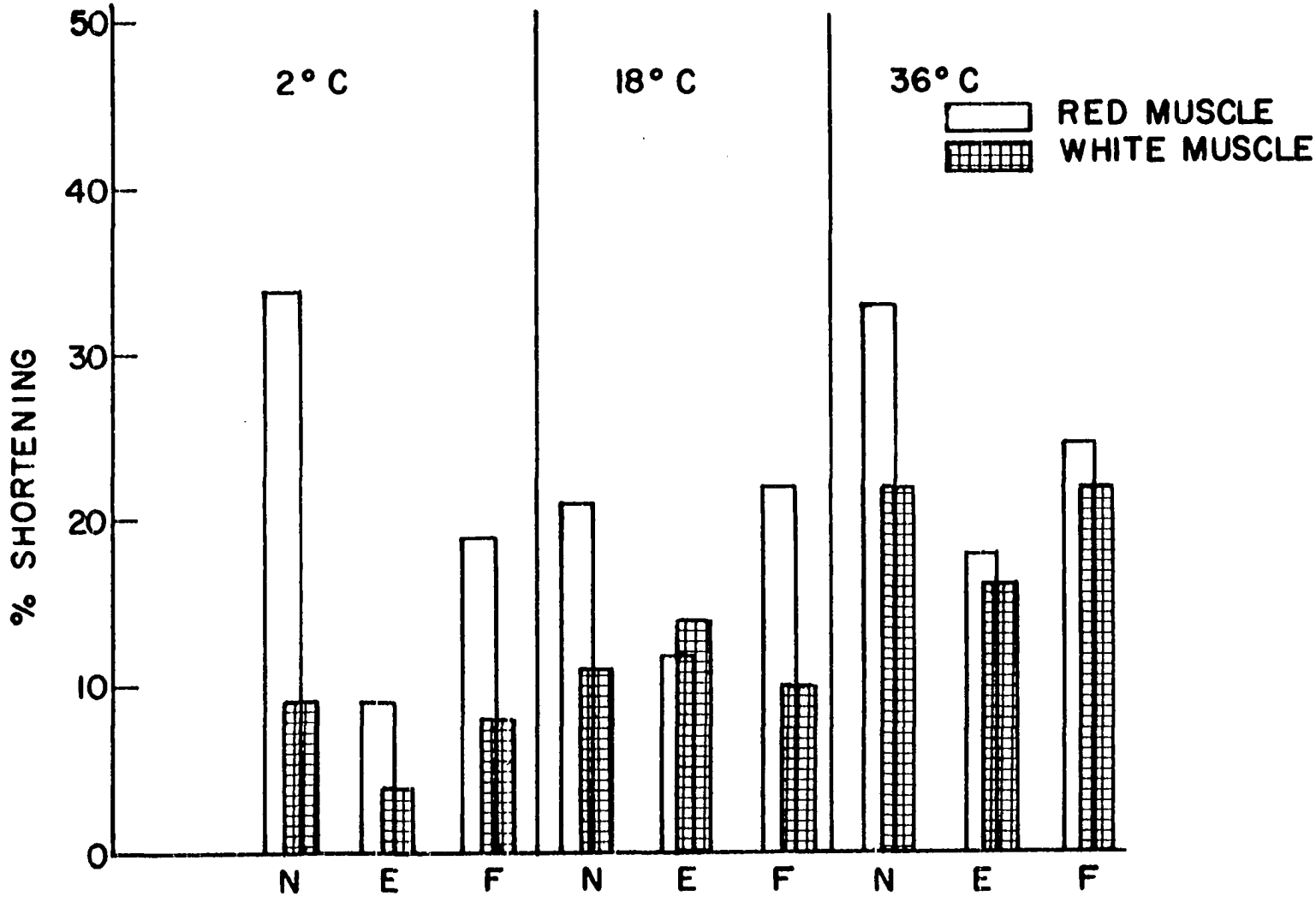
Figure 11. The effect of drugs on post-mortem shortening of excised muscle strips. The drugs used were N - nembutal, E - epinephine and F - fluoro dinitro-benzene. The results shown are averages of nine observations from three birds


50
40
30
20
10
0
% SHORTENING
2° C
18° C
36° C
RED MUSCLE
WHITE MUSCLE
N
E
F
N
E
F
N
E
F

The strips from the FDNB-treated birds were intermediate in extent of shortening. This finding corresponds with the position of the FDNB-treated birds in the time to maximum tension for strips held isometrically. The shortening data present an encapsulated view of the rigor process and can give a relatively good estimation of the time to maximum rigor. As Busch's (1969) data relate, shortening cannot be used to predict exact tension values. Nor does shortening data provide any information regarding the relaxation phase of rigor mortis. It is this ability to describe a relaxation phase that makes the isometric tension method the best available for the study of rigor mortis.

Free Amino Acids

Concurrent with the post-mortem physical changes are a number of chemical changes. The chemical changes are many and diverse. As a complement to the study on the physical characteristic to post-mortem muscle, a biochemical investigation was also undertaken. Of primary interest were the changes in concentration of the various free amino acids. Gross or selective changes in free amino acids may lend support to the hypothesis that proteolysis is a factor responsible for the increased tenderness associated with aging.

Two groups of 12 birds each were used in this study. One group was 17 weeks and the other 27 weeks of age. The birds were dispatched and the muscles excised in the usual manner. Samples of both red and white muscles were taken at 0, 4, and 24 hours post-mortem. The results and significance of the variables studied are shown in several tables which

follow. The complete analysis of variance for each amino acid can be found in the Appendix (Table 20).

Table 9 presents an overall summary of the data and the significance of the variables studied. As can readily be seen, all three factors: Chronological age, muscle color and sampling time do indeed influence the concentrations of free amino acids extracted from the muscle. Only one of the amino acids studied, threonine, was not significantly affected by any of the major variables. Conversely, serine, asparagine, glycine, methionine and arginine were significantly affected by all three treatments and their interactions.

Chronological age

The post-mortem free amino acid concentration of muscles of birds of 17 and 27 weeks of age are shown in Table 10. It can generally be said that the concentration of the free amino acids increase with chonological age. Of the 19 amino acids which were studied, 13 varied significantly with age and 9 of these increased in concentration with increasing age. Only aspartic acid, serine, asparagine and glutamic acid decreased. These results are consistent with other research on muscle tissue in the avian and other species. The younger group of birds in this study were immature and still growing rapidly. As growth occurs there is an increase in the cellular components of the muscle, i.e. total nitrogen, total protein, nonprotein nitrogen and extracellular protein (Simmonds et al., 1964). The extracted free amino acids which were analyzed in this study would comprise a portion of the nonprotein nitrogen fraction. The 27 week

Table 9. Summary of the main effects and interactions studied. Statistical data from which this summary is drawn can be found in the least squares analysis of variance included in the Appendix

Amino acid	µM/g	Age	Color	Sampling time	Age X Color	Sampling time X Age	Sampling time X Color
Aspartic	0.160	**	-	*	-	**	*
Threonine	0.450	-	-	-	*	*	-
Serine	1.447	**	**	*	*	**	**
Asparagine	0.242	**	**	*	*	**	**
Glutamic	0.922	*	**	*	-	**	-
Glutamine	2.250	-	**	*	-	-	*
Proline	0.505	-	**	*	**	*	**
Glycine	1.338	*	**	**	**	*	**
Alanine	2.525	-	**	-	-	*	-
Valine	0.140	*	*	**	*	**	-
Methionine	1.130	**	*	**	**	**	*
Isoleucine	0.135	**	*	**	*	**	-
Leucine	0.375	**	-	**	-	**	-
Tyrosine	0.300	**	-	**	-	**	*
Phenylalanine	0.215	**	-	**	-	**	-
Ornithine	0.055	-	-	**	-	**	-
Lysine	0.268	**	-	**	*	**	**
Histidine	0.125	-	**	**	-	*	**
Arginine	0.300	**	**	**	**	**	**

** Significant at 0.01 level.

* Significant at 0.05 level.

Table 10. Comparison of the free amino acid concentrations of turkey muscles from birds of two age groups[a]

Amino acid	Free amino acid concentration[b]	
	17 weeks of age	27 weeks of age
Aspartic acid	0.191 ± 0.01	0.132 ± 0.01
Threonine	0.435 ± 0.04	0.463 ± 0.04
Serine	1.801 ± 0.09	1.090 ± 0.09
Asparagine	0.272 ± 0.01	0.211 ± 0.01
Glutamic acid	0.987 ± 0.05	0.859 ± 0.05
Glutamine	2.320 ± 0.29	2.176 ± 0.29
Proline	0.494 ± 0.03	0.514 ± 0.03
Glycine	1.210 ± 0.07	1.466 ± 0.07
Alanine	2.299 ± 0.16	2.750 ± 0.16
Valine	0.129 ± 0.01	0.150 ± 0.01
Methionine	0.112 ± 0.01	0.149 ± 0.01
Isoleucine	0.114 ± 0.01	0.154 ± 0.01
Leucine	0.348 ± 0.02	0.440 ± 0.02
Tyrosine	0.282 ± 0.01	0.316 ± 0.01
Phenylalanine	0.187 ± 0.01	0.242 ± 0.01
Ornithine	0.061 ± 0.01	0.051 ± 0.01
Lysine	0.215 ± 0.02	0.321 ± 0.02
Histidine	0.126 ± 0.01	0.126 ± 0.01
Arginine	0.256 ± 0.01	0.342 ± 0.01

[a]Results shown are averages from 12 birds per age group and four samples per bird.

[b]μM per gram of fresh tissue.

old birds were approaching physiological maturity and their rate of growth would have stabilized.

The results of this analysis which show the increasing concentration of free amino acids in turkey muscle with increasing age must only be considered in the context of this experiment. Many other factors such as plane of nutrition, breed and sex, which were controlled in this study, can also significantly influence growth. The results may have been considerably different had any one or all of these factors been varied.

Muscle type

Table 11 presents a comparison of the free amino acid concentrations of the red (B. flexor) and white (P.superficialis) muscles. The most obvious difference between the two muscles is in the concentration of glutamine. The extract from the B. flexor has about twelve times the concentration of this amino acid as the P. superficialis. Red muscle also has approximately four times the amount of glycine, three times the amount of alanine and twice the concentration of proline as white muscle. Eight of the free amino acids studied are significantly different in concentration between red and white muscles. This finding is consistent with research on chicken muscle as reported by Berry and Cunningham (1970) and Miller and May (1965). The muscle color-free amino acid relationship will be discussed with regard to differences in muscle tenderness in a later section of this thesis.

Table 11. Comparison of the free amino acid concentrations of turkey muscles by muscle type (color)[a]

Amino acid	Free amino acids of turkey muscles[b]	
	B. flexor (red)	P. superficialis (white)
Aspartic acid	0.153 ± 0.01	0.169 ± 0.01
Threonine	0.467 ± 0.04	0.430 ± 0.04
Serine	2.180 ± 0.09	0.714 ± 0.09
Asparagine	0.312 ± 0.02	0.171 ± 0.02
Glutamic acid	0.823 ± 0.04	1.023 ± 0.04
Glutamine	4.196 ± 0.29	0.304 ± 0.29
Proline	0.703 ± 0.03	0.305 ± 0.03
Glycine	2.119 ± 0.07	0.557 ± 0.07
Alanine	3.833 ± 0.16	1.216 ± 0.16
Valine	0.132 ± 0.06	0.148 ± 0.06
Methionine	0.117 ± 0.01	0.143 ± 0.01
Isoleucine	0.125 ± 0.01	0.143 ± 0.01
Leucine	0.376 ± 0.02	0.412 ± 0.02
Tyrosine	0.299 ± 0.01	0.300 ± 0.01
Phenylalanine	0.206 ± 0.01	0.225 ± 0.01
Ornithine	0.059 ± 0.01	0.052 ± 0.01
Lysine	0.287 ± 0.02	0.248 ± 0.02
Histidine	0.151 ± 0.01	0.101 ± 0.01
Arginine	0.361 ± 0.01	0.237 ± 0.01

[a]Results shown are the average from 24 birds with two samples per bird per muscle type.

[b]μM per gram of fresh tissue.

Sampling time

Table 12 presents the free amino acid concentration of turkey muscle held for various post-mortem aging periods. Of the 19 amino acids studied, 17 were significantly affected by this treatment. Thirteen were significant at the $P < 0.01$. There does not seem to be any overall general pattern between free amino acid concentration and sampling time. The concentration of 7 of the amino acids studied increased and 6 decreased in concentration with longer aging. Four of the amino acids were inconclusive in pattern.

The muscle samples were held at 20°C. At this temperature, if proteolysis occurs, it would probably occur at a faster rate than at a lower temperature and therefore be more evident. Microbial growth would also be accelerated. At this temperature, and because of the short time period involved, 24 hours, the microbial growth would be primarily limited to surface growth. Samples were intentionally not treated with a bacteriocide. In order to minimize undue influence of proteolysis which may have been attributed to micro-organisms, the samples were drawn from the interior of the muscle. While an attempt was made to minimize the effect of micro-organisms it is recognized that they do undoubtedly play a significant role in flavor and tenderness characteristics. Their effect would be most pronounced in meat aged for long periods, and indeed it may also be important with short aging periods.

The amino acids, valine, isoleucine and tyrosine show the most definite increases in concentration over the 24 hour aging period. This increase in concentration is consistent regardless of muscle type (color)

Table 12. Comparison of the free amino acid concentration of turkey muscles held for various post-mortem aging periods at 20°C[a]

Amino acid	Free amino acid concentration[b] Sampling time post-mortem		
	0 hr	4 hr	24 hr
Aspartic acid	0.186 ± 0.02	0.175 ± 0.02	0.123 ± 0.02
Threonine	0.466 ± 0.05	0.461 ± 0.05	0.420 ± 0.05
Serine	1.657 ± 0.13	2.769 ± 0.13	0.916 ± 0.13
Asparagine	0.261 ± 0.02	0.271 ± 0.02	0.193 ± 0.02
Glutamic acid	0.977 ± 0.06	1.092 ± 0.06	0.701 ± 0.06
Glutamine	2.805 ± 0.40	2.656 ± 0.40	1.290 ± 0.40
Proline	0.516 ± 0.04	0.578 ± 0.04	0.413 ± 0.04
Glycine	1.468 ± 0.10	1.589 ± 0.10	0.957 ± 0.10
Alanine	2.440 ± 0.22	2.948 ± 0.22	2.188 ± 0.22
Valine	0.120 ± 0.01	0.133 ± 0.01	0.166 ± 0.01
Methionine	0.132 ± 0.01	0.077 ± 0.01	0.181 ± 0.01
Isoleucine	0.102 ± 0.01	0.122 ± 0.01	0.188 ± 0.01
Leucine	0.355 ± 0.02	0.340 ± 0.02	0.487 ± 0.02
Tyrosine	0.281 ± 0.01	0.280 ± 0.01	0.339 ± 0.01
Phenylalanine	0.191 ± 0.02	0.169 ± 0.02	0.285 ± 0.02
Ornithine	0.076 ± 0.01	0.056 ± 0.01	0.037 ± 0.01
Lysine	0.191 ± 0.02	0.194 ± 0.02	0.418 ± 0.02
Histidine	0.091 ± 0.01	0.138 ± 0.01	0.148 ± 0.01
Arginine	0.266 ± 0.02	0.239 ± 0.02	0.392 ± 0.02

[a]The results shown are the average from eight birds per sampling time and two samples per bird.

[b]μM per gram of fresh tissue.

or age. Conversely, glutamine and ornithine show a consistent decrease in concentration over the 24 hour aging period. This decrease is likewise consistent over both muscle types and both age groups. Except for glutamine and ornithine it could generally be said that the concentration of the free amino acids increase during post-mortem aging. These findings on turkey muscle are consistent with those of Gardner and Stewart (1966) in their study of the changes in the free amino acids and other nitrogen compounds in stored beef muscle. They found a general increase in free amino acid concentration with aging and noted one exception; the decrease in the concentration of glutamine. Parrish et al. (1969) also found a general increase in the concentration of free amino acids with post-mortem aging. The increase in free amino groups which they detected occurred after most of the increase in tenderness.

Ma et al. (1961) analyzed various muscles from beef and correlated free amino acid concentration and tenderness. They found the concentrations of the two amino acids, leucine and isoleucine, increasing from the less tender to the more tender muscles. In comparing their results with what we obtained in the avian species, our data on the red and white muscles are consistent with their hypothesis. The P. superficialis is more tender and has a higher concentration of leucine and isoleucine than the B. flexor. Relevant to the tenderness-free amino acid observation of Ma et al., it is also noteworthy that both isoleucine and leucine increase throughout the 24 hour storage period. The tenderness of both breast and thigh muscle increases during the first 24 hours post-mortem (Stadelman et al., 1966).

Major statistical interactions

The frequency of the interaction between age and sampling time was numerous and highly significant (Table 13). Thirteen of the 19 amino acids studied were significantly influenced by this interaction at the one percent level and five at the five percent level. The most striking difference observed was that the free amino acid concentration from the older birds tended to increase over the aging period while the concentration from muscles of the younger birds remained stationary or decreased slightly. In the older group of birds, the concentration of seven amino acids increased, one decreased and five were relatively constant over the 24 hour sampling period. For the younger group, five decreased, seven were constant and only one amino acid increased in concentration.

This striking difference between the birds of different age groups suggests that the muscle tissue of the older bird is more labile to proteolytic degradation. The opposite has been found true with regard to the connective tissue portion of muscle. Additional cross-linkages of the collagen molecule tend to make it more stable in older animals (Goll et al., 1964b,c,d). The major sources of the free amino acid in muscle tissue would be the sarcoplasmic and myofibrillar proteins. The connective tissue or stroma proteins would have little if any influence on free amino acid level.

This apparent lability to proteolysis of the sarcoplasmic and myofibrillar proteins of older birds may influence tenderness. In fact, this may account for the reason why older turkeys are consistently more tender with short aging periods than are the younger turkeys.

Table 13. Comparison of the free amino acid concentration of muscles from turkeys of two age groups at three sampling times post-mortem[a]

Amino acid	Free amino acids of turkey muscles[b] Sampling time post-mortem					
	17 weeks of age			27 weeks of age		
	0 hr	4 hr	24 hr	0 hr	4 hr	24 hr
Aspartic acid	0.269	0.221	0.081	0.101	0.129	0.165
Threonine	0.548	0.477	0.279	0.383	0.445	0.560
Serine	2.249	2.363	0.764	1.039	1.174	1.068
Asparagine	0.354	0.339	0.123	0.168	0.201	0.262
Glutamic acid	1.113	1.251	0.595	0.840	0.932	0.806
Glutamine	3.408	2.771	0.789	2.202	2.541	1.786
Proline	0.542	0.622	0.318	0.489	0.535	0.518
Glycine	1.461	1.582	0.588	1.474	1.596	1.327
Alanine	2.512	2.901	1.484	2.363	2.995	2.892
Valine	0.132	0.137	0.118	0.108	0.128	0.213
Methionine	0.130	0.085	0.120	0.135	0.070	0.242
Isoleucine	0.109	0.110	0.120	0.093	0.113	0.255
Leucine	0.376	0.339	0.329	0.334	0.340	0.646
Tyrosine	0.312	0.297	0.238	0.251	0.262	0.435
Phenylalanine	0.189	0.174	0.199	0.192	0.164	0.370
Ornithine	0.096	0.063	0.025	0.056	0.048	0.049
Lysine	0.189	0.185	0.269	0.192	0.203	0.568
Histidine	0.088	0.168	0.122	0.094	0.108	0.175
Arginine	0.275	0.224	0.269	0.258	0.255	0.513

[a]Results shown are the average from 24 birds with two samples per bird per sampling time.

[b]μM per gram of fresh tissue.

Of the 19 amino acids studied, the age X color interactions were significant for 5 amino acids at the 1 percent level and also 5 at the 5 percent level (Table 14). The highest concentrations for the amino acids proline and glycine were found in the red muscles of the older group of turkeys. Conversely, for the younger group, the highest concentration was found in the white muscles. For the amino acids arginine, isoleucine, lycine, methionine and valine the highest concentrations were found in the older group of birds for both muscle types. The only case in which the highest concentration occurred in the young birds for both muscle types was for the amino acid serine. Generally, as pointed out with regard to the major variables studied, the highest concentrations of amino acids are found in the red muscles of the older birds.

Although seven of the amino acids studied showed significant interaction at the 1 percent level between sampling time X color, there are no apparent differences between red and white muscles when compared over the 24 hour sampling period. Table 15 presents this comparison.

The data from the free amino acid study were also subjected to a multivariate analysis. Frequently groups of variables function together to effect the results. The multivariate analysis revealed that four amino acids were of key importance. Variation of serine, glutamine, glycine and alanine as a group explained 78 percent of the total variation associated with age, color and sampling time.

Table 14. Comparison of the free amino acid concentrations of turkey muscles from two age groups[a]

Amino acid	Free amino acids of turkey muscles[b]			
	B. flexor		P. superficialis	
	(17 weeks of age)	(27 weeks of age)	(17 weeks of age)	(27 weeks of age)
Aspartic acid	0.191	0.115	0.189	0.149
Threonine	0.386	0.549	0.483	0.377
Serine	2.708	1.653	0.893	0.535
Asparagine	0.375	0.249	0.169	0.171
Glutamic acid	0.917	0.729	1.057	0.989
Glutamine	4.249	4.143	0.397	0.210
Proline	0.619	0.787	0.369	0.241
Glycine	1.840	2.397	0.581	0.534
Alanine	3.393	4.273	1.200	1.227
Valine	0.113	0.150	0.146	0.150
Methionine	0.079	0.155	0.143	0.143
Isoleucine	0.095	0.155	0.132	0.154
Leucine	0.307	0.446	0.389	0.434
Tyrosine	0.275	0.322	0.289	0.310
Phenylalanine	0.175	0.236	0.201	0.248
Ornithine	0.059	0.060	0.063	0.042
Lysine	0.213	0.362	0.217	0.279
Histidine	0.157	0.145	0.095	0.107
Arginine	0.286	0.437	0.226	0.247

[a]Results are the average from 24 birds. Two samples of each type per bird were analyzed.

[b]μM per gram of fresh tissue.

Table 15. Comparison of the free amino acid concentration of turkey muscles at three sampling times and two muscle types (color)[a]

Amino acid	Free amino acids of turkey muscles[b] Sampling time post-mortem					
	B. flexor			P. superficialis		
	0 hr	4 hr	24 hr	0 hr	4 hr	24 hr
Aspartic acid	0.134	0.192	0.133	0.237	0.158	0.112
Threonine	0.460	0.487	0.455	0.470	0.435	0.385
Serine	2.630	2.690	1.221	0.684	0.847	0.611
Asparagine	0.376	0.376	0.184	0.146	0.165	0.201
Glutamic acid	0.944	0.936	0.589	1.010	1.248	0.812
Glutamine	5.236	4.943	2.408	0.373	0.370	0.168
Proline	0.808	0.774	0.526	0.224	0.383	0.309
Glycine	2.432	2.478	1.197	0.504	0.699	0.468
Alanine	3.903	4.386	3.210	0.972	1.510	1.166
Valine	0.112	0.124	0.158	1.280	0.142	0.174
Methionine	0.133	0.060	0.159	0.132	0.095	0.204
Isoleucine	0.095	0.105	0.175	0.108	0.109	0.201
Leucine	0.352	0.318	0.459	0.358	0.361	0.515
Tyrosine	0.297	0.286	0.314	0.266	0.274	0.360
Phenylalanine	0.186	0.167	0.263	0.196	0.171	0.306
Ornithine	0.076	0.061	0.042	0.076	0.044	0.032
Lysine	0.252	0.226	0.383	0.129	0.162	0.454
Histidine	0.096	0.198	0.160	0.086	0.079	0.137
Arginine	0.359	0.316	0.408	0.173	0.163	0.375

[a]Results shown are the average from 24 birds with two samples per birds per muscle type.

[b]μM per gram of fresh tissue.

SUMMARY

1) A post-mortem pattern of isometric tension development followed by a gradual release of tension occurred in turkey muscle. The pattern is common to both muscle types, birds of several ages and over the temperature range studied (2°C-37°C). The post-mortem tension pattern can conveniently be divided into four parameters: rate of tension development, time to maximum tension, amount of maximum tension and rate of tension release. These can easily be measured and related to the variables studied.

2) The rate of tension development was significantly influenced by temperature ($p < 0.01$) and age ($p < 0.05$). There was also a significant ($p < 0.05$) interaction on tension developed by the variables of temperature and color. The rate of tension development increased with increasing temperature. The only disparity from this generalization occurred with red muscles because of an overriding shortening influence at low temperatures. The rate of tension development for red muscle exceeded white muscle. Muscles from younger birds developed tension more rapidly than muscles from older birds.

3) Age, color and temperature significantly ($p < 0.01$) influenced the time to maximum tension. White muscles developed maximum tension more quickly than the red muscles. Except at lower temperatures where a stimulatory effect has been demonstrated, the time to maximum tension development increased with increasing temperature.

4) Red muscle developed more tension in going through rigor mortis than did white muscle. The differences between muscle types is most

pronounced in the temperature range 0-10°C, where red muscles are stimulated to shorten.

5) There was no significant difference in the rate of tension release for the two muscle types studied. The release of tension was faster at the higher temperatures. A correlation coefficient of 0.80** defines the relationship between rate of tension development and loss of tension. The greater the amount of tension developed during rigor mortis the faster the release of tension.

6) Excised muscle strips normally shorten as they pass through rigor mortis. The shortening which occurs in excised strips closely parallels the tension development which occurs when muscle strips are restrained isometrically. Age, muscle type and temperature significantly ($p < 0.01$) influenced the degree of shortening. Overall shortening is inversely related to age. Red muscle shortened more than white muscle strips at all temperatures studied. There are two ways in which environmental temperature can influence the degree of shortening. Temperature primarily influences the rate of the biochemical reactions which occur post-mortem. A secondary effect of temperature, which is especially noteworthy at the temperature extremes studied, is that of stimulating the muscle to contract. The temperature extremes are stimulatory only for a short period post-mortem, after awhile the muscle gradually loses its irritability.

7) When turkey muscle is rapidly frozen pre-rigor, the muscle upon thawing is subjected to severe contracture with massive exudation. The degree to which this phenomenon, called "thaw rigor", occurs in inversely proportional to storage time and delay prior to freezing. At lower thawing temperatures less shortening occurred. Moreover, red muscle strips

shortened more than white muscle strips.

8) The variable of chronological age, muscle type (color) and post-mortem sampling time have a significant effect on the type and the amount of free amino acids which can be extracted from muscle. The concentration of free amino acids was higher in the older group of birds studied. Several of the amino acids studied occurred in higher concentrations in the red muscles. The most notable concentration difference was the amino acid glutamine which in the B. flexor is about twelve times the concentration of the P. superficialis. With the exceptions of glutamine and ornithine, it can generally be said that the concentrations of the free amino acids extracted increase with post-mortem aging time. Post-mortem aging of muscles from the older birds yielded extracts containing higher concentrations of free amino acids. This suggests that the muscles from older birds are more labile to proteolysis.

CONCLUSIONS

The post-mortem physiochemical characteristics of turkey muscle is significantly influenced by chronological age. Younger birds develop tension faster and take a longer time to reach maximum tension. The amount of tension at maximum is also higher for the younger birds.

The post-mortem phenomena outlined by the isometric tension pattern parallel the changes which occur in excised strips. The muscle which is held isometrically does however lose its ability to maintain tension and this loss can be measured. It is this capability of measuring decline in tension that makes the isometric tension method the preferred technique of depicting rigor mortis. The isometric tension method more closely duplicates muscles attached to the carcass.

The effect of environmental temperature on pre-rigor muscle tissue has been grossly underestimated in the poultry industry. Industry traditionally chills the carcass as rapidly as possible even to the extent of flushing with liquid carbon dioxide and nitrogen. Rapid chilling was instigated because of microbial considerations. Current processing techniques are more rapid and sanitary than in the past. Because of this, some flexibility in post-mortem holding times is not only possible but desirable. Although red muscle is more sensitive to environmental temperature than white muscle, one basic consideration is that white muscle underlies the largest muscle surface area of the avian carcass. Processing temperature by necessity must therefore minimize contraction of both muscle types. From a tenderness standpoint the environmental temperature during

the first hour post-mortem should be in the range of 14°-23°C. After the first hour a gradual decrease to freezing temperature is preferred. Freezing should never occur before four hours post-mortem or the detrimental effects of "thaw rigor" will adversely affect product quality. After four hours post-mortem the effect of environmental temperature is negligible. By this time the muscles are no longer sensitive and generally tension development will have ceased.

Under current processing conditions some turkeys are being frozen pre-rigor and this factor adversely affects tenderness. Often the effect of freezing pre-rigor may be negated by slow thawing. Because of the growing practice of cooking turkey from the frozen state the industry must take steps to minimize pre-rigor freezing.

Another industry practice which may adversely affect product tenderness is hot deboning. Muscle tissue severed anytime prior to maximum rigor will tend to shorten. The extent of shortening depends on environmental temperature and time post-mortem. Shortening can be minimized by controlling temperature between 14° and 23°C and severing the tissues as late in the tension development phase as possible.

The time and temperature factors which must be optimized to produce the most tender bird are most critical for the younger birds.

The free amino acid data of this thesis lend support to the contention that poultry goes through rigor mortis more rapidly than the other red meat animals. There is some factor, perhaps proteolysis, associated with the post-mortem physiochemical changes which causes an increased

extractability of free amino acids with post-mortem aging time. The increased extractability is especially noteworthy in the older birds. The data suggest that tissue from the older birds is more labile.

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APPENDIX

Table 16. Summary of isometric tension data; least squares analysis of variance. Data are derived from 142 observations from approximately 30 turkeys

	d.f.	Mean squares			
		Rate up	Rate down	Time to maximum rigor	Maximum tension
Total	247				
Total reduction	21	887.52	930.33	390.48	8,937.17
MU-YM	1	5,097.15	963.45	2,419.99	63,710.25
Temperature	6	361.45**	1,622.77**	133.45**	4,102.19**
Age	7	180.78*	333.25	50.26**	1,936.22*
Color	1	262.17	1,281.17	119.40**	6,044.38*
Temperature x color	6	181.68*	1,513.52*	111.06**	2,463.48*
Remainder	226	79.09	563.47	11.54	986.94

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 17. Analysis of the factors which influence post-mortem shortening of excised turkey muscle strips. Fifteen birds were used in this experiment

Source	Degrees of freedom	Mean squares
Age	2	585.22**
Birds	9	69.26
Temperature	4	399.03**
Color	1	1,266.33**
Temperature x color	4	53.79
Age x temperature	8	83.50
Age x color	2	121.72
Age x temperature x color	8	21.65
Error	81	42.98
Total	119	

**Significant at 0.01 level.

Table 18. Analysis of the factors which influence the percent shortening during "thaw rigor." Samples were derived from 15 birds

Source	Degrees of freedom	Mean squares
Age	2	19.57
Freezing time	2	10,173.43**
Color	1	115.86
Storage time	2	1,537.85**
Age x freezing time	4	1,286.20**
Age x color	2	1,000.60**
Age x storage time	4	352.15*
Freezing time x color	2	32.34
Freezing time x storage time	4	515.70**
Color x storage time	2	185.21
Age x freezing time x color	4	185.97
Age x freezing time x storage time	8	105.39
Age x color x storage time	4	238.62
Freezing time x color x storage time	4	116.91
Error	116	132.35
Total	161	

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 19. Analysis of the factors which influence the percent shortening during "thaw rigor"

Source	Degrees of freedom	Mean squares
Bird	4	32.00
Color	1	192.53**
Temperature	2	3,547.20**
Color x temperature	2	66.13
Error	20	33.04
Total	29	

**Significant at 0.01 level.

Table 20. Summary of the main effects and interactions of several variables on the free amino acids extracted from turkey muscle. Twenty-four birds of two ages were used in this experiment

	Mean squares					
Source	Age	Color	Sampling time	Age X Color	Sampling time X Age	Sampling time X Color
d.f.	1	1	2	1	2	2
Asp	132**	9.8	43.4*	12.4	160**	73.2*
Thr	29.8	53.8	24.0	694*	503*	22.5
Ser	19,204**	82,579**	8,245**	4,664*	7,364**	7,063**
Asp-NH_2	146**	772**	69.4	155**	296**	241**
Glu	624*	1,540**	1,549**	137	828**	198
Glu-NH_2	822	581,664**	26,857*	62.7	11,692	26,480*
Pro	15.0	5,072	251*	839**	235*	432**
Gly	2,497*	93,650**	4,312**	3,509**	1,682*	3,333**
Ala	7,808	263,006**	5,766	7,052	6,735*	3,155
Val	16.5*	10.0*	21.5**	10.5*	39.6**	0.03
Met	54.5**	26.4*	103**	54.8**	52.9**	7.8*
Ileu	63.7**	12.8*	85.5**	14.4*	65.0**	0.7
Leu	325**	47.3	253**	87.0	370**	8.2
Tyr	44.0**	0.1	40.4**	6.5	193**	20.3*
Phe	113**	13.7	145**	1.8	97.3**	5.7
Orn	4.0	2.0	14.6**	4.5	10.1**	0.5
Lys	431**	58.9	655**	71.8*	265**	126**
His	0.0	97.8**	35.9**	5.3	31.3*	44.5**
Arg	284**	595**	252**	160**	185**	84.7**

* Significant at 0.01 level.

** Significant at 0.05 level.