Chapter 3

# Meat and Fish

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## Chapter Outline



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## I. INTRODUCTION

Meat is defined as the flesh of animals used as food. A more precise definition is provided by the US Food and Drug Administration (Meyer, 1964): meat is that derived from the muscles of animals closely related to man biochemically and therefore of high nutritive value. The more conventional animal species include cattle, pig, sheep, and the avian species chicken and turkey. In fish, however, it is often the white muscle that provides the main nutritional source. The per capita consumption of muscle foods in the USA has changed over the past decade by chicken replacing pork as the second most preferred muscle (Table 3.1). Seafood, particularly fish, accounts for approximately 20% of all animal proteins consumed worldwide (Zabel *et al.*, 2003). In the developing continents, Africa, Asia, and Latin America, the consumption of meat and fish is still extremely low or non-existent, as evidenced by the increasing incidence of malnutrition. This lack of high-grade proteins and the accompanying deficiency in essential amino acids still remains the world's most urgent problem.

This chapter will discuss the dynamic changes involved in the conversion of muscle to meat or edible fish. Following the death of the animal or fish, many chemical, biochemical, and physical changes occur leading to the development of postmortem tenderness. A greater understanding of these changes should make an important contribution to the production of high-quality meat or fish products. While the degradation of myofibrillar and cytoskeleton proteins is desirable for postmortem tenderization of mammalian and avian muscles, such changes can

**TABLE 3.1** US Per Capita Meat Consumption 1970-2008[a]

| Year | Beef | Lamb | Pork | Veal | Chicken | Turkey | Fish | Total Meat |
|---|---|---|---|---|---|---|---|---|
| **1970** | 51.8 | 1.2 | 28.1 | 1.0 | 15.8 | 4.2 | 2.8 | **104.9** |
| **1972** | 52.3 | 1.2 | 28.1 | 0.8 | 16.3 | 4.7 | 2.9 | **106.3** |
| **1974** | 52.5 | 0.8 | 27.4 | 0.8 | 15.6 | 4.5 | 2.7 | **104.3** |
| **1976** | 57.7 | 0.7 | 23.7 | 1.3 | 16.5 | 4.6 | 3.4 | **107.9** |
| **1978** | 53.5 | 0.6 | 24.7 | 1.0 | 17.5 | 4.5 | 3.5 | **105.3** |
| **1980** | 46.9 | 0.6 | 30.4 | 0.6 | 18.9 | 5.3 | 3.3 | **108.0** |
| **1982** | 47.2 | 0.6 | 26.2 | 0.7 | 19.5 | 5.5 | 3.1 | **102.8** |
| **1984** | 48.0 | 0.6 | 27.6 | 0.7 | 20.4 | 5.7 | 3.4 | **106.4** |
| **1986** | 48.4 | 0.6 | 26.4 | 0.8 | 21.3 | 6.7 | 3.7 | **107.9** |
| **1988** | 44.6 | 0.6 | 28.5 | 0.6 | 22.8 | 8.1 | 3.7 | **108.9** |
| **1990** | 41.6 | 0.6 | 27.1 | 0.4 | 24.4 | 9.1 | 3.7 | **106.9** |
| **1992** | 40.6 | 0.6 | 28.7 | 0.4 | 26.8 | 9.2 | 3.6 | **109.9** |
| **1994** | 40.9 | 0.5 | 28.6 | 0.4 | 28.1 | 9.1 | 3.9 | **111.5** |
| **1996** | 41.6 | 0.5 | 26.4 | 0.5 | 28.1 | 9.4 | 3.8 | **110.3** |
| **1998** | 41.3 | 0.5 | 28.1 | 0.3 | 29.1 | 9.1 | 3.5 | **111.9** |
| **2000** | 42.0 | 0.5 | 27.9 | 0.3 | 31.2 | 9.0 | 3.4 | **114.3** |
| **2002** | 42.0 | 0.5 | 28.1 | 0.2 | 32.7 | 9.2 | 3.7 | **116.4** |
| **2004** | 41.0 | 0.5 | 28.0 | 0.2 | 34.2 | 8.8 | 3.4 | **116.1** |
| **2006** | 40.8 | 0.4 | 26.8 | 0.2 | 35.1 | 8.7 | 4.0 | **116.0** |
| **2008** | 38.8 | 0.4 | 26.8 | 0.2 | 33.8 | 9.1 | 3.8 | **112.9** |

[a]*The data is adapted from the USDA/Economic Research Service, www.ers.usda.gov, Feb. 1, 2011. The per capita meat "consumption" is measured in lbs/year and reflects the total amount of meat supply in the United States adjusted for loss. The USDA calculates "consumption" by dividing the total annual supply of a specific meat by the annual population and adjusting that figure for loss by subtracting 1) the estimated amount of meat lost when converting it from the farm to a market ready product, 2) the estimated amount of meat lost at the retail level due to spoilage, and 3) the estimated amount of meat lost when consumers cook it at home. The fish category includes both fresh and frozen fish. http://vegetarian.procon.org/view.resource.php?resourceID=004716#V*

lead to unfavorable changes in fish muscle (Geesink *et al.*, 2000b). Texture remains one of the most important quality attributes affecting consumer acceptance of meat and fish products. In meat products, however, it is tenderness that is important to the consumer, while firmness is key to fish quality (Cheret *et al.*, 2007). Considerable advances have been made over the past two decades with a better understanding of the molecular mechanisms involved in the tenderization and textural changes in both meat and fish (Delbarre-Ladrat *et al.*, 2006; Terova *et al.*, 2011).

## II. THE NATURE OF MUSCLE

While muscles are classified into several types, it is the striated or voluntary muscle which constitutes lean meat. The basic unit of the muscle is the fiber, a multinucleate, cylindrical cell bounded by an outer membrane, the sarcolemma. These fibers associate together into bundles, and are enclosed by a sheath of connective tissue, the perimysium. Fiber bundles are held together by connective tissue and covered by a connective tissue sheath, the epimysium. Connective tissues important to the texture and edibility of the meat and fish include fibrous proteins, collagen, reticulin, and elastin. Fish muscle has much less connective tissue, thus providing less of a problem in tenderization.

### A. Structure

Individual muscle fibers are composed of myofibrils which are 1−2 μm thick and are the basic units of muscular contraction. The skeletal muscle of fish differs from that of mammals in that the fibers arranged between the sheets of connective tissue are much shorter. The connective tissue is present as short transverse sheets (myocommata) which divide the long fish muscles into segments (myotomes) corresponding in numbers to those of the vertebrae (Dunajski, 1979). The individual myofibrils are separated by a fine network of tubules, the sarcoplasmic reticulum. Within each fiber is a liquid matrix referred to as the sarcoplasm, which contains mitochondria, enzymes, glycogen, adenosine triphosphate (ATP), creatine, and myoglobin.

Examination of myofibrils under a phase-contrast light microscope shows them to be cross-striated due to the presence of alternating dark or A-bands and light or I-bands. These structures in the myofibrils appear to be very similar in both fish and meat. The A-band is traversed by a lighter band or H-zone, while the I-band has a dark line in the middle known as the Z-line. A further dark line, the M-line, is observed at the center of the H-zone. The basic unit of the myofibril is the sarcomere, defined as the unit between adjacent Z-lines as shown in Figure 3.1. Examination of the sarcomere by electron microscopy reveals two sets of filaments within the fibrils, a thick set consisting mainly of myosin, and a thin set containing primarily F-actin.

In addition to the paracrystalline arrangement of the thick and thin set of filaments, there appears to be a filamentous 'cytoskeletal structure' composed of connectin and desmin (Young *et al.*, 1980−1981). Connectin is now recognized as the major myofibrillar protein in the 'gap filaments' in muscle and is present throughout the sarcomere of

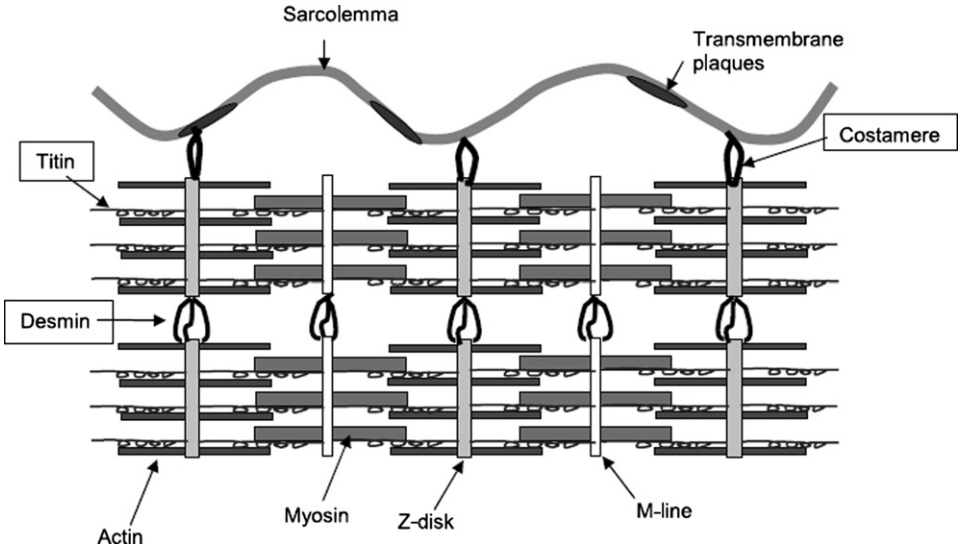



**FIGURE 3.1 Schematic representation of muscle myofibrillar proteins showing the major components of the sarcomere.** Boxes indicate the cytoskeleton structures and proteins susceptible to postmortem cleavage. *(Kemp* et al.*, 2010)*

skeletal muscle (Maruyama *et al.*, 1976a). These gap or G-filaments were reported by Locker and Leet (1976a, b) to span the region between the thick and thin filaments in fibers of overstretched beef muscle. Locker (1984) proposed that each gap filament formed a core to an A-band. Connectin was subsequently characterized as the protein titin, consisting of the three fractions titin-1, -2, and -3, reported by Wang *et al.* (1979) to account for 10−15% of the myofibrillar proteins in chicken breast. Titin-3 is now recognized as a distinct protein and referred to as nebulin (Wang and Williamson, 1980). Desmin, on the other hand, was reported by several researchers to be present in the periphery of each Z-disk in chicken skeletal muscle (Lazarides and Hubbard, 1976; Grainger and Lazarides, 1978). It may have a role in maintaining alignment of adjacent sarcomeres, which unifies the contractile process of the separate myofibrils.

## B. Cytoskeleton

The cytoskeleton of muscle is composed of two elements, gap filaments and intermediate filaments (Stanley, 1983).

### *1. Gap Filaments*

Gap filaments (G-filaments) were originally identified by Hanson and Huxley (1955) as extremely thin elastic 'S-filaments' responsible for keeping the actin filaments together. The model in Figure 3.2, proposed by Hoyle, showed that these filaments were located parallel to the fiber axis extending between the Z-disks, and were referred to as 'gap filaments' (Sjöstrand, 1962). These filaments were found by Maruyama *et al.* (1976b, 1977) to be composed of a rubbery, insoluble protein called 'connectin'. Wang *et al.* (1979) identified a high-molecular-weight protein which was referred to as titin. Subsequent research showed the high-molecular-weight components of connectin to be titin (Maruyama *et al.*, 1981). Titin appeared to be the major cytoskeleton protein in the sarcomere responsible for muscle cell integrity (Wang and Ramirez-Mitchell, 1979, 1983a, b). Locker and Leet (1976b) proposed that each gap filament formed a core within an A-band and linked the two thick filaments in adjacent sarcomeres through the Z-line. Wang and Ramirez-Mitchell (1984), using four distinct monoclonal antibodies to rabbit titin, showed that titin passed from the M-line through the A-band and into the I-band, thereby discounting the central core model.

Wang *et al.* (1979) also identified a large myofibrillar protein in vertebrate skeletal muscle referred to as nebulin. This protein was later isolated from the myofibrils of rabbit psoas and chicken breast muscles using immunological and electrophoretic techniques. It was found to be distinct from titin (Wang and Williamson, 1980; Murayama *et al.*, 1981; Ridpath *et al.*, 1982, 1984). The location of nebulin in the myofibril was at the $N^2$ line. Wang and Ramirez-Mitchell (1983b) presented an alternative model for the G-filaments consisting of an elastic filamentous matrix containing both titin and nebulin as additional sarcomere constituents (Locker, 1984).

Nebulin is a family of giant proteins (600−900 kDa) in skeletal muscle that forms a set of inextensible filaments anchored at the Z-line (Wang and Wright, 1988). For the past two decades it was considered by many investigators to be a molecular ruler that dictated the length of the actin thin filament. Several properties exhibited by nebulin allowed it to function as a molecular ruler (Horowitz, 2006). These included, first, its ability to extend the entire length of the thin filament with its C-terminus anchored in the Z-disc and its N-terminus towards the center of the sarcomere (Wright *et al.*, 1993). Secondly, the length of nebulin can vary by alternative splicing, enabling it to correlate with the thin filament lengths of the different muscles (Kruger *et al.*, 1991; Labeit *et al.*, 1991). Thirdly, nebulin has a unique protein structure that enables it to bind all the major components of the thin filament. The critical role of nebulin in maintaining proper skeletal function was shown in nebulin-deficient mice which died within 2 weeks of birth as a result of muscle weakness (Bang *et al.*, 2006; Witt *et al.*, 2006). In addition to its role as a molecular ruler, recent evidence suggests that nebulin regulates contraction by optimizing the actin−myosin interaction and controlling calcium in the sarcoplasmic reticulum (Root and Wang, 1994, 2001; Ottenheijm *et al.*, 2008; Bang *et al.*, 2009; Chandra *et al.*, 2009). The interaction of the C-terminus of nebulin appeared to be regulated by desmin, suggesting

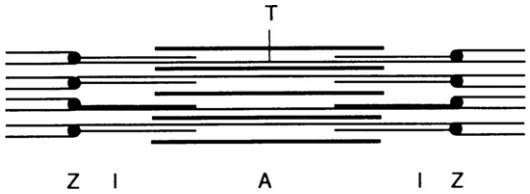



**FIGURE 3.2 Diagram showing proposed model for muscles including a very thin elastic filament (T) extending between Z-disks (Z) and parallel to the A-band (A) and I-band (I).** *(Hoyle, 1967)*

that it may be involved in maintaining the lateral alignment of myofibrils, an important property for coordinated and efficient contraction (Bang *et al.*, 2002; Conover *et al.*, 2009; Tonino *et al.*, 2010). Recently, Pappas *et al.* (2010) failed to find a role for nebulin in actin polymerization as it appeared to regulate thin actin filament lengths by a stabilization mechanism that prevented actin depolymerization. Defects in the nebulin gene are associated with congenital nemaline myopathy, a debilitating condition of muscle weakness (Gokhin *et al.*, 2009). The important role that nebulin plays in muscle health and disease was reviewed by Labeit *et al.* (2011).

### 2. Intermediate Filaments

These filaments linking the myofibrils laterally to the sarcolemma are intermediate in size (10 nm in diameter) between the actin (6 nm in diameter) and myosin (14−16 nm in diameter) filaments (Ishikawa *et al.*, 1968). The protein isolated from these filaments, desmin, also referred to as skeletin, is located in the periphery of the Z-disk in the filamentous form (Lazaride and Hubbard, 1976; Richardson *et al.*, 1981). The cytoskeleton role of desmin is to connect Z-lines of adjacent myofibrils (O'Shea *et al.*, 1981; Robson *et al.*, 1984).

## C. Connective Tissue

The interstitial space in muscle cells is occupied by three proteins, collagen, reticulin, and elastin, together referred to as connective tissue. The endomysium layer surrounding the muscle fibers is composed of fine reticular and collagenous fibrils, while elastin is sparsely distributed in the muscle with the blood, capillary, and nervous systems (Asghar *et al.*, 1984). Bundles of these muscle fibers are surrounded by a thicker connective tissue, the perimysium. These connective tissues appear to unite at the ends of the muscle in the thick tendon fibers as shown in Figure 3.3 (Etherington and Sims, 1981). During muscle contraction, movement is transmitted via the tendon to the skeleton. The limited elasticity of collagen in the tendon permits the translation of muscle contraction into a high degree of movement.

### 1. Collagen

The major protein of connective tissue is collagen, a glycoprotein. It was originally thought to be composed of two polypeptide chains, $\alpha_1$- and $\alpha_2$-chains, which formed a triple helical structure. At least 10 different $\alpha$-chains are now known which appear to be responsible for the different types of collagen so far identified. Of these, collagen I and III are the most abundant types in muscle, with minor quantities of IV, V, VI, XII, XIV, XV, and XIX. These differ from each other in their primary structure and amino acid composition (Asghar *et al.*, 1984). Morphologically, three discrete collagen depots, endomysium, perimysium, and epimysium, make up the three-dimensional network of collagen and elastin fibers embedded in a matrix of proteoglycans (Lepetit, 2008). The endomysium is the layer of connective tissue surrounding each muscle and overlying the basement membrane (McCormick, 1999). The epimysium includes the connective sheath surrounding individual muscles and continuous with the tendon joining other muscles (McCormick, 1999). It is extremely tough and resistant to shear and solubilization. The perimysium accounts for the bulk of the intramuscular connective tissue (IMCT) and plays a key role in determining meat texture differences (Lewis and Purlow, 1990). The subunit of the collagen fiber is the collagen monomer tropocollagen. This is composed of three polypeptide $\alpha$-chains arranged in a pattern which allows the staggered overlap of one polypeptide chain over the other, as shown for the type I tropocollagen monomer in Figure 3.4 (Asghar and Henrickson, 1982).

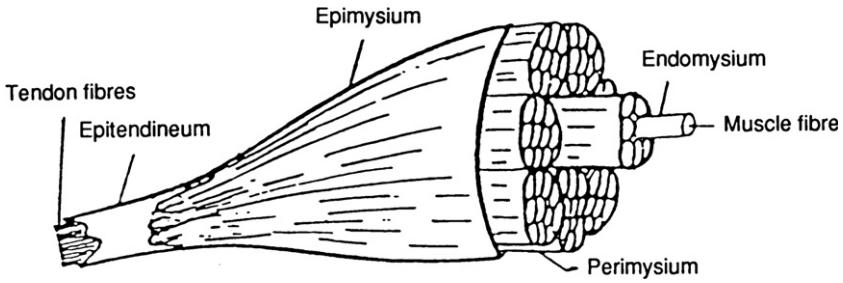



**FIGURE 3.3 Connective tissues of muscle.** *(Etherington and Sims, 1981)*

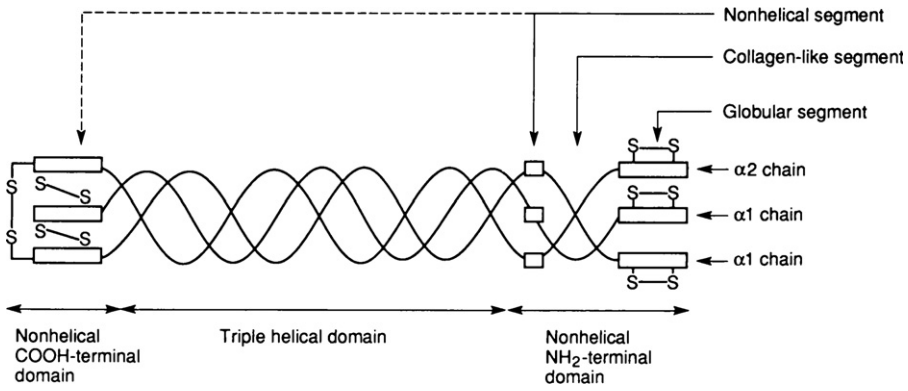



**FIGURE 3.4 Schematic representation of the type I tropocollagen monomer, composed of two identical pro-$\alpha_1$(I) chains (solid lines) and one pro-$\alpha_2$(I) chain (dashed line).** *(Asghar and Henrickson, 1982)*

The major collagen component of both the epimysium and perimysium is type I collagen, while types III, IV, and V are located in the endomysium (Bailey and Peach, 1968; Bailey and Sims, 1977). Since collagen is the principal component of connective tissue, the texture of meat is greatly influenced by it. Bailey (1972) suggested that an acceptable meat texture requires a certain degree of cross-linkages in collagen. A lack or an overabundance of such linkages in collagen produces meat that is either too tender or too tough. The toughness associated with meat from older animals is attributed to the high degree of stable and mature cross-linkages in the collagen fibers (Eyre *et al.*, 1984; Reiser *et al.*, 1992). The relative amounts of these stable cross-links appear to determine the texture of cooked meat (Bailey and Light, 1989; Bailey, 1990).

### a. Collagen and Meat Texture

Attempts to correlate total collagen of muscles with meat texture have resulted in conflicting reports. Dransfield (1977) found a definite relationship between total muscle collagen and toughness. Other studies showed that the qualitative nature of collagen, rather than quantity, ultimately affected texture (Bailey, 1972; Shinomokai *et al.*, 1972; Bailey *et al.*, 1979; Bailey and Sims, 1977). A later study by Light *et al.* (1985) examined the role of epimysial, perimysial, and endomysial collagen in the texture of six bovine muscles. These researchers reported a correlation between both collagen fiber diameter and collagen content of perimysial and endomysial connective tissue and meat toughness. A linear plot was obtained when the number of heat-stable cross-links was plotted against the compressive force (kg) (Figure 3.5) for six muscle perimysia cooked at 75°C using the data of Dransfield (1977). Although not as clear-cut, similar trends were observed for both epimysial and endomysial

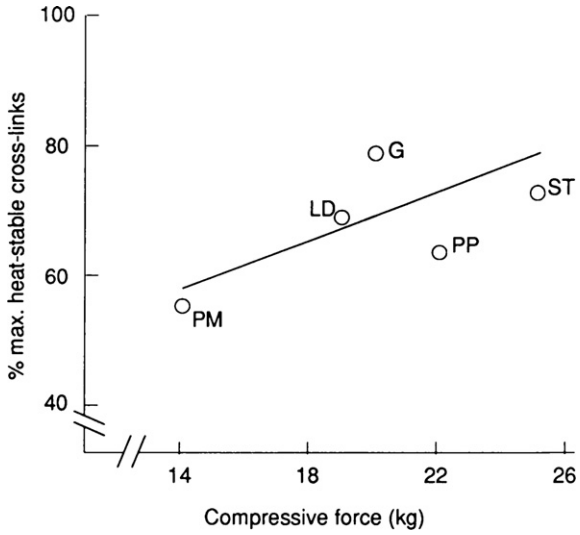



**FIGURE 3.5 Plot of total heat-stable (keto-imine) cross-links in six perimysia versus compressive force estimated after cooking the muscles for 1 hour at 75°C (from data by Dransfield, 1977).** PM: Psoas major; LD: longissimus dorsi; PP: pectoralis profundis; G: gastrocnemius; ST: semitendinosus. *(Light* et al.*, 1985)*

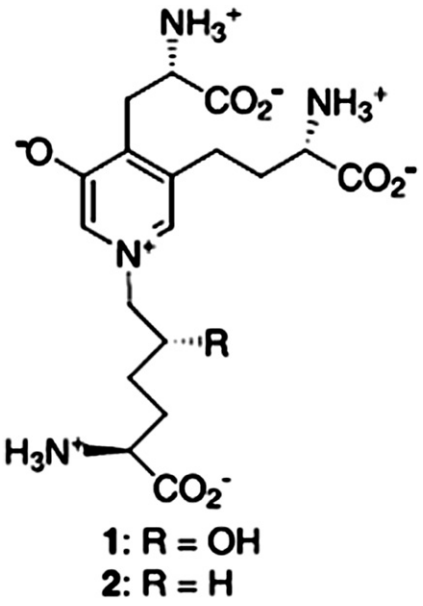



**FIGURE 3.6 Pyridinoline** *(Allevi and Anastasia, 2003).*

muscle samples. Based on these results it was apparent that the cross-links have a crucial role in determining tenderness or toughness of meat. If the primary cause for meat fracture and breakdown, according to Purslow (1985), is via the perimysium or at the perimysial−endomysial junctions, the nature of the cross-links between these fibers could be extremely important.

The toughening of the meat in older animals was attributed to changes in collagen cross-links. These cross-links appeared to be mediated by changes in lysyl oxidase during advanced aging (Bailey and Shimokomaki, 1971; Robins *et al.*, 1973). The toughening of the meat during aging was related to a decrease in the immature collagen cross-link, hydroxylysinoketonorleucine (HLKNL) (Shimokomaki *et al.*, 1972). The main mature cross-link, pyridinoline (PYR) (Figure 3.6) was supposedly derived from two reducible HLKNL and stabilized collagen in the older animals (Fujimoto, 1977; Eyre and Oguchi, 1980; McCormick, 1999). The formation of PYR was found to be related to meat texture and formed bridges in the different types of collagen (Shimokomaki *et al.*, 1990; Nakano *et al.*, 1991; Bosselmann *et al.*, 1995). A study by Coro *et al.* (2002) reported an increase in collagen from 0.448 to 0.568% in Ross chicken breast muscle (pectoralis major) aged from 20 to 540 days. During this period, PYR also increased from 0.009 to 0.101 mol/mol collagen, and was inversely related to collagen solubility. Textural changes measured by shear values, however, showed that a decrease in tenderness was affected more by the formation of PYR cross-links than by an increase in collagen synthesis. A decrease in collagen solubility was also directly related to an increase in PYR concentration. Reviews by Lepetit (2007, 2008) discuss the role of collagen in meat tenderness or toughness.

### b. Collagen and Fish Texture

Fish muscles generally contain only one-tenth of the collagen found in red meats. They are divided by thin membranes, myocommata, into segments or myotomes as shown in Figure 3.7. The myocommata are composed

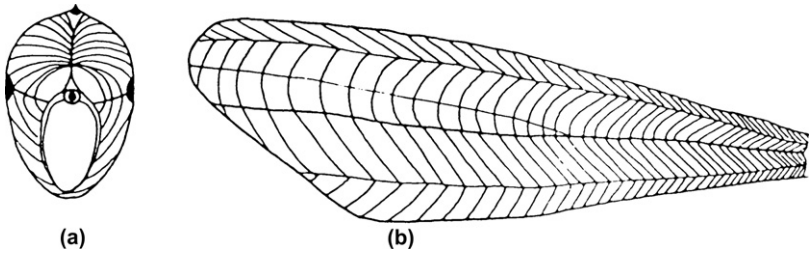



**FIGURE 3.7 The metameric structure of fish muscles.** The patternsof lines on the cross-section (a) and longitudinal section (b) represent the arrangement of sheets of connective tissue in the muscles. *(Dunajski, 1979)*

of connective tissue, with each muscle fiber surrounded by a cell wall or basement membrane containing thin collagen fibrils. The integrity of the fish muscles is maintained by the connective tissue of the myocommata and collagen fibers, which together form the endomysial reticulum. If the myotomes are not connected to the myocommata, slits and holes form in the flesh, which is characteristic of gaping. This results in the deterioration of fish quality as the fish fillets fall apart and become quite unacceptable. Love *et al.* (1972) attributed the development of this problem to the rupturing of the endomysial and myocommata connection brought about by rough handling or bending of the stiffened fish. The development of gaping, accompanied by softening of the tissue, is a consequence of deterioration during storage and is enhanced by rapid and strong muscular contractions during rigor (Love, 1988; Bremner, 1999; Taylor *et al.*, 2002). Examination of this phenomenon in cod and wolfish by Ofstad *et al.* (2006) attributed the increase in gaping during ice storage to the degradation of proteoglycans and glycoproteins, major constituents of the IMCT. They play an important role in the spatial organization of the collagen fibers by anchoring cells to the extracellular matrix in the collagen network. The greater propensity for gaping in cod, compared to wolfish, was attributed to the more rapid detachments occurring between myofibers prior to the myotomes to myocommata detachments (Ofstad *et al.*, 2006). Around 40% of the downgrading of Atlantic salmon (*Salmo salar* L.) during secondary processing was attributed to either soft flesh or gaping (Michie, 2001). In addition, preslaughter stress of salmon was reported to accelerate fish fillet softening, increasing gaping as well as fillet color and drip loss (Kiessling *et al.*, 2004; Erikson and Misimi, 2008; Morkore *et al.*, 2008). A novel tensile testing method, using a mechanical texture analyzer, was recently developed by Ashton *et al.* (2010) for assessing texture and gaping in salmon fillets. The contribution of connective tissue to the texture of cooked fish remains unclear, compared to its role in meat, as a consequence of the smaller amount present. For example, the high level of connective tissue in dogfish requires a cooking temperature of 45°C, which is still substantially lower than the cooking temperature of 92°C for 1 hour needed to obtain the same degree of tenderness for beef.

## III. CONTRACTION OF MUSCLE

While the majority of studies have been conducted on mammalian muscle, it is apparent that similar changes occur in fish muscle. It is generally accepted that contraction and relaxation of striated muscle occurs by the sliding action of the thick filaments over the thin filaments, with the length of the filaments remaining the same (Rowe, 1974). Myosin possesses adenosine triphosphatase (ATPase) activity, which requires the presence of magnesium and calcium ions. It is the regulation of myofibrillar ATPase which determines the contractile response of the muscle. This enzyme catalyzes the hydrolytic cleavage of ATP, thereby providing the most immediate source of energy for muscular contraction:

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{H_3PO_4}$$

$$\Delta G_{298}\ (\text{Standard free energy change at } 25^{\circ}\mathrm{C}) = -11.6\ \text{kcal/mole}$$

In resting muscle the activity of ATPase is very low, resulting in the slow release of adenosine diphosphate (ADP) and inorganic phosphorus at the active sites of myosin and actin. Once muscle stimulation occurs, the head of myosin, containing the actin-combining and enzymatic sites, interacts with actin with the rapid release of ADP and inorganic phosphate ($P_i$). The increase in ATP hydrolysis can be several hundred times that observed in the resting state (Perry, 1979). This is accompanied by a conformational change in the myosin-head, causing a change in the angle it makes with the actin filament. The overall result is that the actin monomer with the myosin head attached moves forward by approximately 5−10 nm (Huxley, 1969). Once ADP and $P_i$ are released from the myosin head, the actin monomer detaches itself to permit a fresh molecule of Mg-$ATP^{2-}$ to be picked up by the enzymatic site on the myosin head and the enzyme−substrate complex is re-established. Muscular contraction is thus characterized by a rapid conversion of ATP to ADP and inorganic-phosphate, and on completion the muscle returns to its resting state. The latter is characterized by the capacity of the substrate−enzyme complex at enzymatic sites in the myosin head to be released once stimulation occurs. The hydrolysis of myosin in the presence of actin has been studied by a number of researchers, although detailed steps remain to be characterized (Chock *et al.*, 1976; Eccleston *et al.*, 1976).

## A. Regulation of Muscle Contraction: Troponin and Tropomyosin

The regulation of muscular contraction appears to involve the release of calcium from the vesicles of the sarcoplasmic reticulum, located in the myofibrils. Calcium is released when the stimulus is received at the muscle fiber by way of the central nervous system. It stimulates myosin ATPase, thus releasing the energy necessary for muscle contraction to facilitate the sliding action of actin filaments which form contractile actomyosin (Huxley, 1964). This was confirmed in studies by Goodno *et al.* (1978), who reported up to 100-fold increase in ATPase activity of the myofibril when calcium ion ($Ca^{2+}$) levels were increased. Calcium regulates actin−myosin interaction by directly binding to the troponin C component of the I-filament.

Troponin is a myofibrillar protein associated with the thin filaments, which appears to control the interaction between actin and myosin. It is an elongated molecule of molecular weight 80 kDa, which is attached to tropomyosin, another myofibrillar protein. They both provide the regulatory system in muscular contraction (Ebashi, 1974). Tropomyosin, a long, coiled α-helix, is located in each of the two long-pitch helical grooves of the actin monomers on thin filaments (Seymour and O'Brien, 1980). Murray and Weber (1974) showed that troponin (Tn) and tropomyosin (TM) interacted with seven actin molecules (Figure 3.8). In contrast, troponin is found at discrete intervals of 38 nm along the thin actin filaments and is associated with the stripes on the I-band. Troponin is composed of three subunits, troponin C (TnC), troponin T (TnT), and troponin I (TnI). It appears to have multiple, compartmentalized functions, with each subunit exerting a particular role including binding tropomyosin or $Ca^{2+}$ or inhibiting actomyosin ATPase (Greaser and Gergely, 1973). When the muscle is stimulated, TnC first binds $Ca^{2+}$ then neutralizes TnI inhibition of actomyosin ATPase which is then translated into conformational changes in protein via tropomyosin. The role of TnT has remained obscure although it has since been reported to enhance actomyosin ATPase activity at high $Ca^{2+}$ and may play a dual role in filament regulation (Dahiya *et al.*, 1994; Potter *et al.*, 1995; Tobacman, 1996). Recent research by Murakami *et al.* (2008) showed that head-to-tail polymerization of tropomyosin was important for binding to actin and for regulating actin-myosin contraction. The 2.1Å solution structure of crystals containing overlapping tropomyosin N and C termini is shown in Figure 3.8A. They emphasized the importance of the molecular swivel generated by TnT at the junction between successive tropomyosin for regulating striated muscle contraction.

Skeletal muscles include a variety of fibers, classified into slow and fast types, composed of complex multiple myofibril proteins. Schiaffino and Reggianni (1996) showed that the major proteins responsible for the different fiber types were myosin heavy-chain isoforms. The fiber types appeared to have different ATPase activities which may impact meat quality traits during the conversion of muscle to meat. Ouali (1992) suggested myofibrils might be the limiting step in the development of meat texture. Later researchers correlated muscle fiber types and size to meat quality traits in pigs (Chang *et al.*, 2003; Ryu and Kim, 2005) and in beef (Crouse *et al.*, 1991; Ozawa *et al.*, 2000; Maltin *et al.*, 2003). There are four calcium binding sites in TnC of fast muscles compared to three calcium binding sites in slow muscles. In the presence of low calcium levels the formation of cross-links is inhibited by the troponin−tropomyosin complex. An increase in calcium levels following stimulation of the muscle results in the binding of calcium to TnC and the formation of the actomyosin complex. Accumulation of calcium in the

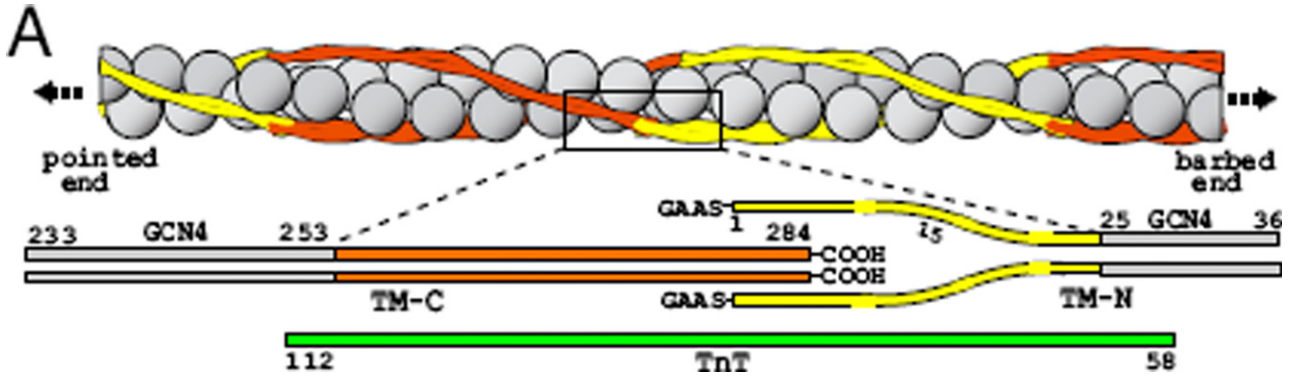



**FIGURE 3.8** Overview of the crystal structure of the overlapping tropomyosin N (TM-N) and tropomyosin C (TM-C) termini junction. Schematic representation of the constructs used (**O**-actin). The C-terminal (TM-C) (residues 254−284, preceded by a 20 fragment of the GCN4 leucine zipper to stabilize dimerization) and N-terminal (TM-N) [residues 1−24, followed by a 12 residue fragment of the GCN4 leucine zipper, and preceded by N-terminal GlyAlaAlaSer-extension (Murakami *et al.,* 2008).

sarcoplasmic reticulum is achieved against a concentration gradient requiring an active transport pumping system involving ATP. This is hydrolyzed by ATPase present in the membranes of the sarcoplasmic reticulum (de Meis and Vianna, 1979).

## B. Mechanism of Tropomyosin Action

Extensive studies using electron micrographs of a myosin subfragment (S-1) suggested that tropomyosin regulated muscle contraction by steric blocking and unblocking of the myosin interaction sites in the muscle thin filaments (Haselgrove, 1972; Huxley, 1972b; Parry and Squire, 1973). This theory was questioned by Seymour and O'Brien (1980), who proposed that tropomyosin was located on the opposite side of the thin filament helix axis from the binding sites of myosin S-1 (Moore *et al.*, 1970). Further studies by Taylor and Amos (1981), based on three-dimensional image reconstructions of electron micrographs of thin filaments decorated with myosin S-1, suggested that the location of the binding sites proposed by Moore *et al.* (1970) was incorrect. Taylor and Amos (1981) clearly demonstrated that tropomyosin was located on the same side of the actin helix. Later research by Mendelson (1982) using high-resolution reconstructions of X-ray scattering of myosin S-1 further supported the model for attachment of myosin S-1 to actin presented by Taylor and Amos (1981). Thus, regulation of muscle contraction appeared to occur by steric blocking of actin−S-1 myosin interaction. Amos *et al.* (1982) presented structural evidence that the head of myosin S-1 interacted with two sites on F-actin. In the active state tropomyosin was thought to occupy a position near the middle of the actin groove, while in the inhibited state it lay on the other side of the groove where it could interface with interactions between S-1 and actin at the first or second site. Based on the three dimensional image reconstructions of actin-myosin complexes, Taylor and Amos (1981) and Amos *et al.* (1982) suggested a globular shape, while research by Garrigos and co-workers (1992) supported a more anisomeric model, in agreement with other researchers (Milligan and Flicker, 1987; Karijama, 1988). For further information on the structural mechanism of ATP-induced dissociation of rigor myosin from actin, the paper by Kuhner and Fischer (2011) is recommended.

Tropomyosins (TPM) are present in a large number ($> 20$) of isoforms encoded by four genes, TPM1(α-TM, $\alpha$-$TM_{fast}$), TPM2(β-TM), TPM3(α-TM,$\alpha$-$TM_{slow}$), and TMP4 (Perry, 2001). The three major isoforms expressed in skeletal muscle are TPM1, TPM2, and TPM3. Oe *et al.* (2007) determined the cDNA sequences in TPM isoforms in bovine skeletal muscles from Holstein cows. Based on the amino acid sequence from two-dimensional gel electrophoresis, they identified three TPM isoforms expressed in five physiologically different bovine skeletal muscles (masseter, diaphragm, psoas major, longissimus thoracis and semitendinosis). The three isoforms identified, TPM1, TPM2, and TPM3, had 93.3%, 99.6%, and 100% amino acid homology to the human sequence, respectively. TPM2 accounted for approximately 50% of the TPM in all of the muscles examined; however, the presence of TPM2 and TPM3 depended on whether the muscle was a fast or slow type. The higher sequence homology between TPM1 and TPM3 suggested that the ratio of these TPMs may be an important factor in determining the contractile properties of skeletal muscles.

The structural basis for troponin and tropomyosin activation of muscle was recently reported by Lehman *et al.* (2009), in which troponin appeared to be involved in both activation and inhibition of muscular contraction. Key structural changes in troponin resulted in marked steric effects that control actin activation of myosin ATPase and its subsequent contraction.

## C. ATP and the Lohmann Reaction

On cessation of the stimulus, calcium ions are removed by a 'relaxing factor' with the sarcoplasmic reticulum acting as a calcium pump (Newbold, 1966). While the primary source of ATP in muscle is derived from ADP by aerobic respiration, it can also be resynthesized from ADP and creatine phosphate (CP) by the Lohmann reaction:

$$\mathrm{ADP} + \mathrm{CP} \xrightarrow{\text{ATP:creatine phosphotransferase}} \mathrm{ATP} + \text{creatine}$$

ATP:creatine phosphotransferase and creatine phosphate are both located in the sarcoplasm. This reaction is important in conditions leading to muscle fatigue, representing an immediate pathway for the resynthesis of ATP. Consequently, muscular activity can continue until adequate amounts of ATP are generated via carbohydrate degradation.

## D. Fish Muscle Contraction

Fish muscle consists of two types, red and white muscle, in which rigor contractions have been shown to differ. While the proportions of these two muscles vary from one species to the next, red muscle never exceeds 10% of the total muscle for any species, for example, tuna. The dark and white muscle content of 16 species of fish was measured by Obatake and Heya (1985) using a rapid direct gravimetric method on the heated fish. With the exception of saury pike, the dark muscle of all the other species of fish never exceeded the 10% level. The ratio of dark muscle to whole muscle accounted for over 12% in the so-called red meat species (e.g. sardine, saury pike, frigate herring, and round herring) compared to less than 3% in the white meat species, such as yellow sea bream and silago. The dark or red muscle is characterized by a high myoglobin content as well as distinct proteins (Hamoir and Konosu, 1965). Obatake *et al.* (1985) found that the dark muscle of fish had higher levels of extractive nitrogen constituents, as well as creatine, compared to white muscles.

Rigor contraction has been shown to be far greater in red muscle of fish compared to white muscle (Figure 3.9) and corresponded more closely with that of mammalian muscle (Buttkus, 1963). The role of contraction, tension, and elasticity associated with the development of rigor mortis in postmortem fish muscles is still poorly understood. Bate-Smith and Bendall (1956), in studies on rabbit muscle, found considerable shortening during the rigor period which was not concomitant with stiffening and rarely occurred at room temperature. In contrast, the red muscle of lingcod and trout consistently produced a postmortem contraction at 20°C, the rate being indicative of the condition of the fish prior to death (Buttkus, 1963). White muscle is generally regarded as the nutritional flesh of fish, therefore most studies have been confined to this tissue. The importance of red muscle in the postmortem changes of fish cannot be ignored, however, since as yet there is no process capable of separating these two muscles in the fish processing industry.

Trucco *et al.* (1982) reported that the visual and tactile estimation of rigidity of fish during the prerigor, full rigor, and postrigor stages originally proposed by Cutting (1939) was still the most reproducible method. Their results, shown in Figure 3.10, indicate that for sea bream (*Sparus pagnes*) it took 10 hours for rigor mortis to develop and approximately 30 hours for its resolution, compared to 55 hours in anchovy. However, resolution of rigor mortis was not apparent until 80 hours. The development of the actomyosin complex during rigor mortis was monitored by the reduced viscosity of the high-strength muscle extract (Crupkin *et al.*, 1979). Thus, the course of rigor mortis could be followed by measuring the viscosity of the extract (Figure 3.11).

Bito *et al.* (1983) developed the rigidity/rigor index to determine the stage of rigor mortis in fish. The upper half of the whole fish was placed on a horizon table on one side, suspending the other half or tail part off the edge. The vertical distance ($L$) between the base of the caudal fin and the table surface was measured over time intervals, with rigor index calculated as:

$$\text{Rigor index } (\%) = [(L_o - L)/L_0] \times 100\%$$

where $L_o$ is the value measured immediately after death, and $L$ values are recorded at different time intervals after death.

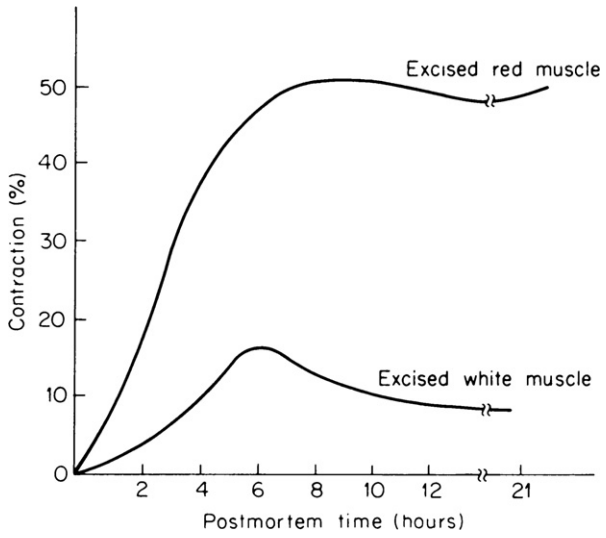



**FIGURE 3.9 Rigor contraction of excised red and white muscle of lingcod at 20°C.** *(Buttkus, 1963)*

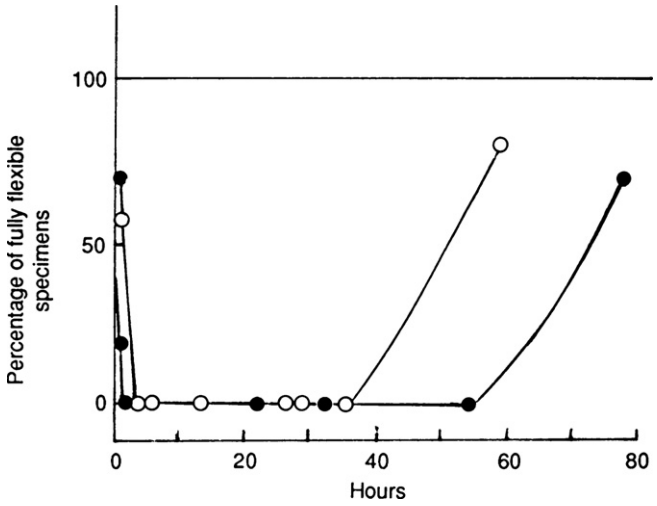



**FIGURE 3.10** **Evolution of rigor mortis in sea bream (○) and anchovy (●).** *(Trucco* et al.*, 1982)* © *Elsevier*

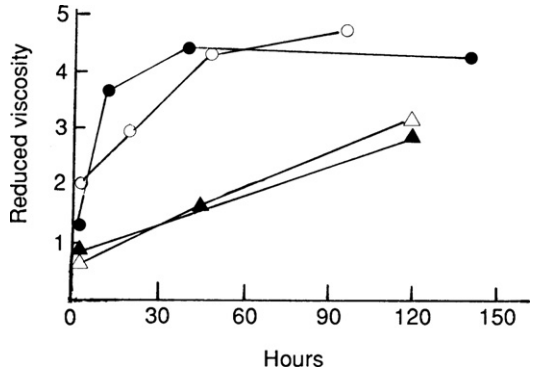



**FIGURE 3.11** **Reduced viscosity of the high ionic strength muscle extract during the development of rigor mortis in hake (○), Patagonian blue whiting (●), tail hake (▲), and grenadier (△).** *(Trucco* et al.*, 1982)* © *Elsevier*

Using the rigor index, Wang *et al.* (1998) examined rigor mortis and ATP degradation in cultivated Atlantic salmon (*Salmo salar*). The data obtained followed a similar pattern to that observed by Crupkin *et al.* (1979) for sea bream using viscosity as an index of rigor. Wang *et al.* (1998) showed that rigor mortis started 8 hours after death, reaching a maximum between 24 and 30 hours (Figure 3.12). Complete dissolution of rigor mortis in Atlantic salmon occurred between 60 and 70 hours. The different stages of fish were classified using the rigor index as: no stiffening during prerigor (rigor index of $< 10\%$), fully stiffened in rigor mortis (rigor index $< 100\%$), and postrigor (rigor index $< 10\%$). The ATP content of the Atlantic salmon muscle decreased from 7.25 to 0.14 μmol/g in rigor mortis, consistent with a decrease in ATP of less than 1 μmol/g reported in plaice by Iwamoto *et al.* (1987) when a rigor index of 100% was also reached. The *K* value (see Section IV.B, below) for Atlantic salmon was also found to increase from 0.7 to 10.6% to 41.1% at prerigor, rigor, and postrigor stages. These values were within the range reported by Erikson *et al.* (1997) for unstressed farmed Atlantic salmon.

## IV. CONVERSION OF MUSCLE TO MEAT AND EDIBLE FISH

A vast array of biochemical and physicochemical reactions takes place from the time that the animal or fish is killed until it is consumed as meat or edible fish. This period can be divided into three distinct stages:

1. The prerigor state, when the muscle tissue is soft and pliable. This is characterized biochemically by a fall in ATP and creatine phosphate levels as well as by active glycolysis. Postmortem glycolysis results in the conversion of glycogen to lactic acid, causing the pH to fall. The extent of pH change varies from one species to another as well as among

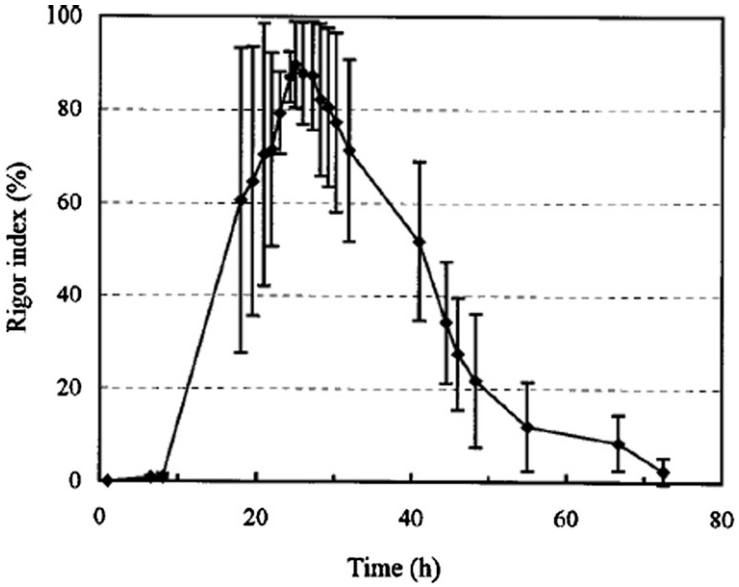



**FIGURE 3.12 Rigor index histories of Atlantic salmon stored at 0°C.** The curve shows the mean of four replicated tests and the error bars show standard deviation. *(Wang* et al., *1998)*

different muscles. Nevertheless, in well-fed, rested animals the glycogen reserves are large so that in the postmortem state the meat produced has a lower pH than meat produced from animals exhausted at the time of slaughter.

2. The development of the stiff and rigid condition in the muscle known as rigor mortis. This occurs as the pH falls and is associated with the formation of actomyosin. The loss of extensibility associated with the formation of actomyosin proceeds slowly at first (the delay period) and then extremely rapidly (fast phase). The onset of rigor mortis normally occurs at 1−12 hours postmortem and may last for a further 15−20 hours in mammals, depending on a number of factors to be discussed later. Fish generally exhibit a shorter rigor mortis period commencing 1−7 hours after death, with many factors affecting its duration.
3. The postrigor state, during which the meat and fish muscles gradually tenderize, becoming organoleptically acceptable as aging progresses. Mammalian meat usually attains optimum acceptability when stored for 2−3 weeks at 2°C following dissolution of rigor.

The importance of rigor mortis in fish is recognized by the fishing industry, since in addition to retarding microbial spoilage, it affords a stiffness to the fish which is generally recognized by the consumer as a sign of good quality. The rigor period, however, is also a distinct disadvantage with respect to the filleting of fish, as it renders the fish too stiff to process. Thus, filleting is carried out following dissolution of rigor, or on trawlers, immediately before the development of rigor.

The principal changes following death are summarized in Figure 3.13. Following the death of the animal or fish, circulation of the blood ceases, which results in a complex series of changes in the muscular tissue. Since blood is an ideal medium for spoilage microorganisms, as much as possible is removed from the animal carcass to ensure that the edibility and keeping quality of the meat are maintained. With respect to fish, only some of the larger species are bled; the blood differs from that of warm-blooded animals and coagulates far more rapidly. The advent of the modern fish-processing trawler brought with it a number of problems, particularly surface discoloration in sea-frozen fillets. This discoloration in the prerigor processed cod fillets developed from surface contamination by the fish blood in the form of methemoglobin (Kelly and Little, 1966). This phenomenon can be prevented in prerigor fish fillets of ocean perch (*Sebastes marinus*) and cod (*Gadhus morhua*) by correct bleeding methods.

The most immediate effect of the stoppage of blood circulation and the removal of blood from the muscle tissue is depletion of the oxygen supply to the tissue and the subsequent fall in oxidation−reduction potential. This results in an inability to resynthesize ATP, as the electron transport chain and oxidative phosphorylation mechanisms are no longer operative.

## A. ATP and Postmortem Changes

The major source of ATP supply to the muscle fibers is lost following the death of the animal or fish, since glycogen can no longer be oxidized to carbon dioxide and water. In its place, anaerobic metabolism takes over, resulting in the

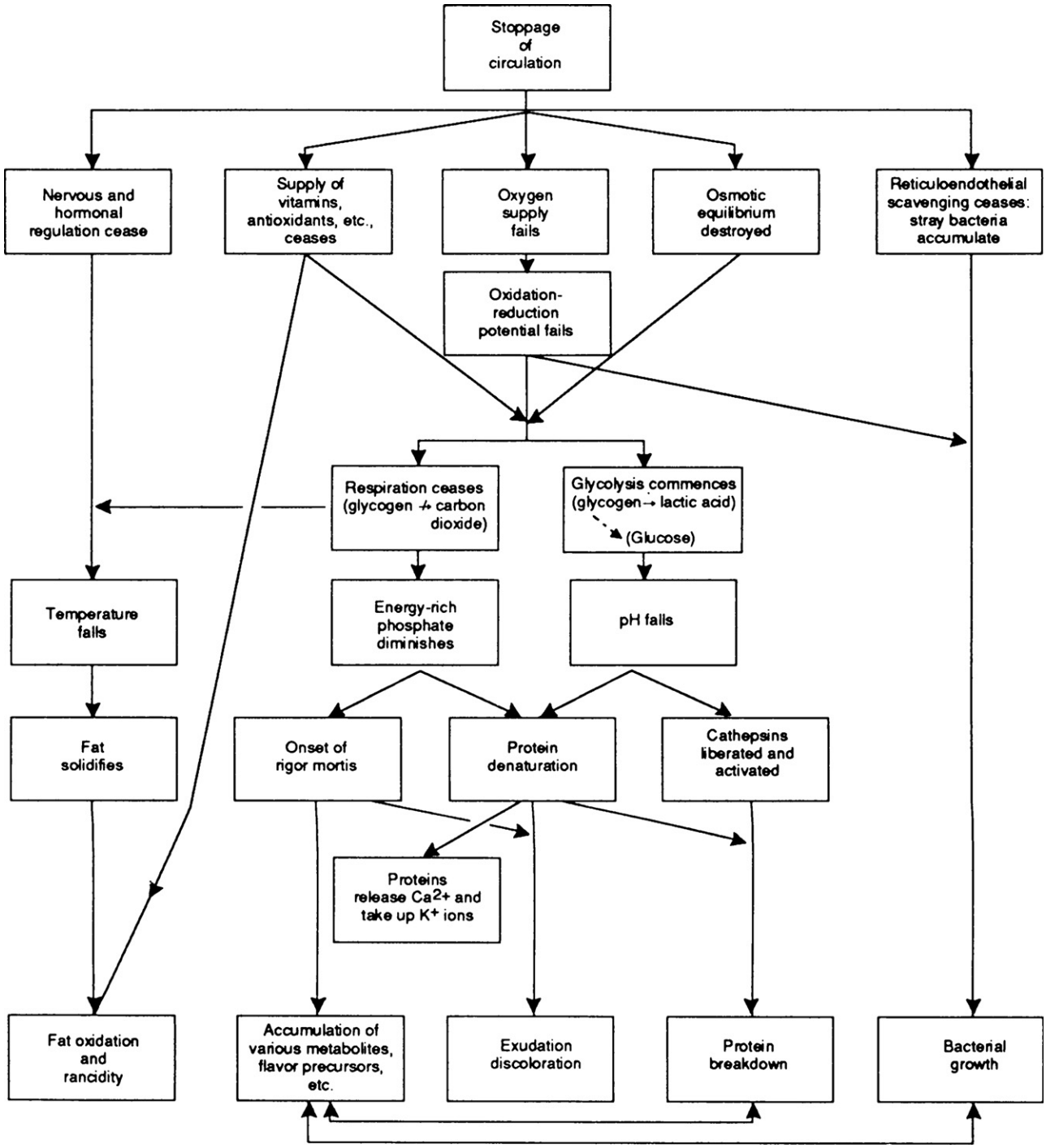



**FIGURE 3.13 The consequences of stoppage of the circulation in muscular tissue. (Lawrie, 1998)** *(Reprinted with permission. Copyright © by Wood Publishing Ltd.)*

conversion of glycogen to lactic acid. Under normal aerobic conditions 39 molecules of ATP are produced for each glucosyl unit of glycogen oxidized compared to only three molecules of ATP for each hexose unit broken down under anaerobic conditions. The time for the first phase development of rigor mortis is determined by the postmortem level of ATP. The level of ATP is also depleted by the non-contractile ATPase activity of myosin which maintains the temperature and structural integrity of the muscle cell (Bendall, 1973). This results in the production of inorganic phosphate, which stimulates the degradation of glycogen to lactic acid. Inorganic phosphate is essential for the

phosphorolysis of glycogen to glucose l-phosphate by muscle phosphorylase, which is the initial step in the degradation of glycogen. In addition to the ATPase of myosin, the sarcoplasmic reticulum has ATPase activity.

The level of ATP is maintained in the muscles after death by an active creatine kinase which catalyzes the resynthesis of ATP from ADP and creatine phosphate (Lawrie, 1966; Newbold, 1966). Thus, in the early postmortem or prerigor period, the concentration of ATP remains relatively constant, whereas there is a rapid decline in creatine phosphate levels. In studies with rabbit muscle of well-rested and relaxed animals the creatine phosphate levels were high immediately postmortem but fell rapidly to one-third of the original before any detectable loss in ATP (Bendall, 1951). A rapid fall in creatine phosphate levels was also observed in poultry muscle accompanied by the liberation of free creatine (De Fremery, 1966). The transitory rise in pH in poultry muscle immediately postmortem was attributed to the presence of creatine. Studies by Hamm (1977) on ground beef muscle showed that creatine phosphate was totally degraded within 1−2 hours postmortem.

As discussed earlier, mammalian muscle is capable of maintaining its ATP level for as long as several hours postmortem, compared to fish skeletal muscle, which generally exhibits a rapid decline in ATP levels (Tomlinson and Geiger, 1962). Some species of fish, however, can maintain a constant ATP level but must be in an unexercised state prior to slaughter. The relationship between ATP and creatine phosphate levels in mammalian muscles appears to be similar to that reported in fish skeletal muscle (Partmann, 1965).

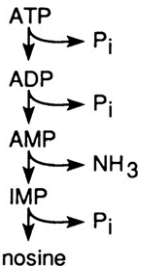
The continued activity of the several ATPases in the muscle cell, including in the sarcoplasmic reticulum, mitochondria, sarcolemma, and the myofibrils, presumably contributes to the depletion of ATP in the muscle. Of these, Hamm *et al.* (1973) concluded that it was the myofibrillar ATPase, rather than membrane or sarcoplasmic reticulum ATPase, which was probably responsible for the degradation of ATP in postmortem skeletal muscle. There is an overall decrease in the ATP level as a consequence of ATPase, activity, a decrease in creatine phosphate, and the inability of postmortem glycolysis to synthesize ATP at an effective rate.

The development of rigor mortis in fish is also related to the reduction in ATP. Depletion of creatine phosphate, adenosine monophosphate (AMP), and glycogen reserves and the subsequent inability to resynthesize ATP results in the formation of the actomyosin complex. This is accompanied by the muscle becoming tough and inextensible. Unlike land animals, rigor mortis in fish terminates far more rapidly. Jones and Murray (1961) reported that the onset of rigor mortis for cod occurred when ATP dropped to 5% of the original level in the rested fish. This was corroborated for a number of Indian fish by Nazir and Magar (1963), although some species were found to enter rigor at much higher phosphate levels, for example, *Mugul dussumieri* and *Harpodon nehereus*. Jones *et al.* (1965) followed the steady decline in ATP during the postmortem period for cod until the point at which rigor developed. The ATP levels for unexercised and exercised cod muscle were 2.35 and 0.82 μmole/g, respectively, which showed the effect of exercise in determining the ATP levels at which rigor was established. A reduction in the time required for rigor mortis development is also associated with excessive struggling by fish during capture, which has been correlated with a reduction in creatine phosphate. The depletion of ATP in cod prior to the onset of rigor was shown by Fraser *et al.* (1961) to be 1.25 μmole/g muscle. Iwamoto *et al.* (1987) found that in spiked plaice (*Paralichthys olivaceus*), the ATP level remained constant for a short time after death as creatine phosphate was degraded prior to ATP breakdown. Using the rigor index method developed by Bito *et al.* (1983), Cappelin and Jessen (2002) monitored the changes in ATP, IMP, and glycogen in 16 different muscle positions in cod. While the fish were considered in rigor, the dorsal and tail muscles were not in rigor because they were still high in ATP.

## B. Postmortem Metabolism of ATP

The development of rigor mortis in animals or fish is a direct response to the decline of ATP. Bendall and Davey (1957) observed that the liberation of ammonia occurred when rabbit voluntary muscle was fatigued or passed into rigor. This was shown to arise from deamination of adenylic acid to inosine-5′-monophosphate (IMP). They postulated the direct deamination of ADP in which ammonia was produced in equimolar proportions to the disappearance of adenosine nucleotides, primarily AMP, during the development of rigor. Tsai *et al.* (1972) reported the presence of ATP, ADP, and IMP in prerigor porcine muscle and traces of AMP. The levels of ATP and ADP declined rapidly in the postmortem muscle, while the concentration of IMP, inosine, and hypoxanthine increased markedly.

Fraser *et al.* (1961) reported an increase in ammonia during the resolution of rigor in cod muscle. The postmortem degradation of ATP follows a similar pattern to that in mammalian muscles, in which ATP is rapidly degraded to ADP by the sarcoplasmic ATPase, and hydrolyzed by myokinase to AMP. AMP is then converted to IMP by deaminase action (Saito and Arai, 1958). Nucleotides, particularly IMP, are recognized as important contributors to the flavor of




**SCHEME 3.1** Postmortem degradation of ATP to inosine in meat and fish.

good-quality fish (Hashimoto, 1965). Scheme 3.1 summarizes the postmortem degradation of ATP in both fish and meat in which inosinic acid is dephosphorylated to inosine.

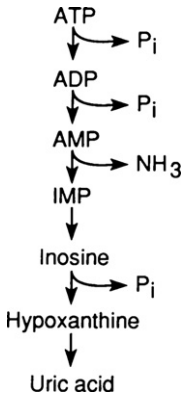
IMP and guanosine-5′-monophosphate (GMP) have been reported to enhance the meaty flavor and suppress sulfurous, fatty, burnt, starchy, bitter, and hydrolyzed vegetable type flavors (Wagner *et al.*, 1963; Kuninaka, 1967). Flavor enhancers, such as monosodium glutamate (MSG), IMP, and GMP are natural components of meat and are believed to make a major contribution to meat flavor (Farmer, 1999). They have been shown to improve flavor and have been used by the Japanese for many years to give 'umami' (Reineccius, 1994; Maga, 1994). When using added IMP, Kurtzman and Sjostrom (1964) concluded that canned chicken-containing noodle soup was not flavor enhanced. However, other products evaluated, including canned beef noodle soup, did show improvement with IMP addition.

At equimolar concentrations, IMP has most effect on aroma, but at concentrations related to those reported in the literature for red meat, ribose, IMP, and glucose-6-phosphate were all found to increase 'meaty' and 'roasted' aromas (Farmer *et al.*, 1996). Cambero *et al.* (2000) reported that flavor properties of beef broths were more influenced by the IMP concentration than GMP and AMP concentration and that small non-amino acid nitrogen compounds are more important than peptidic nitrogen with higher molecular weight than 600 Da. In addition to its flavor-potentiating action, IMP has been reported in early studies to be essential for development of meaty aroma during cooking (Batzer *et al.*, 1960; Koehler and Jacobson, 1967). Farmer *et al.* (1996) reported that at four times (340 mg/100 g) the reported concentration in beef and pork, IMP caused highly or very highly significant increases in both 'meaty' and 'roasted' aroma. They also reported that, in most cases, the addition of twice the reported concentration of IMP gave no significant effect. Mottram and Madruga (1994) observed an increase in many sulfur-containing furans, including 2-methyl-3-(methyldithio)furan, on addition of IMP to beef at 10 times its natural concentration. They affirmed that IMP was a precursor for 2-methyl-3-furanthiol and mercaptoketones, but 2-furanmethanethiol was not derived from IMP. IMP appears to act as a source of these thiols via the Maillard reaction and this reaction appears to involve the intermediate formation of 4-hydroxy-5-methyl-3(2H)-furanone and dicarbonyls, such as butanedione and pentanedione, and their subsequent reaction with hydrogen sulfide or cysteine (Mottram and Madruga, 1994). IMP also appears to suppress the 'green' odor, probably caused by hexanal, and to reduce the formation of *n*-aldehydes and other lipid-oxidation products (Farmer *et al.*, 1996).

Nucleotides and nucleosides are potential precursors of free ribose and ribose phosphate, which have been implicated in Maillard reactions during processing and storage of flesh (Tarr, 1954; Macy *et al.*, 1964). During thermal processing of flesh foods, IMP is degraded into inosine and hypoxanthine, which have been reported to be either flavorless or bitter (Arya and Parhar, 1979).

Macy *et al.* (1970) reported that the concentration of IMP decreases, whereas that of free nucleosides and bases increases during roasting of beef, lamb, and pork. Piskarev *et al.* (1972) reported that both AMP and IMP increase during thermal sterilization of freshly slaughtered meat, but decrease in aged meat. Suryanarayana-Rao *et al.* (1969) reported that IMP, inosine, and hypoxanthine decrease during canning of shrimps, but Hughes and Jones (1966) and Mori *et al.* (1974) reported that hypoxanthine is stable at canning temperatures. Aliani and Farmer (2005a, b) reported IMP to be generally the most abundant nucleotide in commercially available chicken muscle, with average quantities of IMP in chicken breast and leg muscle of 83.7 and 44.6 mg/100 g, respectively. Inosine, an enzymatic breakdown product of IMP, was the second major compound in most chickens (36.2 and 28.5 mg/100 g in breast and leg, respectively), while GMP and hypoxanthine were detected at lower concentrations. AMP was least abundant in most of the chickens analyzed in this study.

The biochemical reactions involved in the formation of these nucleotides, nucleosides, and bases in meat have been studied for several decades.




**SCHEME 3.2** Degradation of ATP in fish muscle.

Early work by Bendall and Davey (1957) demonstrated that these reactions in meat were catalyzed by ATPase, myokinase, and deaminase at 37 and 17°C. In addition to the reactions shown in Scheme 3.1, the presence of ITP and IDP was reported and attributed to the following reaction:

$$\text{ADP} \rightarrow \text{IDP} + \text{NH}_3$$

$$2\ \text{IDP} \rightarrow \text{ITP} + \text{IMP}$$

Small amounts of inosine and hypoxanthine, degradation products of IMP, were also found in postrigor mammalian muscle. The conversion of ATP to IMP occurred by the time the ultimate pH was reached, while the degradation of IMP followed the establishment of the final pH (Lawrie, 1966).

The degradation of ATP in fish muscle also leads to the formation of IMP, which is subsequently hydrolyzed to uric acid (Saito *et al.*, 1959; Kassemsarn *et al.*, 1963; Tarr, 1966) (Scheme 3.2). The presence of 5′-nucleotidase activity was reported in carp muscle by Tomioka and Endo (1984, 1985).

The level of these nucleotides changes drastically following the death of the fish. Many estimates have been made of fish freshness based on the level of nucleotides (Saito *et al.*, 1959), ammonia (Ota and Nakamura, 1952), amines (Karube *et al.*, 1980), and volatile acids (Suzuki, 1953). Of these, the production of nucleotides from ATP appears to be the most reliable indicator in fish. While inosine is comparatively tasteless, its conversion to hypoxanthine gives rise to a bitter substance (Jones, 1965). Conflicting reports refuting this were subsequently presented by a number of Japanese workers, including Hashimoto (1965), who suggested that hypoxanthine was tasteless. The presence of hypoxanthine in fish muscles was proposed as a chemical index of freshness and quality in fish (Jones *et al.*, 1964; Spinelli *et al.*, 1964). Dugal (1967) suggested that an average rate of hypoxanthine formation could be obtained for a group of fish which would reflect the degree of freshness for a particular species of freshwater fish. Watanabe *et al.* (1984) estimated fish freshness by monitoring the level of IMP by an enzyme sensor. Karube *et al.* (1984) developed a multifunctional enzyme sensor system for assessing fish freshness based on measuring the levels of IMP, inosine, and hypoxanthine. This was based on the changes observed in ATP, ADP, and AMP levels in sea bass, saurel, mackerel, and yellowfish following death. Their results, shown in Figure 3.14, indicate a rapid decrease and reduction in ATP and ADP levels 24 hours after death as well as a drop in AMP to less than 1 μmole/g.

IMP increased sharply during the first 24 hours postmortem and then decreased gradually, accompanied by a rise in inosine and hypoxanthine. The changes in these nucleotides varied with the individual fish species. Since ATP, ADP, and AMP were still present in some of the fish varieties for up to 2 weeks they included these nucleotides with hypoxanthine, inosine, and IMP in the overall equation defining fish freshness.

Saito *et al.* (1959) first proposed the term '*K* value' as an indicator of fresh fish defined as the ratio of inosine plus hypoxanthine to the total amount of ATP-related compounds. The *K* value has since been used to express freshness of marine products (Lee *et al.*, 1982; Uchiyama and Kakuda, 1984). Ryder (1985) developed a rapid method for computing *K* values based on the quantitative measurement of ATP and its degradation products using high-performance liquid chromatography (HPLC). Surette *et al.* (1988) monitored the postmortem breakdown of ATP-related compounds in Atlantic cod (*Gadus morhua*) and reported that inosine hydrolysis and hypoxanthine formation resulted from both autolytic and bacterial enzyme activity. These nucleotide catabolites provide a useful index of quality as their presence is affected by spoilage bacteria and mechanical damage during handling. These researchers

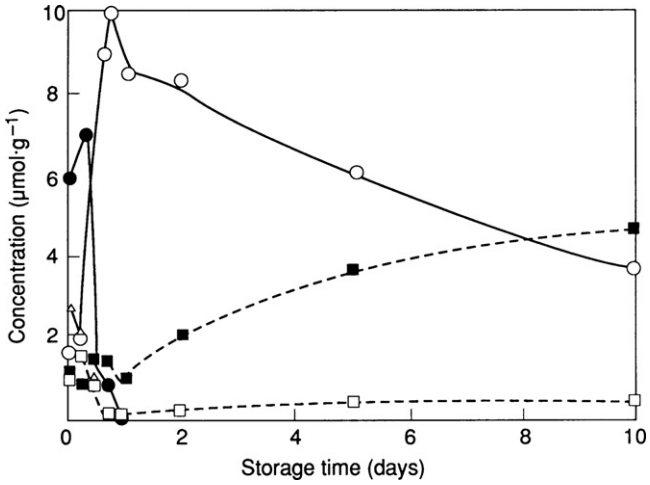



**FIGURE 3.14 Time-course of ATP decomposition and associated reactions in sea bass.** ●: ATP; △: ADP; □: AMP; ○: IMP; ■: HxR + Hx. *(Reprinted with permission from Karube et al., 1984. Copyright © by the American Chemical Society.)*

suggested that a diagnostic kit for measuring catabolites, such as inosine monophosphate (IMP), inosine (INO), and hypoxanthine (Hx), could provide a useful tool for assessing the fresh quality of cod.

$$K = \frac{[\text{Inosine}] + [\text{Hypoxanthine}] \text{ x } 100}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + \text{inosine} + \text{hypoxanthine}}$$

*G* and *P* values were derived from the K value. Burns *et al.* (1985) defined the *G* value as an index for evaluating the shelf-life of lean fish during ice storage, while a second quality indicator, *P*, was introduced to serve as an indicator of spoilage during the early stages of chilled storage.

$$P = \frac{\text{Inosine} + \text{Hypoxanthine}}{[\text{AMP}] + [\text{IMP}] + \text{inosine} + [\text{Hypoxanthine}]}$$

The choice of one indicator over another depends on the degradation patterns of the metabolites (Shahidi *et al.*, 1994).

The applicability of the *K* value for assessing the freshness of edible meat was reported by Nakatani *et al.* (1986). By monitoring the changes in ATP degradation products in beef and rabbit muscles during cold storage they proposed the following new index $K_0$, in which adenosine is represented by AD and xanthine by X:

$$\frac{\text{Inosine} + \text{hypoxanthine} + \text{xanthine} \times 100}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{AD} + \text{INO} + \text{Hx} + \text{X}}$$

Subsequent research by Fujita *et al.* (1988) found that this index could also be used to assess the freshness of both pork and chicken.

Most studies dedicated to understanding the ATP degradation pathway by analyzing the resulting products failed to report the concentrations of ribose and/or ribose-1-phosphate (R1P), which may result from the breakdown of inosine to hypoxanthine, and ribose-5-phosphate (R5P), which can be a product of a direct IMP breakdown to hypoxanthine (Lee and Newbold, 1963). These potential breakdown reactions of IMP are presented in Figure 3.15. It has been suggested that in beef, sugars may contribute to the sweet flavors and aromas upon heating (MacLeod, 1994).

The lack of information on natural concentrations of these key sugars in meat might be explained by the fact that most analytical methods used in recent years were adequate for nucleotides and not for sugars. In order to overcome this problem, a postcolumn derivatization method was employed (Aliani and Farmer, 2002) to determine sugar and sugar phosphates in chicken breast (24.7 mg) and leg (14.1 mg/100 g) from five different local commercial suppliers. Ribose and R5P have been shown to be important in aroma development in heated model systems and also contribute to the desirable browning reactions in meat. The addition of small quantities of ribose to raw meat has been shown to increase the quantities of key odor compounds as well as desirable roasty and meaty notes in cooked meat (Farmer *et al.*, 1999). Aliani *et al.* (2005a, b), using different sensory studies, reported the importance of ribose as a key flavor precursor in chicken meat and suggested that a two- to four-fold increase in the natural concentration of ribose (25 mg/100 g wet weight) may be sufficient to significantly improve the roasted and chicken odors in cooked chicken breast (Aliani and Farmer, 2005a, b).

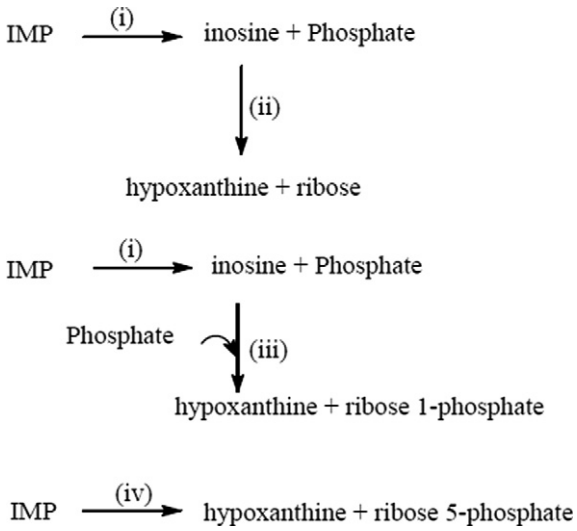



**FIGURE 3.15 Degradation of IMP by three potential pathways.** *(Adapted from Lee and Newbold, 1963)*

Several sugars and sugar phosphates, such as ribose (Aliani and Farmer, 2005b; Mottram and Nobrega, 1998), R5P (Mottram and Nobrega, 1998), glucose, glucose-6-phosphate (G6P) (Farmer *et al.*, 1999), mannose, and fructose (Aliani and Farmer, 2005b; Madruga *et al.*, 2010), are usually found in most types of meat such as chicken (Aliani and Farmer, 2005a), beef (Koutsidis *et al.*, 2008), pork (Meinert *et al.*, 2009), and goat (Madruga *et al.*, 2010), and are believed to display flavor-generating potential (Meinert *et al.*, 2009).

Postmortem conditioning contributed to an increase in ribose concentrations and is likely to have a major effect on flavor formation (Koutsidis *et al.*, 2008). Ribose significantly increased from conditioning day 1 to day 21 at 4°C in beef longissimus lumborum muscle. Ribose has been reported to be the most heat-labile sugar, while fructose is the most stable (Macy *et al.*, 1964). Therefore, ribose is likely to be altered or breakdown during high temperatures. Ribose can be formed from the degradation of R5P (Aliani *et al.*, 2008). R5P has been shown to undergo Maillard reactions significantly more rapidly than similar sugars and sugar phosphates (Sandwick *et al.*, 2005). Madruga *et al.* (2010) reported that all sugars present in goat meat decreased during cooking, with fructose exhibiting the highest percentage loss of 66%. This can be attributed to the involvement of sugars in the Maillard reaction, which produces several pentose and hexose degradation products containing carbonyl groups which are main reactants for the formation of important heterocyclic compounds such as pyrazines, thiazoles, and pyridines in meat volatiles (Madruga *et al.*, 2010). The odors obtained from these reactions are dependent on the amino acid; however, the nature of the sugar is said to dictate the rate of the reaction (Kiely *et al.*, 1960).

## C. Adenosine Nucleotides and Protein Denaturation

During frozen storage, deteriorative changes in fish texture have been reported as a consequence of protein denaturation (Dyer, 1951; Andou *et al.*, 1979, 1980; Acton *et al.*, 1983). The possible effect of adenosine nucleotides on protein denaturation was recently investigated by Jiang *et al.* (1987). These researchers assessed protein denaturation in fish frozen at −20°C by extractability of actomyosin (AM) and monitored the activities of Ca-ATPase and Mg(EGTA)-ATPase [EGTA = ethylene glycol *bis*(2-aminoethylether)tetraacetic acid] in AM. During frozen storage the molecular weight of the myosin heavy chain and actin decreased. The least stable muscle was associated with the lowest levels of ATP, ADP, AMP, and IMP and the highest levels of inosine and hypoxanthine. A correlation of −0.80 was obtained between inosine and hypoxanthine and the Ca-ATPase total activity of AM compared to +0.78 for ATP, ADP, AMP, and IMP (Figures 3.16 and 3.17). These results point to the possible involvement of adenosine nucleotides with protein denaturation.

The rate of ATP breakdown was also found to be much faster in muscles exhibiting pale, soft, and exudative (PSE) or dark, firm, and dry (DFD) conditions as they had much lower levels of ATP and higher levels of IMP compared to normal meats (Tsai *et al.*, 1972; Lundstrom *et al.*, 1977; Essen-Gustavson *et al.*, 1991). A method for the early postmortem detection of PSE and DFD in pork was developed by Honikel and Fischer (1977) using the *R* value (the ratio of the absorption at 250 and 200 nm). Batlle *et al.* (2000) detected the PSE condition in longissimus dorsi muscle

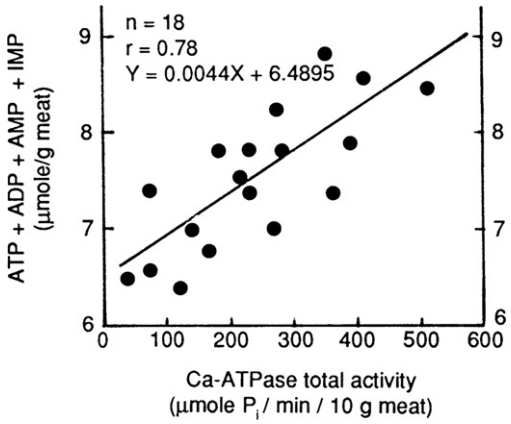



**FIGURE 3.16 Relationship between the sum of the quantity of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and inosine monophosphate (IMP) and Ca-ATPase total activity.** *(Reprinted with permission from Jiang* et al.*, 1987. Copyright © by the American Chemical Society.)*

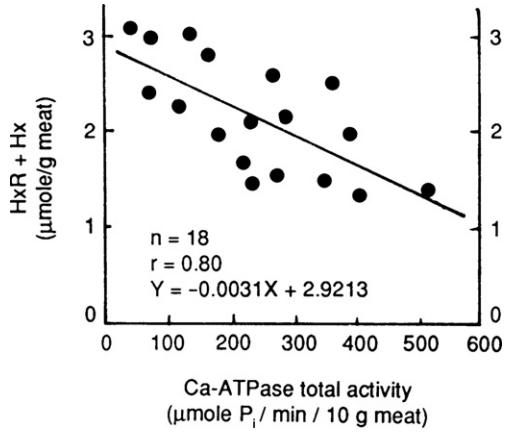



**FIGURE 3.17 Relationship between the content of inosine (HxR) and hypoxanthine (Hx) and Ca-ATPase total activity.** *(Reprinted with permission from Jiang* et al.*, 1987. Copyright © by the American Chemical Society.)*

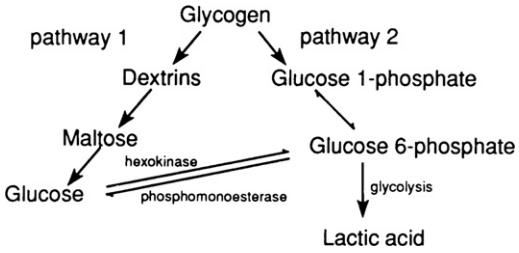
of pork meats by measuring ATP-related compounds 2 hours postmortem. PSE meats were significantly lower ($p < 0.05$) in ATP, but higher in AMP, IMP, and hypoxanthine compared to normal meat. Measurement of $K_o$ values, $R'$ values, and the ratios of IMP/ATP all proved useful indicators of PSE.

## D. Postmortem Glycolysis

Once the supply of oxygen to the muscle tissue is depleted, glycogen, the main carbohydrate of animal and fish muscle, undergoes anaerobic glycolysis to lactic acid. Compared to mammalian muscle, the level of glycogen in fish muscle is reported to be much lower. Tomlinson and Geiger (1962), however, found a close similarity between the muscle glycogen levels for many species of both fish and warm-blooded animals. This was attributed to the excessive struggling normally associated with the capture of fish, resulting in depletion of the glycogen level compared to that in the rested fish.

Postmortem degradation of glycogen in fish muscle suggests that two possible pathways are involved: the hydrolytic or amylolytic pathway, and the phosphorolytic pathway. These are illustrated in Scheme 3.3.

The postmortem conversion of glucose-6-phosphate to glucose by phosphomonoesterase only occurs to a slight extent in fish muscle. Consequently, the hydrolytic pathway appears to be the main one operating in fish. This pathway was first postulated by Ghanekar *et al.* (1956) and has since been confirmed as the main degradative pathway of glycogen to glucose for most fish (Tarr, 1965; Burt, 1966; Nagayama, 1966). In mammalian muscle, however, it is




**SCHEME 3.3** Postmortem degradation of glycogen.

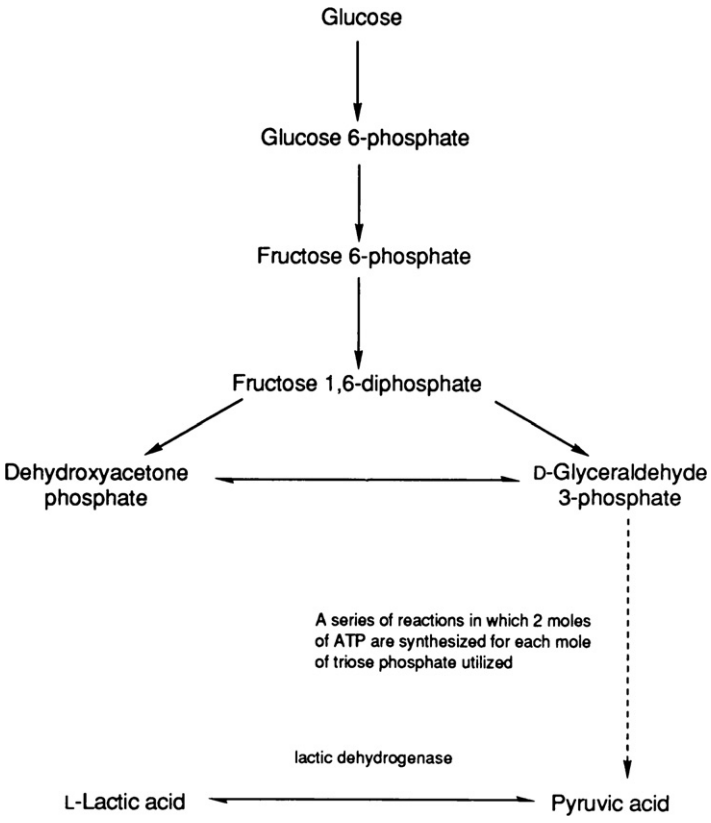
the phosphorolytic pathway that is responsible for glycogen degradation. When stressed, catecholamines released from sympathetic neurons and adrenal medulla accelerate glycogen degradation through activation of glycogen phosphorylase. The activated phosphorylase rapidly removes glucose-1-P units from glycogen molecules (Poso and Puolanne, 2005).

Irrespective of which pathway is involved in the initial breakdown of glycogen, the final pathway of glycolysis is the same for either animal or fish muscle. The enzymes responsible have, in the main, been characterized and identified in mammalian muscle, with many since reported in many species of fish, including rainbow trout (MacLeod *et al.*, 1963; Tarr, 1968). The general reactions involved in the glycolytic pathway are outlined in Scheme 3.4.

The rate of postmortem glycolysis in muscles is affected by temperature, muscle fiber type, and hormone secretions, as well as the intensity of the nervous stimuli in the muscle before and during slaughter (Beecher *et al.*, 1965; Disney *et al.*, 1967; Tarrant *et al.*, 1972a, b; Bendall, 1973). The effect this has on the pH of the muscle will be discussed in the next section.

## E. Postmortem pH

The production of lactic acid causes the pH of the muscle tissue to drop from the physiological pH of 7.2−7.4 in warm-blooded animals to the ultimate postmortem pH of around 5.3−5.5. A direct relationship was demonstrated by




**SCHEME 3.4** Metabolism of glucose to lactic acid.

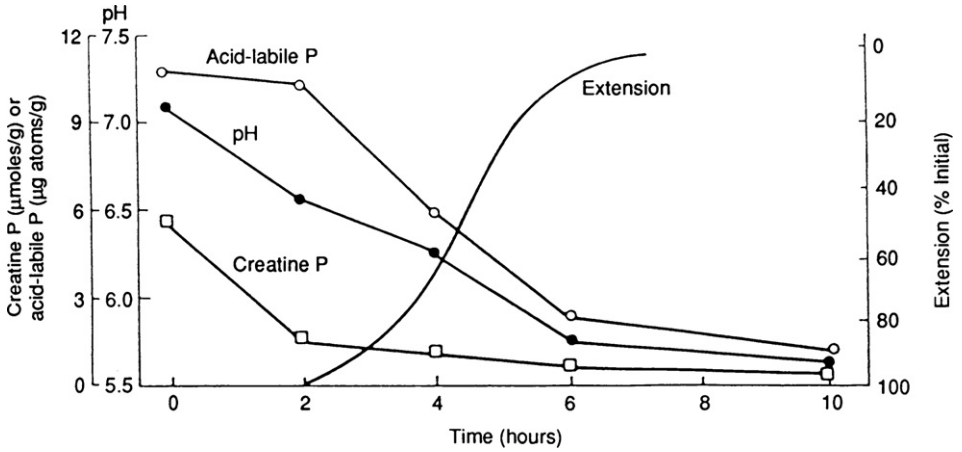



**FIGURE 3.18 Chemical and physical changes in beef sternomandibularis muscle held at 37°C.** Extension changes were recorded on an apparatus similar to that described by Bate-Smith and Bendall (1949) using a load of about 60 g/cm$^2$ and a loading−unloading cycle of 8 minutes on and 8 minutes off. Zero time: 1 hour 45 minutes postmortem. *(Newbold, 1966)*

Bate-Smith and Bendall (1949) between the rate of fall of pH in postmortem rabbit muscle and lactic acid production. It is particularly important to attain as low a pH as possible in the tissue, since in addition to retarding the growth of spoilage bacteria, it imparts a more desirable color to the meat. In the case of frozen fish, however, a higher pH is more desirable to prevent toughness. The final pH can be attained within the first 24-hour postmortem period, the glycolytic pathway being related to ATP production, the net fall of which is directly responsible for the development of rigor mortis. The interrelationship between the creatine phosphate disappearance, fall in the levels of ATP and pH, and the decrease in extensibility as a measure of rigor mortis is shown in Figure 3.18. ATP is the major source of acid-labile phosphorus, while the fall in pH is a measure of glycolysis.

A postmortem pH of 5.3−5.5 is attained in the muscles of well-rested animals fed just before slaughter, when glycogen is at a maximum level. Animals that undergo severe death struggling, however, are fatigued prior to slaughter and characterized by lower glycogen levels, as illustrated in Table 3.2 for chicken breast muscle.

The lower glycogen levels result in a higher final postmortem pH of around 6.0−6.5, producing a dark, dry, and close-textured meat that is much more susceptible to microbial spoilage (Cassens, 1966; Joseph, 1968a, b). This meat, referred to as DFD, represents a serious quality problem, particularly in beef (Tarrant, 1981), and is discussed further in Section K. The final postmortem pH in meat rarely falls below 5.3, although several exceptions have been reported. For example, in pig longissimus dorsi muscle, pH values ranging from 4.78 to 5.1 have been recorded (Lawrie *et al.*, 1958). Meat with a pH of 5.1−5.5 was found to be in an exudative condition with a whitish color and loose texture, while meat at pH 4.78 had abnormal muscle fibers. This is not unexpected, as the isoelectric point of the major meat proteins is around pH 5.5, which would lead to a loss of water-holding capacity (WHC).

The lactic acid concentration in fish is similarly dependent on the initial glycogen stores before death as well as on the treatment of the fish. Fish muscles have been reported to have lactic acid concentrations ranging from 0.29% in haddock (Ritchie, 1926) to 1.2−1.4% in tuna (Tomlinson and Geiger, 1962) during rigor mortis. Most fish exhibit a higher postmortem pH compared to warm-blooded animals of around 6.2−6.6 at full rigor. An exception is in the

**TABLE 3.2 Effect of Slaughter Conditions on the Glycogen Concentration 3 Minutes Postmortem in Chicken Breast Muscle**

| Condition | Initial Glycogen Level (mg/g) |
|---|---|
| Anesthetized | 8.3 |
| Stunned | 6.0 |
| Struggling | 3.4 |

Adapted from De Fremery (1966).

case of flatfish, where a final pH of 5.5, similar to that of mammals, has been reported. The struggling of fish during capture substantially depletes the glycogen stores, resulting in a high pH at rigor of around 7.0, giving rise to a condition known as 'alkaline rigor'. This condition was reported in cod as well as other fish species (Fraser *et al.*, 1961). A review by Wells (1987) noted that the capture, transportation, and handling of live fish is accompanied by substantial biochemical and physiological changes. The extent of such changes was dependent on species as well as environmental conditions. Eliminating stress in fish by allowing them to return to a resting condition during captivity prior to death should be the ultimate goal to avoid the abnormal pH changes incurred during struggling.

Low ultimate pH has also been associated with textural problems in fish such as halibut, Alaska pollack, and tuna (Patashnik and Groninger, 1964; Konagaya and Konagaya, 1979; Suzuki, 1981). Love (1975) noted that while low ultimate pH produced a tough texture, high ultimate pH resulted in a 'sloppy' soft texture in Atlantic cod (*Gadus morhua*). This condition rendered the fish unfit for filleting and produced a poor frozen product (Love *et al.*, 1982; MacCallum *et al.*, 1967). Postmortem changes in soft-textured cod caught off Newfoundland were examined by Ang and Haard (1985), who reported the lowest ultimate pH in cod that had been feeding heavily on capelin before capture. Atlantic cod do not feed during the months prior to spawning in May and June but feed intensely during the postspawning period. These researchers found that the muscles of cod caught during this intense feeding period were characterized by a persistent low ultimate pH up to 100 hours following rigor. Ang and Haard (1985) suggested that the altered metabolic state as a result of the heavy feeding caused the low and stable ultimate pH in the cod muscle, which was responsible for the soft texture in these fish.

## F. Time-Course of Postmortem Glycolysis

The ultimate postmortem pH is dependent on the physiological state of the muscle, the type of muscle, and the species of animal or fish studied. The different rates of fall in pH with time are shown in Figure 3.19 for three species of animal.

Several postmortem changes observed visually in meat are related to the rate of decline in pH and temperature. For example, a rapid decline in pH in beef held at around body temperature resulted in changes in color, decreased WHC, and some muscle protein denaturation (Scopes, 1964; Chaudhry *et al.*, 1969; Lister, 1970; Follett *et al.*, 1974; Locker and Daines, 1975). Earlier work by Cassens (1966) and Briskey *et al.* (1966) studied the rate of glycolysis in porcine muscle by monitoring the decline in pH. In addition to the physiological state of the muscle they observed that certain pigs were predisposed to a rapid postmortem glycolysis. The properties of the meat associated with a fall in pH are summarized in Table 3.3.

Similar changes occur in fish muscle as the pH declines. A low postmortem pH was associated with poor fish texture, low WHC, and high drip loss (Kelly, 1969). A high water content in cod muscle was identified by Love (1975) in those fish with high postmortem pH, which correlated best with the texture of the cooked fish.

## G. Effect of Temperature on Postmortem Glycolysis: Cold Shortening

The rate of postmortem glycolysis varies with temperature, as evident from differences in the final pH of mammalian muscles (Marsh, 1954; Cassens and Newbold, 1966; Newbold, 1966; Newbold and Scopes, 1967). These researchers

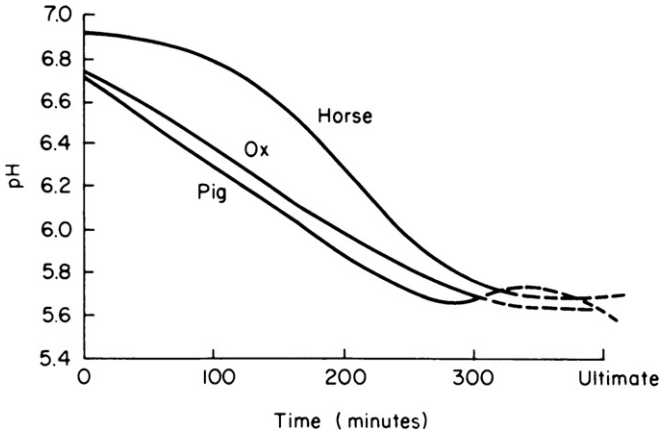



**FIGURE 3.19 The effect of species in a given muscle (longissimus dorsi) and at a given temperature (37°C) on the progress of glycolysis measured by a fall in pH (Lawrie, 1966).** *(Reprinted with permission. © Wood Publishing Ltd.)*

**TABLE 3.3 Relationship Between Type of Fall in pH and Meat Properties**

| Final pH | Type of Decrease | Properties of Meat |
|---|---|---|
| 6.0–6.5 | Slow, gradual | Dark |
| 5.7–6.0 | Slow, gradual | Slightly dark |
| 5.3–5.7 | Gradual | Normal |
| 5.3–5.6 | Rapid | Normal to slightly dark |
| 5.0 | Rapid | Dark to pale but exudative |
| 5.1–5.4 then up to 5.3–5.6 | Rapid | Pale and exudative |

Adapted from Cassens (1966).

all reported the hastening of rigor mortis as temperature is reduced from 5 to 1°C due to increased glycolytic activity and ATP hydrolysis. Stimulation of contractile actomyosin ATPase appeared to be potentiated by the release of $Ca^{2+}$ ions. This phenomenon, known as 'cold shortening', results in toughening of cooked meat (Marsh and Leet, 1966). A 30–40-fold increase in the level of ionic calcium was reported by Davey and Gilbert (1974) in the myofibril region of beef muscle held at 0°C compared to 15°C. Jeacocke (1977) examined the relationship between temperature and postmortem pH decline in beef sternomandibularis muscle. The results illustrated in Figure 3.20 indicate a minimum fall in pH over 10–12°C which increased as the temperature dropped to 0°C, characteristic of cold shortening. This was attributed to an increase in glycolysis due to enhanced ATPase activity in contractile actomyosin. Cornforth *et al.* (1980) subsequently confirmed earlier research by Buege and Marsh (1975) that the mitochondrial content of the muscle was involved in cold shortening. These researchers also proposed a role for the sarcoplasmic reticulum in the reversibility of this phenomenon. This was attributed to the possible effect of temperature on the membrane of the sarcoplasmic reticulum and the subsequent release of $Ca^{2+}$ ions.

Honikel *et al.* (1983) identified two types of shortening taking place in beef muscle. One which occurred above 20°C was referred to as 'rigor shortening' while the other taking place below 15°C was termed 'cold shortening'. In both cases muscle contraction was explained by the release of $Ca^{2+}$ ions into the myofibrillar space in the presence of

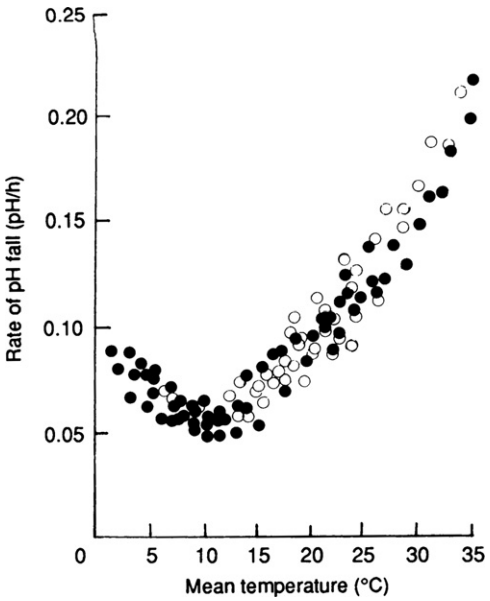



**FIGURE 3.20 The rate of pH fall in beef sternomandibular muscle as a function of the mean temperature of the adjacent thermocouple junction.** The results of 16 different temperature gradients are pooled. (●) Muscles vacuum-packed before insertion into the apparatus; (○) muscles not vacuum-packed. *(Jeacocke, 1977)*

adequate levels of ATP. The uptake of $Ca^{2+}$ ions by the sarcoplasmic reticulum was particularly sensitive to both pH and temperature changes. For example, Honikel *et al.* (1983) found that rigor shortening commenced at pH 6.25 in the presence of 2.4 μmole ATP/g muscle. This represented optimum conditions for the uptake of $Ca^{2+}$ ions by the sarcoplasmic reticulum as observed previously by Cornforth *et al.* (1980) and Whiting (1980). The myofibrillar Mg/Ca-ATPase activity was reported by Bendall (1969) to be independent of pH between 6 and 7. Both theories explain the development of rigor shortening at temperatures of 20°C and higher and at pH below 6.3. In sharp contrast, however, cold shortening developed at pH 7 in the presence of full ATP concentration (4 μmole/g) in the muscle. The occurrence of cold shortening was attributed by Cornforth *et al.* (1980) to the combined effect of the release of $Ca^{2+}$ ions from the muscle mitochondria and the reduced uptake of $Ca^{2+}$ ions by the sarcoplasmic reticulum.

Herring *et al.* (1965) first showed a relationship between cold shortening and sarcomere length. The potential for cold shortening varied with muscle types as red muscle was more susceptible to cold shortening (Bendall, 1973). As a result, pork is less susceptible than beef and lamb to cold shortening, as it is composed mostly of white fibers.

The development of cold shortening is highly undesirable and can be avoided by holding the meat at a minimum temperature of 15°C until the pH drops below 6.0. Lamb carcass, however, should be held for at least 16 hours to ensure that prerigor changes have been completed (McCrea *et al.*, 1971). This represents a delay for the meat-processing industry, which utilizes hot-deboning of beef carcasses which are cut and rapidly refrigerated below 15°C long before the pH falls below 6.0. One technique for rapidly reducing the pH of the carcasses to below 6.0 involves the use of electrical stimulation to accelerate postmortem glycolysis, as discussed in the next section.

## H. Effect of Electrical Stimulation on Postmortem Glycolysis and Tenderness

Electrical stimulation of muscle has long been known to accelerate postmortem glycolysis and hasten the onset of rigor (Harsham and Detherage, 1951; De Fremery and Pool, 1960; Hallund and Bendall, 1965; Forrest and Briskey, 1967). Carse (1973) prevented cold shortening by subjecting freshly slaughtered lamb carcasses to 250 V pulses and attained pH 6.0 within 3 hours compared to 15.4 hours for the unstimulated carcass. This technique facilitated accelerated conditioning of lambs and is commercially used in New Zealand. Similar results were also reported in lamb by Bendall (1976) and Chrystall and Hagyard (1976). The latter researchers monitored the progress of glycolysis in stimulated and unstimulated lamb longissimus dorsi muscles by following the change in pH, as shown in Figure 3.21. Electrical stimulation substantially accelerated postmortem glycolysis, with the final pH 5.5 attained within 8 hours for the stimulated carcass compared to 24 hours for the control. Chrystall and Devine (1978) noted that during electrical stimulation, glycolysis was stimulated as much as 150-fold, which resulted in a marked drop in pH. Even following cessation of the stimulus, the rate of glycolysis can increase by as much as three-fold. The initial effect was attributed by Newbold and Small (1985) to activation of glycogen phosphorylase, which reached a peak following 30 seconds of electrical stimulation. The increased glycolysis and ATP turnover following cessation of electrical stimulation, however, remains unexplained.

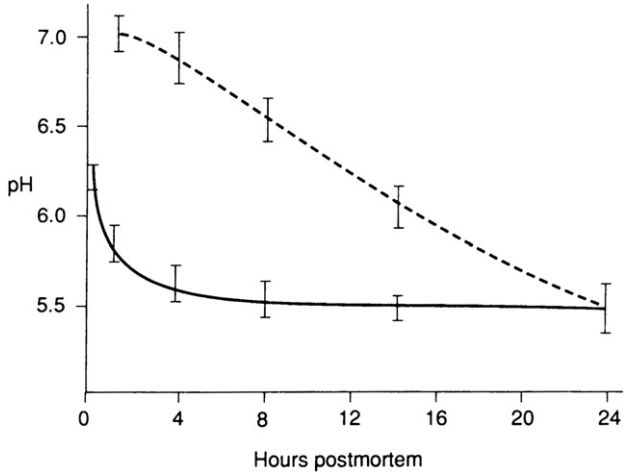



**FIGURE 3.21 Time-course of pH fall in longissimus dorsi muscles of stimulated (– –) and unstimulated (——) animals.** Standard deviation is shown by the vertical bar. *(Chrystall and Hagyard, 1976)*

Horgan and Kuypers (1985) examined postmortem glycolysis in rabbit longissimus dorsi muscles following high-voltage and low-voltage stimulation. As expected, there was an increase in the fall of pH as well as in, for example, phosphorylase activity following electrical stimulation. This was attributed to an increase in phosphorylase kinase activity and a substantial loss in phosphorylase and phosphatase activities. The yield of sarcoplasmic reticulum was reduced, suggesting an increase in the release of $Ca^{2+}$ ions from the stimulated muscle. However, whether the results were due to physical disruption or to fast glycolysis was not determined, as later work by Hopkins and Thompson (2001) reported that free calcium concentration was a function of pH decline. The biochemical and physical effects of electrical stimulation on beef and sheep meat were reviewed by Hwang *et al.* (2003).

Electrical stimulation has been reported to improve tenderness in meat (Savell *et al.*, 1977; McKeith *et al.*, 1980). Its effect was attributed to several factors, including reduction of cold shortening capacity, myofibril fragmentation, and enhanced activity of acid proteases caused by lowering of the pH (Davey *et al.*, 1976; Chrystall and Hagyard, 1976; Savell *et al.*, 1977, 1978). Sonaiya *et al.* (1982) subjected one side of a cow carcass (semimembranosus, longissimus dorsi, and triceps brachii) to electrical stimulation and monitored the pH, temperature, and myofibril fragmentation index over a period of 1 week following treatment. A significant drop in pH occurred in the treated muscles compared to the corresponding untreated muscles. A higher myofibril fragmentation index (MFI) was observed for the electrically stimulated muscles, which is normally associated with the degradation of troponin T and the Z-disk by the calcium-activated factor. However, the appearance of the 30 kDa protein, normally associated with CAF action, did not reach a maximum level until 72 hours post-stimulation, by which time the MFI had decreased. It was apparent from this study that electrical stimulation did not enhance CAF activity as might have been expected. Since the degradation of troponin T was brought about primarily by the action of cathepsins, the effect of electrical stimulation was attributed to the enhanced activity of these proteolytic enzymes at the low pH.

Concern about installation costs and operator safety associated with high-voltage electrical stimulation of meat led to more frequent use of low-voltage electrical stimulation (LV-ES, voltage $< 100$ V) in many countries. Such treatment has been reported to improve tenderness in beef (Taylor and Marshall, 1980; Aalhus *et al.*, 1994). Chrystall *et al.* (1980), working with lambs, recommended that for efficiency, LV-ES treatment be carried out within a short time after slaughter. Mixed results reported on beef tenderness using LV-ES ranged from none to a slight improvement and even negative changes, including poorer quality (Unruh *et al.*, 1986; Redbottom *et al.*, 2001; Hwang *et al.*, 2003). Different muscles were reported to respond differently to stimulation on the same carcass (Devine *et al.*, 1984). A study by Rhee and Kim (2001) examined the effect of LV-ES and temperature conditioning at 2, 16 and 30°C for 3 hours on glycolysis and calpain activity in the loin (m. longissimus) obtained from the Korean native cattle (Hanwoo). LV-ES appeared more effective in accelerating the rate of glycolysis in longissimus compared to conditioning at 30°C, although both accelerated the decline in pH and depletion of ATP and glycogen. The activity of the calpains was found to decrease over 24 hours. The best treatment for accelerating glycolysis and improving calpain activity proved to be a combination of LV-ES and conditioning at 30°C. Hollung *et al.* (2007) later showed that LV-ES not only affected the muscles within the beef carcass differently, but also had a variable effect on Warner–Bratzler (WB) shear force and pH decline, in the same muscle from different animals. Some animals showed no effect by LV-ES on pH decline, while WB shear force values were improved. Thus, LV-ES may be responsible for other mechanisms, besides accelerated pH decline and cold shortening prevention, that must play an important role in meat tenderness.

A novel approach to enhancing meat quality in Australia is based on medium-voltage electrical stimulation (Pearce *et al.*, 2009). The alternating frequencies enhanced stimulation of the muscle, making it more tender than the untreated control after 30 days. No detrimental effects were observed for either drip loss or retail color display.

## I. Prerigor Pressurization

Prerigor pressurization is another accelerated procedure for processing meat that has been developed (Macfarlane, 1973; Kennick *et al.*, 1980; Elkhalifa *et al.*, 1984a, b). Macfarlane (1973) was the first to tenderize prerigor meat using high-pressure treatment (103 MPa, 30–35°C for 1–4 minutes) and observed a rapid fall in pH as well as improved tenderness ratings. Since prerigor is rarely used, they examined the effect of high pressure on postrigor beef tenderness, which is discussed in Section P.

## J. Glycolytic Enzymes

Investigations were conducted to determine the first glycolytic enzyme inhibited as the pH falls, as glycolysis ceases at pH values much higher than 5.3. Newbold and Lee (1965) found that phosphorylase was the limiting enzyme in

minced sternomandibular muscle diluted with an equal volume of 0.16 M potassium chloride, which was consistent with earlier studies by Briskey and Lawrie (1961). Kastenschmidt *et al.* (1968) examined the metabolism of pig longissimus dorsi muscle and confirmed that phosphorylase was the primary control site in postmortem glycolysis. Phosphorylase is one of the key enzymes in glycolysis present in muscles (Scopes, 1970). In addition to this enzyme, Kastenschmidt *et al.* (1968) implicated phosphofructokinase and pyruvic kinase in glycolytic control. On the basis of these and related studies, the muscles in pigs were classified as 'fast' or 'slow' glycolyzing muscles (Briskey *et al.*, 1966; Kastenschmidt *et al.*, 1968).

## K. Pale Soft Exudative and Dark Firm Dry Conditions

Meat quality defects such as PSE and DFD result in unattractive meats that are rejected by consumers (Viljoena *et al.*, 2002). The estimated percentage of PSE and DFD reported for meat in the USA was 16 and 10%, respectively (Cassens *et al.*, 1992). Other countries, including Canada, Portugal, and the UK, reported higher incidences (Fortin, 1989; Santos *et al.*, 1994). Surveys indicate that this problem is increasing rather than decreasing (Wariss, 2000). Associated with these phenomena are the considerable and continuing financial losses suffered by the meat industry in many countries (Adzitey and Nurul, 2011).

### *1. PSE*

The PSE condition, mainly associated with porcine muscles, is due to the rapid drop in pH, to 5.3−5.8 within 1 hour following death, while the muscle temperature is still above 36°C. Although a considerable amount of research has been conducted on PSE, it remains a significant meat quality problem in the pork industry (Barbut *et al.*, 2008). The pale color, soft texture, and poor WHC of PSE meat has caused serious economic losses for the industry (Wang *et al.*, 2005). Early research showed that a combination of high temperature and low pH caused partial denaturation of muscle proteins (Bendall and Lawrie, 1964; Charpentier, 1969; Goutefongea, 1971). The effect of high temperature and low pH on the properties of phosphorylase was examined by Fischer *et al.* (1979); these conditions caused the denaturation of phosphorylase, with the resultant loss of activity, as well as decreased solubility in PSE muscles.

The development of PSE meats has been shown to be stress related. This is prevalent in pigs that are genetically sensitive to stress, which suffer acute preslaughter stessors, although it can occur in normal pigs as well (Topel *et al.*, 1969; Oliver *et al.*, 1988; Honkavaara, 1989; Kuchenmeister *et al.*, 2000). This phenomenon, once characterized as porcine stress syndrome, was shown to be linked to a single autosomal recessive gene. This caused aberrant calcium metabolism in postmortem muscle due to a mutation in the 615 amino acid (Arg615Cys) of the sarcoplasmic reticulum $Ca^{2+}$ release channel. As a result, twice the amount of calcium is released from the sarcoplasmic reticulum compared to normal muscle (Mickelson and Louis, 1996). Pigs with this stress susceptibility were also reported by Kuchenmeister *et al.* (1999) to have a diminished uptake of $Ca^{2+}$ in postmortem muscle. The increased release of sarcoplasmic $Ca^{2+}$ was responsible for enhancing muscle metabolism by accelerating the production and accumulation of lactate in postmortem muscle. Gispert *et al.* (2000) conducted a complete survey of preslaughter conditions, including delivery from the farms to slaughter and frequency of halothane genotypes, on the development of PSE in five Spanish abattoirs. This led to a study by Guardia *et al.* (2004), who identified and assessed both environmental and genetic risk factors for PSE meat under commercial Spanish conditions. Using a polychotomous logistic regression model, they found that a decreased risk of PSE meat with transportation time depended on the stocking density. For example, the risk of PSE meat increased with stocking density for transportation times greater than 3 hours, while the reverse was true for shorter transits.

A similar condition was reported in beef muscle, described as pale, watery beef. Fischer and Hamm (1980) studied postmortem changes in fast-glycolyzing muscles of beef. Significant correlations were obtained in which a lower muscle pH was associated with lower WHC and lower glycogen and higher lactate levels. It was evident that phosphorylase activation occurred in fast-glycolyzing muscles, although the overall effect on beef quality was far less severe than in PSE pork.

Recent reports also identified the development of pale and exudative conditions in poultry meat around the world, characterized by a high $L^*$ value and low WHC (Petracci *et al.*, 2009). Increasing evidence suggests that exposure of birds to thermal stress before slaughter was one factor responsible for PSE in broilers (Barbut, 1998; Mitchell and Kettlewell, 1998). The biological cause of broiler PSE is considered to be the excessive release of $Ca^{2+}$ as a result of a genetic mutation of ryanodine receptors, a class of intracellular calcium channels located in the sarcoplasmic reticulum. Wilhelm *et al.* (2010) found that the 24-hour postmortem pectoralis major muscle classified as PSE had

lower pH, WHC, and shear force values than the control samples. Higher protease activity in the PSE samples, particularly calpain activity, was also observed, which contributed to the eventual collapse of the sarcomere structure. Further work on pectoralis major muscles in turkey by Zhu *et al.* (2011) demonstrated the detrimental effects of elevated postmortem temperate (40°C) and glycolysis, which caused the denaturation of phosphorylase, leading to PSE turkey meat.

### *2. DFD*

Improper handling of cattle before slaughter, causing physiological stress and exhaustion, is the major cause of DFD (Grandin, 1980; Tarrant, 1981). Excessive physical exercise in animals depletes the muscle glycogen, although other factors such as fasting, trauma, and psychological stress have also been implicated (Howard and Lawrie, 1956; Bergstrom and Hultman, 1966; Conlee *et al.*, 1976; Sugden *et al.*, 1976). Tarrant and Sherrington (1980) monitored the final postmortem pH of steer and heifer carcasses at a slaughtering plant in Ireland over a 3-year period. A seasonal effect was noted for the development of DFD with an average incidence of 3.2% in the carcasses examined. Measurement of the ultimate pH of the meat remained the best method for characterizing this phenomenon. The pH limits reported for the development of DFD ranged from 5.8−5.9 as the lower limit for normal meat to 6.2−6.3 as the upper limit for extreme DFD (Fjelkner-Modig and Ruderus, 1983; Tarrant, 1981). The high pH conditions result in negligible denaturation of proteins so that the water remains tightly bound with little or no exudate (Warriss, 2000). The incidence of DFD in Sweden was examined by Fabiansson *et al.* (1984), who classified beef carcasses with a pH less than 6.2 after 24 hours as DFD. The overall incidence of DFD in electrically stimulated and non-stimulated carcasses was 3.4 and 13.2%, respectively. Further studies continue to report on the development of DFD in beef (Viljoena *et al.*, 2002; Kannan *et al.*, 2002; Apple *et al.*, 2005; Zhang *et al.*, 2005).

## L. Postmortem Changes in Meat and Fish Proteins

The decline in pH of the muscle to an acidic state, together with the various exothermic reactions, such as glycolysis, has a profound effect on the muscle proteins of both meat and fish. The retention of moisture by fresh meat is recognized as one the most important quality characteristics. The majority of the water is held within the myofibril, between the myofibrils and the cell membrane (sarcolemma), and between muscle cells or bundles (Offer and Trinick, 1983; Offer and Cousins, 1992). Consequently, any degradation of muscle proteins, particularly by proteases, could affect the WHC of the muscle. This section will discuss the phenomenon of protein lability within the muscle and its effect on meat and fish quality.

Shortly after death, the body temperature in cattle may rise from 37.6 to 39.5°C (Meyer, 1964). Even during refrigeration, meat cools slowly as a result of the various exothermic reactions taking place, such as glycolysis. This phenomenon is known as ‘animal heat’ and was recorded from ancient times. Consequently, postmortem changes in muscle proteins are affected very often by a combination of high temperatures and low pH. Such changes include loss of color and decrease in WHC (Cassens, 1966). In meat, it is the sarcoplasmic proteins that are denatured and become firmly attached to the surface of the myofilaments, causing the lightening of meat color (Bendall and Wismer-Pederson, 1962). In fish, sarcoplasmic proteins are generally more stable than myofibrillar proteins, and are unaffected by dehydration or prolonged cold storage.

### *1. Water-Holding Capacity*

The major postmortem change in meat and fish muscle is the loss of water or exudation. In lean meat, which has around 75% water, the majority of the water is somewhat loosely bound by the meat proteins. In the prerigor state, meat has a high WHC, which falls within the first few hours following death to a minimal level coincident with the establishment of rigor mortis. This minimal level corresponds to the ultimate postmortem pH of 5.3−5.5, which is the isoelectric point of the major muscle proteins. A rapid fall in pH leads to a number of changes including some muscle protein denaturation (Scopes, 1964; Chaudhrey *et al.*, 1969) and loss of WHC (Disney *et al.*, 1967). Tarrant and Mothersill (1977) determined glycolytic rates at several locations in beef carcasses as they affect the properties of the muscles. The postmortem pH decreased with distance from the carcass surface, indicating an increased rate of glycolysis which was accompanied by a greater degree of protein denaturation and drip loss. Drip loss was measured by low centrifugation of intact muscle samples from several muscles, as shown in Table 3.4.

**TABLE 3.4 Drip Losses in Four Muscles in the Round**

| Muscle | Depth (cm) | Percentage Drip ± SE (*n*) |
|---|---|---|
| M. biceps femoris | 1.5 | 8.1 ± 0.7 (6) |
| | 5 | 14.9 ± 0.8 (6) |
| | 8 | 18.2 ± 0.7 (6) |
| M. semitendinosus | 1.5 | 7.5 ± 1.0 (6) |
| | 8 | 16.7 ± 1.2 (6) |

SE: standard error; *n*: number of muscles examined.
Adapted from Tarrant and Mothersill (1977).

The muscles at a depth of 8 cm were paler, softer, and wetter than those at 5 cm or 1.5 cm after 2 days postmortem, which is somewhat characteristic of the PSE condition observed in pork, although not as pronounced. This increased drip loss was attributed to a decrease in the WHC of denatured muscle proteins and sarcolemma disruption. The smallest drip loss at 1.5 cm was attributed to the low temperatures ($< 15°C$) attained before the pH reached 6.0. This explained why rapid cooling of beef carcasses following slaughter minimized the amount of drip loss.

Evidence suggests that reduced degradation of key cytoskeleton proteins, such as the intermediate filament protein desmin, which ties the myofibril to the cell membrane, may allow shrinkage of the myofibril and hence shrinkage of the muscle. Such shrinkage, however, opens up drip channels and increases drip loss (Morrison *et al.*, 1998; Kristensen and Purlow, 2001; Rowe *et al.*, 2001). Consequently, preventing shrinkage, by increasing degradation of desmin, would allow more moisture to remain in the tissue. Melody *et al.* (2004) associated differences in drip loss in porcine longissimus dorsi muscle with decreased proteolysis of desmin (Table 3.5). Since desmin is degraded by μ-calpain (Huff-Lonergan *et al.*, 1996), autolysis and activation of this enzyme might explain the variation in desmin degradation and its influence on drip loss. Examination of porcine longissimus dorsi, semimembranosus, and psoas major muscles by Melody *et al.* (2004) showed that differences in μ-calpain activity, μ-calpain autolysis, and protein degradation could explain differences in both tenderness and WHC for these muscles. These results do not rule out a role for other intermediate filament proteins such as talion and vinculin. For a more detailed discussion on the role that biochemical and structural changes have on WHC, the review by Huff-Lonergan and Lonergan (2005) is recommended.

**TABLE 3.5 Drip Loss Percentage and Desmin Degradation Product of the Porcine Longissimus Dorsi (LD), Semimembranosus (SM), and Psoas Major (PM)**

| Item | LD | SM | PM |
|---|---|---|---|
| **Drip loss (%)** | | | |
| 24 hours of storage | $1.57^{x} \pm 0.384$ | $1.46^{xy} \pm 0.373$ | $1.06^{y} \pm 0.250$ |
| 96 hours of storage | $2.95^{b} \pm 0.623$ | $2.78^{b} \pm 0.551$ | $1.10^{c} \pm 0.214$ |
| **Desmin degradation product[a]** | | | |
| 45 minutes postmortem | $0.126^{c} \pm 0.027$ | $0.177^{c} \pm 0.036$ | $0.286^{b} \pm 0.039$ |
| 6 hours postmortem | $0.165^{c} \pm 0.106$ | $0.188^{c} \pm 0.056$ | $0.415^{b} \pm 0.097$ |
| 24 hours postmortem | $0.306^{xy} \pm 0.081$ | $0.233^{y} \pm 0.071$ | $0.391^{x} \pm 0.057$ |

[a]*Ratios were calculated as the intensity of the desmin degradation product band in each sample over the intensity of the intact desmin degradation product band in the internal designated densitometry standard.*
[b,c]*Within a row, means without a common superscript letter differ ($p < 0.01$);* [xy]*within a row, means without a common superscript letter differ ($p < 0.05$).*
From Melody *et al.* (2004).

In the case of fish muscle, the ultimate pH tends to be higher than meat, hardly falling below 6.0 even in full rigor. However, considerable water losses were reported from exercised fish muscle similar to those in mammalian skeletal muscle (Partmann, 1965). A rapid rise in expressible fluid was observed in cod stored in ice for a 168-hour period (Banks, 1955). A series of papers published by Tomlinson *et al.* (1965, 1966a, b) reported that a decrease in pH in pacific halibut (*Hippoglossus stenolepis*) caused protein insolubility, which resulted in a pale, soft, exudative condition resembling PSE in pork (Briskey, 1964). This condition, known as chalkiness in halibut, is a problem for the fishing industry in the Pacific northwest because of rejection by the consumer. A technical report on chalky halibut investigations from 1997 to 1999 by Kammer (2000) for the International Pacific Halibut Commission also identified water temperature, season, postcapture handling, and sexual maturity as factors affecting chalkiness in Pacific halibut. To alleviate this condition, fish are allowed to remain alive after capture to remove excess lactic acid, so that a normal postmortem pH is attained following death.

A study by Hagen (2008) confirmed the importance of pH to chalkiness in farmed Atlantic salmon (*Hippoglossus hippoglossus* L.). They also implicated the activity of cathepsins B and D in the development of chalkiness. A related study on commercially farmed Atlantic halibut by Hagen *et al.* (2008) also found that cathepsins had a significant impact on protein content and liquid loss. Of the cathepsins examined, cathepsin H was the largest contributor to liquid loss, accounting for approximately 48.8% of the total variance. To obtain the best quality Atlantic halibut, they strongly recommended harvesting in the fall (autumn) or early winter, when liquid loss and cathepsin activities were low.

### *2. Proteomics and Water-Holding Capacity*

Using proteomics, Hwang *et al.* (2004) found a strong relationship between an increase in drip loss in pork longissimus muscle and the high rate of postmortem proteolysis. Three proteins, troponin T, adenylate kinase (the substrate of ATP-dependent proteinase SP-22) and D5-1 protein, were all found to be related to changes in drip loss. Using two-dimensional electrophoresis, Van de Wiel and Zhang (2007) isolated a number of protein markers that could serve as important to the drip loss. These included creatine phosphokinase M-type (CKP), desmin, and a transcription activator (SWI/SNF). A recent proteomic study by Yu *et al.* (2009) examined the relationship between meat quality and heat shock proteins (HSPs) in longissimus dorsi muscle of pigs. Using the enzyme-linked immunosorbent assay (ELISA) technique, Yu *et al.* (2009) observed a tendency for the four HSPs (αβ-crystalline, HSP27, HSP70, and HSP90) to decline as drip loss increased in the longissimum dorsi muscles, suggesting their close relationship with WHC in pork.

## M. Postrigor Tenderness

The most widely used process for tenderizing meat involves postmortem aging of the carcass. An optimum aging period of 8−11 days for choice carcasses from beef was proposed by Smith *et al.* (1978), although longer periods are used by the meat industry. The aging (conditioning or ripening) process is accelerated by raising the temperature. For example, in the Tenderay process (American Meat Institute Foundation, 1960), beef is held at 15°C for a 3-day period in ultraviolet light to control the surface microbial spoilage. This contrasts with fresh pork, which is not aged because of the rapid onset of fat rancidity even at low temperatures. In addition to temperature, both electrical stimulation and pressurization accelerate postmortem aging, as discussed previously (Savell *et al.*, 1981; Koohmaraie *et al.*, 1984).

## N. Mechanism of Postrigor Tenderization

Since the last edition of this book, there have been major advances in our understanding of the biochemistry of meat tenderness (Herrera-Mendez *et al.*, 2006; Luciano *et al.*, 2007; Kemp *et al.*, 2010; Huff-Lonergan *et al.*, 2010). Three proteolytic enzyme systems in muscle appear to play a role in postmortem tenderization. Listed in order of their discovery, they are the lysosomal cathepsins, calpains, and proteosomes or multicatalytic proteinase complexes (MCPs) (Koohmarie and Geesink, 2006). Cathepsins were the first enzymes discovered by De Duve *et al.* (1955) that appeared to be involved in meat tenderness. Calpains were identified later in rat brain by Guroff (1964) and received far greater attention because of their ability to alter Z-line intensity. More recent studies, however, suggested a possible role for proteosomes in tenderization, with some researchers concluding that 20S proteosome was a potential contributor to the tenderization of stored meat (Matsuishi and Okitani, 1997; Dutaud, 1998; Lamare *et al.*, 2002; Sentandreu *et al.*, 2002; Thomas *et al.*, 2004).

### 1. Calcium-Activated Factor: The Calpain Family

A calcium-dependent endogenous protease capable of degrading the Z-disk was first identified in skeletal muscle by Busch *et al.* (1972), and subsequently referred to as the calcium-activated factor (CAF). This factor was active at neutral pH in the presence of calcium ions (Dayton *et al.*, 1975, 1976a, b). It degraded tropomyosin, troponin T, troponin I, filamin, and C-protein, with no detectable effect on myosin, actin, α-actinin, or troponin C (Dayton *et al.*, 1975; Dayton and Schollmeyer, 1980). CAF was found by Olson *et al.* (1977) to cause major changes in the myofibrillar proteins. Using a combination of microscopic and sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), they observed the degradation of the Z-line, fragmentation of the myofibril, and disappearance of troponin T. This was accompanied by the release of a 30 kDa myofibril component from aged beef longissimus dorsi muscle. The presence of a 3 kDa component had been reported earlier during the aging of chicken and beef muscles (Hay *et al.*, 1973; Penny, 1974; Samejima and Wolfe, 1976). Adding calcium ions to minced muscle samples, Cheng and Parrish (1977) increased the rate of troponin T degradation to the 30 kDa component. These results provided convincing evidence of the importance of CAF in the proteolysis and tenderization of postmortem muscle.

The role of CAF in meat tenderization was shown indirectly by Olson *et al.* (1976), who found that postmortem degradation of the Z-disk and myofibril fragmentation in bovine longissimus muscle correlated with tenderness scores as well as Warner−Bratzler shear values (Table 3.6). An objective method was subsequently developed for measuring myofibril fragmentation, referred to as the myofibril fragmentation index (MFI), which correlated with the tenderness scores of beef steaks (Olson and Parrish, 1977). Confirmation of the relationship was provided in later studies on beef loin tenderness and MFI by Culler *et al.* (1978), MacBride and Parrish (1977), and Parrish *et al.* (1979). MacBride and Parrish (1977) introduced the term 'myofibril fragmentation tenderness' to describe tenderness in conventionally aged beef carcasses.

**TABLE 3.6** Effect of Postmortem Storage (2°C) on Myofibril Fragmentation Index (MFI) and Warner−Bratzler (W-B) Shear Force of Longissimus (L), Semitendinosus (ST), and Psoas Major (PM) Muscles

| | Days of Postmortem Storage | | |
|---|---|---|---|
| | 1 | 3 | 6 |
| **MFI**[a] | | | |
| L | 49.6 ± 1.3 | 69.8 ± 1.1 | 76.3 ± 0.9 |
| ST | 48.8 ± 0.8 | 68.2 ± 1.1 | 77.6 ± 1.0 |
| PM | 47.1 ± 0.9 | 49.3 ± 1.1 | 54.7 ± 1.0 |
| **W-B**[b] | | | |
| L | 2.60 ± 0.20 | 2.23 ± 0.17 | 2.13 ± 0.12 |
| ST | 3.27 ± 0.11 | 2.72 ± 0.09 | 2.64 ± 0.11 |
| PM | 2.16 ± 0.12 | 1.94 ± 0.11 | 1.86 ± 0.17 |

Data are shown as mean ± standard error of five carcasses. Means not underscored by the same line are significantly different ($p < 0.05$).
[a] *Absorbance per 0.5 mg myofibril protein × 200;*
[b] *kilograms of shear force per cm*$^2$.
From Olson *et al.* (1976). Copyright © by Institute of Food Technologists.

These calcium-dependent proteases were subsequently referred to as calpains by Murachi (1985). Calpains are a large family of intracellular cysteine proteases of which 14 members have been identified so far (Goll *et al.*, 2003). The three main calpain isoforms found in skeletal muscle are μ-calpain, m-calpain, and skeletal specific calpain, p94 or calpain 3, together with calpastatin, an inhibitor of both μ- and m-calpains (Koohmaraie and Geesink, 2006). Calpain 3/p94 appeared to bind to titin at the $N_2$ line (Sorimachi *et al.*, 1995; Kinbara *et al.*, 1998), a site where proteolysis has been associated with tenderization (Taylor *et al.*, 1995). When not bound to titin, calpain 3 autolyzes rapidly even in the absence of $Ca^{2+}$ (Kinbara *et al.*, 1998; Spencer *et al.*, 2002). Because of the difficulty in purifying active calpain 3, its involvement in tenderization was determined by correlating its expression or autolysis with proteolysis and tenderization. Parr *et al.* (1999a) were unable to find any association between the amount or autolysis of calpain 3 and changes in meat tenderness. In contrast, significant correlations were reported by Illian *et al.* (2004a, b) between the rate of calpain 3 autolysis with meat proteolysis and tenderization. However, further work by Geesink *et al.* (2011), using transgenic mice overexpressing calpastatin, found that activation of calpain 3 by autolysis produced negligible changes in postmortem proteolysis. Thus, calpain 3 appeared to play a minor role, if any, in postmortem proteolysis in muscle. The other two isoforms, however, differed with respect to the amount of $Ca^{2+}$ needed for their activation. For example, μ-calpain required between 5 and 60 μM for half maximal activity compared to 300−1000 μM for m-calpain (Goll *et al.*, 1992). Of the two isomers, μ-calpain appeared to be the one with a major role in the postmortem degradation of myofibrillar proteins associated with tenderization (Geesink *et al.*, 2000a; Hopkins and Thompson, 2002; Koohmaraie *et al.*, 2002). μ-Calpain, because of its limited specificity, is unable to degrade proteins to their constituent amino acids, or degrade myosin or actin, the major myofibrillar proteins. A model proposed by Goll *et al.* (2003) and Neti *et al.* (2009) suggested that calpains release myofilaments from microfibrils, which are then completely degraded to amino acids by proteosome and lysosomes.

### 2. Calpastatin

Calpastatin, an endogenous inhibitor of calpain enzymes, is present in all tissues containing calpains. It is a highly polymorphic protein requiring calcium levels similar to those required to activate calpain (Goll *et al.*, 2003). Calpastatin is susceptible to proteolysis, although the resulting protein fragments retain inhibitory activity. Because of the ability of calpastatin to inhibit calpains, the presence of high levels would naturally prevent or reduce proteolysis of meat and tenderization. This is evident in Figure 3.22, as the random selection of commercial pigs with high levels of calpastatin produced a higher incidence of toughness a few hours following slaughter (Sensky *et al.*, 1998; Parr *et al.*, 1999b).

The genetic component of meat quality is extremely complex since such important traits such as color, flavor, juiciness, fat content, and tenderness are controlled by several different genes throughout the genome, referred to as quantitative trait loci (QTL). A QTL with large effects on pork tenderness was found at the same position on porcine chromosome 2 (SSC2q) (Stearns *et al.*, 2005). Among the genes in this region was calpastatin (CAST). Mutations in CAST, resulting in unregulated calpain activity and increased tenderness, were examined in a number of studies (Parr *et al.*, 1999b; Ciobanu *et al.*, 2004). Comparative analysis of SSC2q by Meyers *et al.* (2007) showed that it was orthologous to a segment of the human chromosome 5 (HSA5) and contained a strong positional candidate gene (CAST). Since some of the CAST polymorphs were associated with meat quality characteristics, it should provide a better understanding of the molecular basis of pork tenderness. Further work by Nonneman *et al.* (2011)

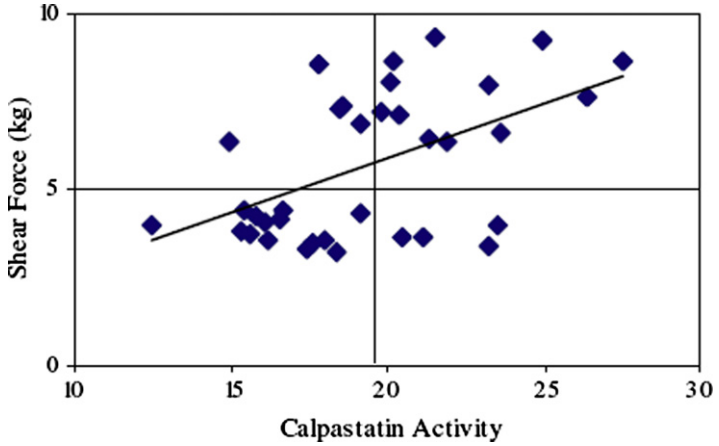



**FIGURE 3.22 Correlation between slaughter (2 hour) calpastatin activity (× $10^7$ fluorescence units/kg) and 8 day shear force in porcine longissimus dorsi (LD).** *(Kemp* et al.*, 2010)*

resequenced the calpastatin regulatory and transcribed regions in pigs with divergently different shear force values. This enabled these researchers to identify possible mutations that could affect tenderness, with a total of 194 single nucleotide polymorphisms (SNPs) identified. Of these, four SNPs were consistently associated with pork tenderness in over 2826 pigs examined from four distinct populations. Nonneman *et al.* (2011) concluded that these calpastatin markers could be used to predict tenderness in pig populations. Markers are also available within calpastatin and μ-calpain genes for selecting beef cattle with the genetic propensity for producing tender meat (Casas *et al.*, 2006).

### 3. Lysosomal Proteases

At one time the lysosomal proteases, the cathepsins, were considered to be the main agents of postmortem tenderness in meat (Bate-Smith, 1948). However, their precise role in postmortem tenderization remains inconclusive, as there is limited degradation of actin and myosin, the primary substrates for cathepsins, during postmortem conditioning (Koohmaraie *et al.*, 1991). In addition, these enzymes must be released from lysosomes in order to access the myofibril proteins (Hopkins and Taylor, 2002). Disruption of the lysosomal membrane, however, can occur under conditions of low postmortem pH and high carcass temperature (O'Halloran *et al.*, 1997). Overall, there appeared to be little association between the activity of cathepsins and meat tenderness (Whipple *et al.*, 1990). Cathepsins include both exopeptidases and endopeptidases and are classified into cysteine (cathepsins B, H, I, and X), aspartic (cathepsins D and E), and serine (cathepsin G) peptidases (Sentandreuet *et al.*, 2002). They exhibit an optimum pH of around 5.5 and are active at 37°C. Schwartz and Bird (1977) showed that rat muscle cathepsins B and D were capable of hydrolyzing myosin. A cathepsin from rabbit muscle was also found to break down myosin, α-actinin, and actin (Okitani *et al.*, 1980). Cathepsin B was also reported to degrade troponin T (Noda *et al.*, 1981) and actin (Hirao *et al.*, 1984). Further research is necessary on the activities of cathepsins B and D, which have yet to be demonstrated in postmortem muscle (Parrish and Lusby, 1983). The combined effects of CAF and cathepsin D on the myofibrils of ovine longissimus dorsi muscles were examined by Elgasim *et al.* (1985). In both cases there was loss of the Z-line, but in the case of CAF it was totally destroyed. The Z-line proteins, α-actinin, desmin, and actin, were degraded or released by either of the enzymes. CAF degraded desmin and released α-actinin without affecting actin. Cathepsin D primarily affected α-actinin, with little or no effect on actin or desmin. Although inconclusive, it appears from this study that these two enzymes might act cooperatively in the degradation of the myofibrillar proteins.

A purified cathepsin L preparation from rabbit muscle was shown by Mikami *et al.* (1987) to degrade titin, nebulin, c-protein, α-actinin, myosin light chains, and tropomyosin in myofibrils prepared from rabbit, chicken and beef muscle. Catheptic damage was most rapid in chicken myofibrils and least rapid in beef myofibrils, consistent with the more rapid conditioning in chicken. Thomas *et al.* (2004) examined the role of proteasomes and cathepsins in ostrich meat tenderization. While cathepsin D remained very active throughout the 12-day postmortem aging period at 4°C, cathepsin B, L, and H activities were minimal during the first few hours postmortem but increased substantially after 12 days. Their results suggested that cathepsin D played a role in ostrich meat tenderization as B, L, and H were not active until day 1. Previous work on ostrich meat by Van Jaarsveld *et al.* (1997) found that cathepsins B, B + L and D activities all remained very stable after 12 days of storage at 4°C. In contrast, only cathepsin H activity was found to decrease significantly, by 40%, during that period. These results appeared to clarify the controversy surrounding the role of cathepsin in postmortem proteolysis discussed by Sentandreu *et al.* (2002).

The presence of cathepsin A in fish muscle was first reported by Makinodan and Ikeda (1976). This enzyme was subsequently purified by Toyohara *et al.* (1982) from carp muscle but did not appear to be directly involved in the postmortem proteolysis of the fish muscle. Later research by Aoki and Ueno (1997) attributed the postmortem degradation in the white muscle of aging mackerel at 0°C to the action of cathepsin L. While there have been a few reports of cathepsins in the muscles of some fish species (Wojtowicz and Odense, 1972; Matsumiya and Mochizuki, 1993; Porter *et al.*, 1996), a more comprehensive examination of their distribution in 24 fish species was carried out by Aoki *et al.* (2000). With the exception of cathepsin D, marine red-flesh fish were higher in cathepsins B, B-like, L, and latent L compared to marine white-flesh fish and freshwater fish. Since Yamashita and Konagay (1990b, 1991) reported that cathepsin L was the main cause of tissue degradation and softening in salmon, the large amounts present in the red flesh of marine fish could explain why they readily degrade and soften during storage.

### 4. Proteasomes

Proteasome, a multicatalytic protease complex, is the main enzyme responsible for protein degradation in the cytosol and nucleus. Proteasomes are involved in many biological processes and are particularly abundant in skeletal muscle

(Robert *et al.*, 1999). The most common form of proteasome is 26S proteasome, which contains a 19S regulatory subunit and a 20S catalytic core. The 20S proteasome, also known as the multicatalytic proteinase complex (MCP), is the catalytic core of protease complexes (Dahlmann *et al.*, 2001). It exists as a free entity or part of a core of larger particles with high proteolytic properties. Although initially, 20S-proteasome was thought unable to degrade proteins or polypeptides, later studies reported it exhibited both endopeptidase and exopeptidase activities (Forster and Hill, 2003; Liu *et al.*, 2003; Orlowski and Wilk, 2003). Several researchers also supported the ability of 20S-proteasome to degrade myofibrils and myofibrillar proteins *in vivo* (Taylor *et al.*, 1995; Matsuishi and Okitani, 1997). Dutaud *et al.* (2006) confirmed the ability of 20S-proteasome to degrade the Z-band in veal with a pH 5.8 or higher. The potential role of both proteasomes and cathepsins in the tenderization of ostrich meat was reported by Thomas *et al.* (2004). Koohmaraie and Gesink (2006) reported that the degradation pattern for myofibrillar proteins incubated with 20S-proteasome differed from that seen in postmortem muscle. The latter, however, does not completely exclude the proteasome from contributing to postmortem proteolysis (Houbak *et al.*, 2008).

### 5. Proteomics and Meat Tenderness

Using proteomics, Morzel *et al.* (2008) demonstrated the important contribution of sarcoplasmic proteins in beef longissimus thoracis muscles to meat tenderness and eating quality. Succinate dehydrogenase (SDH) was found to be the best common predictor of initial and overall tenderness, accounting for 67.6% and 57.8% of variation of these palatability traits. In addition, regression analysis showed that heat shock protein HSP27 in fresh meat and HSP27 in aged beef accounted for up to 91% variation in sensory scores. Based on data collected in biopsies and in samples from bovine longissimum thoracis muscles shortly after slaughter, Jia *et al.* (2009) identified peroxiredoxin-6, an antioxidant enzyme, as a potential protein marker for tenderness in beef.

## O. Cytoskeleton and Meat Tenderness

### 1. G-Filaments

Davey and Graffhuis (1976) cooked beef neck muscle to maximum stretch and found that the G-filaments remained intact as long as the meat had not been aged. The tenderness of myofibrillar proteins involves weakening of the G-filaments. The rapid degradation of titin, a major protein component of the G-filaments, was reported by King (1984) to occur at elevated temperatures as well as at 0 and 15°C. Their results differed from those of Locker and Wild (1984), who found that titin was particularly resistant to aging for up to 20 days at 15°C. Nebulin, however, was found to disappear within 2 days, accompanied by an improvement in tenderness. No significant change in titin was reported, although a new B-band was detected by polyacrylamide electrophoresis (Locker and Wild, 1984). A later study by Lusby *et al.* (1983) examined the aging of beef longissimus dorsi muscle at three storage temperatures (2, 25, and 37°C) over a 7-day period. Nebulin was also found to be rapidly degraded, with the appearance of a new band corresponding to the B-band reported by Locker and Wild (1984). Lusby *et al.* (1983) claimed that titin-1 was converted to titin-2 as the time and temperature increased. However, Locker (1984) pointed out that the photographs of their gels were inconclusive because of a lack of resolution of their bands. Paterson and Parrish (1987) noted some minor differences in the titin content between tough and tender muscles at 7 days postmortem. Native titin T1 (3000 kDa), was later to be degraded early postmortem into a major degradation product, T2 (2000 kDa) (Wang *et al.*, 1979; Kurzban and Wang, 1988) and a subfragment (1200 kDa) (Matsuura *et al.*, 1988) by cleavage in the I-band region (Furst *et al.*, 1988). Using a monoclonal antibody method for titin (9 D 10) developed by Wang and Greaser (1985), Ringkob *et al.* (1988) showed that there was an alteration in the shape of titin within 2 days in postmortem beef muscle. Immunofluorescence microscopy of muscle sections and myofibrils enabled them to monitor changes in the I-band by showing transition from two- to four-band anti-titin staining patterns by the antibody per sarcomere in the muscle after 24–48 hours of storage. A similar observation for titin was made by Fritz and Greaser (1991) in postmortem beef muscle. This pointed to proteolysis of either titin or another protein to which titin may be attached.

A number of researchers used electron microscopy to show the occurrence of myofibril breakage near the Z-line during postmortem storage (Davey and Graafhuis, 1976; Taylor *et al.*, 1995). Boyer-Berri and Greaser (1998) determined whether changes in titin immunofluorescence patterns near the Z-line occurred postmortem and whether such changes were affected by muscle type. Using an antibody against a 56 kDa fragment (FE-RE) of titin located in the Z-line, they examined unaged and aged myofibrils obtained from four muscles (cutaneus trunct, rectus abdominis, psoas major, and masseter from five steers. Their results suggested that during postmortem aging the titin–actin

interactions with the Z-line and N1−line might be weakened as some titin (FE RE) fragments are able to move away from the Z line. This was the first evidence for rearrangement of the Z-line region of titin during postmortem aging that could be related to the breakdown of the Z-line and increased fragility of the myofibril. Such titin changes could also be involved in the development of postmortem tenderness.

### 2. Intermediate Filaments

The degradation of desmin, which plays a role in linking adjacent myofibrils together, may cause physical changes to the postmortem muscle. Robson *et al.* (1984) monitored the changes in three major desmin fractions isolated from bovine skeletal muscle during postmortem aging for up to 7 days at 15°C by SDS−PAGE. Only a slight decrease in the amount of desmin was observed after 1 day, although after 7 days' storage a substantial loss of intact desmin was evident. This change was paralleled by a decline in troponin T (37 kDa band) which disappeared after 7 days' storage and was replaced by a band at around 30 kDa, as observed previously by Olson *et al.* (1977). The degradation of desmin may be carried out by CAF, since this has been shown by O'Shea *et al.* (1979) using purified desmin. Thus, the degradation of desmin and the connecting intermediate filaments may play an important role in meat tenderness during aging (Yamaguchi *et al.*, 1982, 1983a, b).

## P. Effect of Pressurization on Tenderness

### 1. High-Pressure Treatment

Prerigor pressurization has been reported to increase meat tenderness (Macfarlane, 1973; Elgasim, 1977; Kennick *et al.*, 1980). As discussed in Section I, this approach was discontinued and replaced by high-pressure treatment of postrigor meat. However, no improvement in tenderness was initially reported by Bouton *et al.* (1977) in postrigor meat subjected to high-pressure treatment at low temperatures ($< 30$°C). By combining high pressure with heat, they found that an optimum temperature of 55−60°C improved the tenderness of the postrigor meat. Ma and Ledward (2004) treated postrigor beef longissimus dorsi to high pressures of up to 800 MPa over a temperature range of 20−70°C for 20 minutes. While no tenderizing effect was observed below 60°C, a significant decrease in hardness was found at 200 MPa and 60−70°C. A significant improvement in tenderness in postrigor beef sternomandibularis muscle was recently reported by Sikes *et al.* (2010) with high-pressure−high-temperature-treated compared to raw or heat treated beef (200 MPa at 60°C).

Since muscle enzymes important in meat tenderization are susceptible to high pressure, several studies have investigated the effects of such treatment. Elgasim (1977) showed that improvements in tenderness induced by prerigor pressurization could be related to lysosomal activity. An examination of bovine longissimus muscle exposed to different pressure levels by Elgasim *et al.* (1983) suggested the early release of lysosomal enzymes when conditions of pH and temperature were still conducive to rapid enzyme activity. This was based on the Z-line degradation observed in the pressurized muscle 24 hours postmortem. The pH of the muscle was less than 6.0 following pressurization, which was shown previously to facilitate the activity of cathepsin D, a lysosomal enzyme (Eino and Stanley, 1973; Robbins and Cohen, 1976). Since cathepsins are entrapped in lysosomal bodies, participation in meat aging and tenderization requires their release into the cytosol (Goll, 1983). Thus, improvement in tenderness associated with prerigor pressurization may be explained, in part, by the early release of lysosomal enzymes. This was evident in a study by Homma *et al.* (1994), who monitored acid phosphatase as an index of lysosomal membrane disruption. An increase in acid phosphatase activity was accompanied by increased cathepsin B, D, and L activities with pressures of up to 400 MPa. A further rise in pressure to 500 MPa, however, tended to decrease the activity of these cathepsins. Using electron microscopy to examine the membrane integrity of the lysosomes, Jung *et al.* (2000) established a correlation between lysosomal enzyme activities and lysosomal integrity in beef muscle subjected to high pressure. Acid phosphatase activities were always higher in the pressure-treated meat than in the corresponding control, and increased with pressure and time. In the case of cathepsin D, only the higher pressures caused a significant increase in activity. The highest values were observed for cathepsin D when beef muscle was subjected to a pressure of 500−600 MPa for short periods, less than 200 seconds. Using immunoelectron microscopy, Kubo *et al.* (2002) observed the release of cathepsin D from rabbit longissimus thoracis muscle pressurized at 100 MPa for 5 minutes followed by the absorption of cathepsin on the microfibrils. The brief exposure to high pressure appeared to have a far more drastic effect on the muscle than did normal conditioning at 2−4°C for 7 and 14 days.

Homma *et al.* (1995) found that calpain activity decreased in meat subjected to pressures above 100 MPa and was inactivated above 300 MPa. Meat pressurized at 200 MPa still had calpain activity while calpastatin was completely inactivated. The presence of calpain activity in pressurized meat below 300 MPa, however, suggested that it could still play a role in tenderization. Purification of proteasomes from rabbit muscle by Otsuka *et al.* (1998) reported an increase in activity at 150 MPa which decreased at higher pressures. Using electron microscopy, they attributed gaps between the filament structures, the complete loss of the M-line, and partial loss of the Z-line to proteasomes action.

### *2. High-Pressure Shockwaves*

Several patents have reported high-pressure shockwaves as an alternative method for tenderizing beef (Godfrey, 1970; Long, 2000). This technology, referred to as hydrodynamic pressure (HDP) wave technology, involves the underwater detonation of explosives to generate shockwave pressure on meat submerged in a test chamber (Solomon *et al.*, 1997). The high-pressure shockwave generated by explosives resulted in dramatic improvements in the tenderness of beef, pork, lamb, and poultry (Solomon *et al.*, 1997, 1998; Meek *et al.*, 2000; Claus *et al.*, 2001a). The effect of the shockwaves was instantaneous, with improvements in tenderness ranging from 37% to 57%, equivalent to that obtained by aging (Solomon, 1998). As an alternative to explosives, high-efficiency compact sparkers were examined for generating shockwaves for meat tenderization (Schaefer 2004a–d, 2005). Sparkers have a similar effect to explosives, but differ by being electrically driven acoustic sources of high-pressure shockwaves. Some improvements in tenderness using sparkers were reported previously in poultry, beef, and pork (Claus *et al.*, 2001b; Claus, 2002; Sagili and Claus, 2003). A recent study by Bowker *et al.* (2011) showed favorable changes in the tenderness of beef loins using a high sparker efficiency consistent with previous studies using shockwaves.

## Q. Lysosomal Enzymes and Collagen Degradation

The possible effects of lysosomal enzymes on the connective tissue of bovine muscles were examined by Wu *et al.* (1981). They showed that high-temperature conditioning (37°C) resulted in the release of lysosomal enzymes and enhancement of collagen solubilization. The increased breakdown of collagen fibers in the presence of lysosomal glycosidases was attributed to the degradation of the proteoglycan components which normally interfere with the degradation of collagen by collagenase (Eyre and Muir, 1974; Osebold and Pedrini, 1976).

## V. MEAT PIGMENTS

Consumer acceptance of packaged fresh meat is primarily influenced by its color (Pirko and Ayres, 1957). The bright red color of fresh meat, caused by the pigment oxymyoglobin, is preferred by most consumers to meat of a darker or browner color. Metmyoglobin, the brown pigment responsible, develops during storage of the meat and is generally recognized by consumers to indicate lack of freshness. Consequently, a predominance of this pigment in packaged meat products results in their rejection. This discoloration of packaged meats, referred to by the industry as 'loss of bloom', is associated by the consumer with bacterial growth, although this may not always be the case. The annual consumption of meat, particularly beef, is well in excess of 100 lb per capita in North America, purchased mainly as raw meat. Any deleterious changes in the nature of these pigments is of great concern to the meat industry as it affects the consumer market. Approximately 15% of retail beef is discounted in price largely as a result of surface discoloration (Smith *et al.*, 2000), so it is economically important to maintain the bright red color associated with fresh meat.

The major pigment in meat is the purplish-red pigment myoglobin. Hemoglobin, the red blood pigment, was considered at one time to play only a minor role since blood is normally drained from the slaughtered carcass. Solberg (1968) stated, however, that the desirable color of meat is influenced by both myoglobin and hemoglobin. While the amount of hemoglobin reported in meat varies considerably in the literature, the relatively small amount may still be important in terms of color and stability. Myoglobin accounts for 10% of the total iron in an animal prior to slaughter, but in the case of a well-bled carcass it could account for as much as 95% of the total iron (Clydesdale and Francis, 1971). Since the majority of studies carried out on meat color have been concerned primarily with myoglobin, this section will confine its discussion to those changes associated with this pigment.

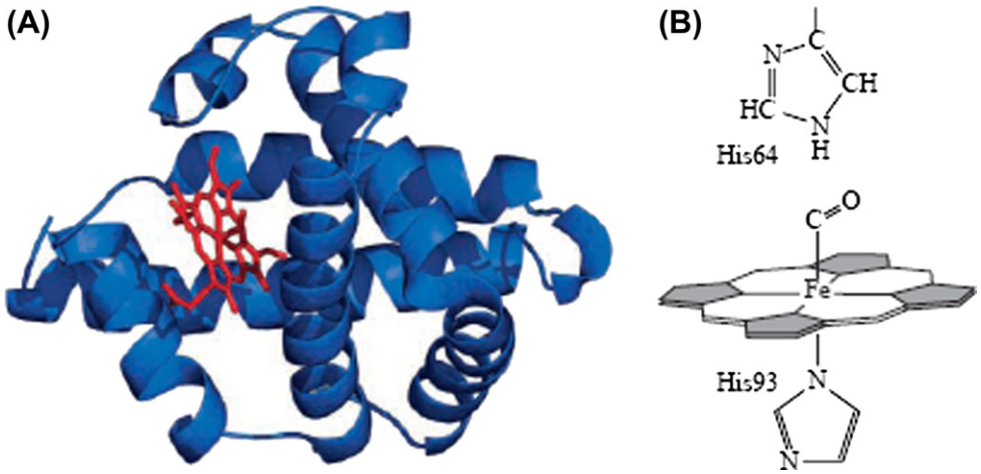



**FIGURE 3.23 Myoglobin consists of a backbone and heme-binding domain.** (A) The backbone of myoglobin consists of eight α -helices that wrap around a central pocket containing a heme group. (B) The protoheme group is bracketed or stabilized by histidine residues above (His64) and below (His93). *(Ordway and Garry, 2004.)*

## A. Myoglobin

Myoglobin is a water-soluble protein composed of a single polypeptide chain, globin, containing eight α-helices. It is connected to a prosthetic heme group, an ion (II) protoporphyrin−IX complex (Pegg and Shahidi, 1997). The latter consists of four pyrrole groups containing a centrally located atom of iron (Figure 3.23). The molecular weights of mammalian myoglobins are generally larger than fish myoglobins, ranging from 14 to 18 kDa (Satterlee and Zecharia, 1972; Fosmire and Brown, 1976; Yamaguchi *et al.*, 1979; Ueki and Uchiai, 2004; Chaijan *et al.*, 2007). The primary factors responsible for the color of meat are the valence state of the iron atom and the ligand bond to the free binding site of the heme (Seideman *et al.*, 1984).

### *1. The Primary Structure of Myoglobins*

Myoglobin stability varies among species as a result of differences in the amino acid sequences of their respective globins as well as secondary structures. Over the past few years the primary structures of myoglobin from goat, turkey, bison, tuna, and sardine have all been established (Chaijan *et al.*, 2007; Joseph *et al.*, 2010, 2011; Suman *et al.*, 2009). The complete primary structure of isolated goat myoglobin obtained by Edman degradation and tryptic and cyanogen bromide peptides was reported by Suman *et al.* (2009). They compared the amino acid sequence of goat myoglobin with homology of known ruminant myoglobin (sheep, cattle, buffalo, and deer) sequences. Goat myoglobin was similar to other livestock myoglobins by having 153 residues. However, the amino acid sequence in goat myoglobin was quite different from that in sheep and other ruminants. As a small ruminant meat animal, goat myoglobin was more similar to sheep than the larger ruminants. The two amino acid substitution differences between sheep and goat myoglobins were the smaller amino acids, threonine and glycine, at positions 8 and 52 in goat myoglobin, compared to the larger amino acids, glutamine and glutamic acid, at positions 8 and 52 in sheep myoglobin (THRgoat8GLNsheep and GLYgoat52GLU sheep) (Figure 3.24). Distal (HIS64) and proximal (HIS94) histidines, important for stabilizing the heme group and coordinating oxygen binding in other meat-producing livestock, were also conserved in goat myoglobin. Using the same techniques, Joseph *et al.* (2011) determined the complete amino acid sequence of turkey myoglobin. The percentage sequence similarities between turkey myoglobin and other myoglobins are summarized in Table 3.7. The amino acid sequence of turkey myoglobin was identical to that of chicken myoglobin, while it shared 92.5% homology with ostrich, 76.5% with pig, and less than 73% with ruminant myoglobins.

### *2. Myoglobin Changes in Raw Meat*

Studies on fresh meat discoloration are concerned primarily with the formation of metmyoglobin in postrigor meat. The color of fresh meat is determined by the relative proportions of the three primary forms of myoglobin (Watts *et al.*, 1966). These include the three oxidative states of myoglobin: oxymyoglobin, deoxymyoglobin, and

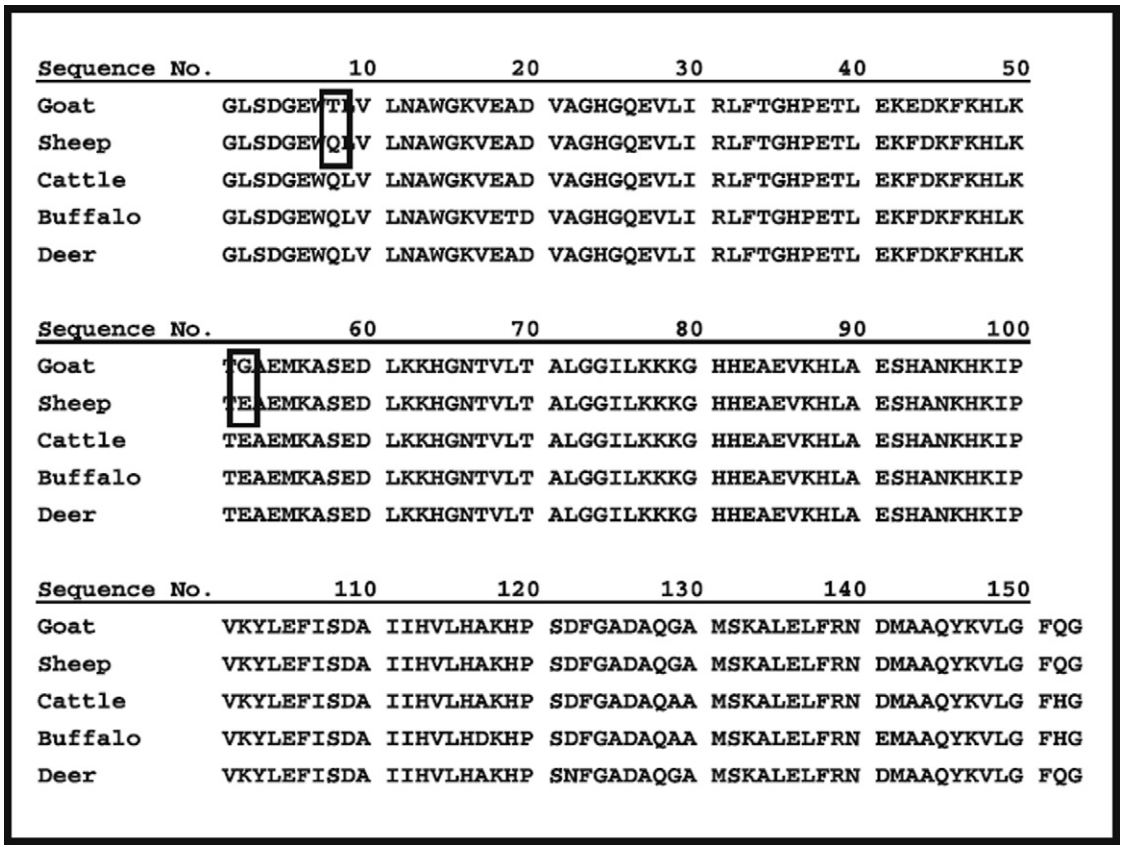



**FIGURE 3.24 Comparison of amino acid sequences of goat myoglobin and other ruminant myoglobins.** Differences between sheep and goat myoglobins at positions 8 and 52 are boxed. Goat = *Capra hircus*; sheep = *Ovis aries*; cattle = *Bos taurus*; buffalo = *Bubalus bubalis*; deer = *Cervus elaphus*. *(Suman* et al.*, 2009)*

metmyoglobin. The predominant form of myoglobin in brightly colored red meat is oxymyoglobin, in which myoglobin is bound to oxygen. Deoxymyoglobin, the reduced form of myoglobin, is a purplish pigment bound to water (or with oxygen removed) and is readily converted to oxymyoglobin. In contrast, the undesirable brownish color associated with discolored meats is metmyoglobin, in which the valency of the iron portion is converted to the ferric form.

**TABLE 3.7** Percentage Sequence Similarities Among Turkey Myoglobin and Other Myoglobins

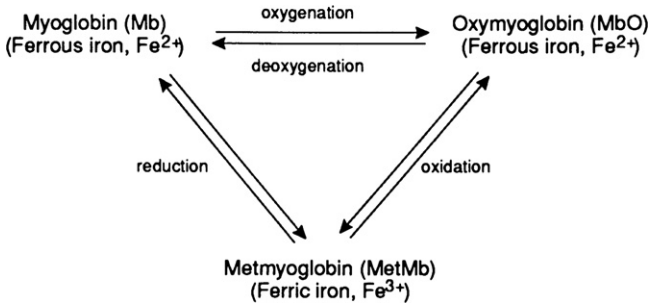
| Species | Turkey | Chicken | Ostrich | Pig | Beef | Buffalo | Sheep | Goat |
|---|---|---|---|---|---|---|---|---|
| Turkey | 100 | | | | | | | |
| Chicken | 100 | 100 | | | | | | |
| Ostrich | 92.8 | 92.8 | 100 | | | | | |
| Pig | 76.5 | 76.5 | 74.5 | 100 | | | | |
| Beef | 72.5 | 72.5 | 70.6 | 88.2 | 100 | | | |
| Buffalo | 71.2 | 71.2 | 69.9 | 86.9 | 98.0 | 100 | | |
| Sheep | 72.5 | 72.5 | 70.6 | 89.5 | 98.7 | 96.7 | 100 | |
| Goat | 71.9 | 71.9 | 69.9 | 88.2 | 97.4 | 95.4 | 98.7 | 100 |

Turkey: *Meleagris gallopavo*; chicken: *Gallus gallus*; ostrich: *Struthio comelus*; pig: *Sus scrofa*; beef: *Bos taurus*; buffalo: *Bubalu bubalis*; sheep: *Ovis aries*; goat: *Capra hircus* (Joseph *et al.*, 2011).

**TABLE 3.8** Comparison of Living Muscle Tissue and Meat

| Condition | Living Muscle | Postrigor Muscle Meat |
|---|---|---|
| Myoglobin | Oxymyoglobin | Myoglobin |
| pH | 7.35–7.43 | 5.3–5.5 |
| Temperature (°C) | 37.7–39.1 | 2–5 |
| Osmotic pressure (% NaCl equiv.) | 0.936 | |
| Oxidation–reduction potential (mV) | +250 | −50 |

From Solberg (1970).




**SCHEME 3.5** Myoglobin changes in fresh meat.

When the animal is slaughtered, oxygen is no longer available to the muscle tissue, resulting in the conversion of oxymyoglobin to deoxymyoglobin. Other changes during this period include pH, temperature, osmotic pressure, and oxidation–reduction potential (Table 3.8). Under these conditions the pigment changes occurring in meat are of great concern to the food technologist. Metmyoglobin, the undesirable brown pigment, is in equilibrium with the other pigment forms, as shown in Scheme 3.5. Further degradative reactions are mediated by bacterial action, causing irreversible damage to the porphyrin ring. This results in the formation of bile pigments, choleglobin, sulfmyoglobin, and oxysulfmyoglobin, as well as other nitrogenous compounds characteristic of meat spoilage.

### *3. Myoglobin Changes and Oxygen Tension*

The formation of oxymyoglobin involves the complexing of oxygen with the heme group of myoglobin. This process, referred to as oxygenation, occurs under high oxygen tensions and favors the formation of the desirable bright cherry-red colored meat pigment. Under low oxygen tensions, however, metmyoglobin is formed (Taylor, 1972). In fresh meat both the reduced and oxygenated forms of myoglobin are present, with the predominating form determining the final color of the meat. The formation of oxymyoglobin involves the covalent binding of molecular oxygen to myoglobin (Clydesdale and Francis, 1971). Under conditions of low oxygen tension, oxygen dissociates from the heme to yield myoglobin. The latter, being unstable, is then oxidized to metmyoglobin (Pirko and Ayres, 1957). The formation of the metmyoglobin is accompanied by the loss of an electron in the iron molecule, resulting in a change from the ferrous ($Fe^{2+}$) to the ferric ($Fe^{3+}$) state (Giddings and Markarkis, 1973; Giddings, 1977a, b).

### *4. Effect of pH, Temperature, and Salt on the Formation of Metmyoglobin*

The formation of metmyoglobin occurs under conditions of high temperature, low pH, ultraviolet light, and in the measure of salt and aerobic bacteria (Seideman *et al.*, 1984). Both high temperatures and low pH cause denaturation of the globin moiety, leaving the heme unprotected so that it undergoes rapid oxidation to metmyoglobin (Walters, 1975; Wallace *et al.*, 1982). The effect of salt is two-fold: it lowers the buffering capacity of the meat and promotes

low oxygen tensions in meat. Either of these effects results in oxidation of myoglobin to metmyoglobin (Brooks, 1937; Seideman *et al.*, 1984).

### 5. Endogenous Meat Enzymes and Metmyoglobin Formation

Freshly stored meat metmyoglobin is formed by two opposing reactions, autoxidation and reduction:

$$\text{Metmyoglobin} \xrightarrow{\text{reduction}} \underset{\text{autooxidation}}{\leftarrow} \text{Myoglobin}$$

Myoglobin is now referred to as deoxymyoglobin, the reduced form of myoglobin ($Fe^{2+}$). These reactions, referred to as metmyoglobin-reducing activity (MRA), are responsible for the valence change in myoglobin from ferrous to ferric in the meat tissue. Differences in MRA between muscles contribute to the ability of some muscles to retain the bright red pigment for much longer periods. Stewart *et al.* (1965) separated these two reactions and showed that the reduction of metmyoglobin was carried out by enzymes. They reported considerable variation in the reducing activity (MRA) of their beef samples, which increased with a rise in pH and temperature. The name methemoglobin reductase is currently used to describe many reducing systems, including those involved with other heme proteins (Bekhit and Faustman, 2005).

Watts *et al.* (1966) concluded that the reduction of metmyoglobin and oxygen in meat was carried out via reduced nicotinamide adenine dinucleotide ($NAD^+$). Succinic dehydrogenase appeared to be one of the enzymes involved, as addition of succinate to meat increased oxygen utilization. Other intermediates of the citric acid cycle and the amino acid L-glutamate were found to stimulate the reduction of metmyoglobin (Saleh and Watts, 1968). The enzymes involved remained potentially active in the postmortem meat and were capable of resuming their activity in the presence of oxygen. This may occur if meat is ground or when cut surfaces are exposed to air, provided suitable hydrogen donors (e.g. $NADH^+$) are still available.

The reduction of metmyoglobin in postmortem meat is due mainly to enzymes in which the mitochondria act as a source of reducing equivalents for the reduction of pyridine nucleotides (MacDougall, 1982). One such enzyme, metmyoglobin reductase, was identified in bovine heart muscle (Hagler *et al.*, 1979). This enzyme required ferrocyanide and NADH and could reduce metmyoglobin back to myoglobin. Arihara *et al.* (1989) established the presence of NADH-cytochrome $b_5$ reductase in bovine skeletal muscle which had an absorption spectrum assembly identical to that of a previously purified erythrocyte cytochrome $b_5$ reductase from bovine red blood cells (Yubisui and Takeshita, 1980; Tamura *et al.*, 1983). Using electrophoretic immunoblotting techniques, Arihara *et al.* (1995) estimated that the amount of NADH-cytochrome $b_5$ reductase in bovine skeletal muscle was 13.8 ± 2.6 g/g tissue, ranging from 9.0 to 12.0 g/g tissue in biceps femoris muscles. Arihara *et al.* (1990) previously located the outer membrane of mitochondria cytochrome *b* on the surface of mitochondria in muscle tissue, and cytochrome $b_5$ in the microsomal fractions. Arihara *et al.* (1995) proposed the two pathways shown in Figure 3.25 to be involved in the

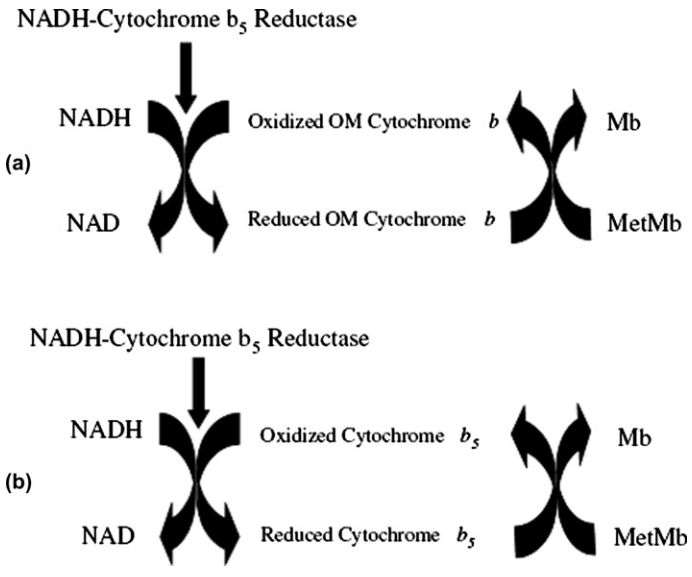



**FIGURE 3.25 Metmyoglobin reduction pathways by NADH-cytochrome $b_5$ reductase: (a) situation at the mitochondria and (b) situation at the sarcoplasmic reticulum.** *(Arihara* et al.*, 1995)*

reduction of MetMb. The enzyme had a much higher affinity for cytochrome $b_5$ and could reduce both MetMb and MetHb. An excellent review of metmyoglobin reduction can be found in Bekhit and Faustman (2005).

## B. Fish Pigments

The molecular weight of fish myoglobin is generally much smaller than that of mammalian myoglobin (Fosmire and Brown, 1976; Yamaguchi *et al.*, 1979; Chaijan *et al.*, 2007). The red color of tuna meat, a rich source of myoglobin, strongly influences acceptance by the consumer. Any discoloration during storage is associated with its conversion to metmyoglobin (Bito, 1965, 1976). The dark muscle of yellowfin tuna (*Thunnus albacores*), also rich in myoglobin, was reported to oxidize more rapidly than mammalian myoglobins (Brown and Mebine, 1969). Levy *et al.* (1985) isolated and characterized metmyoglobin reductase from the dark muscle of tuna which had a similar optimum pH to the corresponding bovine enzyme. The molecular weight of the tuna enzyme was similar to that of the dolphin reductase (30−40 kDa) but half that of bovine reductase (65 kDa). While the isoelectric pH values were different between tuna and bovine reductases, both enzymes were specific for NADH. Thiansilakul *et al.* (2011) recently isolated myoglobin from the dark muscle of eastern little tuna (*Euthynnus affinis*) and found that it was very prone to oxidation and denaturation at pH 3 at temperatures above 60°C. They recommended that processing be carried out under mild conditions to prevent undesirable changes in myoglobin from occurring.

Ochiai *et al.* (2009) showed that the structural stability of different tuna myoglobins (bigeye and bluefin) was affected by slight amino acid substitution. While highly conserved amino acid residues were responsible for the stability of myoglobin, a few non-conserved residues in the non-helical segments and heme pocket region greatly affected the thermal stability of myoglobin.

Earlier research by Miki and Nishimoto (1984) examined the relationship between loss of freshness and discoloration in several fish species, including skipjack, mackerel, and sea bream. In red-muscled fish, percentage metmyoglobin has provided a useful index of discoloration. Miki and Nishimoto (1984) plotted the log of (100 − metmyoglobin%), which represents the ratio of residual oxymyoglobin to total myoglobin against storage times at different temperatures. Their results for skipjack in Figure 3.26 clearly indicate a first order reaction corresponding to changes in freshness. These results are consistent with early work by Matsuura *et al.* (1962), who showed that the rate of autoxidation of isolated myoglobin from fish muscle also followed first order kinetics.

## C. Preservation of Meat Pigments

The accelerated conversion of myoglobin to metmyoglobin under conditions of low oxygen partial pressure is extremely important when packaging fresh meat as color is the primary factor influencing meat purchasing decisions (Mancini and Hunt, 2005). For instance, meat containing 20% metmyoglobin is generally discriminated against by consumers (MacDougall, 1982) and if it exceeds 40% it is either downgraded or rejected (Greene *et al.*, 1971). George and Stratmann (1952) reported that the maximum rate of metmyoglobin formation occurred at around 1−1.4 mm partial pressures of oxygen, decreasing to a constant minimum rate above 30 mm. Consequently, the

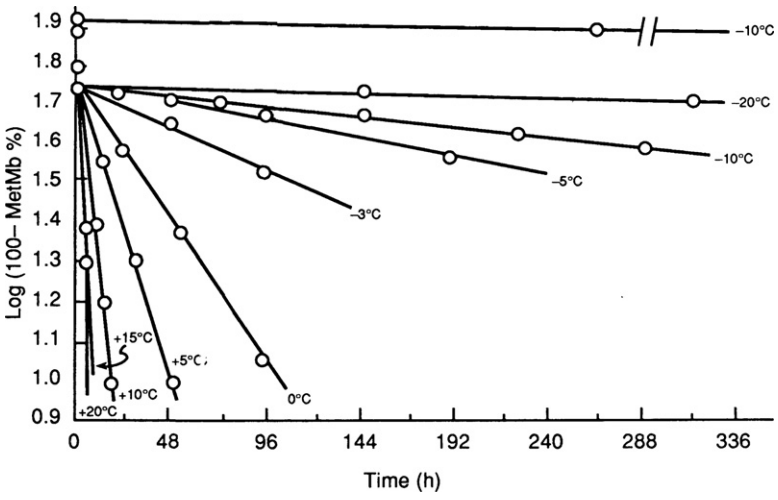



**FIGURE 3.26 Changes in MetMb percentage of skipjack during storage at different temperatures.** *(Miki and Nishomoto, 1984)*

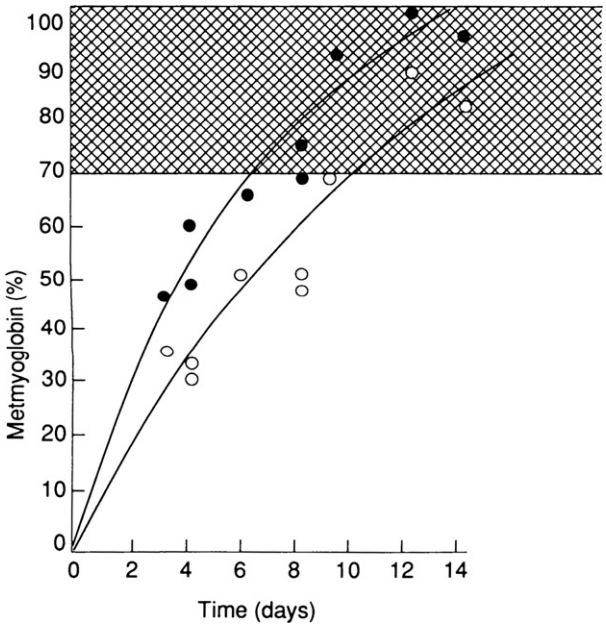



**FIGURE 3.27 Percentage metmyoglobin at the surface of bovine semimembranosus and adductor muscle slices stored at 4 ? 0.5°C in hermetically sealed semirigid polyvinyl-chloride packages containing an air or an enriched oxygen atmosphere.** ●: Air; ○: oxygen enriched; hatched area denotes unacceptable color (Daun *et al.*, 1971). *(Copyright © by Institute of Food Technologists.)*

oxygen permeability of the packaging film is of considerable importance when handling fresh meat products. Landrock and Wallace (1955) suggested that packaging should allow an oxygen penetration of 5 liters of $O_2/m^2$/day/ atmosphere to prevent the formation of metmyoglobin and subsequent browning.

Fellers *et al.* (1963), contrary to previous researchers, reported that oxygen levels higher than normally found in the atmosphere preserved oxymyoglobin. This was later confirmed by Bausch (1966). Thus, high oxygen partial pressures had considerable potential for enhancing the desirable color of meat. Very little deoxygenation takes place as the excess oxygen recombines with myoglobin to form oxymyoglobin. Daun *et al.* (1971) examined the effect of oxygen-enriched atmospheres on packaged fresh meat using a polyvinyl-chloride semirigid tray with a controlled headspace for storing fresh meat. Oxygen flushed into the headspace established an oxygen-enriched atmosphere and the formation of metmyoglobin on the surface of semimembranosus and adductor muscle slices was lower than meat stored under normal air atmosphere. Their results, shown in Figure 3.27, indicated that those samples stored in air were unacceptable, on the basis of color, after 6 days, while meat stored under oxygen-enriched atmospheres took 10 days to reach the same state. One of the major problems that has limited commercialization of this method was the development of fat rancidity under these conditions.

Of the many factors affecting the discoloration of packaged beef, differences between muscles was shown by Hood (1980) to be the most important single factor. Figure 3.28 shows decreased color stability for all four muscles as the temperature increased from 0 to 10°C. The rate of metmyoglobin accumulation was eight times faster in psoas major than in longissimus dorsi. Further work by O'Keeffe and Hood (1982) examined the biochemical factors affecting the rate of metmyoglobin discoloration in beef muscles. Psoas major was found to have a higher level of succinic dehydrogenase activity which, combined with its low myoglobin content, was responsible for a high oxygen consumption rate (OCR), resulting in low oxygen penetration and rapid formation of metmyoglobin compared to the situation in longissimus dorsi. Based on their research, enzymes responsible for metmyoglobin reduction were the main determinants of metmyoglobin formation in beef muscles, with psoas major being low in reducing activity and longissimus dorsi being high. The high-oxygen modified atmosphere packaging (MAP) saturates the meat pigment with oxygen, while carbon dioxide at a level above 20% inhibits bacterial growth (Enfors *et al.*, 1979; Nissen *et al.*, 1996; Sorheim and Dainty, 1996; Luno *et al.*, 2000). Thus, the strong relationship between color stability in the meat from different muscles is due to OCR and myoglobin and metmyoglobin reducing activities (Kropf, 1993; Sammel *et al.*, 2002; Seyfert *et al.*, 2006).

The interrelationship between lipid and pigment oxidation has been studied, since ferric pigments, such as metmyoglobin, are known to enhance lipid oxidation (Younathan and Watts, 1960; Brown *et al.*, 1963). Hutchins

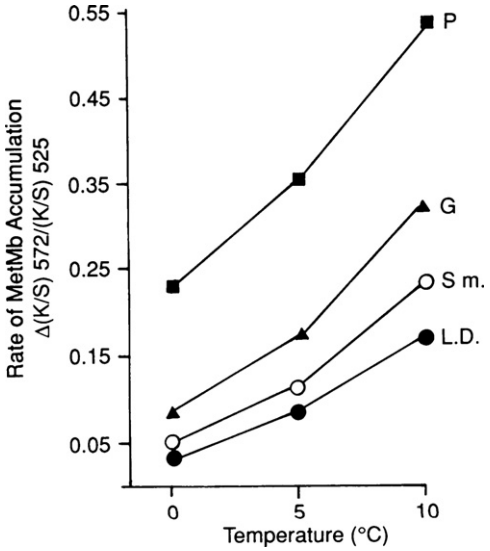



**FIGURE 3.28 Effect of temperature on metmyoglobin accumulation in four bovine muscles from 10 experimental animals.** P: m. psoas major; G: m. gluteus medius; S m.: m. semimembranosus; L.D.: m. longissimus dorsi. *(Hood, 1980)*

*et al.* (1967) reported good correlations between lipid oxidation and metmyoglobin formation in raw meat. Greene (1969) attempted to retard rancidity in refrigerated ground raw beef by adding several antioxidants, including propyl gallate (PG) and butylated hydroxyanisole (BHA). The desirable color of the ground beef was extended in the antioxidant-treated samples. Greene *et al.* (1971) treated samples of raw ground beef with a combination of PG or BHA and ascorbic acid (AA). Based on color scores using a trained panel, a consumer panel, and metmyoglobin measurements, the antioxidant-treated meat had a much longer shelf-life. Among the main limitations of high oxygen MAP are accelerated lipid oxidation and off-flavors (Jakobsen and Berrelsen, 2000; Jayasingh *et al.*, 2002) and premature browning during cooking (Torngren, 2003; John *et al.*, 2004, 2005).

Chapter 11, on lipid oxidation, discusses the myriad of aldehydes responsible for off-flavors and off-odors in food products, including meat (Pearson *et al.*, 1977). Studies showed that monounsaturated aldehydes incubated with oxymyoglobin significantly accelerated metmyoglobin formation (Yin and Faustman, 1993; Chan *et al.*, 1997; Faustman *et al.*, 1999). Among the many classes of aldehydes are the α,β-unsaturated aldehydes, which are extremely reactive with biomolecules (Witz, 1989). The most prominent and studied α,β-unsaturated aldehyde, 4-hydroxy-2-nonenal (HNE), is formed from linoleic acid oxidation in meat (Sakai *et al.*, 1998; Suhr and Kwon, 2005; Gasc *et al.*, 2007). Previous studies showed that a number of factors, including lipid oxidation products such as HNE, reduced the redox stability of oxymyoglobin with the formation of metmyoglobin (Faustman *et al.*, 1999; Alderton *et al.*, 2003). Suman *et al.* (2007) monitored the formation of metmyoglobin when HNE was incubated with porcine oxymyoglobin at pH 7.4 and 5.6, which represent normal physiological and postmortem conditions in the muscle. Their results, in Figure 3.29, show that metmyoglobin formation occurred more rapidly at pH 5.6 and was further enhanced in the presence of HNE. This contrasted with results obtained under alkaline conditions (pH 7.4) where metmyoglobin formation was much lower and not enhanced in the presence of HNE. While HNE can bind with cysteine, histidine, lysine, and arginine residues in proteins, HNE−myoglobin adducts are primarily formed at the histidine residues. Studies conducted separately with myoglobins from horse, cattle, pig, tuna, bison, chicken, and turkey all found that HNE promoted the oxidation of oxymyoglobin (Alderton *et al.*, 2003; Lee *et al.*, 2003a, b; Joseph *et al.*, 2010; Maheswarappa *et al.*, 2010). A recent study by Yin *et al.* (2011) examined the effect of HNE on the oxidation of chicken, turkey, pig, beef, sheep, horse, and deer oxymyoglobins. While HNE increased the oxidation of oxymyoglobin in all species examined, the effect was far greater with beef, horse, sheep, and deer myoglobins than with chicken, pig, and turkey myoglobins. This was attributed to the presence of 13 or more histidine residues in beef, horse, sheep, and deer myoglobins compared with only nine histidine residues in chicken, pig, and turkey myoglobins. Using isotope-labeled phenyl isocyanate, Suman *et al.* (2007) attributed the lower susceptibility of porcine myoglobin to redox instability to the formation of fewer HNE adducts (three) compared to seven in bovine myoglobin. The histidine residues adducted by HNE in porcine myoglobin were HIS 24, 26, and 119, compared to HIS 24, 36, 81, 88, 93, 119, and 152 in bovine myoglobin.

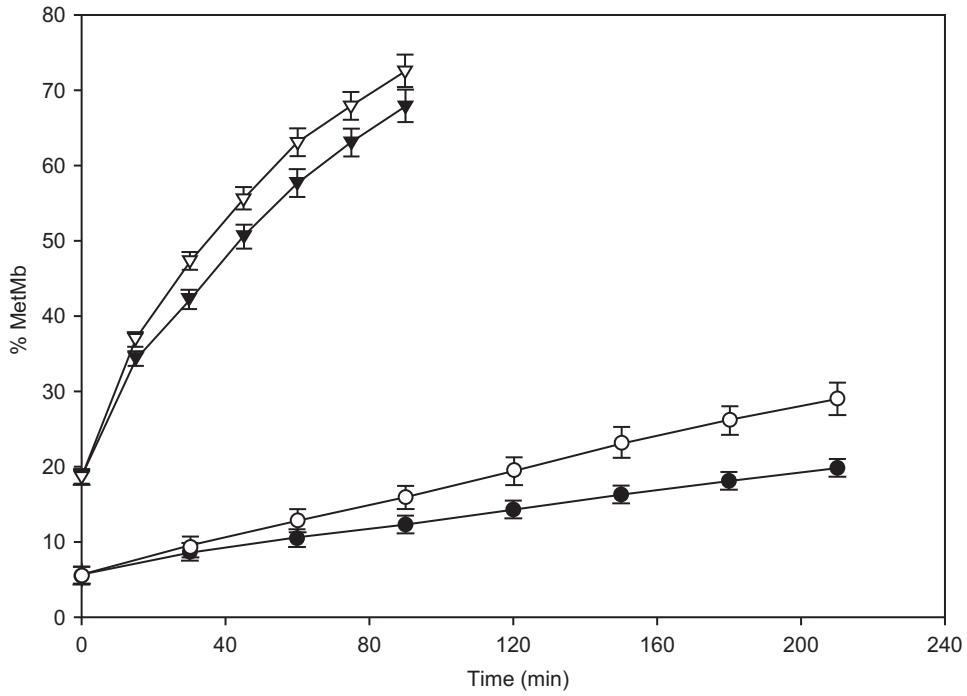



**FIGURE 3.29 MetMb formation during the reaction of porcine OxyMb (0.075 mM) with HNE (0.05 mM) at pH 7.4 or 5.6, at 37°C.** Standard error bars are indicated. τ: pH 5.6, control; ∇: pH 5.6, HNE; ●: pH 7.4, control; ○: pH 7.4, HNE. *(Suman* et al.*, 2007)*

A combination of carbon dioxide and oxygen thus provides an alternative way of extending the shelf-life of meat. While carbon dioxide tended to inhibit spoilage bacteria, oxygen maintained the oxymyoglobin form of the pigment. Taylor and MacDougall (1973) reported the retention of the fresh color in beef for at least 1 week at 1°C in an atmosphere of 60% oxygen and 40% carbon dioxide. Gas packaging of retail cuts of fresh meat in oxygen-free nitrogen or carbon dioxide extended the storage of these products by minimizing oxidative changes and bacterial growth (Partmann and Frank, 1973; Huffman, 1974). O'Keeffe and Hood (1980−1981) examined the effect of anoxic gas packaging of fresh beef in flexible packages or desiccators incorporating a palladium catalyst as an oxygen-scavenging system. No discoloration was observed with meat stored in either carbon dioxide or nitrogen for up to 3 weeks at 0°C. Some differences observed between meat samples were attributed to differences between the animals.

Most fresh retail beef in the USA is displayed in Styrofoam™ trays covered with oxygen-permeable films to permit the rapid oxygenation of the surface myoglobins to form the desirable red color or bloom. Unfortunately, this only lasts for 1−7 days, depending on the muscle or cutting method, before browning or metmyoglobin formation occurs (Maudhavi and Carpenter, 1993). Extending this period for a further 3−4 days is possible using a modified atmosphere containing oxygen, carbon dioxide, and nitrogen (Manu-Tawiah *et al.*, 1991). However, a minimum of 21 days is required for color stability to meet the 7 days needed for packaging and distribution, 7 days for retail display, and 7 days in the home. This extension is possible by adding 0.5% carbon monoxide to the MAP in place of oxygen (Sorheim *et al.*, 1999). In the presence of carbon monoxide, myoglobin forms carboxymyoglobin, a very stable bright red pigment with an almost identical absorption spectrum to oxymyoglobin (El-Badawi *et al.*, 1964; Cornworth, 1994). The addition of low levels of carbon monoxide to MAP systems presents no danger to the consumer and this practice is widely used in Norway for packaging beef (Sorheim *et al.*, 1997). In 2001, a Scientific Committee on Food for the European Commission examined the use of carbon monoxide as a component of packing gases in MAP for fresh meat (SCF/CS/ADD/MSAd/204 Final). The committee concluded that there was no health concern from using 0.3−0.5% carbon monoxide in a gas mixture with carbon dioxide and nitrogen for fresh meat, as long as the storage temperature did not exceed 4°C. Care should be taken, however, to avoid inappropriate storage conditions as carbon monoxide could mask microbial spoilage. Cornforth and Hunt (2008) discussed the advantages and disadvantages of low-oxygen packaging of fresh meat with carbon monoxide and factors that should be taken into consideration when developing a packaging technology that protects both consumers and the meat industry.

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