**MINISTRY OF PUBLIC HEALTH OF UKRAINE**

**O. O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY**

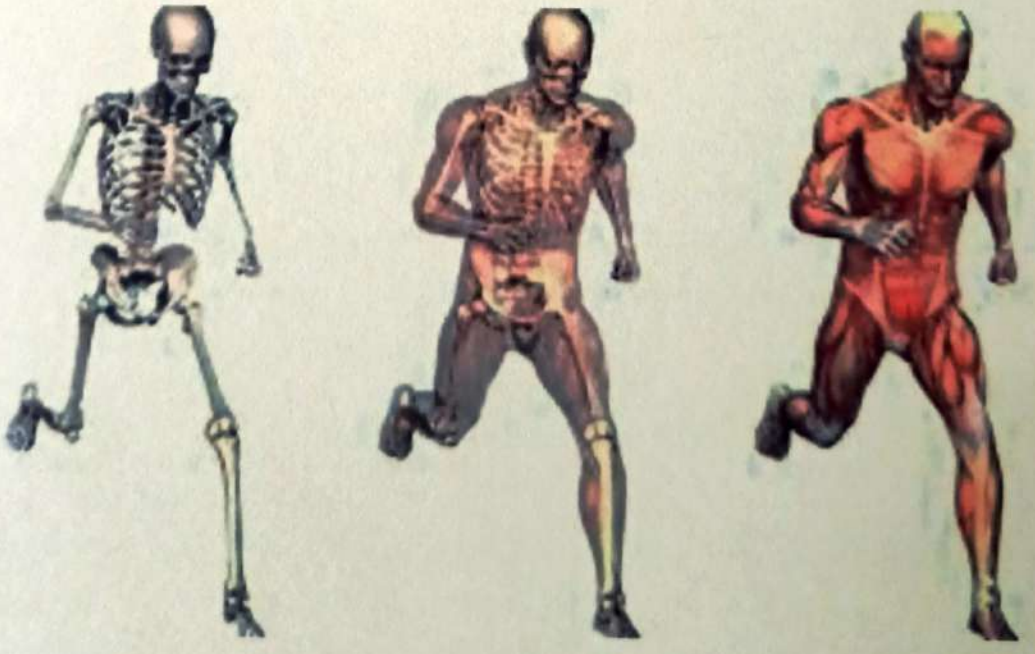
**Department of Bioorganic and Biological Chemistry**

*Methodical recommendations for consideration of the topic*

# *"Biochemistry of muscle tissue"*

*on "Biological and bioorganic chemistry"*

***FOR STUDENTS OF THE 2ST YEAR OF STUDY OF MEDICAL and STOMATOLOGICAL FACULTIES***



Kyiv-2019

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**O.O. BOHOMOLETS**

**KYIV NATIONAL MEDICAL UNIVERSITY**

***DEPARTMENT OF BIOLOGICAL CHEMISTRY***

# BIOCHEMISTRY OF MUSCLE TISSUE

## Guide for practical work on Biological Chemistry

**Methodical instructions are made for students of the 2nd year of medical medical-psychological, dental faculties and FPTAFU**

**Kyiv - 2019**

**Biochemistry of muscles. Molecular physiology of muscle contraction. Biochemical changes in the muscles in pathology.**

***Relevance of the topic.*** Muscles are a system in which the transformation of ATP energy into mechanical energy of contraction and movement occurs. The study of muscle biochemistry opens up possibilities for explaining the molecular mechanisms of diseases that affect muscles. And also help to develop effective treatments for these diseases.

**Teoretical questions.**

**1.** The organization and biochemical composition of skeletal muscles; structure of the Sarcomere.
**2.** Proteins of the Myofilaments: Myosin, Actin, Tropomyosin, Troponin. Structure of Thick and Thin Filaments.
**3.** Muscle extractives. Carnosine, anserine and other histidine compounds in muscle extractives.
**4.** Molecular mechanisms of Muscle Fiber Contraction and Relaxation.
**5.** The energy metabolism of Muscle. Sources of ATP.
***6.*** Biochemical features of the metabolism in the Smooth Muscle. Regulation of the Smooth Muscle contration**.**
**7.** Biochemical features of the metabolism in the Cardiac Muscle Tissue. Features of bioenergetic processes in the myocardium.
**8.** Laboratory Diagnosis of Myocardial Infarction.
**9.** Pathobiochemistry of muscles –Progressive Muscular Dystrophies and Congenial Myopathies.

## Chapter 1. THEORETICAL REVIEW.

### *1. The organization and biochemical composition of skeletal muscles; organization of the Sarcomere.*

Skeletal muscles comprise about 40% of the mass of the average human body and are formed of long multinucleate, cylindrical cells called muscle fibers.
The plasma membrane of muscle cells is known as the sarcolemma. Each muscle is made up of bundles of these cells (forming muscle fibers), embedded in a matrix of connective tissue known as the endomysium. A complete muscle consists of numerous fasciculi surrounded by a thick outer layer of connective tissue.

Within the sarcolemma is the sarcoplasm (cytoplasm), containing all the usual subcellular elements plus long prominent myofibrils. Each myofibril is composed

of bundles of filamentous contractile proteins. A single myofibril is composed of many short structural units, known as sarcomeres, which are arranged end to end. The proteins at the junctions between sarcomeres form the Z line, and thus a sarcomere extends along a myofibril from one Z line to the next Z line. Sarcomeres are composed mostly of actin thin filaments and myosin thick filaments. Sarcomeres represent the minimal contractile unit of a muscle. It is the coordinated contraction and elongation of millions of sarcomeres in a muscle that gives rise to mechanical skeletal activity.

The sarcomeres (which are approximately 2.3 μm long) consist of several distinct regions, discernible by electron microscopy, which provided critical insights into the mechanism of muscle contraction (Figure 1.). The ends of each sarcomere are defined by the Z disc. Within each sarcomere, dark bands (called A bands because they are *a*nisotropic when viewed with polarized light) alternate with light bands (called I bands for *i*sotropic). These bands correspond to the presence or absence of myosin filaments. The I bands contain only thin (actin) filaments, whereas the A bands contain thick (myosin) filaments. The myosin and actin filaments overlap in peripheral regions of the A band, whereas a middle region (called the H zone) contains only myosin. The actin filaments are attached at their plus ends to the Z disc, which includes the crosslinking protein α-actinin. The myosin filaments are anchored at the M line in the middle of the sarcomere.

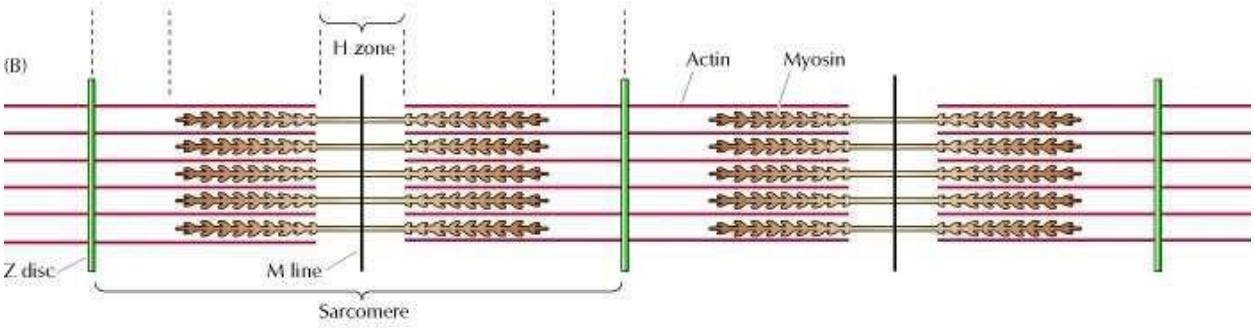
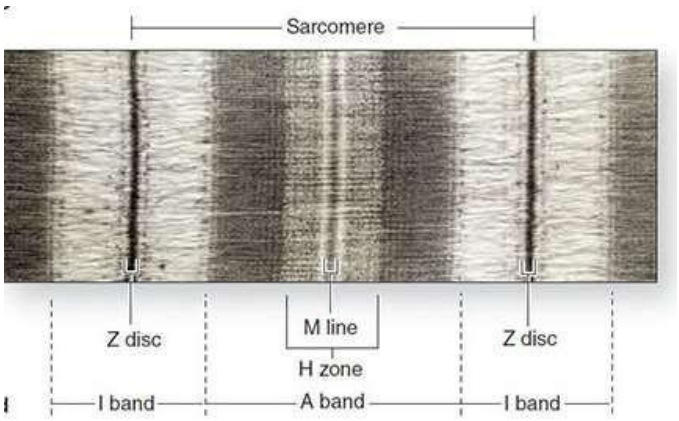



Diagram showing the organization of actin (thin) and myosin (thick) filaments in the indicated regions. (A, Frank A. Pepe/Biological Photo Service.)




**Organizational details of a typical striated skeletal muscle** Myofibrils are a series of sarcomeres separated by Z discs (also called Z bands) which contain thick and thin filaments.Thick filaments are myosin bundles that span the A line and are bound to proteins of the M line (M band) and to the Z discs across the I bands by the large protein called titin. Transmission electron micrograph (TEM) showing the molecular organization of the sarcomere. Image from Junqueira's Basic Histology, 12th ed by Anthony L. Mescher, McGraw-Hill Professional Division, reproduced with permission.

**Figure 1. Structure of the sarcomere**

Two additional proteins (**titin** and **nebulin**) also contribute to sarcomere structure and stability (Figure 2.). Titin is an extremely large protein (3000 kd), and single titin molecules extend from the M line to the Z disc. These long molecules of titin are thought to act like springs that keep the myosin filaments centered in the sarcomere and maintain the resting tension that allows a muscle to snap back if overextended. Nebulin filaments are associated with actin and are thought to regulate the assembly of actin filaments by acting as rulers that determine their length.

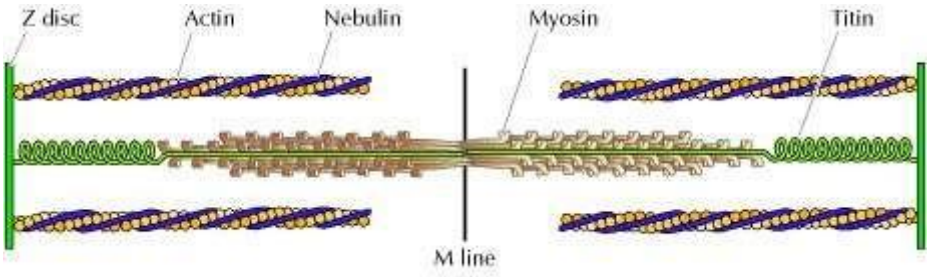



**Figure 2. Titin and nebulin**

Molecules of titin extend from the Z disc to the M line and act as springs to keep myosin filaments centered in the sarcomere. Molecules of nebulin extend from the Z disc and are thought to determine the length of associated actin filaments.

## *2. Proteins of the Myofilaments: Myosin, Actin, Tropomyosin, Troponin. Organization of Thick and Thin Filaments*.

Currently, muscle tissue proteins are divided into three main groups: sarcoplasmic, myofibrillary and stromal proteins. The former accounts for about 35%, the latter - 45% and the third - 20% of the total amount of muscle protein. These groups of proteins sharply differ from each other in their solubility in water and salt solutions with different ionic strength.

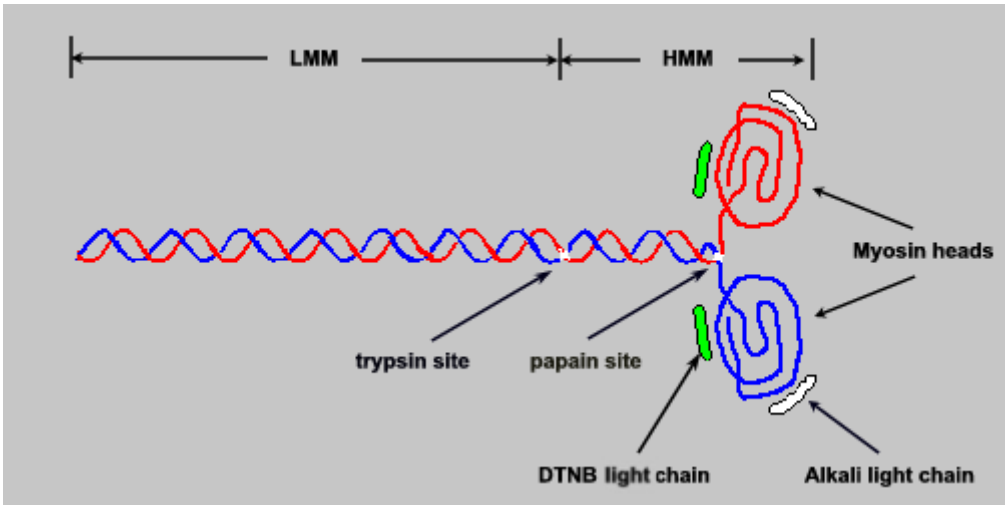
Sarcoplasmic proteins are extracted from the muscles with saline solutions with low ionic strength. These include myoglobin, proteins, enzymes of glycolysis, oxidative phosphorylation, enzymes that catalyze the creatine phosphate and myokinase reactions, proteins involved in the exchange of Ca2 +.

The biochemical basis of muscle activity is related to the enzymatic and physical properties of actins, myosins, and the accessory proteins that constitute the thin and thick filaments. The proteins of the thick and thin filaments can be separated into an ***actin, a myosin*** (see below), and several accessory proteins or protein complexes. The accessory proteins/complexes include the ***α- and β - actinins, the tropomyosins, and the troponin complex*** (see next section). These proteins form the basis of the molecular structure of myofibrils: α-actinin - enters the Z-line and fixes thin threads there; β-actinin - regulates the length of thin filaments; M-protein - enters the M-line and fixes thick filaments there; C-protein - regulates the length of the thick filaments; desmin is contained between the Z-lines of the neighboring myofibrils, ensuring the coincidence of the boundaries of all their sarcomeres. Solubilized myosin molecules are long thin (fibrous) proteins with a molecular weight of about 500,000 daltons.

The muscle actins were initially characterized as being either α (alpha), β (beta), or γ (gamma) actins. The α-actins are the predominant actins of the contractile apparatus of muscle cells. The β- and γ-actins are involved in the regulation of cell motility functions.

Both the actins and the myosins (specifically the heavy chain myosins) possess ATPase activity.

### *Myofilament Compositions.*



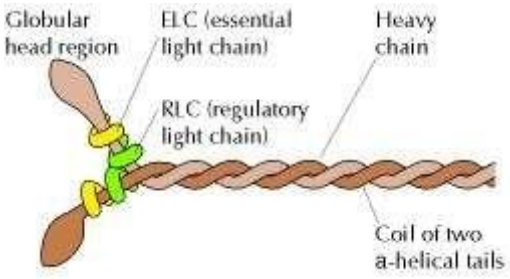




**Figure 3. Myosin II**

The type of myosin present in muscle (**myosin II**) is a very large protein (about 500 kd) consisting of two identical heavy chains (HC - about 200 kd each) and two pairs of light chains (LC - about 20 kd each) (Figure 3.). Each heavy chain consists of a globular head region and a long α-helical tail. The α-helical tails of two heavy chains twist around each other in a coiled-coil structure to form a dimer, and two light chains associate with the neck of each head region to form the complete myosin II molecule. The LC proteins were originally characterized by their chemical sensitivities and so are also referred to as alkali light chains. The alkali light chains are not phosphorylate, the other two light chains in a myosin are phosphorylated. The phosphorylated LC proteins are, therefore, the regulatory light chains of the myosin molecule. They bind $Ca^{2+}$. The phosphorylation of the LC proteins is catalyzed by one of four myosin light chain kinases, MLCK (also identified as MYLK). The myosin light chain kinases are also regulated by $Ca^{2+}$ binding to their calmodulin subunits. Phosphorylation of myosins by MLCK serves to regulate the ATPase activity of the myosin molecule as well as its assembly into thick filaments. The different myosin LC proteins, along with

different myosin HC proteins constitute the varying myosins from cardiac, skeletal, embryonic, and smooth muscle.

The human genome contains a large family of myosin protein encoding genes. The large myosin superfamily is divided into eighteen different classes identified as myosin I through myosin XVIII.

The LC proteins of a myosin molecule are called myosin light chains. Humans express 13 different myosin light chain genes.

***Myosin Structural Characteristics.*** Several functionally important landmarks exist on the myosin molecule. Near the midpoint of the long linear region is a site defined by its ready susceptibility to proteolytic trypsin digestion. Short-term treatment with trypsin splits the myosin molecule into two fragments: light meromyosin ((LMM; molecular weight 125,000) - a fragment of 90 nm in length is formed from the tail portion (C-terminal portion of the molecule) and heavy meromyosin (HMM; molecular weight 350,000) is formed from the rest of the "heads". LMM does not possess ATPase activity and does not bind actin. TMM catalyzes the hydrolysis of ATP and binds actin.

A second proteolytic landmark, susceptible to papain digestion, has also been considered a hinge point. Papain cleaves a site very close to the globular head regions; these then separate to form two subfragments, each known as a SF-1 and SF-2. The ATPase activity of the myosin complex is associated with the SF-1 units.

***Organization of Thick Filaments***.

The thick filaments of muscle consist of several hundred myosin molecules, associated in a parallel staggered array by interactions between their tails. (Figure 4.). The globular heads of myosin bind actin, forming cross-bridges between the thick and thin filaments. It is important to note that the orientation of myosin molecules in the thick filaments reverses at the M line of the sarcomere. The polarity of actin filaments (which are attached to Z discs at their plus ends) similarly reverses at the M line, so the relative orientation of myosin and actin filaments is the same on both halves of the sarcomere. As discussed later, the motor activity of myosin moves its head groups along the actin filament in the direction of the plus end. This movement slides the actin filaments from both sides of the sarcomere toward the M line, shortening the sarcomere and resulting in muscle contraction.

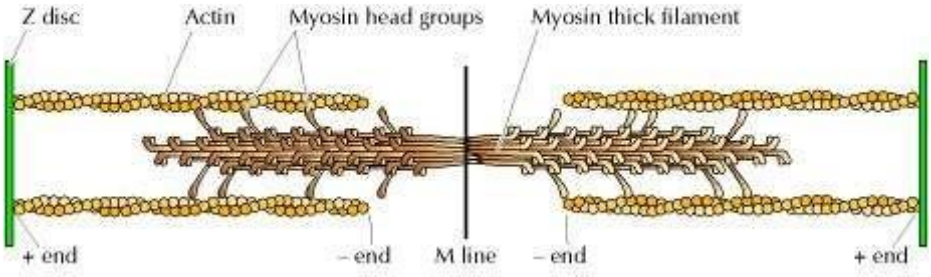



**Figure 4. Organization of myosin thick filaments**

In addition to binding actin, the myosin heads bind and hydrolyze ATP, which provides the energy to drive filament sliding. This translation of chemical energy to movement is mediated by changes in the shape of myosin resulting from ATP binding. The generally accepted model (the swinging-cross-bridge model) is that ATP hydrolysis drives repeated cycles of interaction between myosin heads and actin. During each cycle, conformational changes in myosin result in the movement of myosin heads along actin filaments.

***Organization of Actin Thin Filaments and its activation.***

Thin filaments are composed of many subunits of the globular protein G-actin (G for globular: 42 kD) and several accessory proteins (tropomyosins and the troponin complex). In thin filaments, G-actin is polymerized into long fibrous (filamentous) arrays known as F-actin (F for filamentous). A pair of linear F-actin arrays is helically wound to form the backbone structure of one complete thin filament. Each G-actin subunit has one ADP/ATP binding site. Once polymerized, the actin is capped and the thin filament stabilized by a protein known as β-actinin. Each G-actin molecule contains a myosin head-binding site. In skeletal and cardiac muscle, accessory proteins of the thin filament physically regulate the availability of this site for binding myosin.

Thus, the tropomyosins and the troponin complex control contractile events. Tropomyosin binds lengthwise along actin filaments and, in striated muscle, is associated with a complex of three troponins: troponin I (TnI), troponin C (TnC), and troponin T (TnT). In the absence of $Ca^{2+}$, the tropomyosin -troponin complex blocks the binding of myosin to actin. Binding of $Ca^{2+}$ to TnC shifts the complex, relieving this inhibition and allowing contraction to proceed. (B) Cross-sectional view.

***Troponin.*** Troponin is a complex of three regulatory proteins (troponin C, troponin I and troponin T) that is integral to muscle contraction in skeletal and cardiac muscle, but not smooth muscle. Discussions of troponin often pertain to its functional characteristics and/or to its usefulness as a diagnostic marker for various heart disorders.

The TnC protein of the complex is the $Ca^{2+}$-binding subunit, with similarity to calmodulin, whose role is to effect the $Ca^{2+}$-dependent regulation of muscle contraction. When TnC binds calcium, the whole troponin complex undergoes the conformational changes that move the attached tropomyosin away from the myosin binding sites on actin. This conformational change abolishes the inhibitory action of the TnI protein of the complex. In addition, the conformational change permits nearby myosin heads to interact with myosin binding sites, and contractile activity ensues.

The TnI protein of the complex is an inhibitory protein that block actin and myosin interactions. The function of the TnI protein is to inhibit the ATPase activity of the actin-myosin complex of the thin filaments that control muscle fiber contraction, thereby, resulting in the relaxation of striated muscle.

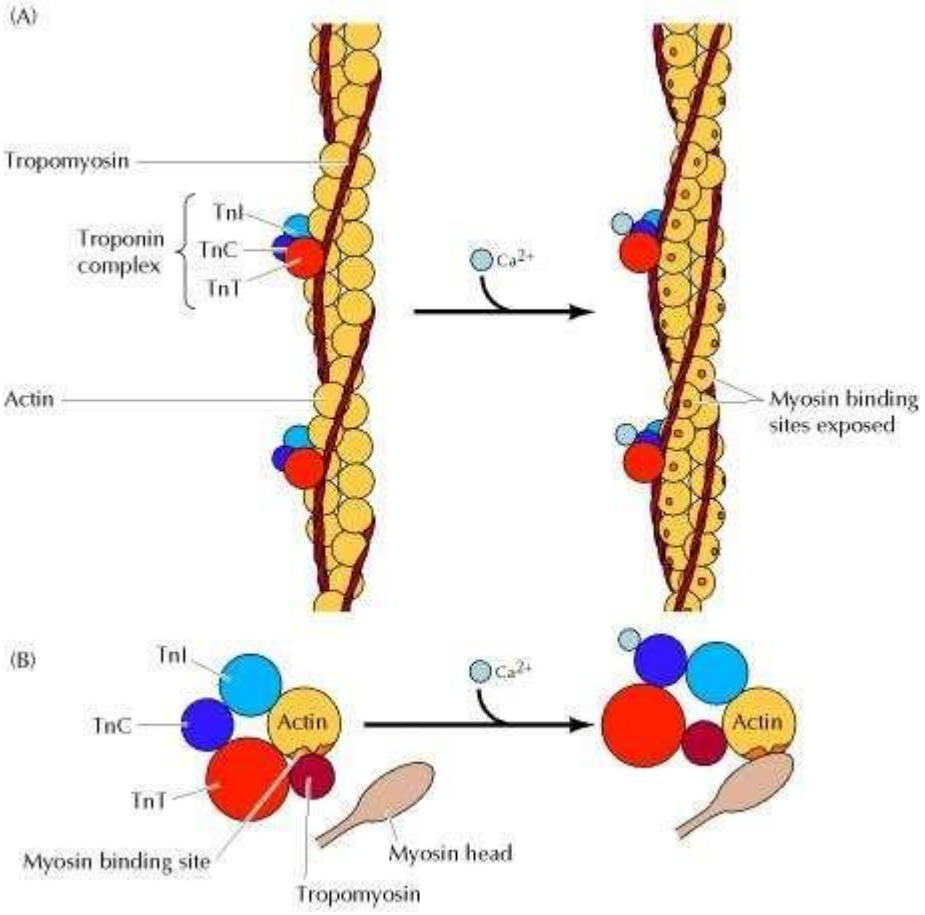



**Figure 5. Association of tropomyosin and troponins with actin filaments**

The TnT protein of the complex binds tropomyosin, thereby regulating troponin complex interaction with thin filaments. The entire troponin complex is attached to one end of each tropomyosin molecule and to actin, physically linking these later two proteins.

It has been established that troponin (its subunits Tn-T and Tn-I) is able to phosphorylate with the participation of c-AMP-dependent protein kinases. The

question of whether troponin phosphorylation in vitro is related to the regulation of muscle contraction remains open.

Stroma proteins in the striated musculature are represented mainly by collagen, elastin and some other connective tissue proteins. These are proteins of the walls of blood vessels, nerves, as well as sarcolemma and some other structures. The total amount of stromal proteins is approximately 15–20% of all human muscle proteins.

### *3. Muscle extractives. Carnosine, anserine and other histidine compounds in muscle extractives.*

The functions and properties of muscles are determined by their biochemical composition, where the largest part relative to the wet mass is water - 73-78%. Accordingly, the dry residue is - 22-27%, where proteins account for 17-21%.

The next in importance among organic substances is glycogen, which accounts for from 0.5 to 3%. Phospholipids are contained in the amount of 0.02-1.0%, cholesterol - 0.02-0.23%. Extractives are substances that dissolve in water and weak acids and therefore are extracted as solutions from muscles (or other tissues). Extractive substances include nitrogen and nitrogen-free substances. Nitrogen-containing extractive substances include various nucleotides (adenyl, cytidyl, uridyl, etc.), among which ATP / ADP and AMP are of great importance for muscle function. The extractive substances of the muscles also include creatine, creatine phosphate, creatinine, anserine, glutamine and other amino acids, glutamine.

Carnosine featuring the characteristic imidazole-ring, its a dipeptide molecule, made up of the amino acid, β-alanine and histidine. It is highly concentrated in muscle and brain tissues. Carnosine and carnitine has been proven to scavenge reactive oxygen species (ROS) as well as alpha-beta unsaturated aldehydes formed from peroxidation of cell membrane fatty asids during oxidative stress. It also buffers pH in muscle cells, and acts as a neurotransmitter in the brain. It is also a zwitterion, a neutral molecule with a positive and negative end.

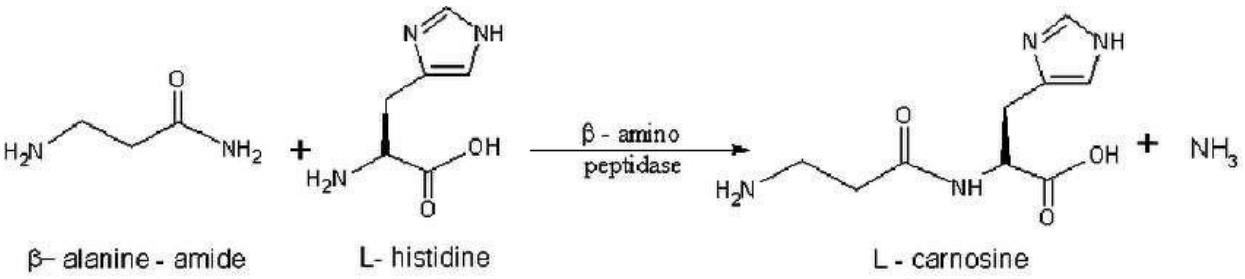
Like carnitine, carnosine is composed of the root word carn, meaning "flesh", alluding to its prevalence in animal protein. A vegetarian (especially vegan) diet provides less carnosine, compared to levels found in a diet including meat.

Carnosine can chelate divalent metal ions.

Carnosine can as well as appearing to reduce the telomere shortening rate. It is also considered as a geroprotector.

Carnosine acts as an antiglycating agent, reducing the rate of formation of advanced glycation end-products (substances that can be a factor in the development or worsening of many degenerative diseases, such as diabetes, atherosclerosis, chronic renal failure, and Alzheimer's disease), and ultimately reducing development of atherosclerotic plaque build-up. Chronic glycation end-productsis speculated to accelerate aging, making carnosine a candidate for therapeutic potential.

Anserine is methylated carnosine (methyl carnosine):




Histidine-containing dipeptides (HCD) are peptides consisting of a histidine (or a methylated form of histidine) and the atypical amino acid beta-alanine. HCD are predominantly and abundantly present in skeletal muscle, but they are also measurable in other tissues such as brain, kidney and liver, although in concentrations 10- to 1000-fold lower. Human skeletal muscles only possess the featuring the characteristic imidazole-ring, is a dipeptide molecule, made up of the amino asid beta-alanine and histidine. It is highly concentrated in muscle and brain tissue. (beta-alanyl-L-histidine), whereas rodent muscles contain carnosine along with its methylated variant anserine (beta-alanyl-N-π-methylhistidine). When beta-alanine is ingested, it turns into the molecule carnosine, which acts as an acid buffer in the body. Carnosine is stored in cells and released in response to drops in pH. Increased stores of carnosine can protect against diet-induced drops in pH (which might occur from ketone production in ketosis, for example), as well as offer protection from exercise-induced lactic acid production.

The physiological effect of histidine dipeptides was studied by the Russian biochemist S.E. Severin in the 60s and investigated so far by many scientists. Carnosine increases the amplitude of skeletal muscle contraction and activates the work of ionic pumps of muscle cells, stimulates the ATP-ase activity of myosin. The content of histidine peptides in smooth and cardiac muscles is many times lower than in skeletal muscles. They create up to 40% of the buffer capacity of fast muscles and allow you to accumulate a lot of lactate. Excess lactate in the absence of histidine peptides leads to acidosis and contracture.

Of the free amino acids in the muscles, the concentration of glutamate and its amide, glutamine, is high. Glutamic acid is involved in the neutralization of ammonia in the muscles. When interacting with ammonia, it turns into glutamine, a transport form of ammonia. Nitrogen compounds that play an energetic role in muscles, such as creatine and creatine phosphate, make up together 0.2-0.55%, creatinine - 0.003-0.005%, ATP - 0.25-0.50%. Carnosine and anserine (0.2-0.3%). Lactic acid in the normal range is 0.01-0.02%, but this figure increases dramatically during hypoxia. Inorganic substances account for only 1.0-1.5%. These are cations K +, Na +, Ca2 +, $Mg^{2}$+,Cu2 +, Mn2 +, Zn2 +, etc. and anions, among which the greatest phosphates and sulfates.

### *4. Molecular mechanisms of Muscle Fiber Contraction and Relaxation. Role of calcium in muscle contraction control.*

The sequence of events that result in the contraction of an individual muscle fiber begins with a signal—the neurotransmitter, Ach-from the motor neuron innervating that fiber. The local membrane of the fiber will depolarize as positively charged sodium ions ($Na^{+}$) enter, triggering an action potential that spreads to the rest of the membrane will depolarize, including the T-tubules. This triggers the release of calcium ions ($Ca^{++}$) from storage in the sarcoplasmic reticulum (SR). The release of $Ca^{2+}$ from the sarcoplasmic reticulum increases the concentration of $Ca^{2+}$ in the cytosol from approximately $10^{-7}$ to $10^{-5}$ *M*. The increased $Ca^{2+}$ concentration signals muscle contraction via the action of two accessory proteins bound to the actin filaments: tropomyosin and **troponin** (Figure 5). Tropomyosin is a protein that winds around the chains of the actin filament and covers the myosin-binding sites. Tropomyosin binds to troponin to form a troponin-tropomyosin complex. The troponin-tropomyosin complex prevents the myosin "heads" from binding to the active sites on the actin microfilaments. Troponin also has a binding site for $Ca^{++}$ ions. When the concentration of $Ca^{2+}$ is low, the complex of the troponins with tropomyosin blocks the interaction of actin and myosin, so the muscle does not contract. At high concentrations, $Ca^{2+}$ binding to troponin C shifts the position of the complex, relieving this inhibition and allowing contraction to proceed. The $Ca^{++}$ initiates contraction, which is sustained by ATP (Figure 6.). As long as Ca++ ions remain in the sarcoplasm to bind to troponin, and as long as ATP is available, the muscle fiber will continue to shorten.

Muscle contraction usually stops when signaling from the motor neuron ends, which repolarizes the sarcolemma and T-tubules, and closes the voltage-gated

calcium channels in the SR. Ca++ ions are pumped back into the SR, which causes the tropomyosin to reshield the binding sites on the actin strands. A muscle may also stop contracting when it runs out of ATP and becomes fatigued (Figure 7.).

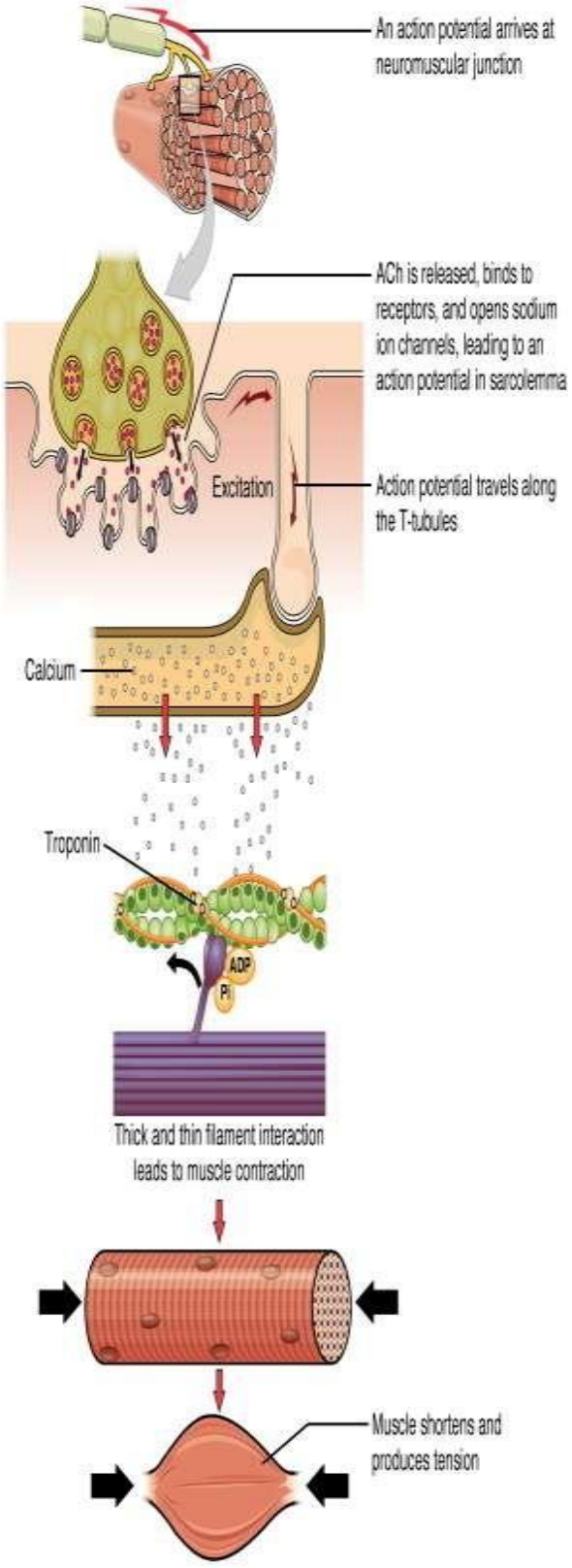


Figure 6. Contraction of a Muscle Fiber.

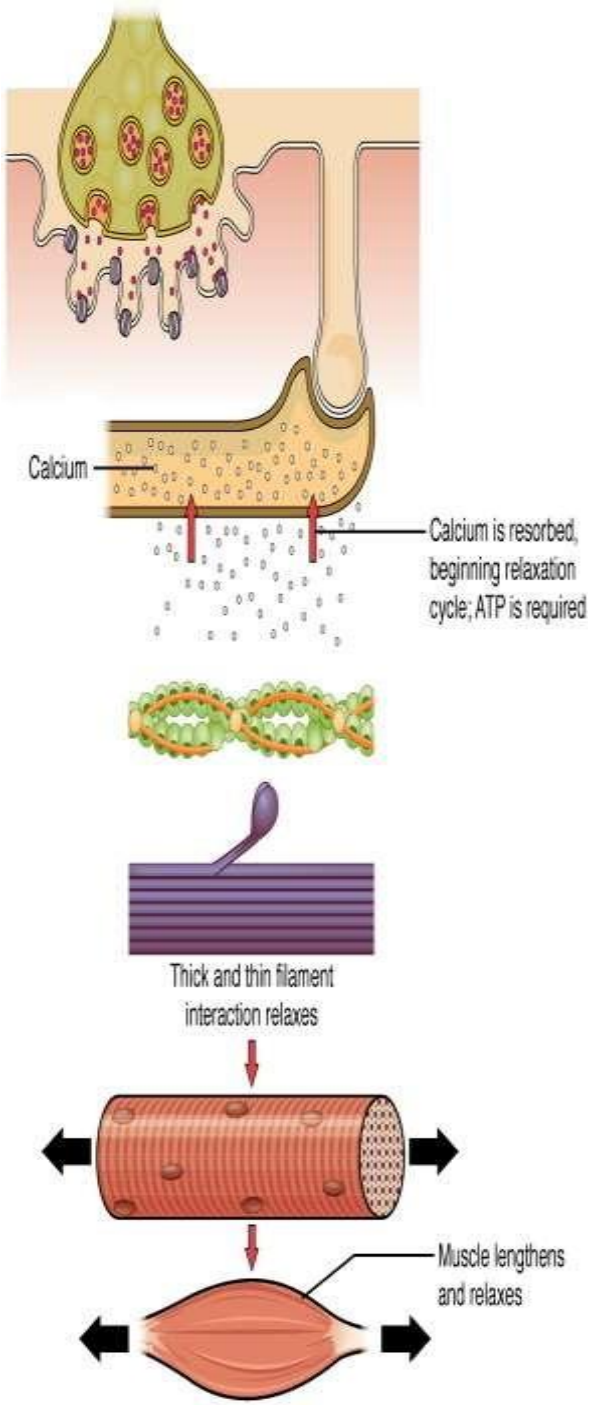



Figure 7. Relaxation of a Muscle Fiber.

**The molecular events of muscle fiber. The Sliding Filament Model of Contraction**

The basis for understanding muscle contraction is the **sliding filament model**, first proposed in 1954 both by Andrew Huxley and Ralph Niedergerke and by Hugh Huxley and Jean Hanson (Figure 8.). During muscle contraction, the actin is pulled along myosin toward the center of the sarcomere until the actin and myosin filaments are completely overlapped. In other words, for a muscle cell to contract, the sarcomere must shorten. However, thick and thin filaments - the components of sarcomeres - do not shorten. Instead, they slide by one another, causing the sarcomere to shorten. The mechanism of contraction is the binding of myosin to actin, forming cross-bridges that generate filament movement.

***Note that the actin and myosin filaments themselves do not change length, but instead slide past each other.***

***Biochemical mechanisms of muscle contraction.***

According to their biochemical essence, the cross-bridges that develop tension during muscle contraction, are temporary links formed between two kinds of filaments by S1 heads of myosin and specific sites of actin. Thus, the biochemical basis of muscle activity is related to the enzymatic and physical properties of actin, myosin and the accessory proteins that constitute the thin and thick filaments.

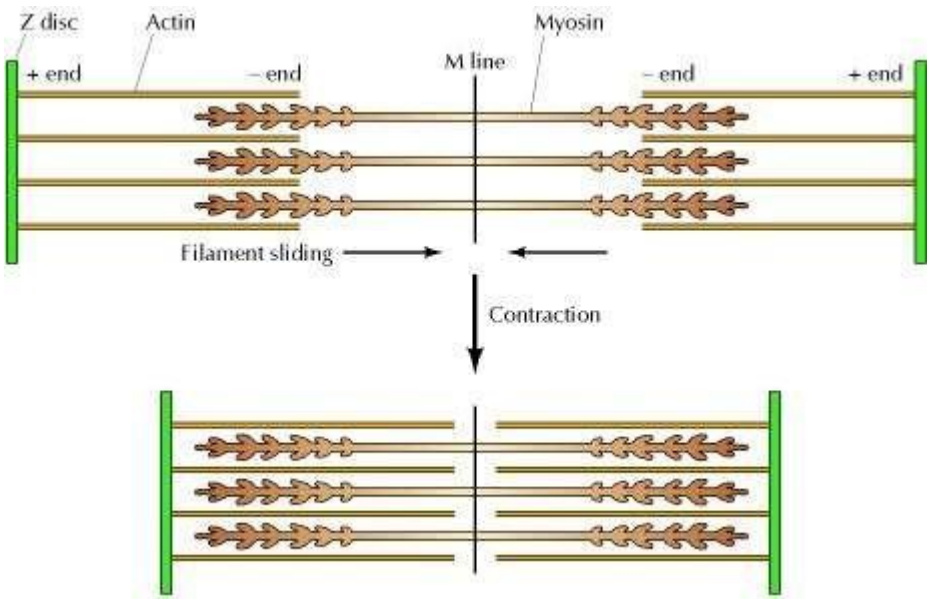


**Figure 8. Sliding-filament model of muscle contraction**

### *Myosin and the power stroke of contraction.*

The cyclic formation and dissociation of complexes between actin and S1 myosin heads, designated above as interfilamental cross-bridges, lead to a reciprocal sliding of the thin and thick filaments:

(a) The active site on actin is exposed as calcium binds to troponin.

(b) The myosin head is attracted to actin, and myosin binds actin at its actin-binding site, forming the cross-bridge.

(c) During the power stroke, the phosphate generated in the previous contraction cycle is released. This results in the myosin head pivoting toward the center of the sarcomere, after which the attached ADP and phosphate group are released.

(d) A new molecule of ATP attaches to the myosin head, causing the cross-bridge to detach.

(e) The myosin head hydrolyzes ATP to ADP and phosphate, which returns the myosin to the cocked position.

Each cycle requires energy, and the action of the myosin heads in the sarcomeres repetitively pulling on the thin filaments also requires energy, which is provided by ATP. Note that each thick filament of roughly 300 myosin molecules has multiple myosin heads, and many cross-bridges form and break continuously during muscle contraction. Multiply this by all of the sarcomeres in one myofibril, all the myofibrils in one muscle fiber, and all of the muscle fibers in one skeletal muscle, and you can understand why so much energy (ATP) is needed to keep skeletal muscles working. In fact, it is the loss of ATP that results in the rigor mortis observed soon after someone dies. With no further ATP production possible, there is no ATP available for myosin heads to detach from the actin-binding sites, so the cross-bridges stay in place, causing the rigidity in the skeletal muscles.

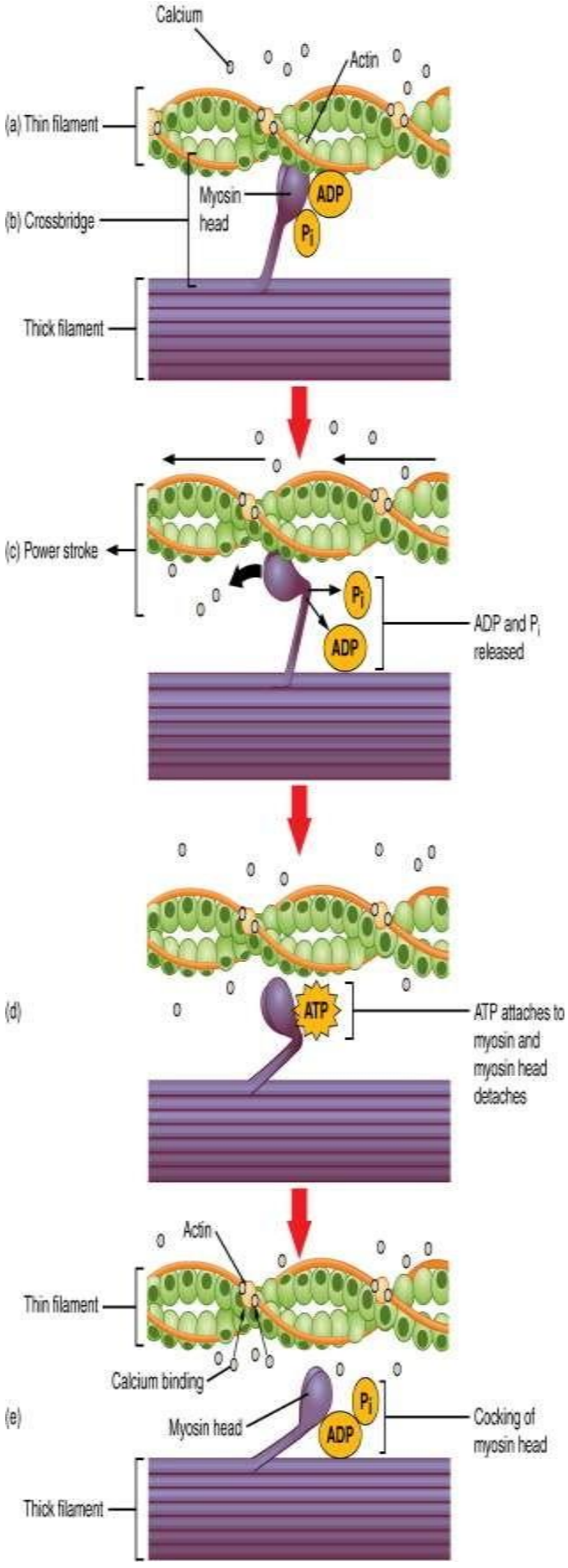



**Figure 9. Model for myosin action.**

***Key Concepts:***

- Striated muscles contain repeating sarcomeres of overlapping arrays of long, thin actin and thicker myosin filaments.
- Myosin filaments carry projections, the myosin heads, which are enzymes that can bind to actin and can split and make use of the energy from ATP.
- During muscle contraction myosin heads bind to actin, change their configuration on actin along with releasing the products of ATP hydrolysis and cause relative sliding of the actin and myosin filaments.
- Vertebrate striated muscle contraction is controlled (regulated) by the action of the proteins troponin and tropomyosin on the actin filaments.
- Nervous stimulation causes a depolarisation of the muscle membrane (sarcolemma) which triggers the release of calcium ions from the sarcoplasmic reticulum.
- Calcium ions bind to troponin and thus cause or allow the tropomyosin strands on the actin filament to move so that the part of the actin surface where myosin heads need to bind is uncovered.
- Contraction (force generation) then occurs and only stops when the sarcoplasmic reticulum pumps calcium out of the muscle interior, troponin loses its bound calcium and tropomyosin shifts back to its off position.

### *5. The energy metabolism of Muscle. Sources of ATP.*

ATP supplies the energy for muscle contraction to take place. In addition to its direct role in the cross-bridge cycle, ATP also provides the energy for the active-transport $Ca^{++}$ pumps in the SR. Muscle contraction does not occur without sufficient amounts of ATP. The amount of ATP stored in muscle is very low, only sufficient to power a few seconds worth of contractions. As it is broken down, ATP must therefore be regenerated and replaced quickly to allow for sustained contraction. *There are three mechanisms by which ATP can be regenerated: creatine phosphate metabolism, anaerobic glycolysis, fermentation and aerobic respiration.*

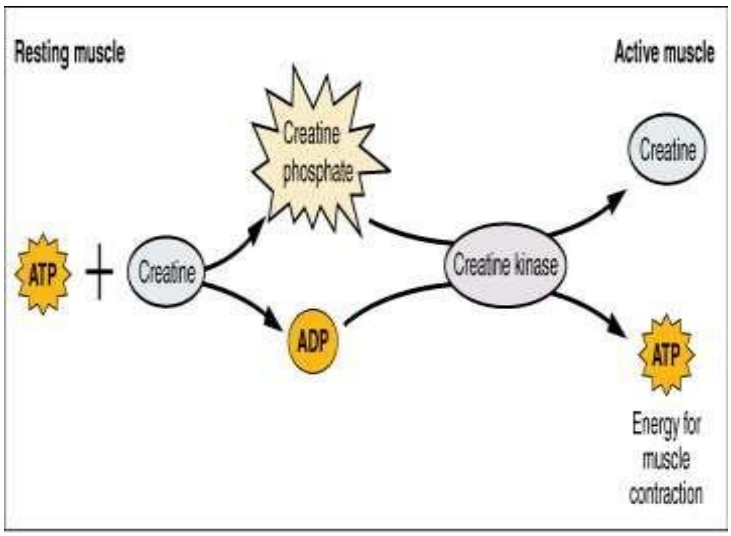
*Creatine phosphate* is a molecule that can store energy in its phosphate bonds. In a resting muscle, excess ATP transfers its energy to creatine, producing ADP and creatine phosphate. This acts as an energy reserve that can be used to quickly create more ATP. When the muscle starts to contract and needs energy, creatine phosphate transfers its phosphate back to ADP to form ATP and creatine. This reaction is catalyzed by the enzyme creatine kinase and occurs very quickly; thus, creatine phosphate-derived ATP powers the first few seconds of muscle

contraction. However, creatine phosphate can only provide approximately 15 seconds worth of energy, at which point another energy source has to be used (Figure 10.). As the ATP produced by creatine phosphate is depleted, muscles turn to glycolysis as an ATP source.

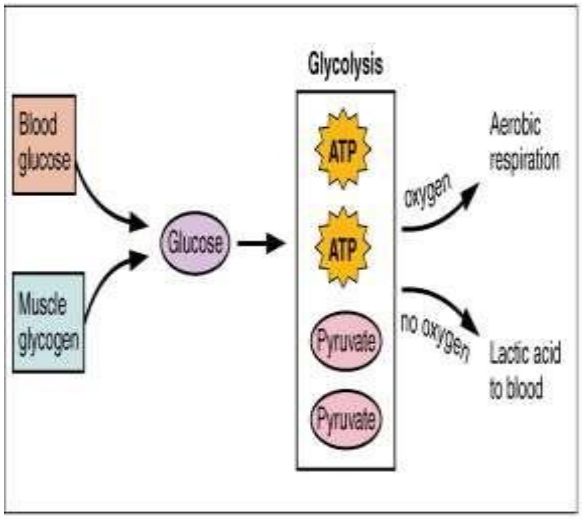
*Glycolysis* is an anaerobic (non-oxygen-dependent) process that breaks down glucose to produce ATP; however, glycolysis cannot generate ATP as quickly as creatine phosphate. Thus, the switch to glycolysis results in a slower rate of ATP availability to the muscle. The glucose used in glycolysis can be provided by blood glucose or by metabolizing glycogen that is stored in the muscle. The breakdown of one glucose molecule produces two ATP and two molecules of *pyruvic acid***,** which can be used in aerobic respiration or when oxygen levels are low, converted to lactic acid (Figure 10b). This conversion allows the recycling of the enzyme $NAD^+$ from NADH, which is needed for glycolysis to continue. This occurs during strenuous exercise when high amounts of energy are needed but oxygen cannot be sufficiently delivered to muscle. Glycolysis itself cannot be sustained for very long (approximately 1 minute of muscle activity), but it is useful in facilitating short bursts of high-intensity output.

*Aerobic respiration* is the breakdown of glucose or other nutrients in the presence of oxygen ($O_2$) to produce carbon dioxide, water, and ATP. Approximately 95 percent of the ATP required for resting or moderately active muscles is provided by aerobic respiration, which takes place in mitochondria. The inputs for aerobic respiration include glucose circulating in the bloodstream, pyruvic acid, and fatty acids. Aerobic respiration is much more efficient than anaerobic glycolysis, producing approximately 36 – 38 ATPs per molecule of glucose versus four from glycolysis. However, aerobic respiration cannot be sustained without a steady supply of $O_2$ to the skeletal muscle and is much slower (Figure 10c). To compensate, muscles store small amount of excess oxygen in proteins call myoglobin, allowing for more efficient muscle contractions and less fatigue. Aerobic training also increases the efficiency of the circulatory system.

Thus, the energy supply of different types of muscular work is different. Therefore, there is a specialization of muscle fibers and the provision of energy in different muscle cells is fundamentally different. There are white and red muscle fibers. Skeletal muscle fibers are grossly divided into two type; slow twitch (type I – red muscle) and fast twitch (type II – white muscle). Type II fibers are further divided into type IIa and type IIb fibers. Type IIa fibers are intermediate fast twitch fibers and can utilize both aerobic and anaerobic metabolism for ATP production. Type IIb fibers are the classic fast twitch fibers. Slow twitch muscle fibers primarily utilize fatty acid oxidation and contain a high concentration of mitochondria and store appreciable amounts of oxygen as oxymyoglobin.




(a)

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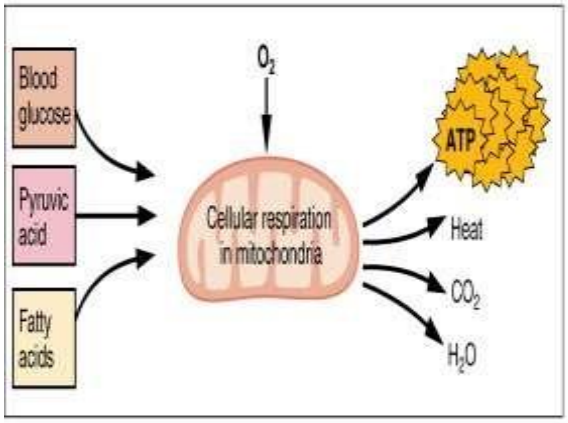


(c)

Figure 10. Muscle Metabolism.

These two facts are the reason that slow twitch fibers are red in color. Fast twitch fibers primarily utilize glucose oxidation to pyruvate for ATP production, contain less mitochondria and myoglobin than slow twitch fibers, and thus, are white muscle fibers. Because slow twitch fibers prefer to oxidize fatty acids they are also referred to as *oxidative fibers,* whereas fast twitch fibers that utilize glucose are referred to as *glycolytic fibers*. Slow twitch fibers are capable of continuous extended contractions and therefore, do not fatigue quickly. Fast twitch fibers are used for short rapid bursts of energy and as such fatigue more quickly than slow twitch fibers.

### ***6. Biochemical features of the metabolism in the Smooth Muscle.*** *Regulation of* ***the Smooth Muscle contration.***

*Structural organization and smooth muscle contraction.* Smooth muscle (so-named because the cells do not have striations) is found around organs in the digestive, respiratory, reproductive tracts. (Figure 11.ab). Smooth muscle is also present in the eyes, where it functions to change the size of the iris and alter the shape of the lens; and in the skin where it causes hair to stand erect in response to cold temperature or fear. Smooth muscle fibers are spindle-shaped (wide in the middle and tapered at both ends, somewhat like a football) and have a single nucleus; they range from about 30 to 200 $\mu$m (thousands of times shorter than skeletal muscle fibers), and they produce their own connective tissue, endomysium. Although they do not have striations and sarcomeres, smooth muscle fibers do have actin and myosin contractile proteins, and thick and thin filaments. These thin filaments are anchored by dense bodies. A *dense body* is analogous to the Z-discs of skeletal and cardiac muscle fibers and is fastened to the sarcolemma. Calcium ions are supplied by the SR in the fibers and by sequestration from the extracellular fluid through membrane indentations. Because smooth muscle cells do not contain troponin, cross-bridge formation is not regulated by the troponin-tropomyosin complex but instead by the regulatory protein *calmodulin.* In a smooth muscle fiber, external $Ca^{++}$ ions passing through opened calcium channels in the sarcolemma, and additional $Ca^{++}$ released from SR, bind to calmodulin. The $Ca^{++}$ - calmodulin complex then activates an enzyme called myosin (light chain) kinase, which, in turn, activates the myosin heads by phosphorylating them (converting ATP to ADP and $P_i$, with the $P_i$ attaching to the head). The heads can then attach to actin-binding sites and pull on the thin filaments. The thin filaments also are anchored to the dense bodies; the structures invested in the inner membrane of the sarcolemma, that also have cord-like intermediate filaments attached to them. When the thin filaments slide past the thick filaments, they pull

on the dense bodies, structures tethered to the sarcolemma, which then pull on the intermediate filaments networks throughout the sarcoplasm. This arrangement causes the entire muscle fiber to contract in a manner whereby the ends are pulled toward the center. (Figure 12).

Smooth muscle is organized in two ways: as single-unit smooth muscle, which is much more common; and as multiunit smooth muscle. The two types have different locations in the body and have different characteristics. Single-unit muscle has its muscle fibers joined by gap junctions so that the muscle contracts as a single unit. This type of smooth muscle is found in the walls of all visceral organs except the heart, and so it is commonly called *visceral muscle*. Because the muscle fibers are not constrained by the organization and stretchability limits of sarcomeres, visceral smooth muscle has a *stress-relaxation response*. This means that as the muscle of a hollow organ is stretched when it fills, the mechanical stress of the stretching will trigger contraction, but this is immediately followed by relaxation so that the organ does not empty its contents prematurely. This is important for hollow organs, such as the stomach or urinary bladder.

*Multiunit smooth muscle* cells rarely possess gap junctions, and thus are not electrically coupled. As a result, contraction does not spread from one cell to the next, but is instead confined to the cell that was originally stimulated. Stimuli for multiunit smooth muscles come from autonomic nerves or hormones but not from stretching. This type of tissue is found around large blood vessels, in the respiratory airways, and in the eyes.

Although smooth muscle contraction relies on the presence of $Ca^{++}$ ions, smooth muscle fibers have a much smaller diameter than skeletal muscle cells. T-tubules are not required to reach the interior of the cell and therefore not necessary to transmit an action potential deep into the fiber. Smooth muscle fibers have a limited calcium-storing SR but have calcium channels in the sarcolemma (similar to cardiac muscle fibers) that open during the action potential along the sarcolemma. The influx of extracellular $Ca^{++}$ ions, which diffuse into the sarcoplasm to reach the calmodulin, accounts for most of the $Ca^{++}$ that triggers contraction of a smooth muscle cell.

Muscle contraction continues until ATP-dependent calcium pumps actively transport $Ca^{++}$ ions back into the SR and out of the cell. However, a low concentration of calcium remains in the sarcoplasm to maintain muscle tone. This remaining calcium keeps the muscle slightly contracted, which is important in certain tracts and around blood vessels.

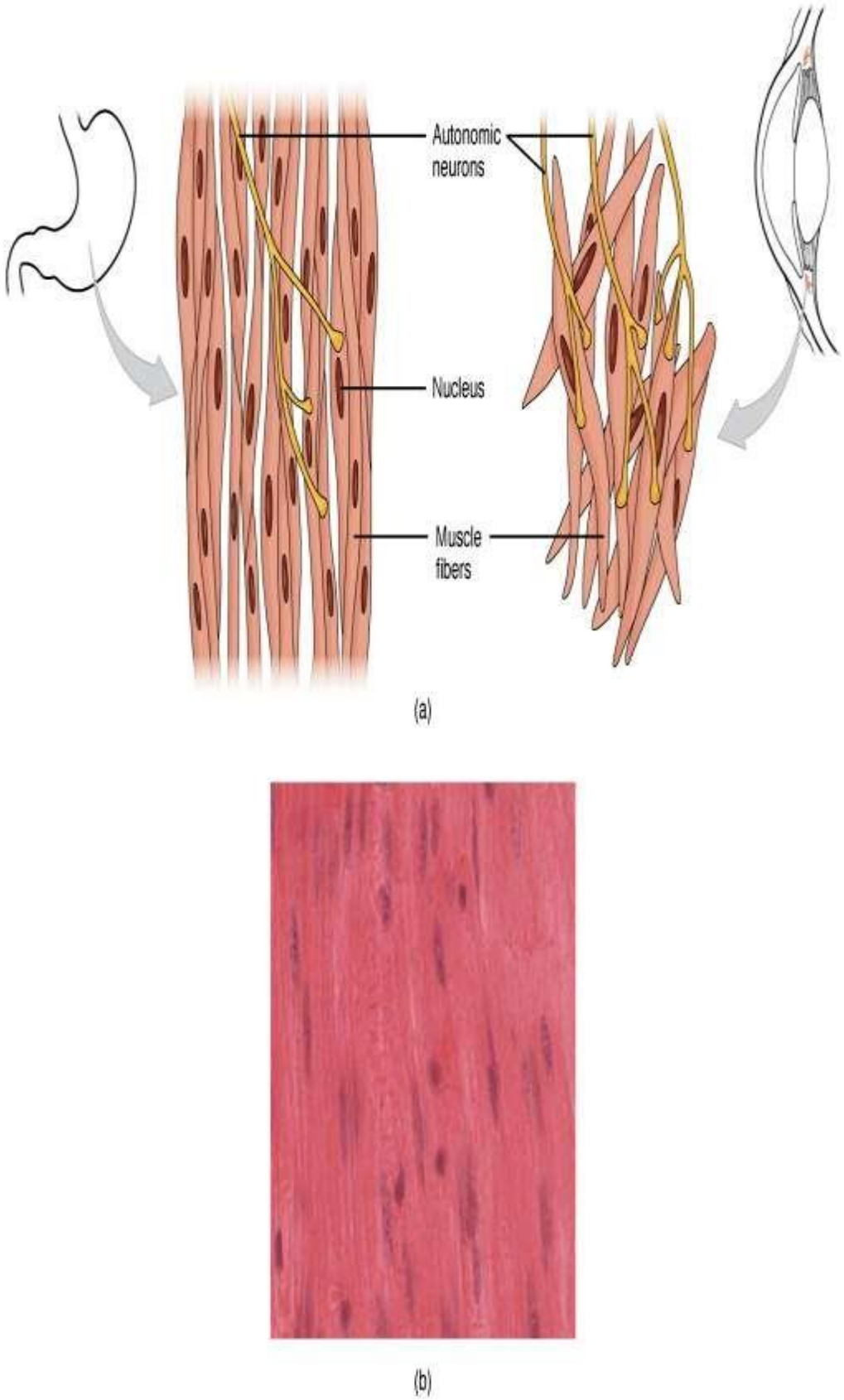


Figure 11. Smooth Muscle Tissue. LM × 1600. (Micrograph provided by the Regents of University of Michigan Medical School © 2012).

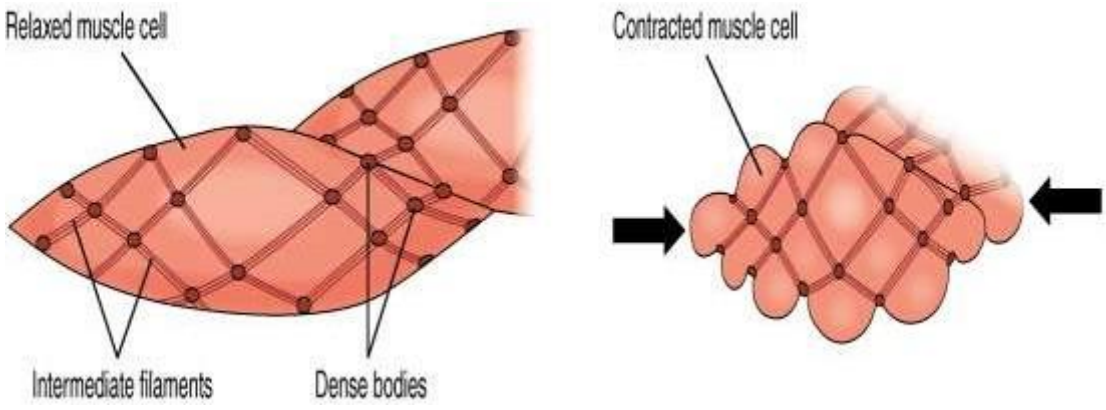


Figure 12. Muscle Contraction. The dense bodies and intermediate filaments are networked through the sarcoplasm, which cause the muscle fiber to contract.

Because most smooth muscles must function for long periods without rest, their power output is relatively low, but contractions can continue without using large amounts of energy. Some smooth muscle can also maintain contractions even as $Ca^{++}$ is removed and myosin kinase is inactivated/dephosphorylated. This allows for the maintaining of muscle "tone" in smooth muscle that lines arterioles and other visceral organs with very little energy expenditure. Smooth muscle is not under voluntary control; thus, it is called involuntary muscle.

*Regulation of muscle contration.* The regulation of actin-myosin contraction in striated muscle, discussed earlier, is mediated by the binding of $Ca^{2+}$ to troponin. In nonmuscle cells and in smooth muscle, however, contraction is regulated primarily by phosphorylation of one of the myosin light chains, called the regulatory light chain (Figure 13.).

$Ca^{2+}$ binds to calmodulin , which in turn binds to myosin light-chain kinase (MLCK). The active calmodulin-MLCK complex then phosphorylates the myosin II regulatory light chain, converting myosin from an inactive to an active state. Phosphorylation of the regulatory light chain in these cells has at least two effects: It promotes the assembly of myosin into filaments, and it increases myosin catalytic activity, enabling contraction to proceed. The enzyme that catalyzes this phosphorylation, called *myosin light-chain kinase,* is itself regulated by association with the $Ca^{2+}$-binding protein calmodulin. Increases in cytosolic $Ca^{2+}$ promote the binding of calmodulin to the kinase, resulting in phosphorylation of the myosin regulatory light chain. Increases in cytosolic $Ca^{2+}$ are thus responsible, albeit indirectly, for activating myosin in smooth muscle and nonmuscle cells, as well as in striated muscle.

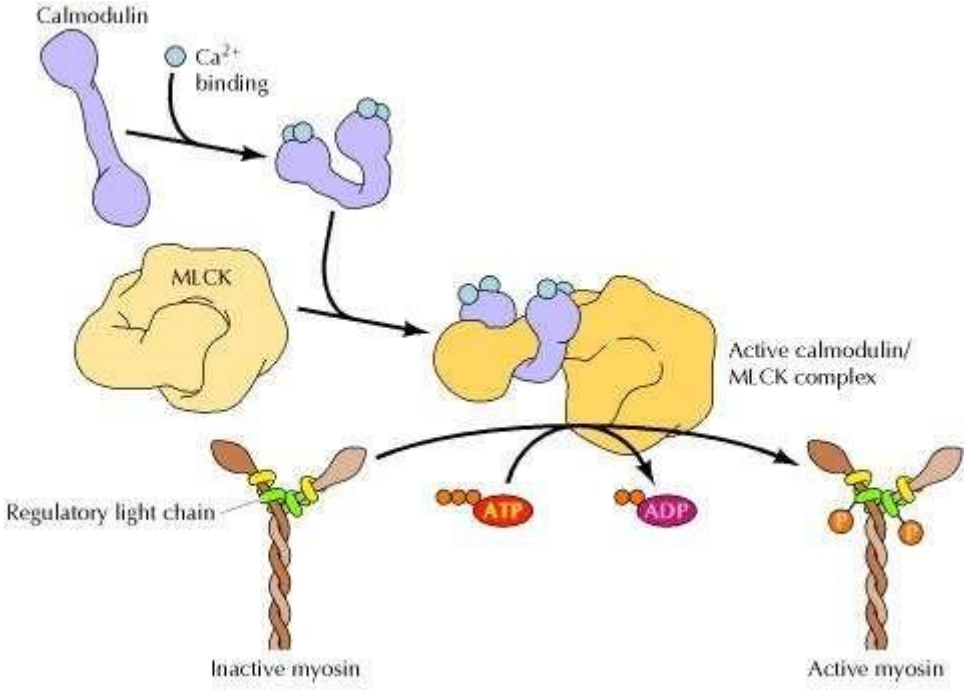


Figure 13. Regulation of myosin by phosphorylation.

*Hyperplasia in Smooth Muscle* Similar to skeletal and cardiac muscle cells, smooth muscle can undergo hypertrophy to increase in size. Unlike other muscle, smooth muscle can also divide to produce more cells, a process called *hyperplasia*. This can most evidently be observed in the uterus at puberty, which responds to increased estrogen levels by producing more uterine smooth muscle fibers, and greatly increases the size of the myometrium.

*Key Concepts:*

- Smooth muscle is found throughout the body around various organs and tracts. Smooth muscle cells have a single nucleus, and are spindle-shaped. Smooth muscle cells can undergo hyperplasia, mitotically dividing to produce new cells. The smooth cells are nonstriated, but their sarcoplasm is filled with actin and myosin, along with dense bodies in the sarcolemma to anchor the thin filaments and a network of intermediate filaments involved in pulling the sarcolemma toward the fiber's middle, shortening it in the process.
- $Ca^{++}$ ions trigger contraction when they are released from SR and enter through opened voltage-gated calcium channels. Smooth muscle contraction is initiated when the $Ca^{++}$ binds to intracellular calmodulin, which then activates an enzyme called myosin kinase that phosphorylates myosin heads so they can form the cross-bridges with actin and then pull on the thin filaments.

- Smooth muscle can be stimulated by pacesetter cells, by the autonomic nervous system, by hormones, spontaneously, or by stretching. The fibers in some smooth muscle have latch-bridges, cross-bridges that cycle slowly without the need for ATP; these muscles can maintain low-level contractions for long periods.

### *7. Biochemical features of the metabolism in the Cardiac Muscle Tissue. Features of bioenergetic processes in the myocardium.*

Cardiac muscle is striated muscle that is present only in the heart. Highly coordinated contractions of cardiac muscle pump blood into the vessels of the circulatory system. Similar to skeletal muscle, cardiac muscle is striated and organized into sarcomeres, possessing the same banding organization as skeletal muscle (Figure 14). However, cardiac muscle fibers are shorter than skeletal muscle fibers and usually contain only one nucleus, which is located in the central region of the cell. Cardiac muscle fibers also possess many mitochondria and myoglobin, as ATP is produced primarily through aerobic metabolism. Cardiac muscle fibers cells also are extensively branched and are connected to one another at their ends by intercalated discs. An *intercalated disc* allows the cardiac muscle cells to contract in a wave-like pattern so that the heart can work as a pump. Intercalated discs are part of the sarcolemma and contain two structures important in cardiac muscle contraction: gap junctions and desmosomes. A gap junction forms channels between adjacent cardiac muscle fibers that allow the depolarizing current produced by cations to flow from one cardiac muscle cell to the next. This joining in cardiac muscle allows the quick transmission of action potentials and the coordinated contraction of the entire heart. The remainder of the intercalated disc is composed of desmosomes. A *desmosome* is a cell structure that anchors the ends of cardiac muscle fibers together so the cells do not pull apart during the stress of individual fibers contracting (Figure 15). Contraction in each cardiac muscle fiber is triggered by $Ca^{++}$ ions in a similar manner as skeletal muscle, but here the $Ca^{++}$ ions come from SR and through voltage-gated calcium channels in the sarcolemma. Pacemaker cells stimulate the spontaneous contraction of cardiac muscle as a functional unit, called a syncytium. Unlike skeletal muscle, a large percentage of the $Ca^{++}$ that initiates contraction in cardiac muscles comes from outside the cell rather than from the SR.

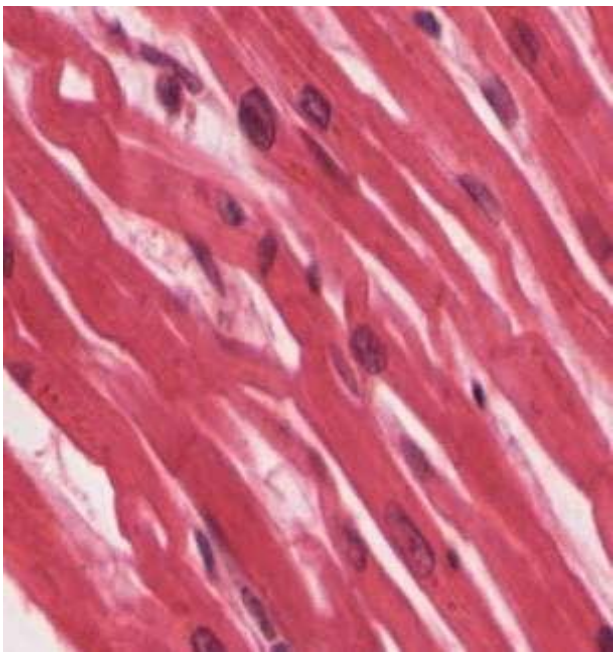

Figure 14. Cardiac Muscle Tissue. LM × 1600. (Micrograph provided by the Regents of University of Michigan Medical School © 2012)

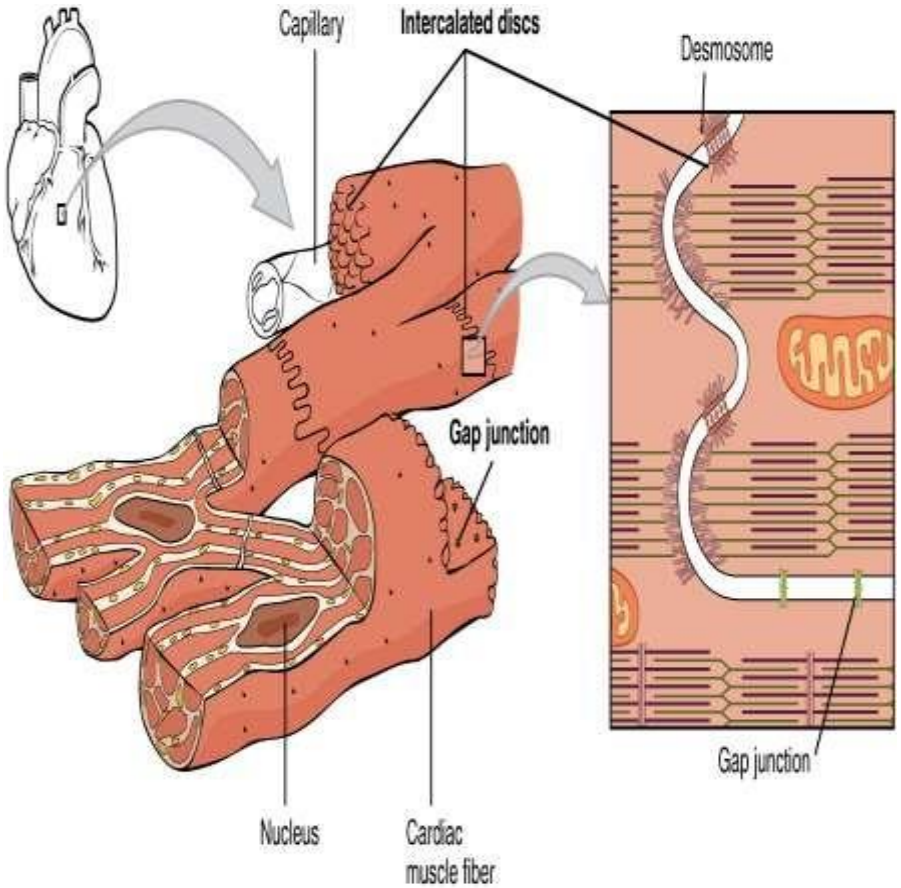


Figure 15. Cardiac Muscle. Intercalated discs are part of the cardiac muscle sarcolemma and they contain gap junctions and desmosomes.

*Features of bioenergetic processes in the myocardium*. The main feature of the energy metabolism of the heart muscle is that it has an aerobic character.
The main source of energy for the myocardium is adenosine triphosphate (ATP). About 30 kg of ATP is produced and used in the heart per day. Energy processes in cardiomyocytes occur in mitochondria (30–40% of the volume of the cardiac cell). With aging, the number of mitochondria decreases, the process of energy formation is disturbed.
The primary energy substrate in the heart muscle is fatty acids, glucose, lactate (lactic acid), ketone bodies and, to a lesser extent, amino acids. Under normal conditions, the main substrate for the formation of energy in the myocardium is free fatty acids (FFA), which serve as a source of 60-80% of ATP. But the oxidation of FLC compared with glucose requires 10% more oxygen. Saturated fatty acids and oleic acid, oxidized in the mitochondria, supply ATP cells.
*Biochemical changes in myocardial infarction.* Ischemic heart disease is caused by an imbalance between the myocardial blood flow and the metabolic demand of the myocardium. Reduction in coronary blood flow is related to progressive atherosclerosis with increasing occlusion of coronary arteries. For ischemic myocardium, reduced oxidative phosphorylation and increased anaerobic metabolism are characteristic. The content of ATP and creatine phosphate in the cell decreases sharply as a result of impaired oxidative phosphorylation in mitochondria.

One of the first manifestations of this condition is a violation of membrane permeability. Violation of the integrity of the membrane contributes to the release of ions from the cell, including K + ions, as well as enzymes. The lack of energy resources and the violation of the ionic composition cause inhibition of the functional activity of muscle cells and their gradual death.

## *8. Laboratory Diagnosis of Myocardial Infarction*

A number of laboratory biomarkers for myocardial injury are available. None is completely sensitive and specific for myocardial infarction, particularly in the hours following onset of symptoms. Timing is important, as are correlation with patient symptoms, electrocardiograms, and angiographic studies.The following biomarkers have been described in association with acute myocardial infarction:

*Troponins:* Troponin I and T are structural components of cardiac muscle. They are released into the bloodstream with myocardial injury. They are highly specific for myocardial injury--more so than CK-MB--and help to exclude elevations of CK with skeletal muscle trauma. Troponins will begin to increase following MI within

3 to 12 hours, about the same time frame as CK-MB. However, the rate of rise for early infarction may not be as dramatic as for CK-MB. Troponins will remain elevated longer than CK--up to 14 days. This makes troponins a superior marker for diagnosing myocardial infarction in the recent past--better than lactate dehydrogenase (LDH). However, this continued elevation has the disadvantage of making it more difficult to diagnose reinfarction or extension of infarction in a patient who has already suffered an initial MI. Troponin T lacks some specificity because elevations can appear with skeletal myopathies and with renal failure. (Kost et al, 1998) (Kumar and Cannon, Part I, 2009)

*Kinase - Total:* The total CK is a simple and inexpensive test that is readily available using many laboratory instruments. However, an elevation in total CK is not specific for myocardial injury, because most CK is located in skeletal muscle, and elevations are possible from a variety of non-cardiac conditions. (Chattington et al, 1994)

*Creatine Kinase - MB Fraction:* Creatine kinase can be further subdivided into three isoenzymes: MM, MB, and BB. The MM fraction is present in both cardiac and skeletal muscle, but the MB fraction is much more specific for cardiac muscle: about 15 to 40% of CK in cardiac muscle is MB, while less than 2% in skeletal muscle is MB. The BB fraction (found in brain, bowel, and bladder) is not routinely measured.

The creatine kinase-MB fraction (CK-MB) is part of total CK and more specific for cardiac muscle that other striated muscle. It tends to increase within 3 to 4 hours of myocardial necrosis, then peak in a day and return to normal within 36 hours. It is less sensitive than troponins. (Saenger and Jaffe, 2007) (Kumar and Cannon, Part I, 2009)

The CK-MB is also useful for diagnosis of reinfarction or extensive of an MI because it begins to fall after a day, so subsequent elevations are indicative of another event. (Chattington et al, 1994)

*Copeptin:* Arginine vasopressin (AVP) is secreted as a prohormone from the posterior pituitary and then cleaved to form a C-terminal part called copeptin. A rapid increase in copeptin can be associated with stroke, sepsis, or acute myocardial injury. In conjunction with troponin, copeptin has high negative predictive value to rule out myocardial injury.

***9. Biochemical changes in the muscles in pathology.***

Common to most muscle diseases (progressive muscular dystrophies, muscle atrophy as a result of their denervation, tenotomy, polymyositis, some avitaminosis, etc.) are a sharp decrease in muscle myofibrillary proteins, an increase in the concentration of stroma proteins and some sarcoplasmic proteins, including myoalbumin.

*Progressive muscular dystrophies and congenital myopathies.* Muscular dystrophies are *genetically transmitted* diseases characterized pathologically by degeneration and loss of myofibers and clinically by inexorably progressive weakness and, many of them, by elevated CK. The pattern of weakness, tempo of evolution, and mode of inheritance vary among different dystrophies. Over 30 genes causing muscular dystrophy are known presently. Muscular dystrophies are clinically classified into the following groups:

- Dystrophinopathies (Duchenne and Becker muscular dystrophies)
- Limb-Girdle dystrophies
- Myotonic dystrophy
- Distal myopathies
- Emery-Dreifuss muscular dystrophy
- Congenital muscular dystrophies

Some of these groups contain several entities with different inheritance patterns. The most common muscular dystrophy in children is Duchene muscular dystrophy. In adults, the most common dystrophies are myotonic dystrophy and the limb girdle dysytrophies.

*Duchenne muscular dystrophy (DMD)* is a progressive weakening of the skeletal muscles. It is one of several diseases collectively referred to as "muscular dystrophy." DMD is caused by a lack of the protein dystrophin, which helps the thin filaments of myofibrils bind to the sarcolemma. Without sufficient dystrophin, muscle contractions cause the sarcolemma to tear, causing an influx of $Ca^{++}$, leading to cellular damage and muscle fiber degradation. Over time, as muscle damage accumulates, muscle mass is lost, and greater functional impairments develop. DMD is an inherited disorder caused by an abnormal X chromosome. It primarily affects males, and it is usually diagnosed in early childhood. DMD usually first appears as difficulty with balance and motion, and then progresses to an inability to walk. It continues progressing upward in the body from the lower extremities to the upper body, where it affects the muscles responsible for breathing and circulation. It ultimately causes death due to respiratory failure, and those afflicted do not usually live past their 20s. Because DMD is caused by a mutation in the gene that codes for dystrophin, it was thought that introducing healthy myoblasts into patients might be an effective treatment. Myoblasts are the

embryonic cells responsible for muscle development, and ideally, they would carry healthy genes that could produce the dystrophin needed for normal muscle contraction. This approach has been largely unsuccessful in humans. A recent approach has involved attempting to boost the muscle's production of utrophin, a protein similar to dystrophin that may be able to assume the role of dystrophin and prevent cellular damage from occurring.

***Practical part.***

***Task 1. Determination of Creatine in urine by Jaffe method.***

***The principle of the method.*** Creatine reacts with pyric acid in an alkaline medium to form a red tautomer of pyratin creatinine, which causes the appearance of persistent orange-red staining. The intensity of staining is proportional to the concentration of creatinine.

***The course of work.*** Prepare an experimental sample: In a cylinder of 100 ml make 0.5 ml of urine and 3 ml of saturated solution of picric acid. Mix thoroughly and add 0.2 ml of 10% sodium hydroxide solution, stand for 10 minutes at room temperature and bring distilled water to 100 ml.

Prepare a control test: add up to 3 ml of the solution of picric acid to 0.2 ml of 10% sodium hydroxide solution and bring the volume of the mixture to 100 ml with distilled water. Prepare a standard sample: add 3 ml of citric acid solution and 0.2 ml of 10% sodium hydroxide solution to a standard solution of 0.5 ml (containing 8.8 mmol / l or 1 g / l creatinine), stand for 10 minutes at room temperature and bring distilled water to 100 ml.

Calculation: Creatinine concentration (mol / day) = (Eop / Est) × 8.8

Norm: 4.4 - 17.6 mole / day, or 0.5-2 g / day.

***Clinico - diagnostic value.*** The content of creatinine in the urine depends on the nature of the nutrition, increasing when eating meat. Increasing its excretion with urine is observed with increased muscular work, febrile states, pneumonia, avitaminosis E, thyrotoxicosis, etc.; reduction of excretion in the urine is observed at muscle atrophy, leukemia, kidney amyloidosis, starvation.

***Task 2. Quantitative determination of creatine in urine.***

***Principle of the method.*** Creatine in the urine is determined by the same method as creatinine, having previously converted creatine into creatinine in an acid medium when heated.

***Material support:*** preliminary urine, saturated solution of picric acid, 10% solution of sodium hydroxide, concentrated hydrochloric acid, FEC, 100 ml measuring cylinders, measuring pipettes, glass sticks, water bath.
***The course of work:*** In one test tube 0.5 ml of urine (experiment) is measured, and in the second one - 0.5 ml of distilled water (control). In both test tubes add 0,1 ml of concentrated hydrochloric acid and place them in a boiling water bath for 3 minutes. After cooling, in both test tubes add 0,2 ml of 10% sodium hydroxide and 3 ml of saturated solution of picric acid, mix the contents of the samples and leave for 5 minutes Then, the contents of the test tubes are quantitatively transferred into the measuring cylinders of 100 ml by flushing the tubes three times with 10 ml of distilled water. Make volumes to the label - 100 ml. Measure the extinction on FEC in a cuvette with a layer thickness of 1 cm with a green filter against the control. Calculation: Creatinine concentration (mole / day) = (Eop / Est) × 8.8

This creatinine is the sum of creatine and actually creatinine. When determining the amount of creatine, there is a difference between the values of creatinine from Experiment 2 and Experiment 1. This difference is multiplied by 1.16 - the conversion factor for the creatinine level corresponds to the amount of creatine, i.e., the ratio of the molecular weights of creatine and creatinine is 131: 113 = 1.16.

Explain the result. Make conclusions

***Clinical and diagnostic value.*** Normal excretion of creatine with urine is in men 0 - 0.3 mmol / day, in women 0-0.61 mmol / day. In the urine of a healthy adult with normal exercise, creatine, as a rule, is absent. Its appearance in urine - creatinuria - is observed with increased muscle load, in the period of growth of children (up to 14-17 years), during pregnancy, in the early postnatal period, in carbohydrate and protein starvation, in the elderly, when healing significant fractures, surgical interventions. Creatinuria is observed with increased tissue disintegration (burns, cancer, tuberculosis), avitaminosis E, diabetes mellitus, parenchymal hepatitis.

### *Situational challenges*

***Task number 1.*** The man runs 1 km. What sources of energy for the work of muscles are used at different stages?
***The standard of the answer****:* At the first stage (the first few seconds), creatine phosphate is the creatine kinase reaction. The rate of cleavage of creatine phosphate in the working muscle is directly proportional to the intensity of the work performed and the magnitude of muscle contraction. At the second stage -

anaerobic glycolysis (30-150 seconds). When the amount of ADP is increased, hexokinase and glycogen phosphorylase are active, this includes the reactions of anaerobic glycolysis. The final product of glycolysis, lactate, changes the pH of the medium, as a result of which the enzymes of aerobic glycolysis and the respiratory chain in the mitochondria are activated. In the third stage - aerobic glycolysis. The energy effect of aerobic glycolysis is 19 times more anaerobic.

***Task number 2.*** In plasma, the patient has a significantly increased level of ASAT, LDH1 and LDH2, and MB-creatine kinase. The development of a pathological process can be suspected in this case?

***The standard of the answer:*** Myocardial infarction.

AsAt, LDH1 and LDH2 and creatine kinase are organ-specific enzymes of the myocardium. An increase in the activity of these enzymes in the blood plasma indicates a necrotic process in the myocardium, a myocardial infarction. The increase in QA activity is noted 2-3 hours after the infarction, LDH1 and LDH2 after 12 hours and ASAT in 4-6 hours.

***Task number 3.*** Why can smooth muscles contract over a wider range of resting lengths than skeletal and cardiac muscle?

***The standard of the answer:*** Smooth muscles can contract over a wider range of resting lengths because the actin and myosin filaments in smooth muscle are not as rigidly organized as those in skeletal and cardiac muscle.

***Task number 4.*** During intense physical exercise in humans, energy metabolism is provided for some time by glycolysis, and during resting-gluconeogenesis. How are these two processes in the Core cycle interconnected?

***The standard of the answer:*** When physical activity in the process of glycolytic energy exchange, lactic acid is accumulated. During the rest she diffuses in the blood and is fond of the liver and heart. In myocardium under the action of LDH1 lactic acid is oxidized to pyruvate. In the liver, 15% of it is oxidized by aerobic activity, and 85% is converted into glucose in glucose, which is released into the blood and enters the muscle, where it is used for the synthesis of glycogen (Cory cycle).

## *Krok 1 Tests with restractor A*

**1.** A 60-year-old man consulted a doctor about an onset of chest pain. In blood serum analysis showed a significant increase in the activity of the following enzymes: creatine kinase and its MBisoform, aspartate aminotransferase. These changes indicate the development of the pathological process in the following tissues:
**A. *Cardiac muscle**
**B.** Lungs
**C.** Skeletal muscles
**D.** Liver
**E.** Smooth muscles

**2.** A patient has myocardial infarction. The first several hours of such medical condition will be characterized by significant increase of activity of the following enzyme in his blood serum:
**A. *Creatine phosphokinase**
**B.** Lactate dehydrogenase4
**C.** Aspartate aminotransferase
**D.** Lactate dehydrogenase5
**E.** Alanine-aminotransferase

**3.** One of the coats of a hollow organ has anastomotic fibers with nuclei. The fibers consist of cells that form intercalated disks at the places of contact. What tissue forms this coat?
**A. *Cross-striated cardiac muscle**
**B.** Cross-striated skeletal muscle
**C.** Unstriped muscle
**D.** Loose fibrous connective tissue
**E.** Dense irregular connective tissue

**4.** For biochemical diagnostics of cardiac infarction it is necessary to determine activity of a number of enzymes and their izoenzymes in the blood. What enzyme assay is considered to be optimal for confirming or ruling out cardiac infarction at the early stage, after the patient develops thoracic pain?
**A. *Creatine kinase MB isoenzyme**
**B.** Creatine kinase MM isoenzyme
**C.** LDH1 isoenzyme
**D.** LDH5 isoenzyme
**E.** Cytoplasmic isoenzyme of aspartate
Aminotransferase

**5.** There is increased activity of AST, LDH1, LDH2, and CPK in the patient's blood. Pathological process most likely occurs in the:
**A. *Heart**
**B.** Skeletal muscles
**C.** Kidneys
**D.** Liver
**E.** Adrenal glands

**6.** A 1-year-old child with the symptoms of affection of limb and trunk muscles had been admitted to a hospital. Examination revealed muscle carnitine deficiency. The biochemical basis of this pathology is a disruption of the following process:
**A. *Transport of fatty acids to mitochondria**

**B.** Regulation of *Ca*2+ level in mitochondria
**C.** Substrate phosphorylation
**D.** Utilization of lactic acid
**E.** Oxidative phosphorylation

**7.** A 34-year-old patient has low endurance of physical loads. At the same time skeletal muscles have increased concentration of glycogen. This is caused by the reduced activity of the following enzyme:
**A. *Glycogen phosphorylase**
**B.** Glucose-6-phosphate dehydrogenase
**C.** Phosphofructokinase
**D.** Glycogen synthase
**E.** Glucose-6-phosphatase

**8.** A patient has been delivered to a hospital with a provisional diagnosis of progressing muscle dystrophy. This diagnosis can be confirmed by the increased concentration of the following substance found in urine:
**A. *Kreatine**
**B.** Pyruvate
**C.** Carnosine
**D.** Troponin
**E.** Hydroxyproline

**9.** After prolonged exercising people usually experience intense muscle pain. What is its most likely cause?
**A. *Accumulation of lactic acid in muscles**
**B.** Intensified disintegration of muscle proteins
**C.** Accumulation of creatinine in muscles
**D.** Increased muscle excitability
**E.** Increased concentratioon of ADP in muscles

**10.** A sportsman needs to improve his sporting results. He was recommended a drug containing carnitine. What process is activated by this compound in the first place?
**A. *Transport of fatty acids**
**B.** Transport of amino acids
**C.** Transport of calcium ions
**D.** Transport of glucose
***E.*** Transport of vitamin *K*

**11.** Rheography of an 18 year old student during exercise showed redistribution of blood flow between organs. The peak blood flow will be observed in the following vessels:
**A. *Skeletal muscles**
**B.** Liver
**C.** Cerebrum
**D.** Kidneys
**E.** Gastrointestinal tract

**12.** A non trained man has usually muscular hypoxy after a sprint. What metabolite accumulates in the muscles as a result of it?
**A. *Lactate**
**B.** Ketone bodies
**C.** Glucose 6-phosphate

**D.** Oxaloacetate
**E.** –

**13.** Myocyte cytoplasm contains a big number of dissolved metabolites of glucose oxidation. Name one of them that turns directly into lactate:
**A. *Pyruvate**
**B.** Oxaloacetate
**C.** Glycerophosphate
**D.** Glucose 6-phosphate
**E.** Fructose 6-phosphate

**14.** Unskilled people usually have muscle pain after sprints as a result of lactate accumulation. What biochemical process may it be connected with?
**A. *Glycolisis**
**B.** Gluconeogenesis
**C.** Pentose-phosphate cycle
**D.** Lypogenesis
**E.** Glycogenesis

**15.** A traumatology unit received a patient with crushed muscular tissue. What biochemical indicator of urine will be raised in this case?
**A. *Creatinine**
**B.** Total lipids
**C.** Glucose
**D.** Mineral salts
**E.** Uric acid

**16.** Characteristic sign of glycogenolisis muscle pain during physical work. Blood examination usually reveals hypoglycemia. This pathology is caused by congenital deficiency of the following enzyme:
**A. *Glycogenphosphorylase**
**B.** Glucose6-phosphatedehydrogenase
**C.** α-amylase
**D.** γ-amylase
**E.** Lysosomalglycosidase

**17.** A 50-year-old woman diagnosed with cardiac infarction has been delivered into an intensive care ward. What enzyme will be the most active during the first two days?
**A. *Aspartate aminotransferase**
**B.** Alanineaminotransferase
**C.** Alanineaminopeptidase
**D.** LDH4
**E.** LDH5

**18.** A 46-year-old female patient has continuous history of progressive muscular (Duchenne's) dystrophy. Which blood enzyme changes will be of diagnostic value in this case?
**A. *Creatine phosphokinase**
**B.** Lactate dehydrogenase
**C.** Pyruvate dehydrogenase

**D.** Glutamate dehydrogenase
**E.** Adenylate cyclase

**19.** When blood circulation in the damaged tissue is restored, lactate accumulation stops and glucose consumption decelerates. These metabolic changes are caused by activation of the following process:
**A. *Aerobic glycolysis**
**B.** Anaerobic glycolysis
**C.** Lipolysis
**D.** Gluconeogenesis
**E.** Glycogen biosynthesis

**20.** A patient is diagnosed with cardiac infarction. Blood test for cardiospecific enzymes activity was performed. Which of the enzymes has three isoforms?
**A. *Creatine kinase**
**B.** Lactate dehydrogenase
**C.** Aspartate transaminase
**D.** Alanine transaminase
**E.** Pyruvate kinase

**21.** 6 hours after the myocardial infarction a patient was found to have elevated level of lactate dehydrogenase in blood. What isoenzyme should be expected in this case?
**A. *_LDH_1**
**B.** *LDH*2
**C.** *LDH*3
**D.** *LDH*4
**E.** *LDH*5

**22.** A patient suffering from stenocardia was taking nitroglycerine which caused restoration of blood supply of myocardium and relieved pain in the cardiac area. What intracellular mechanism provides restoration of energy supply of insulted cells?
**A. *Intensification of ATP resynthesis**
**B.** Reduction of ATP resynthesis
**C.** Increased permeability of membranes
**D.** Intensification of oxygen transporting into the cell
**E.** Intensification of RNA generation

**23.** 12 hours after an accute attack of retrosternal pain a patient presented a jump of aspartate aminotransferase activity in blood serum. What pathology is this deviation typical for?
**A. *Myocardium infarction**
**B.** Viral hepatitis
**C.** Collagenosis
**D.** Diabetes mellitus
**E.** Diabetes insipidus

**24.** A patient presents high activity *LDH*1,2, aspartate aminotransferase, creatine phosphokinase. In what organ (organs) is the development of a pathological process the most probable?
**A. *In the heart muscle (initial stage of myocardium infarction)**
**B.** In skeletal muscles (dystrophy, atrophy)
**C.** In kidneys and adrenals
**D.** In connective tissue
**E.** In liver and kidneys

**25.** As a result of exhausting muscular work a worker has largely reduced buffer capacity of blood. What acidic substance that came to blood caused this phenomenon?
**A. *Lactate**
**B.** Pyruvate
**C.** 1,3-bisphosphoglycerate
**D.** 3-phosphoglycerate
**E.** –

**26.** After a sprint an untrained person develops muscle hypoxia. This leads to the accumulation of the following metabolite in muscles:
**A. *Lactate**
**B.** Ketone bodies
**C.** Acetyl CoA
**D.** Glucose 6-phosphate
**E.** Oxaloacetate

**27.** A 49-year-old driver complains about unbearable constricting pain behind the breastbone irradiating to the neck. The pain arose 2 hours ago. Objectively: the patient's condition is grave, he is pale, heart tones are decreased. Laboratory studies revealed high activity of creatine kinase and *LDH*1. What disease are these symptoms typical for?
**A. *Acute myocardial infarction**
**B.** Acute pancreatitis
**C.** Stenocardia
**D.** Cholelithiasis
**E.** Diabetes mellitus

**28.** A considerable increase of activity of MB-forms of CPK (creatinephosphokinase) and LDH-1 was revealed on the examination of patient's blood. What is the most likely pathology?
**A. *Miocardial infarction**
**B.** Hepatitis
**C.** Rheumatism
**D.** Pancreatitis
**E.** Cholecystitis

**29.** The calcium canals of cardiomyocytes have been blocked on an isolated rabbit's heart.What changes in the heart's activity can result from it?
**A. *Decreased rate and force of heart beat**
**B.** Decreased heart beat rate
**C.** Decreased force of the contraction
**D.** Heart stops in systole

**30.** The gluconeogenesis is activated in the liver after intensive physical trainings.What substance is utilized in gluconeogenesis first of all in this case:
**A. *Lactate**
**B.** Pyruvate
**C.** Glucose
**D.** Glutamate
**E.** Alanine

*Glossary*

***acetylcholine (ACh)*** - neurotransmitter that binds at a motor end-plate to trigger depolarization.

***actin*** - protein that makes up most of the thin myofilaments in a sarcomere muscle fiber.

***action potential*** - change in voltage of a cell membrane in response to a stimulus that results in transmission of an electrical signal; unique to neurons and muscle fibers.

***autorhythmicity*** - heart's ability to control its own contractions

***calmodulin*** - regulatory protein that facilitates contraction in smooth muscles

***hyperplasia*** - process in which one cell splits to produce new cells

***desmosome*** - cell structure that anchors the ends of cardiac muscle fibers to allow contraction to occur

***intercalated disc*** - part of the sarcolemma that connects cardiac tissue, and contains gap junctions and desmosomes

***latch-bridges*** - subset of a cross-bridge in which actin and myosin remain locked together

***Myofibril*** - long, cylindrical organelle that runs parallel within the muscle fiber and contains the sarcomeres

***Myosin*** - protein that makes up most of the thick cylindrical myofilament within a sarcomere muscle fiber

***Sarcomere*** - longitudinally, repeating functional unit of skeletal muscle, with all of the contractile and associated proteins involved in contraction

***Sarcolemma*** - plasma membrane of a skeletal muscle fiber

***Sarcoplasm*** - cytoplasm of a muscle cell

***sarcoplasmic reticulum (SR)*** - specialized smooth endoplasmic reticulum, which stores, releases, and retrieves $Ca^{++}$

***stress-relaxation*** *response* - relaxation of smooth muscle tissue after being stretched

***T-tubule*** - projection of the sarcolemma into the interior of the cell

***thick filament*** - the thick myosin strands and their multiple heads projecting from the center of the sarcomere toward, but not all to way to, the Z-discs

***thin filament*** - thin strands of actin and its troponin-tropomyosin complex projecting from the Z-discs toward the center of the sarcomere

***troponin*** - regulatory protein that binds to actin, tropomyosin, and calcium

***tropomyosin*** - regulatory protein that covers myosin-binding sites to prevent actin from binding to myosin

***visceral muscle*** - smooth muscle found in the walls of visceral organs

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