GENERAL STRUCTURE, CONGENITAL DEFECTS AND DEGENERATIVE DISEASES OF MUSCLE

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Skeletal Muscle
General Overview

Normal skeletal muscle / Histology (H&E stain).

Skeletal muscle is composed of elongated myofiber cells 1-40 mm long by 10-100μ in diameter.

Muscle cells are multinucleated (100-300 nuclei/cell), non-branched with parallel arrangement, and separated from each other by thin connective tissue (endomysium, perimysium, epimysium).

Note in this picture the well defined striations provided by the A (anisotropic) and I (isotropic) bands (arrows).
Skeletal Muscle General Overview

Normal skeletal muscle / Histology (H&E stain).

In this cross section, each fiber is surrounded by a well delineated, thin (not visible) connective tissue known as endomysium.

Nuclei in skeletal muscle are located at the periphery of the fiber which appear in this screen as a dense dark dot (arrowheads).

Groups of muscles bundles are grouped together by slightly thicker connective tissue known as epimysium, seen in this slide as a thin band of connective tissue (black arrows).

Muscle interstitium in this section appears as empty spaces (asterisk).
Skeletal Muscle
General Overview

Normal skeletal muscle / Histology / Thin-1µ section, toluidine blue stain.

The skeletal muscle is highly vascularized and contains numerous capillaries. Note again the parallel arrangement of fibers and prominent striations. Also note a small capillary vessel branching in the interstitium (arrows) of the musculature.

Striation results from specific bands and lines seen only by electron microscopy. These bands are called "A" (anisotropic) and I (isotropic) bands and the lines are the M and Z lines. You do not need to remember or recognize these bands and lines. What you must remember is that the functional / structural unit of skeletal muscle is the sarcomere. Each sarcomere extends from "Z" to "Z" line.
In addition to being highly vascularized, skeletal muscle is well innervated with axons reaching every muscle fiber.

The interface between a muscle fiber and the axon is referred to as the Motor-end plate.

Note a nerve ending (asterisk) emitting thin axons to each individual muscular fiber (arrows).

Remember that the type of muscle fiber (I or II) in a given muscle depends exclusively on the type of motor neuron innervation.
Skeletal Muscle
Types of fibres

• **Type I fibers (Red)**
  - Energy from oxidation (O₂)
  - Slow-twitch
  - Slow fatigue
  - Rich myoglobin / mitochondria

• **Type II fibers (White)**
  - Energy from glycolysis
  - Fast twitch
  - Fast fatigue
  - Low myoglobin / mitochondria
  - Rich glycogen
Types of Muscle Fibers

Normal skeletal muscle / Different types of fibers / histochemistry.

Note that routine HE staining does not allow differentiation of fiber types (Fig a).

Histochemistry techniques are used to show fiber types (Fig b).

There are two main types of muscle fibers.

*Type "I"* oxidative phosphorylation (aerobic, oxygen), rich in mitochondria and myoglobin, slow twitch but fatigue resistant (e.g., chicken leg, red meat).

*Type "II"* anaerobic glycolysis, less mitochondria and myoglobin, rapid twitch but prone to fatigue (i.e., chicken breast, white meat).
Proper fixation is a critical element in the histopathologic evaluation of muscle.

Hypercontracted fibers (asteriks), resulting from improper fixation, are often mistaken for swelling and hypereosinophilia which are two of the most important microscopic changes seen in degenerating myofibers.

These hypercontracted fibers are artifacts produced when fresh muscle is fixed before rigor mortis has disappeared. The fixative acts as an irritant and when muscle is placed in fixative it contracts while fixing.
Postmortem Examination

Degenerated versus normal muscle.

Abnormal muscle may appear pale or darker on postmortem examination. The abnormal color is not only due to alterations of myofibers (degeneration or necrosis), but also to other concurrent changes such as hemorrhage and myoglobin stain.

In this case note the abnormal pale appearance of degenerated muscle in a horse. Degenerated muscle appears pale (asterisk) while the normal musculature appears red.

While doing a postmortem examination, remember to describe the degree, location and extension of the discolored muscle.

Care should be taken not to mistake myodegeneration with fat.

Expect pale muscle in anemic animals and veal calves.
Postmortem Examination

Degenerated versus normal muscle.

In other instances, abnormal muscle may be darker than normal on postmortem examination. The abnormal color is generally the result of hemorrhage and myoglobin stain.

Degenerated muscle fibers appear slightly darker than normal (asterisks). Darker color is due to discrete congestion and hemorrhage.

Sometimes it is difficult to decide which muscle color is normal. If in doubt, check the corresponding musculature in the other side or in other animals of the same species and age.
Degenerated versus normal muscle.

Injection Site

Note a necrotic center (asterisk) surrounded by a thick band of fibrosis. This type of lesion is a typical injection site frequently seen at a postmortem.

Microscopically a lesion like this would look rather severe yet in the context of the whole animal it is just an incidental finding.
Note the musculature is poorly developed in the hind leg. Also there is rigid flexion and extension of hind limbs. Arthrogryposis is a muscular developmental lesion originating from a problem in the central or peripheral nervous system.

If muscle innervation during development is defective, muscle fails to develop (hypoplasia). Also note in this lamb the meconium staining (yellow discoloration of skin) suggesting fetal anoxia.

**Arthrogryposis** is a common congenital condition in aborted fetuses and stillborns. Rigidity of joints is caused by muscle hypoplasia which results from lack of muscle innervation during gestation.

Arthrogryposis is frequently seen in dysraphism, spina bifida, syringomyelia, hydromyelia, etc. In domestic animals, abnormalities in the development of the central nervous system are associated to ingestion of a toxicant during gestation, or congenital viral infections (i.e., BVD).
**Arthrogryposis Calf**

Note that the hind leg musculature is poorly developed (double-arrows), and the rigid flexion and extension of the hind limbs. Animals affected with Arthrogryposis generally lack a tail due to concurrent sacrococcygeal agenesis. It is imperative to check the brain and spinal cord in animals with Arthrogryposis.

**Figure A:** Note a cleft spinal cord (arrows)

**Figure B:** Note the abnormal cavitations (syringomyelia) in the cord (arrowheads)
Muscle Atrophy (Acquired lesion)

Muscular atrophy is the reduction of muscle size resulting from loss of sarcomeres or a decreased number of myofillaments. Muscle atrophy is reversible providing the source of injury is removed. Histologically, there is a reduction in myofiber diameters but not in the amount of connective tissue (abnormal myofiber to endomysium ratios).

There are three main types of muscle atrophy:

**Denervation Atrophy:** (Neurogenic atrophy). There is a lack of tonic stimuli and muscle cells become atrophic. Causes of denervation atrophy include localized loss of nerve function (neuritis) or generalized loss of the entire motor unit (CNS). After denervation, muscles become rapidly atrophic and 50% of muscle mass could be lost in just few weeks.

**Disuse Atrophy:** There is intact innervation (tonic stimuli) but there is reduced movement because of pain, fracture, ankylosis, tenotomy, etc. Lesions are localized to affected groups of muscles.

**Malnutrition Atrophy:** It occurs in cachexia, senility, cancer and chronic inflammation.
Denervation atrophy

Equine Laryngeal hemiplegia

Laryngeal hemiplegia in horses is a typical example of denervation atrophy. Damage or a primary axonopathy to the left laryngeal recurrent nerve causes unilateral atrophy of the laryngeal muscles. Horses suffering of this condition are often referred to as “roarers.” This horse was euthanatized because of chronic respiratory problems.

Note the reduced muscle mass and pale discoloration in the left laryngeal musculature (l). In contrast, note the normal musculature in the right side (r). Microscopic examination of affected muscles revealed small and angular fibers often replaced by adipocytes (fat cells).

Due to the association of laryngeal hemiplegia with guttural pouch infection, these saccular dilations of the Eustachian tubes should be carefully examined at necropsy. In many cases a primary axonopathy involving the laryngeal nerve occurs without involvement of the guttural pouches.
Denervation atrophy

Canine / Polyradiculoneuritis / (Coonhound Paralysis)

This is moderate atrophy of musculature due to polyradiculoneuritis causing progressive ascending paralysis and muscle atrophy. The pathogenesis of Coonhound paralysis remains controversial, but is known to occur in dogs seven days after being bitten by raccoons or other wild animals. The lesions consist of lymphoplasmacytic neuritis (allergic neuritis) affecting primarily the ventral roots of spinal nerves and other peripheral nerves. Fortunately, this condition is only transient and most dogs recover if nursing is adequate.

Ascending paralysis due to a lymphoplasmacytic neuritis.

Although difficult to appreciate in this picture, the dog had also moderate atrophy of the musculature.
Disuse Atrophy

Equine

In Disuse Atrophy, muscle innervation remains intact (tonic stimuli) but reduced muscle movement causes atrophy. Common causes of disuse atrophy include: joint pain, bone fracture, ankylosis, tenotomy, etc. Lesions are localized generally to the affected muscles.

The photograph shown here belongs to a horse with severe muscular atrophy involving the left leg. Note complete loss of muscle mass (white asterisk). Muscle atrophy was localized to this limb and it was caused by lack of movement (disuse atrophy) as a result of chronic joint injury resulting in partial ankylosis.

Microscopic examination of atrophic muscle reveals a reduction in the number and size of the myofibers. Note the different size fibers (arrows). In severe atrophy, myofibers are replaced with fat cells (black asterisk).
Malnutrition Atrophy Dog

Muscle atrophy occurs in cachexia, senility, cancer and chronic inflammation.

In severe malnutrition, muscles become the source of nutrients (protein out of muscle). It may start from 24-48 hours following starvation in animals with few fat reserves. In malnutrition atrophy, there is tonic stimulus and movement is intact.

In cancer and chronic inflammation, muscular atrophy is associated with the release of interleukin 1 (cachectin) by cancer-fighting macrophages.

Lesions are generalized but essential muscles (i.e., diaphragm, tongue, etc.) are less affected.

Figure: Carcass of dog with starvation due to owner negligence. Note the extensive atrophy of scapular muscles (arrow) and loss of intercostal muscles (asterisks). The lungs are visible. Owner was charged by the police.
Malnutrition Atrophy
Duck

Domestic and wild poultry are often affected with muscle atrophy particularly in chronically sick birds. It is customary to evaluate the pectoral musculature (breast) to assess body condition. Birds with severe malnutrition or with chronic debilitating diseases exhibit prominent keel bone (arrows) due to marked atrophy of the pectoral muscles (asterisk).

These tissues belong to a wild duck with chronic lead poisoning. Emaciation resulted from lack of peristaltic movement caused by lead in the nervous system. The proventriculus (p) and ventriculus or gizzard (v) were dilated and impacted with dry ingesta.

In the past, lead toxicity was common in waterfowl because lead shots were used for hunting and wasted pellets accumulated at the bottom of lakes and ponds. Presently, loons and other piscivorous birds accidentally consume lead sinkers lost by fishermen.
Muscle Hypertrophy

*Muscle Hypertrophy* occurs in response to an increase work demand (physiologic / compensatory). There is an increase in the size but not in the number of muscle fibers. Hypertrophic muscle fibers are thickened due to formation of new myofilaments and sarcomeres. Muscular hypertrophy can be enhanced by anabolic steroids.
Muscle Degeneration and Necrosis

Degeneration is a common sequel to myofiber injury regardless of its cause. Common causes of muscle degeneration include: chemical irritants, abnormal metabolism, trauma, infections. Myofiber degeneration can be reversible, however, if injury progresses beyond the "point of no return," degeneration becomes irreversible and necrosis will follow. Only in severe cases can muscle degeneration be detected grossly.

Degenerated muscles may appear pale or dark. This picture shows the pale degenerated muscle (d) in a foal with white muscle disease. Normal muscle (n) is shown for comparison.

Caution: Degeneration should not to be mistaken with pale muscles found in veal calves, anemia, exsanguination, fat (tongue), etc.
Muscle Degeneration and Necrosis

In contrast to previous slide, degenerated muscle appears darker than normal. A darker color occurs when degenerated muscle coexists with hemorrhage or with extensive release of myoglobin (rhabdomyolysis) into the interstitium.

This picture illustrates the leg musculature of a cow that had been down for several days causing physical damage to the muscle which progressed to degeneration and necrosis. Affected tissues appear notably darker (asterisks) than the remaining normal musculature.
Muscle Degeneration and Necrosis

Histopathology

Histologically, degenerating muscle fibers exhibit a variety of microscopic changes including cell swelling and hypereosinophilia (asterisks), as well as loss of striation, fragmentation and rupture of fibers. Ruptured fibers typically produce the formation of so-called “retraction caps.” These retraction caps appear as concavities at the free end of ruptured fibers (arrows).

Myofiber calcification is also a frequent finding particularly in some conditions such as white muscle disease (see next slide).
Degeneration and Calcification

Gross lesions (Fig a):

Note generalized pale musculature and white streaks of degenerated muscle fibers (arrows) intertwined with normal muscle. The glistening appearance of degenerated muscle fibers is due to calcification.

Microscopic lesions (Fig b)

Note the segmental swelling and hypereosinophilia (asterisk) of the degenerated muscle fiber. Also note in the same fiber a segment undergoing dissolution of the sarcoplasm (#). In addition to degeneration, there is focal mineralization or calcification of affected fibers which appear dark purple (arrows).

Degeneration and calcification are commonly seen in the muscle of calves and lambs with White Muscle Disease.
Muscle Repair

Skeletal muscle has the remarkable ability to repair providing that the sarcolemmal tube and myosatellite cells remain intact. Macrophages clean cell debris within 12 hours of necrosis. The sarcolemmal tubes (endomysium + basal lamina) serve as scaffolding for the myoblasts and as a barrier to prevent fibroblasts from getting into the sarcoplasm. Myosatellite cells and myoblast undergo mitosis and prove the elements necessary for the formation of new sarcomeres at the edges of ruptured myofibers. Eventually the edges become bridged by newly formed sarcomeres.

Note the degenerated sarcoplasm (asterisk) and intact basal lamina (arrows). Large numbers of myosatellite cells and myoblasts are seen in this picture as dark nuclei (square)

If sarcolemmal tubes are disrupted regeneration can occur but is generally complicated with fibrosis (scars).
Myopathies
(Degenerative Diseases of Muscle)

• **Nutritional:**
  – White Muscle Disease

• **Metabolic:**
  – Porcine Stress Syndrome
  – Malignant Hyperthermia

• **Exertional:**
  – Azoturia
  – Captive Myopathy
  – Compartment Syndrome

• **Traumatic:**
  – Downer Cow
  – Crush Syndrome (HBC)
White Muscle Disease (Nutritional Myopathy)

White Muscle Disease (WMD) is a very common condition causing important economic losses in farm animals. It has variable morbidity and mortality affecting up to 50% of animals. WMD typically affects rapidly growing, young, well-thrift sheep, cattle, pigs, and is less common in foals and goats. It is also seen in captive minks (WMD / steatitis).

WMD is associated to Vitamin/Selenium deficiency but it is exacerbated by other factors such as exercise, environment (climatologic conditions may be involved), nutrition and some toxicants. The occurrence of WMD is unpredictable and the theory of a geographic predisposition has been recently challenged. WMD is occasionally found in neonates. WMD in pigs may be independent or coexist with other Vitamin E or Selenium deficiency syndromes (mulberry heart, hepatosis dietetica).

The pathogenesis of WMD is related to the oxidation of cell membrane lipids (lipoperoxidation) by free radicals due to lack of oxygen radical-scavengers such as tocopherol (Vitamin E) and selenium containing enzymes (glutathione peroxidase /reductase). Membrane peroxidation induces a positive influx of Ca++ into sarcoplasm and mitochondria. A considerable amount of energy is required to remove Ca++ out of the cell. Once cell energy is exhausted, myofibers degenerate and Ca++ accumulates up 50 times normal amounts. Intracellular enzymes such as CK leak out from the cell into serum; finally degenerated myofibers undergo necrosis.
White Muscle Disease

Since muscle activity relates to the production of free radicals, muscles with higher physiological activity such as diaphragms, intercostal, tongue and heart are more severely affected (type I fibers).

**Gross Pathology.** It is difficult to see in mild cases but in severe cases (fatal), affected muscles appear pale with calcifications (Top).

The heart is also affected appearing as a pale myocardium. Note pale discolored myocardium (arrows) in the left (L) ventricular wall. For still unknown reasons, WMD always affects the left side of the bovine heart while in sheep degeneration and calcification is typically seen in the right side.

Von Kossa stain is often used to confirm calcification (insert top). Calcified fibers appear dark.
**White Muscle Disease (Nutritional Myopathy)**

**WMD Foal:**

As previously described, the pale muscle (W) was obtained from a foal few days old that died of WMD. Note the normal color of equine muscle (N).

**WMD Pig**

Pigs are also susceptible to develop WMD. WMD in pigs may be independent or coexist with other Vitamin E or Selenium deficiency syndromes such as *mulberry heart* and *hepatosis dietetica*.

The musculature of this pig with WMD was slightly pale. However, because of the natural pale color of porcine musculature histopathological examination was required in this case.
Porcine Stress Syndrome (PSS)  
Malignant Hyperthermia  
Pale Exudative Pork

• **Hereditary:**
  – Pigs (Landrace, Pietran, Hampshire, Yorkshire)
  – Dogs and Human beings

• **Pathogenesis:**
  – *Intracellular defect in* Ca$^{++}$ *metabolism* (uptake, storage and release)
  – Exacerbated by stress and physical activity
  – Muscle contraction $\rightarrow$ increase blood lactate $\rightarrow$ acidosis $\rightarrow$ hyperkalemia $\rightarrow$ hyperthermia $\rightarrow$ shock

• **Lesions:**
  – Pale/wet muscles (type II fibers)
  – Pulmonary edema
  – Rapid autolysis
Porcine Stress Syndrome (PSS)

*Porcine Stress Syndrome (PSS)*, also known as *Porcine Malignant Hyperthermia* and *Pale Soft Exudative Pork* is a hereditable, life-threatening, hypermetabolic syndrome. It is most common in Landrace, Pietran, Hampshire, and Yorkshire. Clinically, PPS is characterized by respiratory and metabolic acidosis, myoglobinemia, hyperkalemia, high blood lactate, hyperthermia, cardiovascular collapse and death. Stress such as fighting, exercise, heat can trigger PSS.

The pathogenesis is related to an inherited defect in the intracellular uptake, storage and release of Ca ions. Excessive Ca\(^{++}\) in the cell (consumption of ATP) progresses to degeneration and necrosis of fibers.

Denaturation of sarcoplastic proteins leads to movement of intracellular water into the interstitium (see gross lesions).

**Gross Pathology:**

Muscles with a higher proportion of type II fibers (longissimum, psoas; semitendinosus) are pale and wet (an interstitial edema). To facilitate postmortem diagnosis, several longitudinal cuts are made in affected muscles to promote interstitial fluid escape (water oozes and drips). Extra-muscular lesions include a severe pulmonary edema (froth in the trachea) and accumulation of clear fluid in abdominal and thoracic cavities. **Histopathology:** Early myofiber degeneration, necrosis, and interstitial edema.
Exertional Myopathies

*Exertional Myopathies* comprise a group of diseases which result in severe muscle degeneration following strenuous exercise.

Lesions are similar to WMD but affect mainly major muscle masses with predominantly glycolytic type II fibres.

Postulated pathogeneses includes rapid utilization of glycogen, accumulation of lactic acid, alteration in protein structure, loss of water, an interstitial edema, compressive circulatory disturbances, ischemia, degeneration, necrosis, and in severe cases, myoglobinemia and myoglobinuria.

The most notable types of exertional myopathies are Azoturia and Tying-Up in horses, Greyhound Myopathy in dogs, capture myopathy in wild mammals, Compartment Syndrome in poultry.
Exertional Myopathy
(Azoturia, Paralytic Hemoglobinuria, Monday Morning Disease)

- Pathogenesis?
  - Resting + carbohydrate-rich diet + exercise?
  - Polysaccharide storage disease?
- Profuse sweating and hind limb weakness
- Myoglobinemia $\rightarrow$ nephrosis $\rightarrow$ hemoglobinuria $\rightarrow$ renal failure $\rightarrow$ death
- Lesions:
  - Dark musculature (myoglobin stain)
  - Dark kidneys and red-wine urine

Tying-Up

- Riding, racing (tense, nervous)
- Mild, transient, microscopic diagnosis
Exertional Myopathy
(Azoturia, Paralytic Hemoglobinuria, Monday Morning Disease)

Azoturia is a disease of horses associated to strenuous exercise after a long resting period and a diet rich in carbohydrates. Although carbohydrate-rich theory is not universally accepted anymore. It is clinically characterized by muscle weakness, diaphoresis (sudoresis), reluctance to move, myoglobinuria, and in severe cases, recumbency, renal failure, acidosis and death.

Gross Pathology: Muscles of the extremities (gluteal/lumbar) are swollen, edematous, dark (myoglobin stain), and the kidneys appear dark-black. In horses that survive, affected muscles become pale and atrophic.

Histopathology: In acute cases, there is segmental degeneration and necrosis with little or no calcification, and in chronic cases there is fibrosis and atrophy.

Note extensive areas of dark discoloration caused by the release of myoglobin into the interstitium.
Capture Myopathy

Capture Myopathy is an acute and often fatal muscle condition most often seen in wild ruminants. This form of myopathy is generally preceded by a chase, struggle or transport. The *pathogenesis* is possibly similar to Azoturia.

Gross lesions include muscle hemorrhage and degeneration. If the struggle is severe, tendons may also rupture.

This carcass belonged to an elk that died of Capture Myopathy. Note extensive hemorrhages of the musculature and the subcutaneous tissue (*top*).

Also, note in bottom picture a detached tendon (*arrow*) likely the result of strenuous struggle during capture.
Compartment Syndrome

It is an exertional myopathy characterized by ischemic necrosis of muscles surrounded by heavy aponeurosis. Typically it is found in the supracoracoid muscle of domestic poultry.

During exercise, muscle volume increases. In groups of muscles with heavy aponeurosis, increased muscle volume in a non-expandable compartment causes vascular compression, ischemia and infarction.

Note the greenish color of the supracoracoid pectoral muscle due to degeneration and necrosis (arrow). Ischemic degeneration and necrosis of the supracoracoid muscle occurs after brief but vigorous flapping of wings (chasing or birds with neurologic signs).
Compartment Syndrome (Deep Pectoral Myopathy)

Ischemic Necrosis of the Suprascoracoid Muscle (Frozen Specimen):

Note a well-delineated lesion in the suprascoracoid muscle (arrows) deep in the pectoral musculature.

Ischemic Necrosis of the Suprascoracoid Muscle (Cooked Specimen):

Note the darker appearance of necrotic muscle (asterisk). Lesions appear a few hours after exercise and sometimes not grossly detected until "supper time."

*Like on the postmortem table, a good pathologist never eats anything on the dinner table before proper examination.*
**Downer Syndrome**

It is an ischemic necrosis of ventral and limb muscles following prolonged recumbency (disease/anesthesia).

It occurs mainly in mature large animals (cows > sheep, pigs, horses)

There is a vicious circle of prostration > muscle injury > edema > compression > veno-occlusion > ischemia > injury > prostration 6 >...

Lesions appear as early as 6-12 hours.

Note the dark areas of necrosis and hemorrhage (asterisks) involving several muscle groups.

These are serial transverse sections of a leg from a cow with a history of being down for several weeks. Necrosis is due to physical trauma, compressive ischemia and thrombosis. See the next slide for a close-up view of the muscle necrosis and thrombosis.
Downer Syndrome

A close up of areas of muscle necrosis in the leg (asterisks).

Close examination of the affected leg revealed a large thrombus in a major vessel (arrows).
Downer Syndrome

If an animal survives an episode of extensive muscular necrosis such as downer cow, affected muscle will partially repair but leave scars of fibrous tissue.

Note fibrotic muscle (asterisk).
The End