MUSCLE CONTRACTION AND TENDERNESS

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Research efforts in more recent years have been attracted by the terrific influence muscle contraction state has in affecting tenderness of muscle. Early observations by Lowe and Stewart (1946), Ramsbottom and Strandine (1949), Koonz, et al. (1954), and Paul and Bratzler (1955) were that a muscle which was cut or excised in a pre-rigor condition would be tougher than expected when compared to muscles chilled while attached to the carcass. Locker (1960) suggested that the toughening that occurred might have been due to shortening of the muscle during the onset of rigor mortis. It thus becomes apparent that the degree of contraction as measured by sarcomere length in which a muscle enters the completion of rigor mortis could be influenced by numerous factors including temperature, tension imposed and post-mortem glycolytic rate.

Bendall (1951) studied rigor shortening extensively within the temperature range of 17-37°C. He found adenosine triphosphate (ATP) started to break down when 70% or more of the creatine phosphate (CP) had disappeared. At 17 to 37°C., active shortening occurred after the ATP concentration had begun to fall below some critical level which was pH dependent being lower at a lower pH. Shortening during the onset of rigor was termed an irreversible contraction and depended on the disappearance of ATP. More recently, Locker and Hagyard (1963) examined the shortening of muscle which accompanies or precedes the onset of rigor at temperatures from 0 to 43°C. Their investigations revealed a "cold-shortening" phenomenon which occurred when fresh excised bovine muscle was exposed to temperatures from 0 to 8°C. The shortening which resulted was on the order of magnitude of 48% of the initial length at 0°C. Their results indicated that shortening was minimal at 15-20°C. and was progressively greater at temperatures both above and below this range. One might expect shortening concomitantly with toughening, therefore, in muscles which were exposed in a pre-rigor condition to temperatures well above or below the intermediate range of 15 to 20°C.

Additional studies on the effect of temperature on rigor mortis and physical and chemical changes post-mortem in bovine muscle were conducted by Cassens and Newbold (1966, 1967 a,b). They found the delay phase of rigor mortis was shorter at lower temperatures in the range of 1-15°C. However, the onset phase was prolonged by low temperatures down to 5°C., but it was shorter at 1°C. than at 5°C. The change in extension was completed sooner at 1°C. (and at pH 6.2) than at 5°C., indicating rigor had commenced earlier and was more fully developed sooner at 1° than at 5°C. However, at 1°C., an appreciable amount of ATP (60% of the initial level) still remained upon completion of rigor (as measured on the kymograph) and the pH (at 6.2) was above the ultimate value (5.78). They showed the conditions at 1°C. under which cold-shortening occurred resembled more closely thaw-rigor than normal rigor. It was suggested that the conditions of low temperature might have caused an ion imbalance and the release of sufficient calcium ions from the sarcoplasmic reticulum to activate the actomyosin type of odenosine triphosphatase (ATPase).

At the higher in-vivo end of the temperature scale, accelerated disappearance of ATP and a high rate of glycolysis have also been shown to result in shortened muscle. A high correlation was found by Sink, et al. (1965) between the mean sarcomere length in the pig longissimus after 24 hr. and the duration of the delay phase of rigor mortis. Greater shortening was associated with a diminished duration of the delay phase. Similarly, de Fremery (1966) found that severe mechanical feather plucking, pre-rigor freezing and thawing, elevated temperature, muscle excision and electrical stimulation accelerated ATP and glycogen disappearance as well as the onset of rigor mortis and induced toughness in poultry muscle. Thus, the shortening at high temperature is probably due to a rapid glycolytic rate while that at low temperature is probably due to the cold-stimulation of muscle.

Muscles are believed to exert tension during the onset of rigor mortis and this may aid in explaining the shortening which occurs if muscles are unrestrained. Busch, et al. (1967) found the force of tension of bovine muscle at 2°C. was five times that at 16°C., but that at 37°C. was less than half that at 2°C. At both 16° and 2°C. tension was developed in the presence of 5 mM ATP, and yet marked differences in tension were observed. They concluded that shortening at 2° was different from that at 37°C. At 2° it was "cold-shortening" via the sliding of filaments while at 37°C. they theorized it was due to thermal shrinkage of collagen fibers caused by a low pH and high temperature. However, no sarcomere length measurements were presented of myofibrils of muscles which shortened at 37°C.

Much evidence is accumulating on the effect of "cold-shortening" on tenderness of muscle. Locker (1960) studied the effect of shortening on the eating quality of psoas major muscle. Pre-rigor excision resulted in a 20-30% shortening and there was a corresponding decrease in sarcomere length. He concluded that relaxed muscles were more tender than partly contracted muscles and that this effect was especially significant in muscles of low connective tissue content. However, since some other relaxed muscles were also tough, connective tissue may have had a dominant effect. Herring, et al. (1965a) extended this work on muscle contraction state and tenderness to the semitendinosus (a muscle high in connective tissue) as well as the psoas major. They found "cold-shortening" was greater at 1° than at 5°C. and there were corresponding decreases in tenderness at 1°C. Pre-rigor excised muscles held at the ends were decidedly more tender than portions of the same muscle allowed to shorten during rigor mortis. Pre-rigor excision of muscle resulted in three types of shortening: 1) that associated with the excision itself, 2) that associated with "cold-shortening", and 3) that associated with the onset of rigor mortis.

Weidemann, et al. (1967) observed that pre-rigor cooking of unrestrained muscle produced contraction bands or clots and very short sarcomeres. Stretched muscle reached its ultimate tenderness by one day post-mortem while muscle restrained at the excised length required three days and shortened muscle remained tough during the period of study.

From studies on sarcomere lengths of various muscles from the suspended carcass, Locker (1960) surmised that the variations observed may have been due to strains which were induced. Herring, et al. (1965b) in an extension of their earlier work studied the effect of carcass positioning on the inherent muscle tension during the onset of rigor mortis. The

process of vertical suspension of the bovine carcass (versus horizontal placement) increased tension in certain muscles (psoas major, rectus femoris, latissimus dorsi) as judged by longer sarcomeres when compared to the same muscles from opposite sides held in the horizontal position. This increase in sarcomere length resulted in improved tenderness for these muscles. There was also with vertical suspension of the carcass a release of tension and shortening in certain other muscles including the longissimus dorsi, gluteus medius, adductor, biceps femoris and semitendinosus. These latter muscles had a lower tenderness rating than the same muscles from the contralateral side. Furthermore, shortening of the myofibril was related to an increase in the cross-sectional area of the muscle fiber.

Additional studies were then conducted to determine whether it was more important to prevent "cold-shortening" of muscle post-mortem or whether they should be stretched to achieve maximum tenderness (Herring, et al. 1967b). It was shown using the semitendinosus that sarcomere length was curvilinearly related with fiber diameter. Shear force was curvilinearly related with sarcomere length and the shortening which occurred to sarcomere lengths below 2.0μ resulted in extremely tough muscle from both young (A maturity) and old (E maturity) animals. Shortened muscles also had a higher relative percent cross-sectional area of muscle fibers and a lower relative percent of endomysial and perimysial material than stretched or relaxed muscle. Therefore, marked shortening of muscle with an increased fiber size and a concomitant greater overlap of myofilaments appeared to be responsible for the extreme toughness, termed "actomyosin toughness", which was observed. On the other hand, stretching muscle to sarcomere lengths greater than 2.5μ resulted in only a small improvement in tenderness in the strips of muscle used in the experiments.

Marsh and Leet (1966) also found a complex relationship existed between "cold-shortening" and tenderness. Shortening of the sternomandibularis muscle of up to 20% of the initial excised length did not exert a significant effect on tenderness. However, toughness increased markedly with further shortening beyond this point and a peak shear of several times the original value was received at a shortening of about 40% of the excised length. With yet further shortening, via thaw rigor, "cold-shortening", etc., the muscle became progressively more tender until at 55-60% shortening it was cleaved about as easily as that in which less than 20% shortening had occurred. On the excised muscle strips, delayed chilling was effective in reducing the amount of ultimate "cold-shortening". When muscle strips were restrained, the "cold-shortening" effect did not occur and no toughening was found. The decreased shear force at greater than 40% muscle shortening was difficult to explain. They theorized it was due in some degree to internal disorganization or disruption which was accompanied by exudation and also the sarcolemma may have been ruptured.

Marsh and Leet (1966) also showed that partial insulation of the sternomandibularis muscle from the cold prevented the localized shortening except in that portion of the muscle exposed to the cold air. It was suggested, therefore, that activation of "cold-shortening" could occur in muscle in the carcass that was covered with less fat, that which was not under tension and that which was not in close proximity to bone. Marsh, et al. (1968) then studied the effects of carcass freezing before the completion of rigor mortis on tenderness of ovine longissimus muscle. Very

significant toughness developed in the longissimus muscle of carcasses which were exposed to low temperature (-18°C. with moving air) within about 16 hr. of slaughter. They termed this type of toughness as "processing toughness". It is another term for "actomyosin-toughness" which is due to the effect of "cold-shortening" and/or thaw-rigor contracture. Since the longissimus muscle fibers are attached at only one end to bone, this muscle is allowed to shorten readily under adverse temperature conditions. This type of toughness is not due to the lack of aging, as contracted muscle is tough even after aging up to 10-13 days post-mortem (Goll, et al., 1964; Herring, et al., 1967b). The introduction of earlier or faster freezing of carcasses pre-rigor should be preceded by an extensive and well-controlled study designed to detect this type of toughness.

The effect of the degree of muscle tension pre-rigor was also studied by Buck and Black (1967). They concluded that shear force was more closely related to the intrafiber molecular arrangement (sarcomere length) than to the diameter of the fiber. Gillis and Henrickson (1967) also studied the effect of tension to change the contraction state of semimembranosus and semitendinosus muscles. Increases in tension resulted in an increase in sarcomere length and tenderness and a decrease in fiber diameter. Pre-rigor shortening increased fiber kinkiness.

Contraction state has also been reported to be significantly related to tenderness ($r = .40$) in the longissimus muscle by Cooper, et al. (1967). In a study of A, B and E maturity groups, A & B maturity groups were more tender than the E maturity group, part of which was believed to be due to the longer sarcomeres observed in the former groups. Herring, et al. (1967b) also observed shorter sarcomeres in E than A maturity animals in the semitendinosus muscle.

In related studies to muscle shortening, Cook (1967) found that maintaining muscle fibers in a stretched state during rigor at 7°C. resulted in increased extractability of protein (in NaCl) from muscle tissue. He suggested that such protein solubility was positively related to water-holding capacity. On the other hand, Herring, et al. (1967a) found myofibrillar protein solubility to be more highly influenced by post-mortem physiological conditions than to contraction state of the muscle fiber when using KI as the extracting solution.

The effect of post-mortem aging on sarcomere length was studied by Gothard, et al. (1966). State of contraction after 7 days' aging appeared to be more closely related to tenderness than did state of contraction at time of maximum rigor mortis. They found considerable lengthening of sarcomeres during the post-mortem aging period. Similarly, Biró (1965) studied the morphological changes occurring in myofibrils during aging post-mortem. The initial (pre-rigor) relaxation state of biceps brachii muscle was followed by contracted myofibrils prepared during rigor mortis. Subsequent relaxation (post-rigor) appeared to be less distinct than in fresh samples.

The mechanism of tenderization that occurs with post-mortem aging of muscle is also receiving considerable attention. Recently, studies were initiated at Wisconsin (Herring, 1968) to evaluate certain properties of the proteins of the myofibril in relation to tenderness. Natural actomyosin was selected for these studies and its biochemical and physicochemical

properties were examined in relation to the effects of post-mortem aging and to extremes in inherent tenderness due to animal variation.

The onset of rigor mortis was associated with an increase of actomyosin ATPase activity at various ionic strengths and with either calcium or magnesium as activating cation. The ATPase activity decreased with additional aging of muscle post-mortem, however, there was generally not a return to zero-hr. activity. The onset of rigor mortis is usually complete by 9 hr. in bovine muscle and the 12- or 24-hr. time periods should have been reasonably close to the complete development of rigor mortis. The mechanism whereby the ATPase activity of natural actomyosin extracted from muscle 12-24 hr. post-mortem increased compared to that from fresh muscle could have been due to a number of possibilities. Those suggested are 1) stronger binding between myosin and actin leading to enzyme activation, 2) increased F-actin content of natural actomyosin resulting in activation of the myosin ATPase, or 3) conformational changes at the active sites of actin and/or myosin. Actomyosin ATPase activity was lower for tough muscle than tender muscle at zero-hr., but was higher at 24 hr. than that from the tender group. Activity then decreased more by ten days for the tough group than it did for the tender group. Changes in ATPase activity in the tender group were apparently less affected by aging.

Actomyosin prepared from muscle aged 12-24 hr. also had an increased rate of turbidity rise (superprecipitation -- an *in vitro* measure of muscle contraction) in 50 mM KCl when compared to actomyosin from the zero-hr. time period. Also, actomyosin from tough muscle underwent a more rapid rate of turbidity rise in each of the respective aging periods than did actomyosin from tender muscle. However, the situation in 100 mM KCl was somewhat different than that observed with 50 mM KCl. Actomyosin from tender muscle actually superprecipitated at a more rapid rate than did that from tough muscle. This seemed somewhat perplexing. However, if one takes into account the effect of the regulatory protein α-actinin to enhance super-precipitation at low KCl, but slightly delay it at higher KCl concentrations an explanation was tenable. Perhaps natural actomyosin from tough and tender muscle varied in content or character of this or some other regulatory protein. An alternative explanation could be that there was a stronger interaction of actin and myosin at low ionic strength for tough muscle than for tender muscle, but a weaker interaction at high ionic strength.

Some other physicochemical properties of natural actomyosin were also studied. Reduced viscosity was lowest for natural actomyosin prepared from zero-hr. muscle and it then increased markedly for actomyosin prepared from muscle 12-24 hr. post-mortem. Viscosity then generally decreased with further aging for 5-10 days. Natural actomyosin prepared from muscle aged 12 hr. may have been more asymmetric or have had a higher axial ratio than that obtained from either zero-hr. or 5 or 10-day aged muscle. Alternatively, it may have had higher ratio of actin to myosin which would have resulted in a higher reduced viscosity. Reduced viscosity was higher for actomyosin from tough muscle than that from tender at all aging periods. The reduced viscosity of actomyosin from tender muscle varied little with aging while reduced viscosity of actomyosin from tough muscle was markedly affected by aging muscle post-mortem as it had decreased markedly. It was also observed that actomyosin from tough muscle had a more pronounced gel character and

this was probably reflected in the obvious viscosity differences. It should be mentioned that α-actinin has been shown to promote gelatin of reconstituted actomyosin.

The interaction of natural actomyosin with ATP was studied with the viscometric method. The reduction in viscosity caused by ATP in 0.5 M KCl and 1 mM Mg is generally believed to be due to dissociation of actomyosin to actin and myosin. The actin-myosin interaction (as measured by viscosity decrease) appeared stronger for actomyosin prepared from tender muscle 12-24 hr. post-mortem than that from zero-hr. muscle, while actomyosin from 10-day aged muscle was most easily dissociated by low concentrations of ATP. Results were similar with actomyosin prepared from tough muscle. These data point out the reversibility of the strength or nature of the interaction of myofibrillar proteins as influenced by post-mortem aging of muscle and may partially explain the mechanism of muscle tenderization which occurs during this period.

Analytical ultracentrifugation was also used to study natural actomyosin. The main component of natural actomyosin had a characteristic "double peak" and an extrapolated sedimentation coefficient of 92S. A more polydisperse peak sedimented at a slightly faster rate ahead of the main component of the "double peak". This double character is probably an effect of the actin moiety as pure F-actin solutions sediment with two peaks, the leading one being some type of aggregate or dimer of F-actin. A small polydisperse peak visible in all preparations and sedimenting at 3-4S was also evident. It may represent the presence of some depolymerized or G-actin.

In the diagram representing actomyosin from tender muscle prepared 24-hr. post-mortem the appearance of a minor component with a sedimentation coefficient of about 12S was shown to occur. This component apparently appeared as a result of aging as it had not appeared at 12-hr. post-mortem. However, it was observed that for natural actomyosin prepared from tough muscle that the 12S component had not appeared until 5 days post-mortem and was even more prevalent after 10 days' aging. The other peaks were somewhat similar to those shown for natural actomyosin from tender muscle, but details will appear in a later publication.

In summary, tenderness of bovine muscle is related to certain biophysical and biochemical properties of the myofibril. Post-mortem contraction state is affected by temperature, tension and glycolytic rate and the state of contraction highly affects tenderness within a certain muscle. Fundamental changes in the physicochemical properties of myofibrillar proteins were associated in a reversible manner with the onset and passing of rigor mortis and with the improvement of tenderness due to aging of muscle post-mortem. Furthermore, natural actomyosin from tough muscle when compared to that from tender muscle differed in its properties, indicating fundamental differences in composition may have existed between the two groups.

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