Chapter 3

STRUCTURE AND MUSCLE PHYSIOLOGY

The Science of Poultry and Meat Processing

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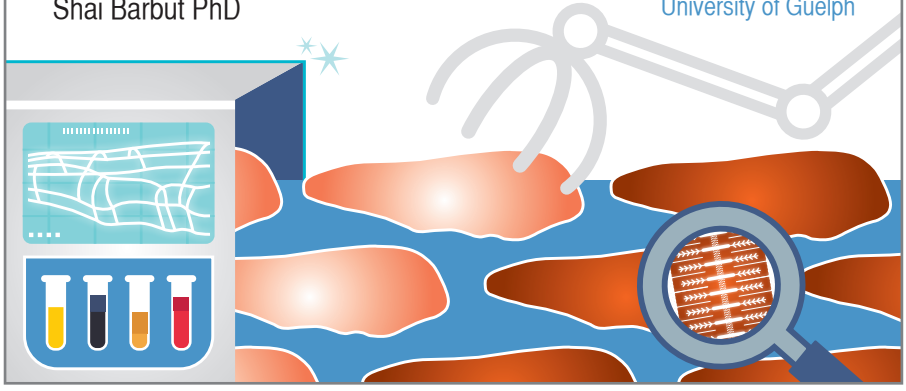
University of Guelph



Chapters

* Topics focussing on poultry. Rest of the chapters are related to both red meat and poultry.

Preface

The aim of The Science of Poultry and Meat Processing book is to provide students and industry personnel with a comprehensive view of the modernized primary poultry meat industry and further processing of both red meat and poultry. An emphasis is placed on basic concepts as well as recent advancements such as automation (e.g. increasing poultry line speed from 3,000 to 13,000 birds per hour over the last 40 years) and food safety (e.g. HACCP in primary and the further processing areas). The book also includes chapters explaining basic muscle biology, protein gelation, heat and mass transfer, microbiology, as well as meat colour and texture to help the reader understand the underlying scientific concepts of meat processing. The Science of Poultry and Meat Processing book is based on over two decades of university teaching experiences, and is designed to be used as a course textbook by students, as well as a resource for professionals working in the food industry. The book is available online, at no cost, to any interested learner. Using this format has also allowed me to include many colour pictures, illustrations and graphs to help the reader.

The book is dedicated to my past and current students who have inspired me to learn more and conduct challenging research projects. I see this as an opportunity to give back to the field that I have received so much from as a student and as a faculty member. Looking back, I have learned a great deal from my MSc and PhD advisor, Dr. A. Maurer, who was the student of Dr. R. Baker - the father of poultry processing in North America. I would also like to thank Dr. H. Swatland with whom I worked for almost 20 years, for the many challenging scientific discussions.

Writing The Science of Poultry and Meat Processing book was a long process, which also included having all chapters peer reviewed. I appreciate the help of my colleagues, but I still take responsibility for any inaccuracy in the book. If you have comments or **suggestions,** I would appreciate hearing from you (sbarbut@uoguelph.ca), as I am planning to revise and update a few chapters on a yearly basis.

I would like to thank the many people who have helped me during the writing process. To Deb Drake who entered all of the material for the book, to Mary Anne Smith who assisted in editing, and to ArtWorks Media for the design and desktop publishing of the book. I greatly appreciate the help of my colleagues who reviewed chapters and provided useful discussions. They include Mark B., Ori B., Sarge B., Gregoy B., Joseph C., Mike D., Hans G., Theo H., Melvin H., Myra H., Walter K., Roland K., Anneke L., Massimo M., Johan M., Erik P., Robert R., Uwe T., Rachel T., Jos V., Keith W., and Richard Z. I would also like to thank my family for their love and support during the entire process.

About the Author

Shai Barbut is a professor in the Department of Food Science at the University of Guelph in Ontario, Canada. He received his MSc and PhD at the University of Wisconsin in meat science and food science. He specializes in primary and further processing of poultry and red meat. His research focuses on factors affecting the quality of meat, as well as protein gelation with an emphasis on structure / function relationships, rheological properties and food safety aspects. He has published over two hundred peer reviewed research papers and is the author of the Poultry Products Processing – An Industry Guide textbook. He is a fellow of the Institute of Food Technologists and has received awards from the Meat Science Association, Poultry Science Association, and the Canadian Institute of Food Science and Technology. He is involved in a number of government committees as well as academic and industrial research projects.

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STRUCTURE AND MUSCLE PHYSIOLOGY

3.1 Introduction

The bird body has a unique structure as compared to mammals and other species because it has been adapted for flight. This includes not only wings but also the development of a light skeleton as well as air sacs that move air in only one direction through the lungs. The development of feathers and skin without sweat glands are other unique features. In this chapter, the basic overall structures of major meat producing poultry (chicken, duck, turkey, geese, pigeon) will be presented as well as the basic overall skeletal structure and muscle layout. Later, the discussion will focus on the tissue types that compose the carcass: connective, epithelial, nerve, and muscle.

Muscle structure and contraction will be described in greater detail as a basis for understanding meat quality aspects and their effects on post-slaughter changes during rigor mortis, deboning, packaging, and storage. Overall, muscle tissue represents the major edible part of the animal that is important to both meat processors and consumers. The differences between white and red muscle fibers (related to white and dark poultry meat) will also be highlighted as well as meat quality issues that can be traced to handling live birds. Two examples are pale, soft, and exudative (PSE) meat related to pre-slaughter stress and cold shortening, which is related to fast cooling during rigor mortis.

3.2 Body and Bone Structure

As indicated above, the body shape of a bird is adapted for flight and is aerodynamic to minimize airflow resistance when flying. The overall structure of a chicken skeleton is shown in Fig 3.2.1 and is typical of many avian species, although the relative size of certain body parts may vary depending on the bird's living environment. In the case of a chicken, the legs are fairly developed (Fig. 3.2.2) and adapted for walking since ancestors of the domestic chicken (jungle

fowl) lived in jungles or open spaces where standing, walking, and running represent a major proportion of total activity. The wings can be used for flying but just for a short duration to escape from predators. Thus, the wings of a chicken are not as developed as those of a migratory duck. The breast muscles that support the wings are fairly developed and even more so in the meat-type birds that have been selected for heavy musculature, especially breast muscles. The names of the different parts are shown in Figure 3.2.2.

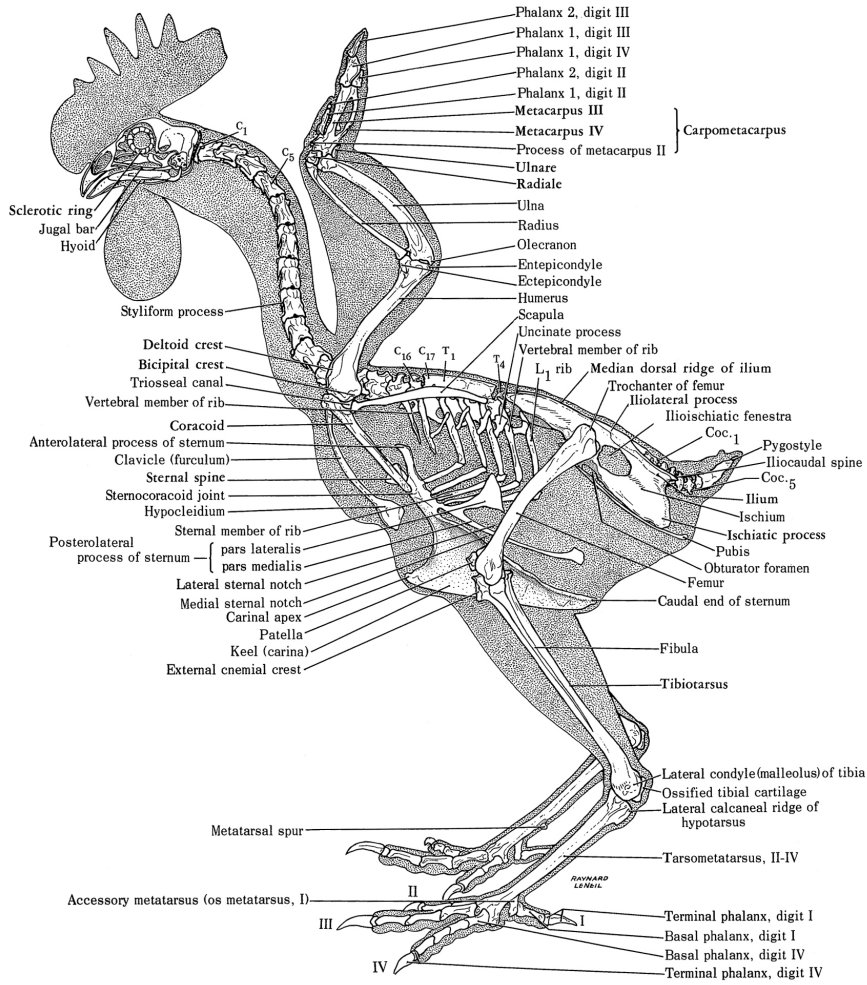

Figure 3.2.1 A lateral view of the skeleton of a Leghorn chicken. Abbreviations: C., cervical vertebra; Coc., coccygeal vertebra; L., lumber vertebra; T., thoracic vertebra. From Lucas and Stettenhiem (1972).

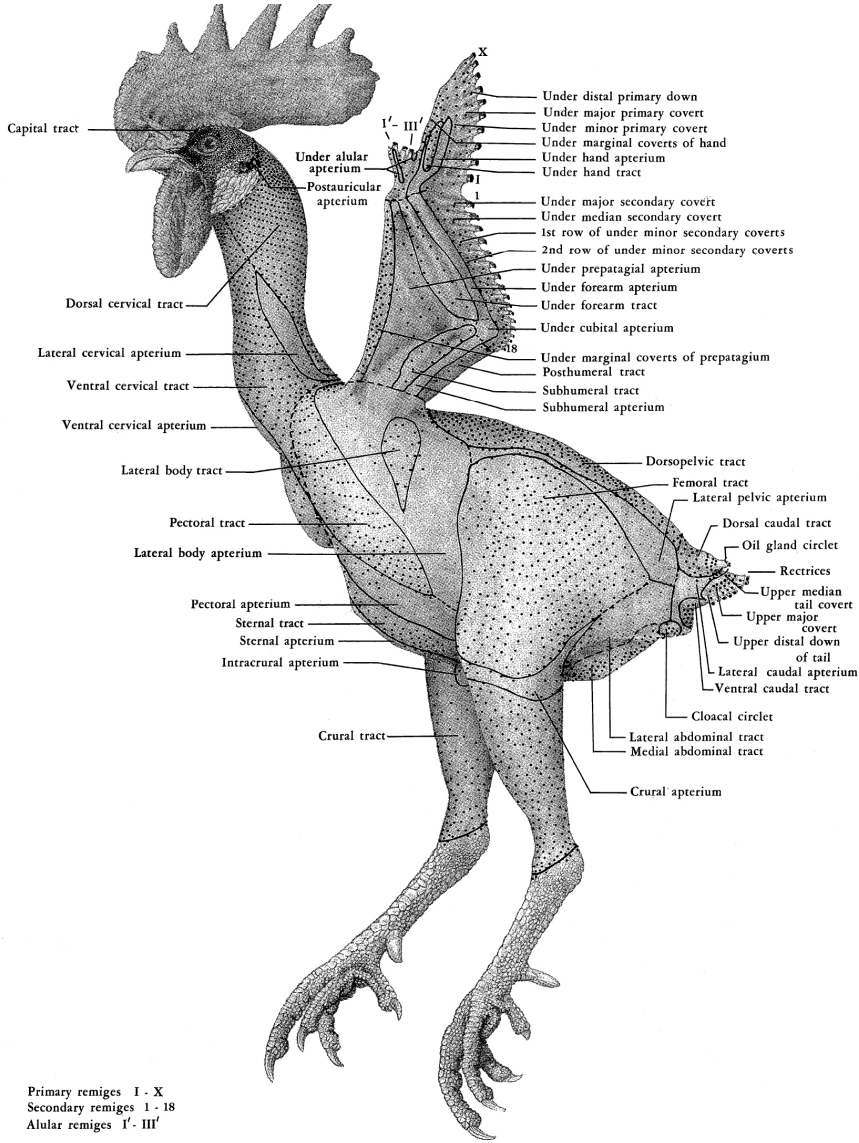

Figure 3.2.2 Left lateral view of the chicken showing the different regions. Abbreviations: reg(s)., region(s); s., synonym. From Lucas and Stettenhiem (1972).

In ducks (Fig. 3.2.3), the feet are adapted for swimming by a web between the toes that serves as a paddle. The beak is wide and has evolved to fit a marsh-type environment where it strains water and can catch small fish.

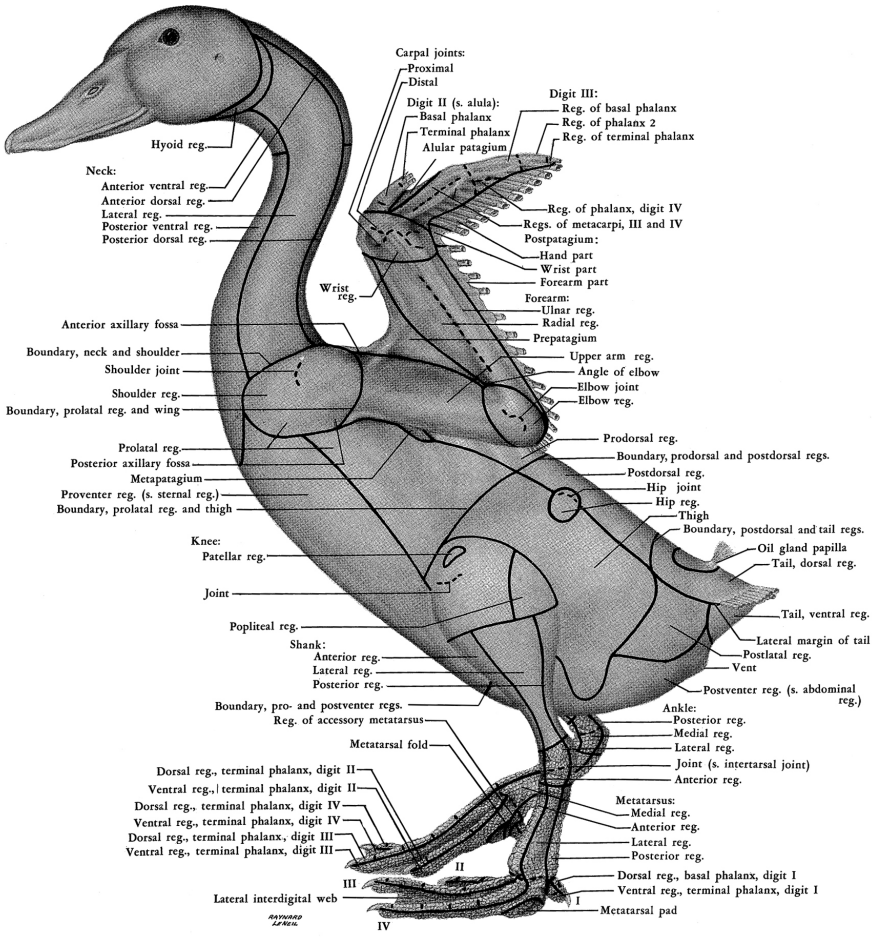

Figure 3.2.3 Lateral view of the White Peking duck showing the different regions. Abbreviations: mar., margin; reg(s)., region(s); s., synonym. From Lucas and Stettenhiem (1972).

In domesticated turkeys (Fig. 3.2.4), intense selection for heavy musculature has resulted in an almost flightless bird. The legs and toes of chickens and turkeys are structured to allow walking and perching on branches as opposed to swimming as in ducks and other waterfowl.

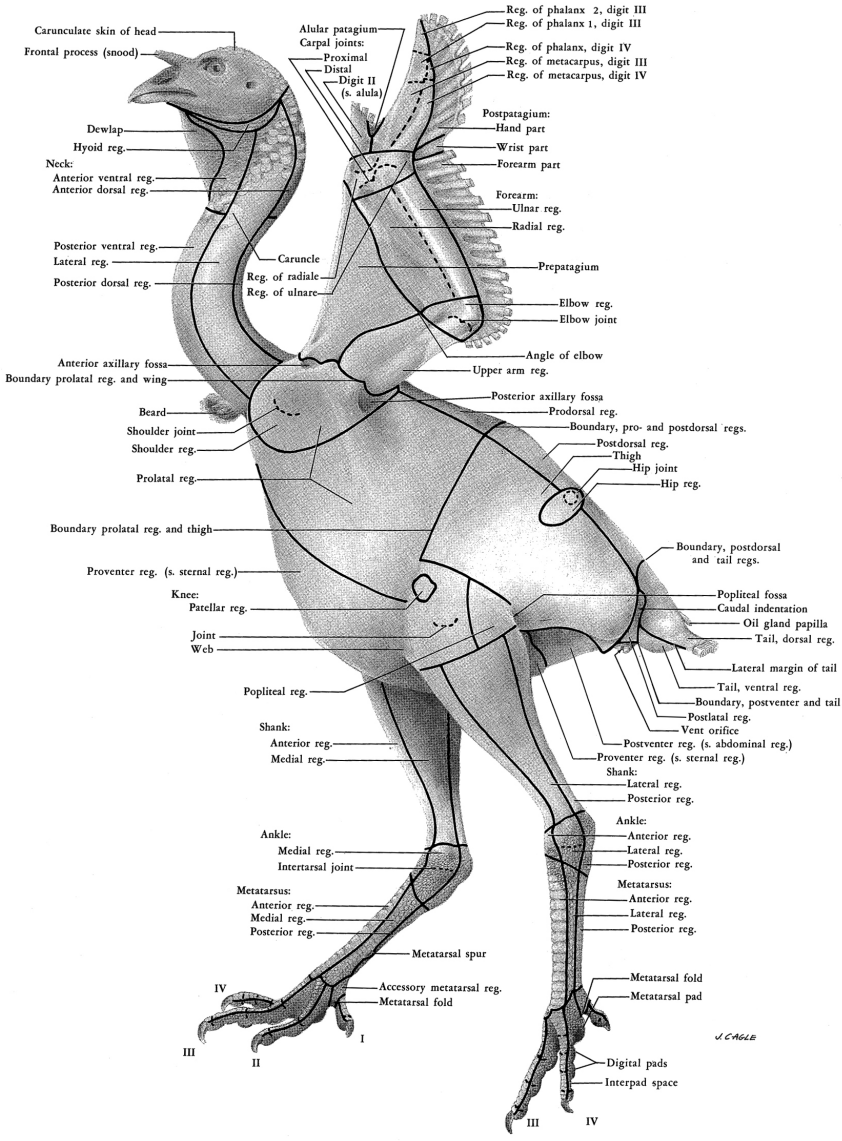

Figure 3.2.4 Lateral view of a turkey showing the different regions. Abbreviations: reg(s)., region(s); s., synonym. From Lucas and Stettenhiem (1972).

In pigeons, large wings relative to body size are used for long distance flying and gliding (Fig. 3.2.5).

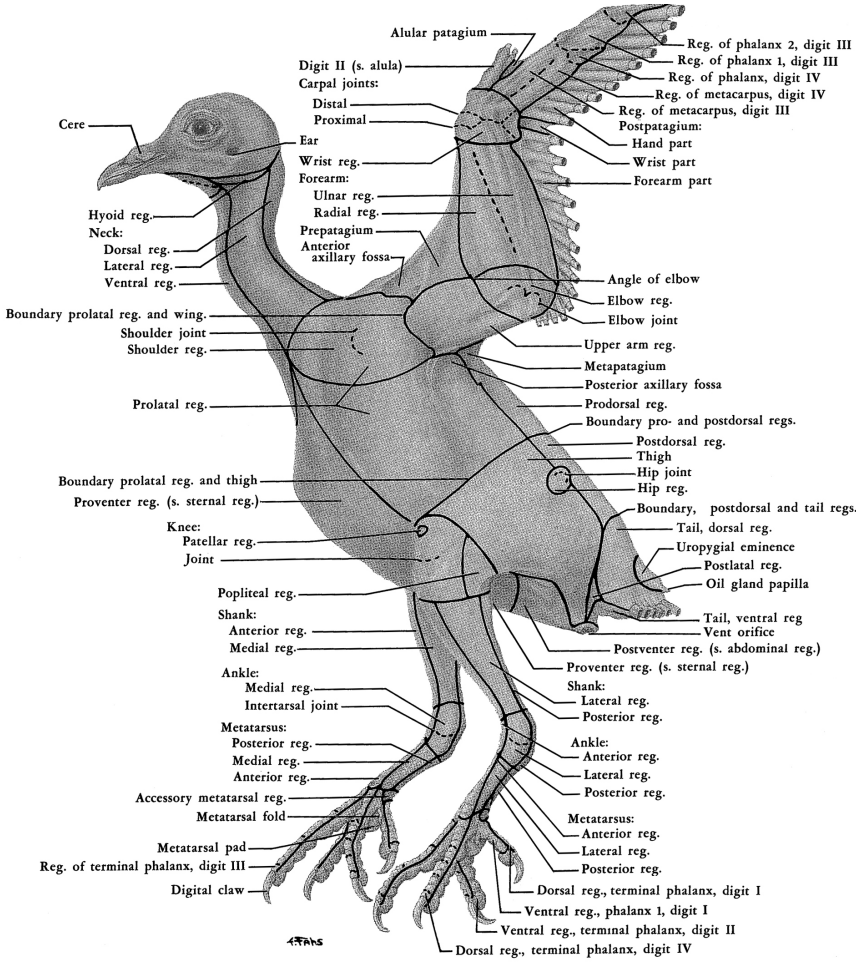

Figure 3.2.5 Lateral view of the common pigeon showing the different regions. Abbreviations: reg(s)., region(s); s., synonym. From Lucas and Stettenhiem (1972).

It is interesting to note that the overall bone structure of a bird's wing resembles the basic bone structure of limbs in mammals; however, major evolutionary modifications have occurred to allow flying.

An additional point worth mentioning is that the number of vertebrae in the axial skeleton varies both between and within bird species; the neck of a chicken can have 16 or 17 vertebrae (Lucas and Stettenheim, 1972). The respiratory system

of birds is unique (Fig. 3.2.6) because oxygen rich air flows efficiently through the lungs and air sacs in only one direction. This is different from the mammalian respiratory system where airflow is bidirectional. There are nine air sacs in the domestic chicken: single clavicular sac, two cervical, two cranial thoracic air sacs, two caudal thoracic and two abdominal air sacs (Grist, 2004). Overall the air sacs are extensions of the bronchi and some connect to the larger long bones to form the pneumatic bones (i.e., makes the bones lighter and this is advantageous during flight).

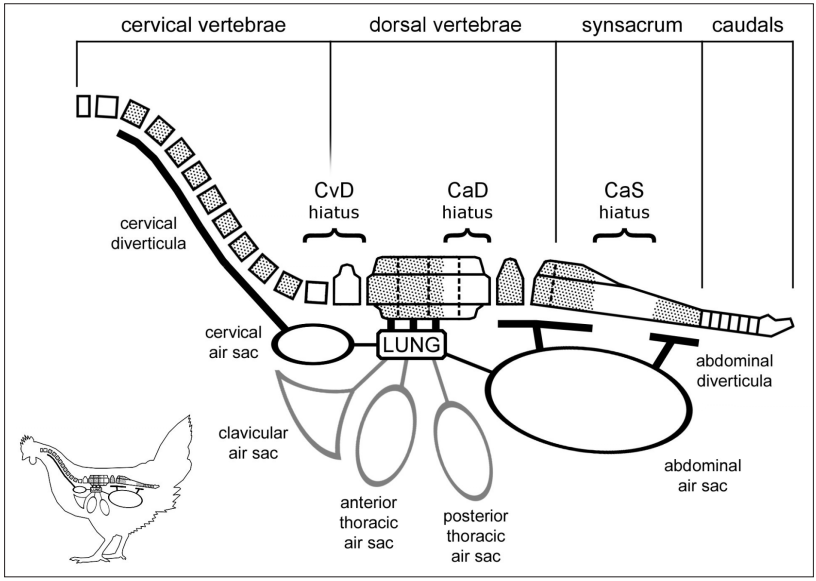

Figure 3.2.6 Respiratory system (lungs and air sacs) in chicken. From Wedel (2009). With Permission.

There are four major tissue types in animals that are related to embryonic development. They include the connective, epithelial, nervous and muscle tissues.

3.3 Connective Tissue

Connective tissue provides a supporting frame (skeleton) to connect and hold different parts of the body. It consists of bones, ligaments, connective tissue covering muscle bundles and fibers, adipose tissue, and blood. The tissue responsible for building bones and cartilage is called "supportive connective

tissue" because it provides strong structural support. Tissue that surrounds muscles, muscle bundles and fibers is called "connective tissue proper". The two types of supportive connective tissue show a number of similarities in their composition and functionality. Usually, both consist of few cells and a lot of extracellular substance. The tissue can range from very soft to very tough such as bones that contain embedded fibers and mineral crystals (calcium salts). In bones, the extracellular substance is tougher than in other connective tissues such as cartilage, where the extracellular substance is more rubbery and soft.

Blood and lymph nodes are also part of the connective tissue system. Blood especially has a large proportion of extracellular material in which cellular components are suspended (cell component usually represents about 40% of total blood volume or even lower in some fast growing breeds). The red blood cells, also known as erythrocytes (i.e., which have a distinct nucleus), transfer gases such as oxygen from the lungs to the body and carbon dioxide from the body to the lungs. The white blood cells, leukocytes, are part of the body's defense system against infections.

Connective tissue proper – consists of fibers with special helix structure of the collagen molecule which provides both strength and elasticity (Fig. 3.3.1). Tropocollagen molecules are the basic structural units of the collagen fiber. They are composed of three α chains that form a triple helix. There are about a dozen types of collagen molecules that have different functional properties and, accordingly, can be found in different locations in the body. The different types of collagen result from at least 20 different α chains that can be combined in different ways to form the triple helix. During filament assembly, the tropocollagen molecules are aligned longitudinally, end to end, and laterally in a slightly overlapping stagger as shown in Figure 3.3.1. This unique spacing and overlapping of tropocollagen molecules results in a collagen fiber that has a striated appearance (Aberle et al., 2012). Not all types of collagen form fibers. Type I and III form large and fine fibers, respectively, Type IV is non-fibrous and forms a chicken wire-like sheath that surrounds individual muscle fibers (basal lamina), and Type V and VIII form microfilaments. In general, the number of collagen fibrils within a muscle depends on its expected load, stress, and activity. Another factor that contributes to overall strength is the formation of intermolecular cross-linkages among the collagen fibrils. In young animals, there are few cross-linkages but as the animal ages the number increases and the bonds become more difficult to break.

Elastin is another major connective tissue protein with a different structure. As compared to collagen, it has a rubbery texture and its fibers can be easily stretched before returning to their original length. Elastin is commonly found in ligaments and arteries and provides structure to certain organs.

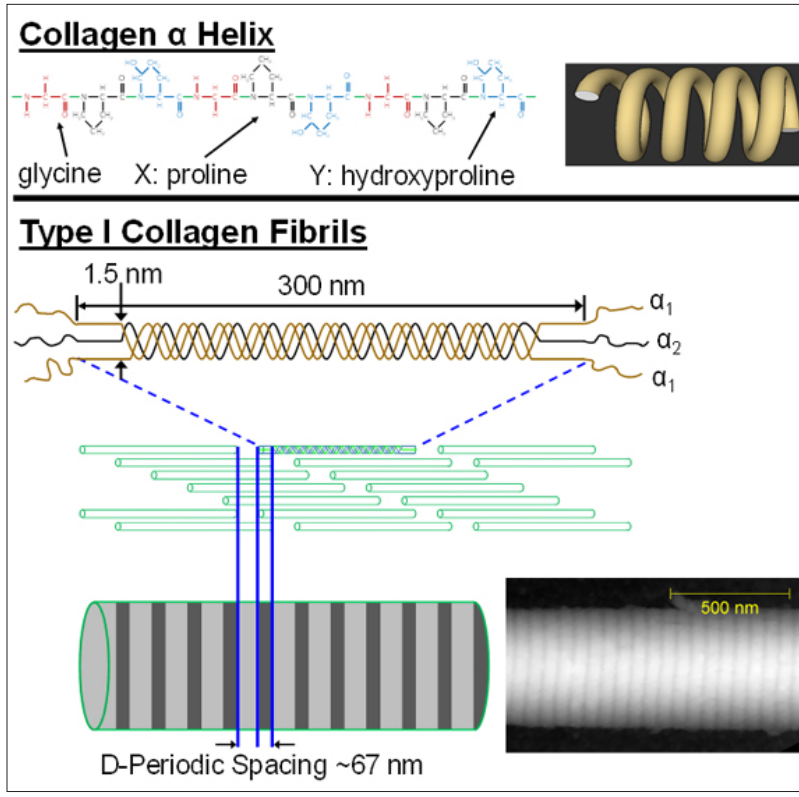

Figure 3.3.1 Microstructure of a collagen fibril which shows how the fibril, participating in muscle movement, can stretch. A striation pattern is seen at 64-67 nm intervals due to the parallel layout of the tropocollagen molecules. The striation can be seen after negatively staining a sample with heavy metals and viewing with an electron microscope. Note: collagen has a relative high content of the unique amino acid hydroxyproline, which can be also used to quantify the amount of collagen in muscle food. From http://www.iupui.edu/~bbml/boneintro.shtml.

Connective tissue proteins usually represent about 1.0% of the total muscle composition. In the meat industry the amount of connective tissue is commonly assessed by the quantity of hydroxyproline, an amino acid that is unique to collagen. Older animals are known to have tougher meat because of increased cross-linking in the collagen fibers. Both processors and consumers should know that collagen can be broken down by exposure to heat, especially prolonged, moist heat that can break some/all of the cross bridges and eventually turns collagen into gelatin. Some of the collagen becomes soluble during cooking (starting melting point ≈ 67°C). As exposure time and temperature increase, more collagen will be converted into gelatin, which becomes apparent when the meat cools and its juices

have a jelly-like consistency. Elastin, on the other hand, cannot be broken down by heat. Therefore, areas high in elastin should be either discarded or tenderized by mechanical means (needles or small blades).

Bones are also part of the connective tissue. An illustration of a chicken skeleton is shown in Figure 3.2.1. The bird skeleton is unique because, although it provides great strength, it is relatively light (i.e., important for the flying bird) as compared to the heavy bone structure needed to support a red meat animal. Bone is an active tissue where building and degradation occur all the time. It consists of an organic matrix and inorganic salts. The former contains the collagen fibers and the so-called ground substance that consists of proteins and sugar complexes. The latter is primarily made up of calcium salts (calcium phosphate and calcium carbonate), which form crystals deposited within the collagen fibers of the organic matrix. The structure consists of bone cells distributed within the matrix and arranged in small cylindrical elements called lacuna (Fig. 3.3.2). These structures form a network of canals between the cell cavities that are important in delivering cell nutrients.

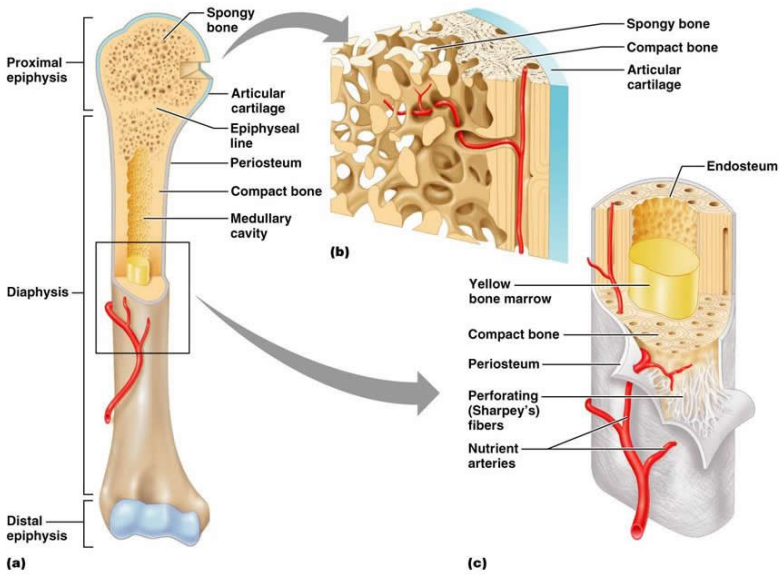

Figure 3.3.2 Overall structure of a long bone. http://classes.midlandstech.edu/carterp/Courses/bio210/chap06/lecture1.html.

The overall structure of a bone (e.g., ulna, femur) is shown in Figs. 3.3.2 and 3.3.3. The long shaft, called a diaphysis, is filled with marrow while the outside consists of a hard, compact bone structure consisting of an organic matrix and inorganic salts. Both ends of the bone, called epiphyses, are enlarged to allow sufficient surface area to connect with other bones via a cartilage-mediated medium. The epiphyseal growth plate is a region underneath the epiphyseal cartilage that separates the diaphysis and epiphysis and is the region responsible for bone elongation (see review by Howlett et al., 1984). The central hollow part of the bone contains the bone marrow that produces new red blood cells. As indicated above, bone tissue a dynamic system in terms of calcium deposition and withdrawal. For example, the laying hen is used as a research model to study osteoporosis in humans because of the fast calcium turnaround during the laying period. Bone growth can be a challenge in fast growing breeds. Summers et al. (2013) have reviewed problems that can occur during the growing of meat and egg type birds.

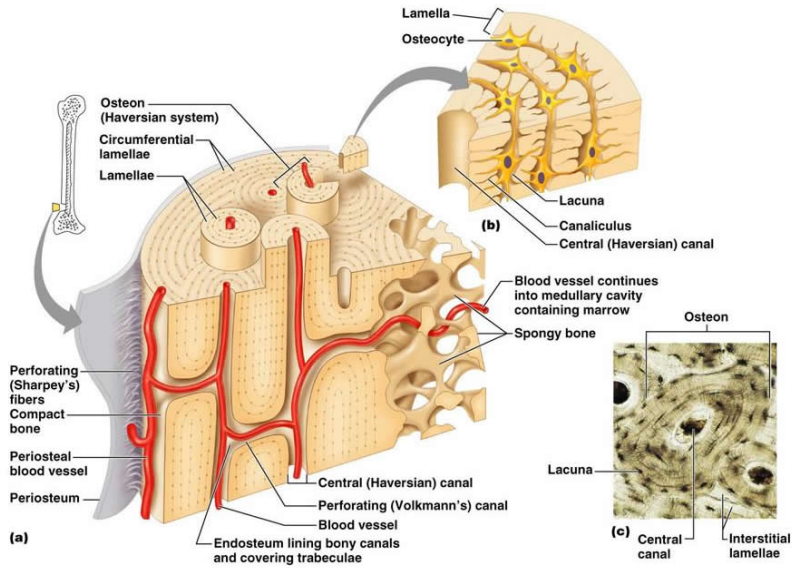

Figure 3.3.3 Structure of a long bone and its microstructure. http://classes.midlandstech.edu/carterp/Courses/bio210/chap06/lecture1.html

Cartilage is another connective tissue that has a strong structure and is used to connect and support different skeletal elements. Cells within cartilage are called chondrocytes and are found in clusters located in small cavities within the extracellular material. The interlacing collagen forms a delicate network of cartilage. Cartilage can differ in the relative amount of collagen fibers and extracellular material. This results in the formation of cartilage with different properties, of which there are three main categories. The first is hyalin cartilage, which is found between individual vertebrae, on the surfaces of joints and bones, and on the dorsal tips of vertebrae. The second type is fibrocartilage, which is found in tendons and within joint ligaments. Fibrocartilage has numerous collagen fibers and can resist repetitive stress. The third type is elastic collagen, which consists of a number of branched elastin fibers that provide elastic characteristics.

Adipose tissue consists mainly of cells and functions to protect sensitive organs (cushioning), store fat (energy) and insulate parts of the body. Adipose tissue is the main means of energy storage for the animal and is used in response to certain needs. For example, migrating birds can largely increase of their adipose tissue mass just before migration. Adipose tissue is usually found enclosed in areas surrounded by a sheath of collagen fibers. Young adipose cells are called adipoblasts. After they mature and fill with fat, however, they are called adipocytes. Adipoblasts grow from 1-2 μm to a size of up to 100 μm by accumulating small lipid droplets that fuse to form a large fat globule. Adipose tissue development is related to age of the animal and the amount of available nutrients. In young animals, the first fat deposit usually appears in the visceral area. Later, subcutaneous fat (under the skin) is developed, followed by a limited amount of intermuscular fat that is deposited in between muscles. As compared to red meat animals, poultry is fairly unique because intramuscular fat, also known as marbling, does not appear in certain locations (breast fillets/*Pectoralis* muscle). In any case, the adipose tissue has a fairly dynamic metabolism, meaning that stored lipids are constantly mobilized (i.e., when a bird lays an egg, it needs to mobilize a large amount of nutrients which includes fat, calcium, etc.).

3.4 Epithelial Tissue

From an embryonic development standpoint, epithelial tissue is designed to serve as the interface between the body and the outside world. Hence, it consists of the skin and the lining of the digestive system. It also contains some other specialized components that will be described below.

The skin (Fig. 3.4.1) serves as a protective layer that prevents microorganisms from entering the body and protects the body from environmental stresses such as drying. It also protects the body against mechanical damage and serves a major role in insulation and heat regulation. In general, the two major parts of the skin are the epidermis, which is the ectodermal portion, and the dermis, which is the mesodermal portion. A unique structure of poultry skin is the feathers (Fig. 3.4.2), which are a complex derivative of epithelial tissue. Feather size varies greatly with the longest tail feathers of a roaster being about a 1,000 times longer than the feathers on its eyelids (Lucas and Stettenheim, 1972).

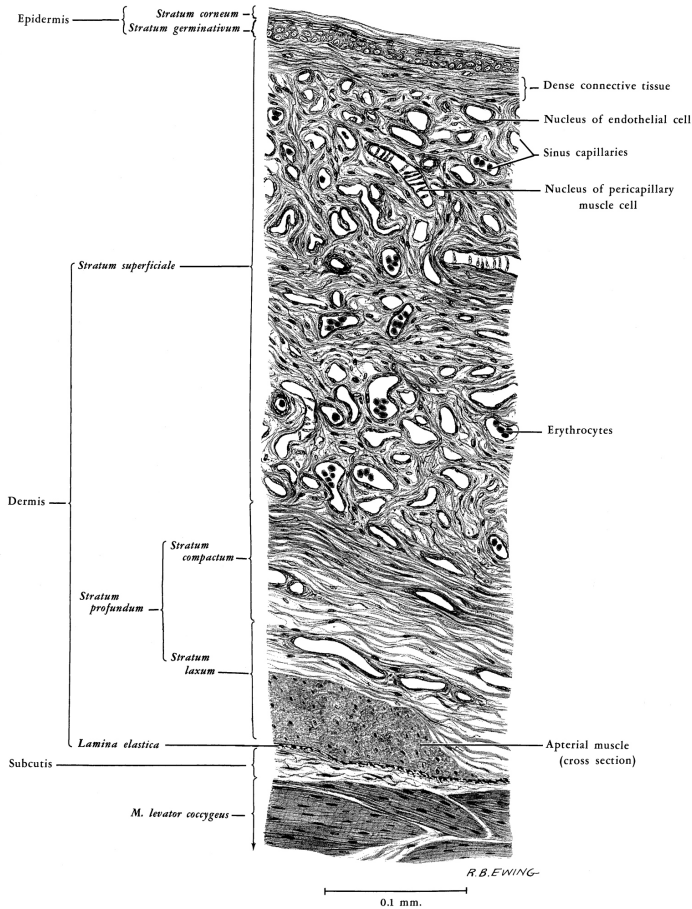

Figure 3.4.1 Structure of the skin section stained with hematoxylin and eosin. Abbreviation: M., *Musculus*. From Lucas and Stettenhiem (1972).

The predominant feathers on a bird's body are called contour feathers and are composed of a shaft with plates or vanes on either side. The feather develops in a follicle (Fig. 3.4.3) and both the follicle and its feather are tubes of modified integument that have a gradient from dermis to keratinized, highly flattened epidermis. The wall of the follicle appears to be drawn upward in some way by the sheath of the growing feather. In a full-growth feather the epidermis of the follicle has a single layer of germinative cells, which are low cuboidal cells that contain large nuclei (Lucas and Stettenheim, 1972). Animal skin has pigmented cells that contain melanin that can make it appear darker. The overall colour of poultry skin, however, is also determined by plant pigments that are absorbed from the diet and deposited in the skin (e.g., xanthophyll in corn can make the skin appear yellow; see discussion in Chapter 16).

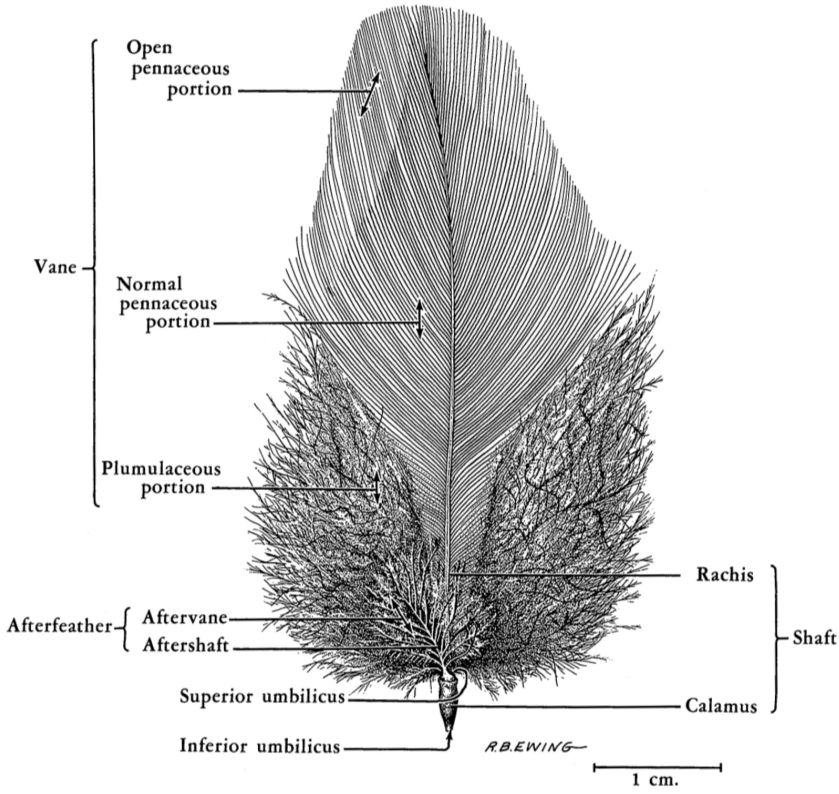

Figure 3.4.2 A structure of a feather from the middle of the dorsal tract of a White Leghorn chicken. From Lucas and Stettenhiem (1972).

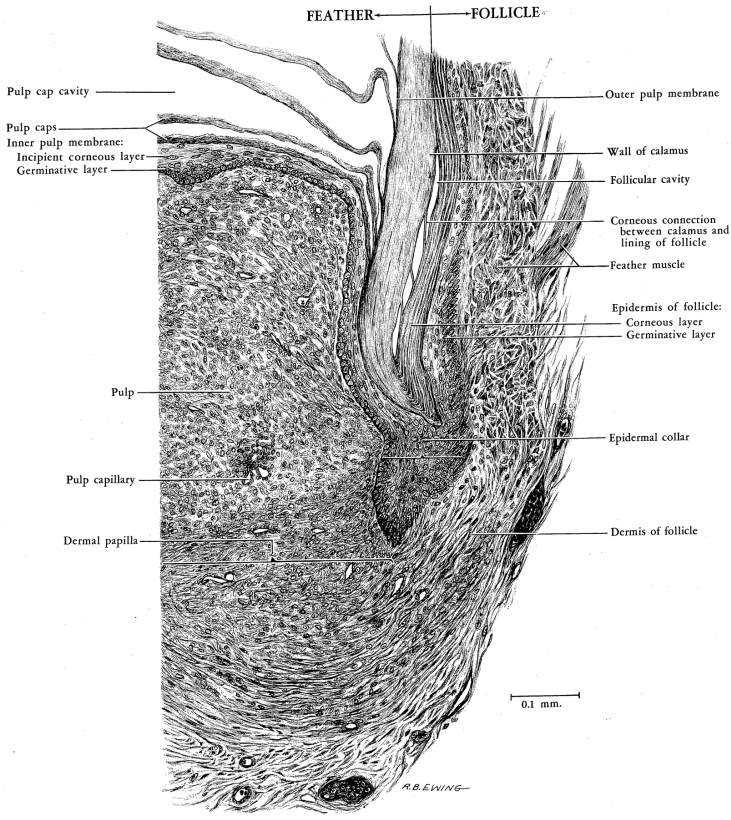

Figure 3.4.3 A microscopical section through a feather follicle of a White Leghorn chicken. From Lucas and Stettenhiem (1972).

Epithelial tissue is commonly characterized by cell shape and the number of cell layers (Fig. 3.4.4). Epithelial cells are usually laid down with little extracellular material. Cell shape can vary from elongated, columnar-type cells to very thin, flat cells called squamous cells. In addition, cuboidal cells also form single or multiple layers on external or internal surfaces of the body.

Other organs that contain epithelial tissue are the lining of the digestive system, liver, and kidney. In organs such as the liver and kidney, the cells secrete different enzymes than those of the digestive system, where they absorb nutrients from the gut and are usually columnar in shape to increase the number of cells in contact with food and make nutrient absorption more efficient.

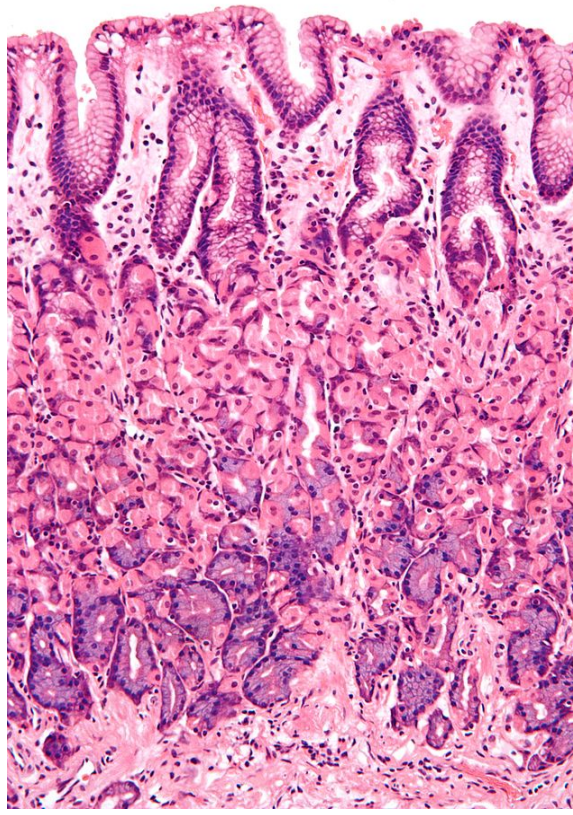

Figure 3.4.4 Classification of cells found in epithelial tissue is based on their shape. Showing here columnar epithelium cells lining at the top of the gut wall, as these many cells can efficiently absorb nutrients. The skin on the other hand has usually flat thin cells for protection. From http://en.wikipedia.org/ wiki/Simple_columnar_epithelium.

3.5 Nervous Tissue

Nervous tissue serves as the communication system within the body. While it represents a small part of the edible meat (usually less than 1%), understanding its structure is essential to understanding muscle contraction (discussed later in the chapter), post-mortem changes, and meat quality issues. The two main structural components are the central nervous system (brain and the spinal cord) and the peripheral system that consists of the nerve cells that reach all parts of the body.

The nerve cell, or neuron (Fig. 3.5.1), is the basic building block of nervous tissue and has a distinct structure consisting of a cell body with an elongated fiber-type structure called an axon. A nucleus is found within the polyhedrally shaped cell body. A few short branched structures called dendrites come out from the body. Motor neurons are those that reach muscle fiber and have a long, single axon that branches when it reaches the muscle. The junction points are called the motor end plates, or neuromuscular junctions (i.e., one nerve reaches a number of muscle fibers and triggers them all concurrently). The action potential, an electrical pulse of about 80 mV that goes from the cell body through the axon to the motor end plates, is transferred to the muscle or other nerves (i.e., their dendrite portion) via a synapse, which is a physical gap between the two cells or structures. Therefore, a chemical transmitter called acetylcholine is used, in most synapses, to convey the message across the gap (e.g., to the muscle). It should be noted that within the brain and other locations, other chemical messengers are used. Certain toxins can block acetylcholine and cause serious problems to the animal. An example of a serious toxin that is of importance in the food industry is the toxin produced by *Clostridium botulinum* (see Chapter 15).

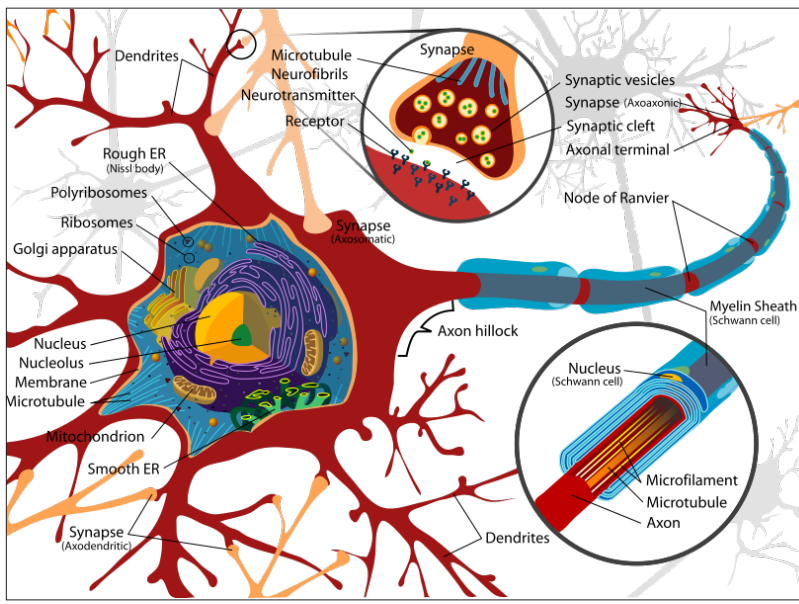

Figure 3.5.1 A schematic drawing of a neuron with motor end plates. From http://en.wikipedia.org/wiki/File:Complete_neuron_cell_diagram_en.svg

In the muscle, nerve trunks consisting of a group of axons can be observed as fine silvery lines because they are covered with a sheath of dense connective tissue. This arrangement helps protect the axons and provides strength to the structure. Small, peripheral nerve fibers are covered with Schwann cells that help speed up the electrical pulse going through the nerve whereas large fibers are often covered with a myelin sheath which is coming from the Schwann cells. Therefore, nerve fibers are commonly referred to as myelinated and non-myelinated fibers.

3.6 Muscle Tissue

The skeletal muscles of a chicken are shown in Figure 3.6.1. They range in size from small muscles (e.g., those that control the eye lids) to very large muscle (e.g., flight muscles). Muscle tissue is considered most important in terms of poultry meat consumption and therefore will be described in detail. So-called white and dark meat in chickens and turkeys represent breast and leg meat, respectively. However, in the migratory duck, the breast meat appears red due to its high myoglobin content, as will be explained later in the chapter.

Muscles are used for various functions in the live animal. The shape and structure is designed to allow for the performance of a specific task ranging from locomotion (flying) to pumping (heart muscle for circulating blood), to moving food along the digestive tract. These three major activities are related to the three types of muscles found in the body: skeletal (movement), cardiac (pumping blood) and smooth (involuntary activities).

a. Skeletal Muscle – Skeletal muscles are mostly voluntary muscles that the animal can either partially or fully control. These muscles are anchored by tendons to bones and are used to move and maintain posture. Although posture control is often maintained as an unconscious reflex, the muscles responsible react to conscious control as well. These muscles comprise 40 – 50% of the average body mass of an adult bird. The muscles range from very large muscles such as the leg muscle (*Biceps femoris*) and flight muscle (*Pectoralis major*), to very small muscles such as those that control eye movement.

Skeletal muscles are also known as striated muscles because of their striated appearance when viewed under a light microscope. As seen in Figure 3.6.2, striations are the result of the repetitive microstructures in the fibers' building blocks (sarcomeres) and their components.

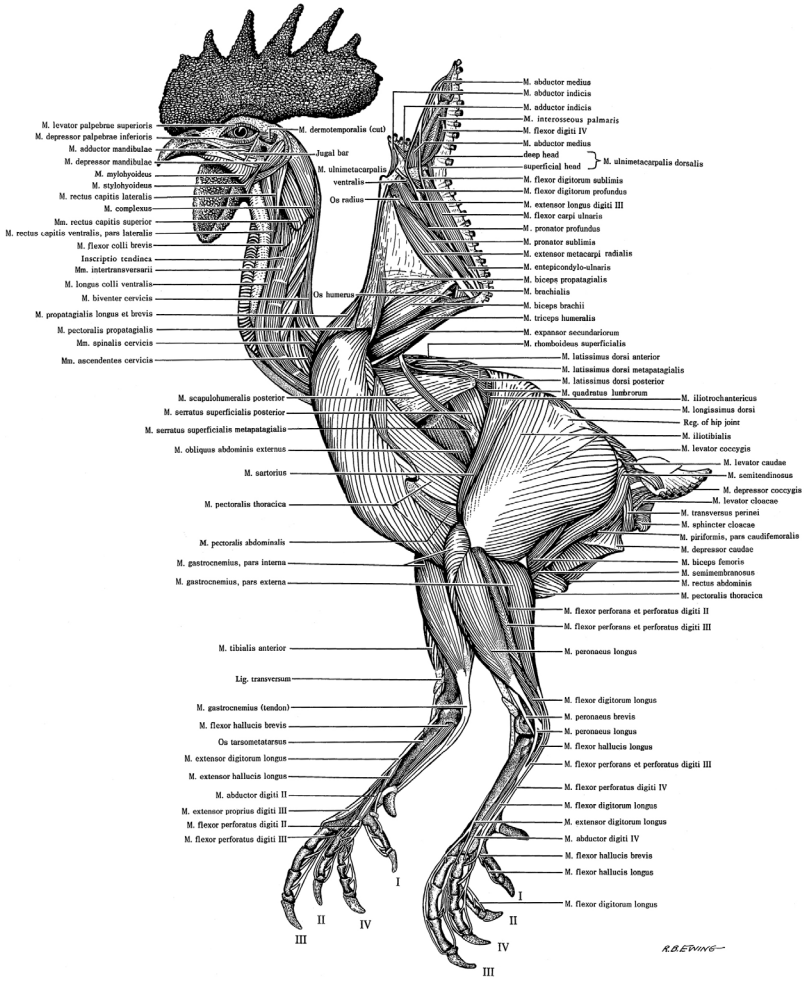

Figure 3.6.1 Lateral view of the superficial musculature of a single comb White Leghorn chicken. Abbreviations: *Lig., Ligamentum; M(m)., Musculus*(i); Reg., Region. From Lucas and Stettenhiem (1972).

Figure 3.6.2 also shows a schematic diagram of whole muscle that is broken down into its components. A large muscle such as the *Pectoralis major* is composed of numerous muscle bundles covered by epimysium. Each muscle bundle (Fig. 3.6.2) is separated from the others by a connective tissue layer called perimysium. As previously indicated, connective tissue provides structural organization,

anchors the different components, and transmits the power generated by sarcomere contraction. Blood vessels and nerves can also be seen in a cross section of the muscle. They supply energy to the active muscle and control its movement.

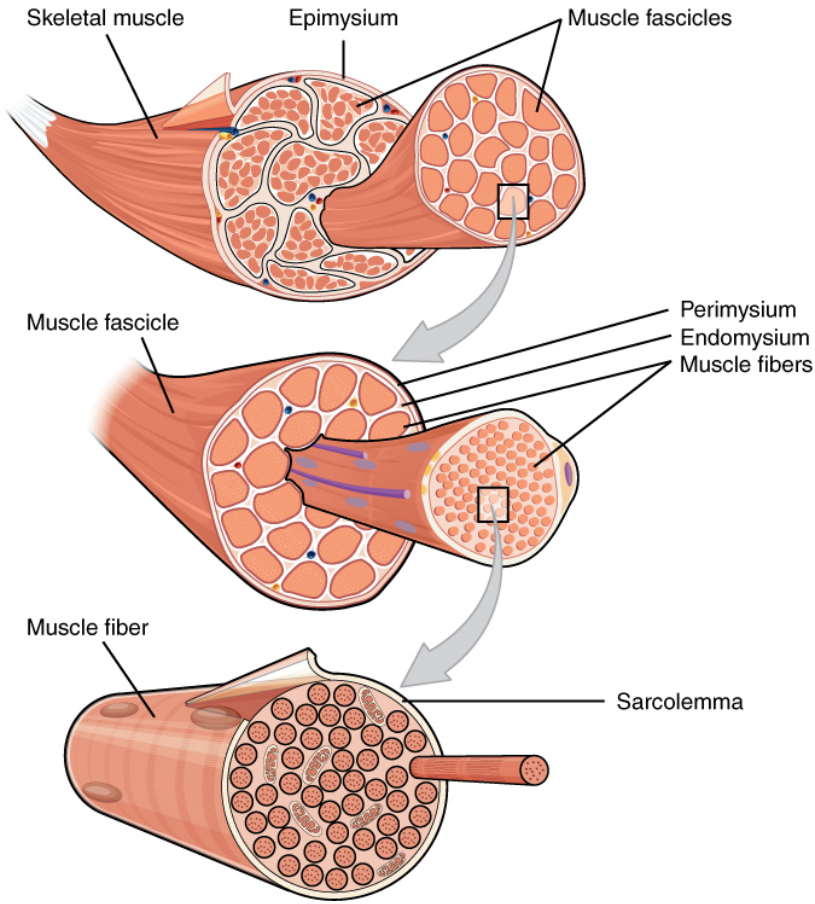

Figure 3.6.2 Schematic diagram showing skeletal/striated muscle structure, starting from a cross section of a whole muscle (size range 0.1 to 0.5 m), including the different layers of connective tissue, going down to the muscle fascicle/bundle, and muscle fiber. A single striated myofibril is coming out from the muscle fiber. It contains the many sarcomeres (smallest contracting unites of the muscle; size range 1.5 to 4.0 microns). Their structure is shown in Figure 3.7.1. They contain the thick and thin filaments with a unique stacked arrangement that produces the light and dark striation of skeletal and cardiac muscles. From http://commons.wikimedia.org/wiki/File:1007_Muscle_Fibes_%28large%29.jpg.

The muscle bundle is composed of smaller muscle fibers that are covered by a thinner layer of connective tissue called endomysium. Skeletal muscles have elongated fibers that are usually multinucleated (Fig. 3.6.3), which seems to permit better control over these long cells. Each fiber consists of numerous myofibrils (Fig. 3.6.2) that have myofilaments inside them forming the sarcomeres. The dark area in a stained muscle preparation is the result of thin and thick filaments overlapping and is called the anisotropic or A-band. Within the A-band is an area without thin filaments that is slightly lighter in colour and is called the H-zone. The area with only thin filaments is referred to as the isotropic or I-band. Sarcomeres are connected through a "backbone" called the Z-line. During muscle contraction, the thick filaments slide toward the Z-line and shorten the sarcomere which causes movement, as will be explained later in the chapter.

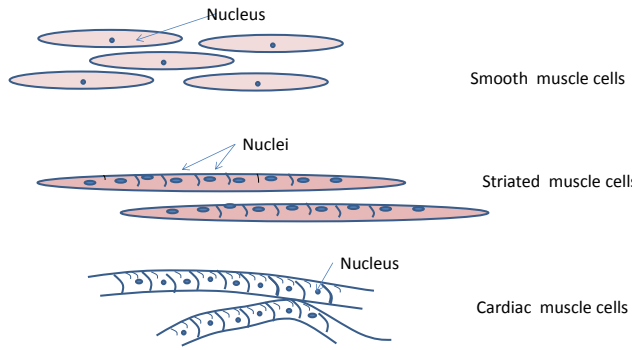

Figure 3.6.3 Smooth, striated (skeletal), and cardiac muscle cells.

b. Cardiac Muscle – Cardiac muscle is an involuntary muscle over which the animal has no direct control. The cells have a striated appearance like a skeletal muscle, but have only one or two nuclei per cell (Fig. 3.6.3) and have a dark red colour as a result of its extensive blood supply. The average length of the cell is about 50-100 μm, and its width is about 15 μm. Cardiac muscle has a unique rhythmic contraction that is triggered by the sinoatrial node and that starts early on in embryonic development. The heart is controlled by the sympathetic and parasympathetic nervous systems, which are partly outside the central nervous system.

Another unique structural characteristic of cardiac muscle is that the fibers run in a mesh-like pattern and are branched. This allows the heart chambers to contract (reduce volume), and pump blood forward. Microscopic examination reveals unique structures called intercalated disks that appear as dense lines, at regular intervals, along the longitudinal axis of a cardiac fiber. They provide a cohesive link between the fibers and facilitate the transmission of contraction force from one fiber to the other.

c. Smooth Muscle – Smooth muscle cells are part of the involuntary systems in the body (i.e., the digestive system, the walls of arteries, and parts of the reproductive system). The fibers have a single, centrally located nucleus and are relatively long and narrow with an average length of a few hundred μm and a diameter of 3-12 μm (Fig. 3.6.3). This muscle does not have a striated appearance like skeletal and cardiac muscle because the repetitive structure of the sarcomere is not as well organized, hence the name smooth muscle. In terms of layout within the body, some areas show different layers of smooth muscle. For example, in the digestive system, a cross section reveals smooth muscle layers that are positioned both perpendicular and parallel to the cut surface. This allows the digestive system to both decrease the gut diameter and elongate to move food down the tube-like structure.

White and Red Fibers – Skeletal muscles can also be divided based on fiber type. In the poultry meat industry there is a difference between white and dark meat. White meat refers to breast muscle from chickens/turkeys, whereas dark meat refers to the leg meat. This classification is based on the overall colour of the meat, which is generally relative to the proportion of red and white fibers within the muscle. Most muscles contain a mixture of red and white fibers; very few muscles are composed of all white or all red fibers.

Red, white, and intermediate fibers have different functions and therefore have different proportions of certain sub-structures (e.g., mitochondria) and metabolic rates (Table 3.6.1). It should be pointed out that these differences are judged on a relative scale and variation can exist within each characteristic. Intermediate fibers (not described in the table) have intermediate characteristics. Muscles with a high proportion of red fibers are used for long term activities such as supporting the skeleton in an upright position. Because of their unique metabolism they are less easily fatigued.

Table 3.6.1 Relative comparisons between red and white muscle fibers in poultry.

Characteristic	Red fiber	White fiber
Myoglobin (conc.)	high	low
Colour	red	white
Contraction speed	slow	fast
Mitochondria (number)	high	low
Mitochondria (size)	large	small
Glycogen content	low	high
Clycolytic activity	low	high
Lipid content	high	low
Oxidative metabolism	high	low
Fiber diameter	small	large

A constant oxygen supply is important and, together with a high proportion of enzymes involved in oxidative metabolism, the fibers can function for extended periods of time. They also have a higher myoglobin (see structure in Chapter 16) content, which results in a darker/redder appearance. Compared to the white fibers, red fibers contract at a slower rate but have the capacity to operate for a longer period of time. The presence of more and larger mitochondria, as well as higher lipid content, allow the fibers to generate energy on site and contract for a longer period of time.

On the other hand, white fibers have less myoglobin and a lower oxidative activity compared to red fibers (Table 3.6.2). Glycolytic metabolism, which predominates in white fibers, can occur with or without oxygen, i.e., aerobic or anaerobic metabolism, respectively. Muscles with relatively high content of white fibers show lower capillary density since they do not rely on fast nutrient transfer. White fibers contract more rapidly and in shorter bursts compared to red fibers and they are more easily fatigued. In some of the active, wild-type birds, such as ducks and geese who fly long distances during their migration, the breast muscle appears red because of the higher proportion of red fibers (i.e., the muscle can operate for a few days while the bird crosses a large body of water).

Table 3.6.2 Total heme, myoglobin, and hemoglobin content in chicken muscles. From Kranen et al. (1999).

Muscle	n	Total heme	Hemoglobin	Myoglobin
		(mg/g)		
Heart	9	3.75 ± 0.64^{a}	2.67 ± 0.65^{a}	1.08 ± 0.41^{a}
Adductor	8	1.39 ± 0.31^{b}	0.83 ± 0.21^{b}	0.56 ± 0.17^{b}
Pectineus	8	0.10 ± 0.04^{e}	0.09 ± 0.04^{d}	0.01 ± 0.00^{c}
Sartorius	6	0.79 ± 0.12^{c}	0.67 ± 0.11^{b}	0.12 ± 0.02^{d}
Pectoralis	10	0.24 ± 0.04^{d}	0.24 ± 0.04^{c}	ND

$^{a-e}$ Per parameter, means with no common superscript differ significantly as analyzed by t test ($P < 0.05$).
Values are means ± SD of the numbers (n) of samples indicated.
ND = not detectable.

3.7 Muscle Proteins and Muscle Contraction

3.7.1 Muscle Proteins

The physical structure of muscle is mainly comprised of proteins made of amino acid chains. Muscle proteins represent 18 - 20% of lean muscle weight whereas water is about 75% and fat 5%. Muscles contain over 50 different proteins but there are about five present in major proportions. Table 3.7.1 shows the three major protein groups based on their water and salt solubility (Asghar et al., 1985). Proteins can also be grouped in other ways but for meat scientists this is the most common division. In the lab, protein separation is achieved by homogenizing a piece of lean muscle tissue (e.g., 1:1 meat to water) in a high speed mixer/homogenizer. The homogenate is then placed in a test tube and centrifuged to separate the aqueous phase, which contains the water soluble proteins (Table 3.7.1; sarcoplasmic proteins). After decanting the aqueous top layer, a salt solution (commonly using 0.6 M sodium/potassium chloride) is added to the bottom layer, mixed well (or homogenized), and centrifuged. This separates the salt soluble proteins into the top layer (Table 3.7.1; myofibrillar proteins) and the non-soluble proteins into the bottom layer (Table 3.7.1; stromal proteins).

Table 3.7.1 The major proteins in an average muscle divided into three groups according to their solubility (see text) and their relative percentage in the wet muscle (based on 19% total protein).

Group	Protein	%
Sarcoplasmic		(5.5)
	Myoglobin	0.2
	Hemoglobin	0.6
	Cytochromes	0.2
	Glycolytic enzymes	2.2
	Creatine kinase	0.5
Myofibrillar		(11.5)
	Myosin	5.5
	Actin	2.5
	Tropomyosin	0.6
	Troponin	0.6
	C-protein	0.3
	α-actinin	0.3
	β-actinin	0.3
Stromal		(2.0)
	Collagen	1.0
	Elastin	0.05
	Mitochondrial	0.95

a. Sarcoplasmic Proteins – Distributed within the cellular fluid (i.e., sarcoplasm), they consist of myoglobin (the oxygen carrying molecule that gives this fraction its distinctive red colour; see structure in Chapter 16) as well as different enzymes. Sarcoplasmic proteins represent about 30% of the muscle's proteins.

b. Myofibrillar Proteins – These proteins are the building blocks of muscle and are also known as contractile or cytoskeletal proteins. The main proteins are myosin and actin (Table 3.7.1), which make up the thick and thin filaments, respectively. More detailed description on their structure and function can be found below. Overall, this group represents about 55% of the muscle proteins.

c. Stromal Proteins – Neither water nor salt soluble, these proteins comprise about 12% of muscle protein. The two major proteins are collagen and elastin, which are part of the connective tissue. They form structural components such as membranes that surround cells, muscle bundles (Fig. 3.6.2), ligaments, and tendons and they cushion joints by providing an intermittent material.

The following section briefly describes the major myofibrillar (salt soluble) proteins involved in muscle contraction, their unique structure, and their three-dimensional arrangement.

a. Myosin – Myosin represents the largest proportion of myofibrillar proteins (45%) and forms the thick filaments in muscle. It is an elongated, rod-shaped protein (Fig. 3.7.1) with a very high molecular weight of around 450,000 Daltons. The structure has two heavy and two light chains, which can be separated when myosin is subjected to a specific proteolytic enzyme activity. The heavy chains consist of the myosin heads, and the light chains consist of the tails. The heads possess a unique ability to split adenosine triphosphate (ATP) molecules into adenosine diphosphate and phosphate (ADP + PO_4), which generates the energy needed for contraction. During contraction the heads form cross bridges with the actin molecules while using energy to change their orientation and cause movement (described further below).

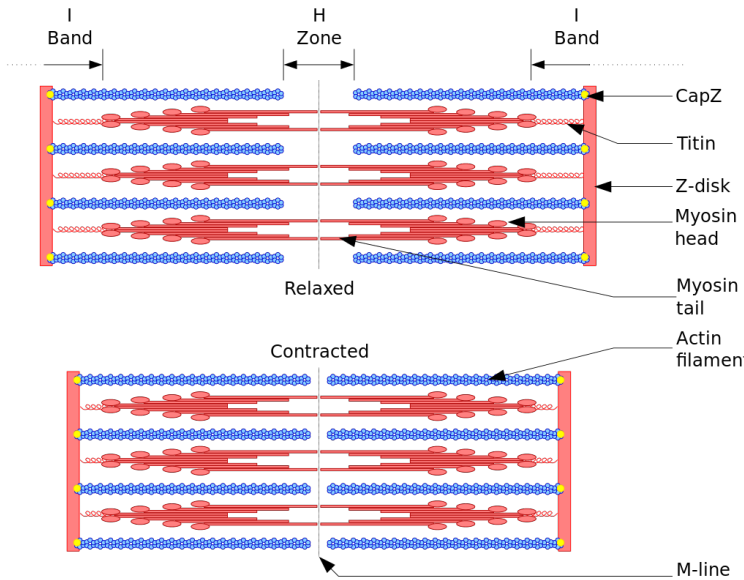

Figure 3.7.1 The microstructure of the major proteins participating in the sarcomere structure (smallest contracting unit) and muscle contraction. Thick filaments made by the myosin protein. Thin filaments made by actin, troponin and tropomyosin. The titin protein which connects the thick filaments to the Z-disk. From http://de.wikipedia.org/wiki/Muskelkontraktion#/media/File:Sarcomere.svg

b. Actin – Actin is the building block of the thin filament. It has a lower molecular weight of 42,000 Daltons and consists of two chains of F-actin that are formed from individual G-actin molecules (Fig. 3.7.1). The formation of the double helix in the thin filament takes place at a specific salt concentration, which favors the formation of the chain.

c. Tropomyosin – This protein is wrapped around thin filaments (Fig 3.7.2) and is a rod-like protein that surrounds the helical structure of actin. It constitutes about 5% of the myofibrillar proteins. There is one tropomyosin molecule for every seven actin molecules. Overall, it "lies" alongside the actin molecule and is positioned in the groove of the helical structure of the actin double helix.

d. Troponin – Troponin is another protein that is wrapped around the thin filament. It is a globular protein and constitutes about 5% of the myofibrular proteins. It is also present in the groove between the two actin filaments, where it "lies" within the tropomyosin strands. The troponin units are positioned in a repetitive pattern along the actin filament (Fig. 3.7.2). Overall, there are three types of troponin molecules:

- Troponin C – binds Ca^{++}
- Troponin I – inhibits ATP
- Troponin T – binds tropomyosin.

3.7.2 Muscle Contraction

Muscle contraction, and the movement it produces, is the result of a complex chain of events. This section provides an overview of the steps and processes involved. However, the reader should be aware that there are many textbooks devoted entirely to muscle contraction and that although we know a lot about the subject we certainly do not understand it all. While numerous individuals have received a Nobel Prize for their work in this area, more discoveries are expected. Muscle movement is the result of thousands to millions of sarcomeres (the smallest contractile unit) moving in unison to produce tension. During this process, stored chemical energy (from the food we eat that has been stored as high-energy bonds in the form of adenosine triphosphate - ATP) is converted into physical movement.

The Sliding Filament Theory is currently the most comprehensive theory used to explain muscle movement and is based on how the thick myosin filaments slide between the thin actin filaments towards the Z-lines (Fig. 3.7.2). During this process, one can measure a shortening of the sarcomeres and a conversion of energy rich ATP into ADP.

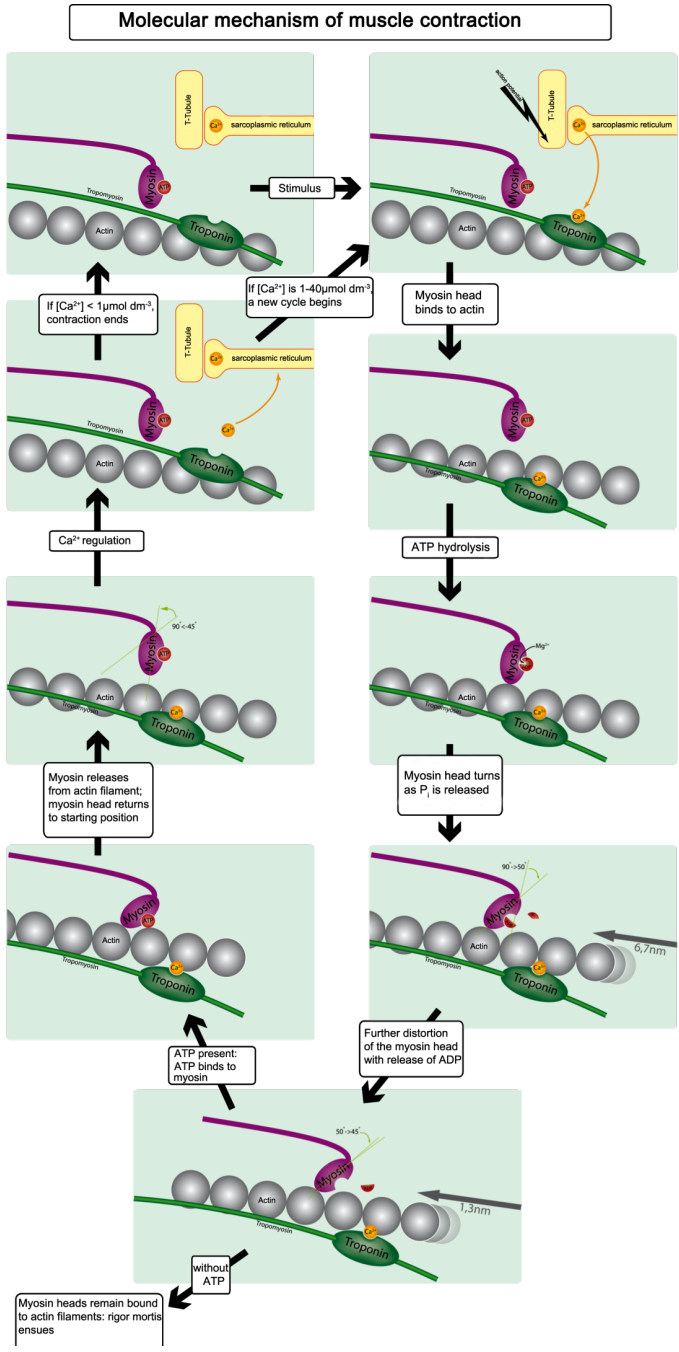

Figure 3.7.2 Illustration of the sliding filament theory. From http://de.wikipedia.org/wiki/Muskelkontraktion#/media/File:Muskel-molekular.png

As mentioned earlier, the myosin heads have a site capable of splitting the ATP, thereby releasing the energy needed to bend or twist the heads so they can pull the myosin molecule towards the Z-line (Pollack, 1990). The trigger for this process comes from the brain and is transferred via the nervous system (Fig. 3.5.1). The signal travels through the nerve by depolarizing the membrane, which quickly changes the internal electrical potential from about -80 mV to +20 mV. During the rest time, the cell establishes and maintains a potential difference (also called the resting action-potential) between the inside and outside of the cell. This is achieved by three mechanisms which include: active pumping of the Na^+/K^+ ions out of the cell, selective permeability to prevent entry of the Na^+, and using large anion proteins trapped on the inside of the cell membrane. When a message is passed through the cell, there is a quick reverse of the electrical potential (also called depolarization). The polarization change takes about 1 millisecond before the original resting potential is restored. The entire event from resting potential to the next resting potential is called Action Potential (i.e., depolarization, repolarization, hyperpolarization).

When the signal arrives at the nerve ending (Fig. 3.5.1; motor end plates), the message is transferred across the synaptic gap to the muscle by the neurotransmitter acetylcholine, which is released from the nerve ending and causes the muscle cell membranes to depolarize. This chemical messenger is broken down very quickly by the enzyme acetycholinesterase to prevent continuous signaling. Electrical depolarization in the muscle cell membrane is transferred to the myofibrils via a special arrangement of T-tubules within the sarcoplasmic reticulum, which causes a calcium release and a chain of events that result in muscle contraction (Fig. 3.7.3). The individual steps of the contraction process are outlined below:

a. calcium is released from the sarcoplasmic reticulum's terminal cisternae into the sarcoplasm
b. free calcium is quickly bound by troponin-C
c. tropomyosin translocates to uncover actin binding sites
d. actin and myosin molecules form cross bridges (Fig. 3.7.2)
e. the myosin head is energized via myosin-ATPase activity, which converts ATP $\rightarrow$ ADP + Pi
f. the repeated formation and breaking of cross bridges results in sliding of the thick filaments towards the Z line and, hence, sarcomere shortening.

During the relaxation phase:

a. signal from the nerve diminishes
b. the sarcolemma and T-tubules are re-polarized, preparing them for the next signal
c. the calcium pump in the sarcoplasmic reticulum actively resequesters calcium
d. cross bridges are broken and cannot reform
e. the tropomyosin molecules cover the actin binding sites
f. passive sliding back of the filaments as the sarcomeres return to their resting state.

An illustration of these steps is shown in Fig. 3.7.3.

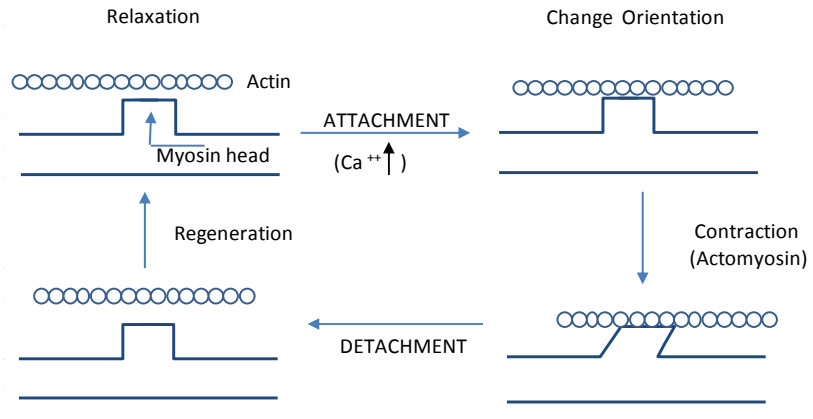

Figure 3.7.3 A schematic illustration of the steps involved in muscle contraction.

Overall, calcium concentration in the sarcoplasm controls muscle contraction. During rest, the concentration of free calcium is below 10^{-8} moles/liter and when calcium is released it increases to around 10^{-5} moles/liter. This causes the troponin-C to bind calcium, which in turn triggers movement of the tropomyosin-troponin system away from the myosin binding sites on the actin molecules. During relaxation, calcium is resequestered and its concentration goes back to around 10^{-8} moles/liter.

3.8 Rigor Mortis Changes and Meat Quality

The sections above described the structure and mechanism of muscle contraction in living tissue. In the living animal, organs work in harmony and the internal environment is kept within a very narrow range of temperature, pH, oxygen, and CO_2 concentration through a process called homeostasis. The body employs thousands of nerve sensors sensitive to physical pressure, temperature, gas concentration, blood pressure, etc., to collect data about external and internal conditions. This information is processed and corrective actions are taken as needed (e.g., fluffing the feathers, running to find a shelter, increasing breathing rate to get rid of heat, etc.).

When the animal is slaughtered and bled, oxygen and nutrient supply to the muscles is stopped and many homeostatic mechanisms are disrupted. Stress conditions prior to slaughter also affect homeostatic conditions, which can later influence meat quality. Stress can arise from activities such as catching the birds, loading, transportation, unloading, and immobilization. Immobilization of poultry, which refers to rendering the bird unconscious, is usually the first step in the process. In most countries, regulations require the use of humane immobilization methods to minimize animal pain and distress at subsequent slaughter. Electrical stunning and controlled atmosphere stunning (CAS; by CO_2, Argon) immobilization are commonly employed (see Chapter 8). A proper immobilization method should also focus on reducing stress, such as wing flipping before and during stunning, in order to minimize hemorrhages in the muscles and incidences of broken bones. The next step after stunning is known as exsanguination or bleeding. This step represents the beginning of the major changes seen during the post-mortem phase. Blood removal is required as an excessive amount of blood left in the muscle will result in an overall dark appearance or dark spots. Usually, around 40-50% of the total blood volume is removed (Chapter 5) and the remainder is contained within the vital organs. This occurs because the peripheral blood vessels constrict when the blood pressure drops in an attempt to maintain blood pressure. Blood removal stops communication between muscles and vital organs. In the living, healthy animal, oxygen is shuttled from the lungs to tissues via red blood cells. Once the oxygen supply is cut off, the normal aerobic tricarbocylic acid (TCA) cycle stops (Fig. 3.8.1) and energy metabolism switches to an anaerobic pathway to provide the muscle with energy. It should be remembered that such anaerobic pathways can only be carried out in the living cell for a certain period of time. In living tissue, lactic acid is produced (i.e., via an anaerobic metabolism pathway) and then must be transported to the liver to be resynthesized into glucose or to the heart where it

is broken down into water and CO_2 via a specialized enzyme system (Aberle et al., 2012). When circulation stops, lactic acid accumulates in the muscle until most of the glycogen stored in the muscle (about 1% of the resting muscle weight) is depleted or until the pH becomes too low for glycolysis enzymes to operate.

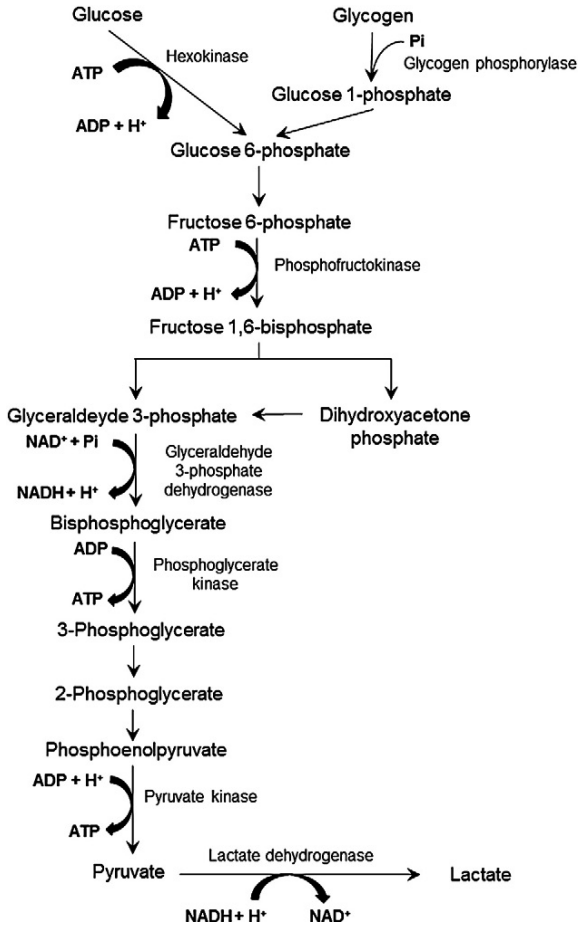

Figure 3.8.1 Aerobic tricarbocylic acid (TCA) cycle stops. From Scheffler et al. (2011).

pH decline during post mortem (Fig. 3.8.2) and its final, lowest point, called the ultimate pH, can vary between different meat producing animals. In poultry pectoral muscle, the drop in pH occurs more than twice as fast as it does in beef and pork (Aberle et al., 2012). The rate and ultimate pH can have major effects on meat quality and colour development. A normal pH reduction pattern is shown by the middle line in Figure 3.8.2. This represents a gradual decrease from the neutral pH of the living breast muscle to about 5.8. In some animals glycogen storage has been depleted prior to slaughter (e.g., due to extended activity or struggling). This results in low lactic acid production, and the pH drop will be minimal and the ultimate pH will stay high. The resulting meat is known as dark, firm, and dry (DFD). The dry appearance results from a high ultimate pH, which is further away from the isoelectric points of the muscle proteins and, therefore, exhibits higher water holding capacity (see Chapter 13). On the other extreme, the meat's pH can drop very quickly at the beginning of the post-mortem process, which results in the so-called pale, soft, and exudative (PSE) meat (Barbut et al., 2008). In this case, a rapid drop in the pH within the first hour, while the meat temperature is still high (e.g., >35°C), can cause protein denaturation. The partially denaturated proteins cannot hold water very well and the surface appears wet, hence, exudative meat. The colour of the meat is pale as a result of more light reflected from the looser muscle structure as compared to the tight structure of the DFD meat (Swatland, 2008).

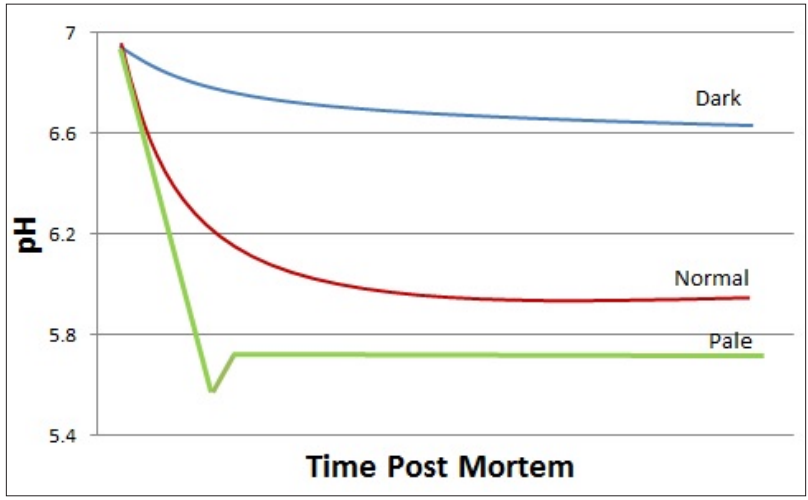

Figure 3.8.2 Rate and extent of pH decline during post-mortem of chicken breast muscle.

Rigor mortis, which means "stiffness of death" in Latin, follows the depletion of energy from the muscle, and results in its temporary toughening. This state does not take place immediately after slaughter, but rather a certain time afterwards (Fig. 3.8.3). It occurs due to the gradual depletion of glycogen and other energy sources such as creatine phosphate within the cell. Dunn et al. (1993) suggested that when the muscle pH drops below 6.3, calcium can no longer be efficiently sequestered by the sarcoplasmic reticulum. As a result, cytoplasmic calcium concentration starts to rise, exposing more myosin sites to actin. In the presence of ATP the muscle starts to build some active tension (as explained by the sliding filament theory) and consequently becomes less extensible (onset of rigor mortis). When all the energy sources have been depleted, the actomyosin cross-bridges (between the thick and thin filaments; Fig. 3.7.2) can no longer be separated and the muscle becomes inextensible with a stiff texture and the muscle has developed full rigor. The time between slaughter and the onset of rigor mortis is called the delay-phase. This is seen in Fig. 3.8.3 as the initial low tension force. After a certain period of time, the muscle becomes flexible again (decline of the curve seen in Fig. 3.8.3) as a result of proteolytic enzymes that slowly breakdown the sarcomere components. Some of the major structural changes during the so-called aging process include the degradation of the Z-line (leading to fragmentation of the myofibrils and the connective tissue) and degradation of individual proteins such as titin, nebulin and desmin (Scheffler and Gerrard, 2007; Scheffler et al., 2011). The proteolytic enzymes responsible for the degradation fall into two major categories: calpains and cathepsins. These enzymes vary in their calcium requirement for activation. Calcium is released from the sarcoplasmic reticulum and mitochondria during postmortem aging. Since the enzymes are activated by calcium, calcium infusion has been suggested as a way to improve tenderness. This actually works and is used more in the red meat industry where tough meat is a bigger problem. Experiments have also shown that chelating the calcium ion inhibits these enzymes and delays tenderness development.

The rate of pH decline is significantly affected by post-mortem temperature and so it is a critical factor in obtaining high quality meat. At high temperature, pH decline is very fast. The combination of high temperature (>35°C) and low pH values will cause protein denaturation, particularly affecting myosin (Bilgili et al., 1989; Scheffler et al., 2011). An optimal temperature for the post-mortem process is between 15-20°C. Muscle temperature reduction should commence as soon as possible after slaughter to also help control microorganism growth. On the other hand, reducing the temperature too quickly to below 5°C can cause meat tenderness problems in poultry meat (Dunn et al., 1993). Temperature reduction to sub-zero temperatures, prior to the completion of rigor mortis, results in a condition known as thaw rigor. This is caused by a severe muscle contraction that

takes place during thawing, and is triggered by an excessive calcium release from the sarcoplasmic reticulum into the sarcoplasm (Aberle et al., 2012; Bilgili et al., 1989). Such a severe contraction of the muscle structure pushes water out of the meat and toughens the muscle. An unrestrained muscle (i.e., dissected and not attached by ligaments to bones) with this condition can shorten by over 50% of its original length after thawing. A microscopic examination of such muscles reveals a severe contraction of the sarcomeres and almost the complete disappearance of the I-band.

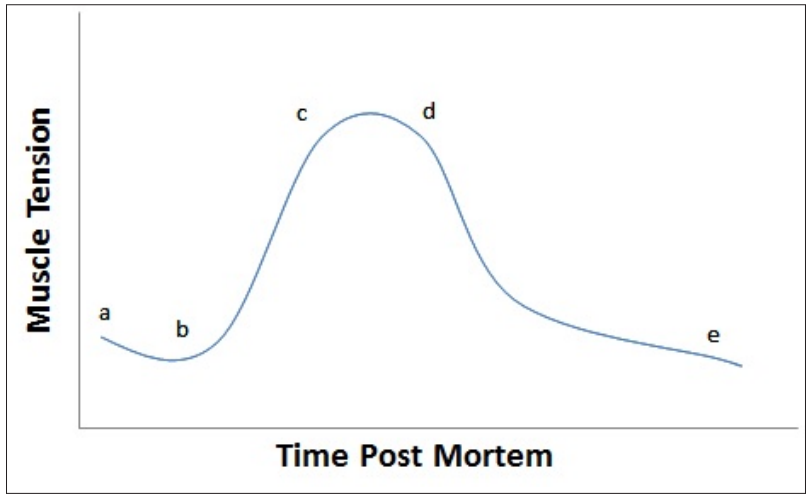

Figure 3.8.3 Development of rigor mortis expressed as muscle tension over time. The regions represent: delay time a-b; development of rigor mortis b-c; full rigor development c-d; and rigor resolution d-e. Time for each section depends on factors such as specie, degree of exercise prior to slaughter, stunning method and temperature. Adapted from Aberle et al. (2012).

Cold shortening is a less severe shortening that can occur when the temperature is reduced below 5°C but above freezing prior to the onset of rigor mortis (i.e., in the presence of ATP). The condition is more common than thaw rigor and damage to the muscle is less severe; however, it can still cause significant toughening and moisture loss problems.

Increasing the muscle temperature above 50°C (higher than normal body temperature), during the rigor process will also result in excessive shortening known as heat rigor. This is the consequence of rapid ATP and creatine phosphate depletion. However, this problem is not commonly seen in the meat industry.

The information presented above is used to illustrate the point that conditions before and during rigor can have major effects on meat quality. This includes maintaining an adequate temperature during the rigor mortis process to prevent shortening and/or toughening of the muscle. It is commonly suggested that the temperature be kept at 18 ± 2°C so it is above 15°C, but still lower than body temperature (≈39°C for broilers). Since the rigor process in poultry is much faster than in beef (1-3 hrs vs. 12-24 hrs, respectively. Note that the 1-3 hrs applies to processes which include electrical stimulation as described below. If no electrical stimulation is used, the range is longer and can be 3-8 hrs), poultry carcass chilling in modern processing plants starts about 30-60 min after slaughter and reaches 5-15°C (see Chapter 5) when rigor is completed or almost completed.

Electrical stimulation can be used after slaughter to speed up the rigor process and overcome some of the problems associated with pre-rigor deboning that might be encountered during rapid chilling. Originally, the process was developed for the red meat industry to allow accelerated processing (i.e., deboning the meat at an earlier stage compared to non-electrically stimulated carcasses). The process includes passing an electric current through the carcass and triggering muscle contraction by stimulating the nervous system (Sams, 1999; Aberle et al., 2012; Barbut, 2014). Such contractions deplete the energy within the muscle and cause a rapid onset of the rigor mortis process. High voltage applied during bleeding of chickens can induce excessive muscle contraction. This can cause physical damage to the sarcomere structure by tearing off some of the sarcomeres, which can actually add to the tenderization effect of electrical stimulation. However, one should be careful not to damage the muscle structure too much. Electrical stimulation of defeathered carcasses at a moderately high level can eliminate risks of rupturing the sarcomeres but accelerate ATP depletion to an extent that allows for filleting at 3.5 hrs post-mortem without the risk of getting tough meat (i.e., this is actually a common process used by the poultry industry). Besides accelerating the rigor mortis process and allowing deboning at an earlier stage (see Chapter 9), electrical stimulation can also be helpful in preventing or minimizing cold shortening problems (Sams, 1999). As mentioned in Chapter 1, the use of electrical stimulation for poultry is becoming very popular because it makes the deboning of broiler meat within 3.5 hrs possible on the line. Additional discussion on the procedure and the equipment used can be found in Chapter 5.

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