Muscle Contraction

Introduction

Contractility and movement are basic properties of all animal cells. These two properties have reached their highest expression in specialized muscle cells. Contractility is not only expressd by muscles but also is found in amoeboid movement, chromosome and spindle activities during mitosis etc. Muscle physiology is one of the most interesting areas of animal physiology. The fibrillar nature of the muscles was first described by Bowman (1840).

Muscles are specialized for contractile ability. Muscle cells can change their shapes very rapidly and reversibly, exerting a mechanical force on neighbouring cells or environment. This change is regulated by the central nervous system and is initiated by the synaptic transmission. However, in certain types of muscles contraction can be produced by the excitation of the motor nerve action.

Although all muscles function on the same molecular basis, they differ considerably in rate of shortening, amount of force produced and so on. There is a wide variety of muscle types because there is a wide variety of functions to be served by muscles including movement of an animal through environment, maintainance of body posture and orientation, circulatory movements, gastrointestinal tract movements, etc. For survival in environment, different animals require different muscular outputs, and the basic contractile machinery has been adapted to various needs.

Muscle cells, in general contain special organelles, called *myofibrils* or *myofilaments*, composed of strands of protein molecules, the *actin* and *myosin* which can be differentiated into many kinds. A muscle cell is elongated, and the myofibrils are arranged through the horizontal axis.

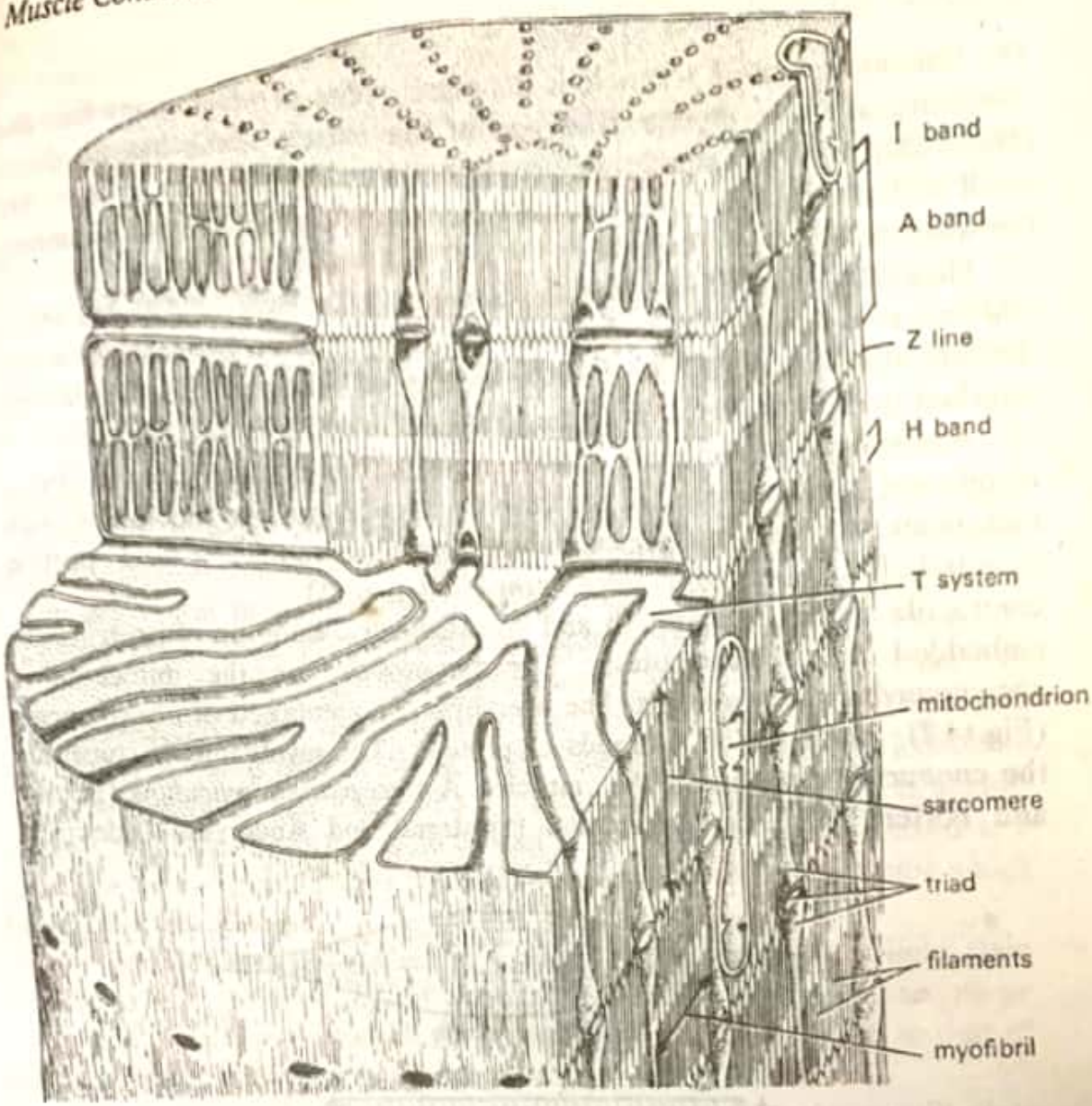


Fig.14.1. Internal structure of the striated muscle fibre. Plasma membrane extends into the T-system and endoplasmic reticulum.

Muscle cells are capable of changing their shapes very rapidly and reversibly, exerting on the neighbouring cells or environment, a mechanical force. This change is usually controlled by the central nervous system and is initiated by the synaptic transmission. However, in certain types of muscles contraction can be produced by the excitation of the motor nerve action.

Muscle cells organized in the form of muscles are very important for the functioning of many systems and occur in varied shapes such as flat sheets, cylinders, thin strands, hollow tubes or a loose network. Movement of the different organs, and the organism as a whole, posture of the animal, shape, functioning of the heart, blood vessels, acquisition of food by the movement of the mouth parts, swallowing, movement of the alimentary canal, expulsion of the secretory products of the glands, and functioning of the sense organs are all controlled by the activity of the muscles. Even in protozoans, contractile elements in the form of myonemes occur, while sponges have contractile epithelial cells or myocytes for the closing of osculum.

General Structure of Muscles

The functional unit of a muscle is the muscle fibre of which many fuse and constitute a whole muscle. The size of the muscle fibres has no direct relationship with the muscle they constitute. Some of the muscle fibres are small and short, while other muscles consist of large muscle cells running the whole length of the muscle.

Generally muscle fibres are covered over by a layer of collagen fibers and connective tissue. Near the ends, they form tendons by means of which they are attached to the bones. In the absence of bones, muscles are directly attached to the epidermis or septa through connective tissue fibres.

According to Bowman (1940), muscle fibres are covered by a membrane; *sarcolemma*, consisting of a typical plasma membrane with trilaminate structure and an outer basement membrane. The fluid portion in muscle fibre is called as *sarcoplasm* (Rollet, 1891)

contractile elements, *myofibrils* and *myofilaments*, made up of proteins are embedded in the sarcoplasm. The *sarcosomes* are the mitochondria accompanying the myofibrils. The myofibrils are composed of myofilaments (Fig.14.2) made up of a strands of protein. The myofilaments constitute the *contractile elements* of the muscles. A *sarcoplasmic reticulum* (Bennet and Porter, 1953) or *sarcotubules* (Sjostrand and Andersson-Cedergren,

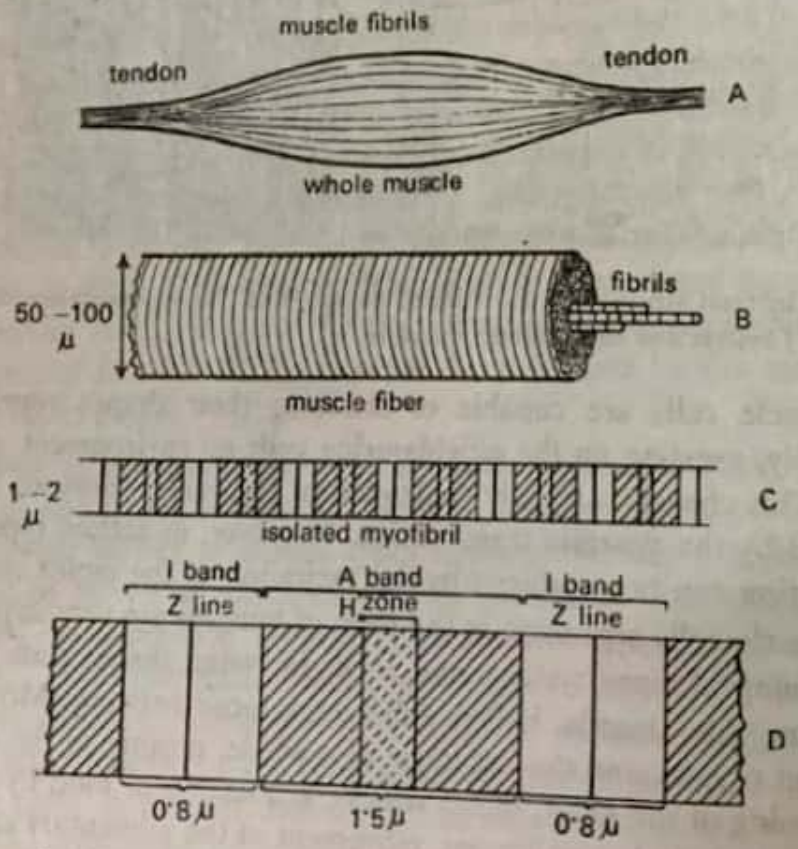


Fig. 14.2 Diagrams showing the arrangement of the contractile filaments in striated (skeletal) muscle. A – Whole muscle. B – Whole muscle consists of muscle fibres. C – Each muscle fibre is made up of striated myofibrils. D – The appearance of myofibril under polarized light.

1957), arranged in a definite fashion in relation to the myofibrils, is present in the muscles.

Muscles may be differentiated into smooth and striated types, though in strict sense these terms are very narrow to accommodate all the different varieties of muscles that exist. The *smooth muscles* include visceral muscles of vertebrates and a few varieties of invertebrates. *Striated muscles* denote all muscles whose fibres contain fibrils showing a periodic structure of repetitive parallel arrays of myofilaments. In this category are the skeletal muscles of frog, tail muscles of ascidian larvae, muscles of the crayfish gut, the muscle bundles of coelenterates etc.

Striped or striated muscles

The striated muscles exhibit an alternating arrangement of dark and light bands crossed by thin dark lines. Under the polarizing microscope, the dark bands are found to be doubly refracting; and the light bands are singly refracting (Weismann, 1913). Under the high power of a light microscope the striated pattern is seen as a regular alternation of isotropic 'I'-bands or light bands, through which light passes equally in all directions and anisotropic 'A'-bands or dark bands, possessing different refractive indices in different directions. The length of a A-band of a vertebrate fibril is usually about 1.5 microns and that of an I-band is 0.8 micron. In the I-band is a dark line known as the 'Z'-line, derived from the term *zwischenscheibe* which bisects the I-band.

In the middle of the A-band is another zone which takes a light stain known as the 'H'-band or 'H'-zone, derived from the German name "Hensen's" line. The portion of the muscle fibre from one Z-line to that of adjacent I-band is termed as the *sarcomere*.

Examination under electron microscope reveals that the myofibril is made up of two kinds of small filaments, one twice as thick as the other. The dense A-band consists of the overlapping thick and thin filaments; the lighter I-band consists of thin filaments, while the H-band consists of the

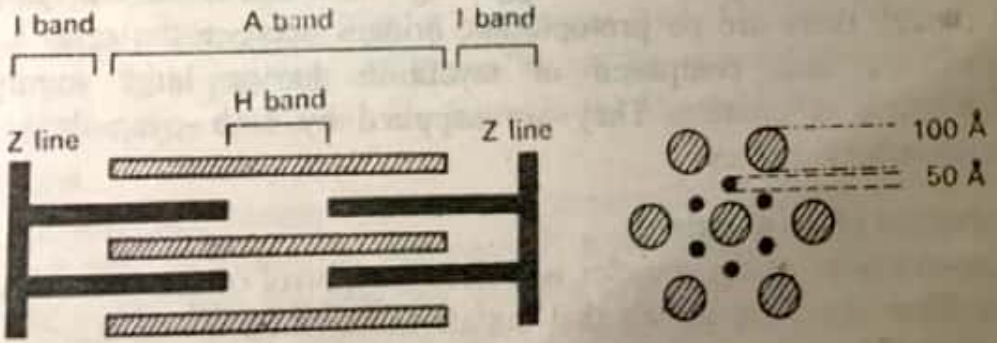


Fig. 14.3. Arrangement of thick and thin filaments. Left-Longitudinal view. Right-Cross section through A-band.

thick filaments only. The thin filaments about in the middle of their length pass through a narrow zone of dense material, the Z-line. The thicker filaments are about 100 Å in diameter and 1.5 microns long and the thinner ones are 50 Å in diameter and 2 microns long. Each thin filament lies in between three thick ones (Fig. 14.3). A myofibril of 1 micron diameter contains about 5,000 filaments in each cross section of an A-band.

Unstriated or smooth muscles

Classic or visceral smooth muscle. The smooth muscles of the stomach, gut, urinary bladder, uterus, the retractor muscles of the extrovert of the sipunculid worms, penis muscles of molluscs and the pharynx are composed of small muscle fibres (20-40 μm in length) with usually only one nucleus. In longitudinally arranged parallel filaments, as they do not form regular arrays, no optical pattern of dense and light bands are visible.

Helical smooth muscle. In this type, the myofibrils are helically arranged, and though the filaments are arranged longitudinally, do not show a periodic structure. A majority of the cephalopod molluscs and the somatic muscles of annelids are examples of this type of muscle.

Paramyosin muscle. Due to the presence of paramyosin or tropomyosin (Bailey, 1957; Kominz, *et al.*, 1957) in addition to actin and myosin, the tonic muscles of molluscs are termed as paramyosin muscles. Paramyosin in these muscles is in the form or ribbons of diameters ranging from 15 to 150 mμ. The paramyosin crystallizes during the contracted phase of the muscle and under this condition the ribbons show an axial periodicity of 15 mμ.

Cardiac muscle

The cardiac muscle fibres are short, cylindrical cells with a single nucleus. They are striated both longitudinally and transversely and are enclosed in it. These muscle fibres are branched and interdigitate. The two adjacent fibres unite with each other through the extensive series of their membrane folds. The junctions, which always occur at 2-lines, are called intercalated discs. The cardiac muscles are not structural syncytium, but the impulse spreads, quickly in the muscle meshwork suggesting that it is a functional syncytium, even though there are no protoplasmic bridges between the cells. Cardiac muscles are also composed of myofibrils having large number of mitochondria in contact. They are supplied by both sympathetic and parasympathetic nerves.

Innervation of the muscle

The contraction of the muscles is under the control of the nervous system. *Motor fibres* are those nerves that initiate the contraction of muscles, while *inhibitory fibres* prevent the contraction. Some of the nerve fibres regulate the continuously contracting muscles as the heart muscles. These are referred to as regulatory fibres. The supply of all these fibres to a muscle is

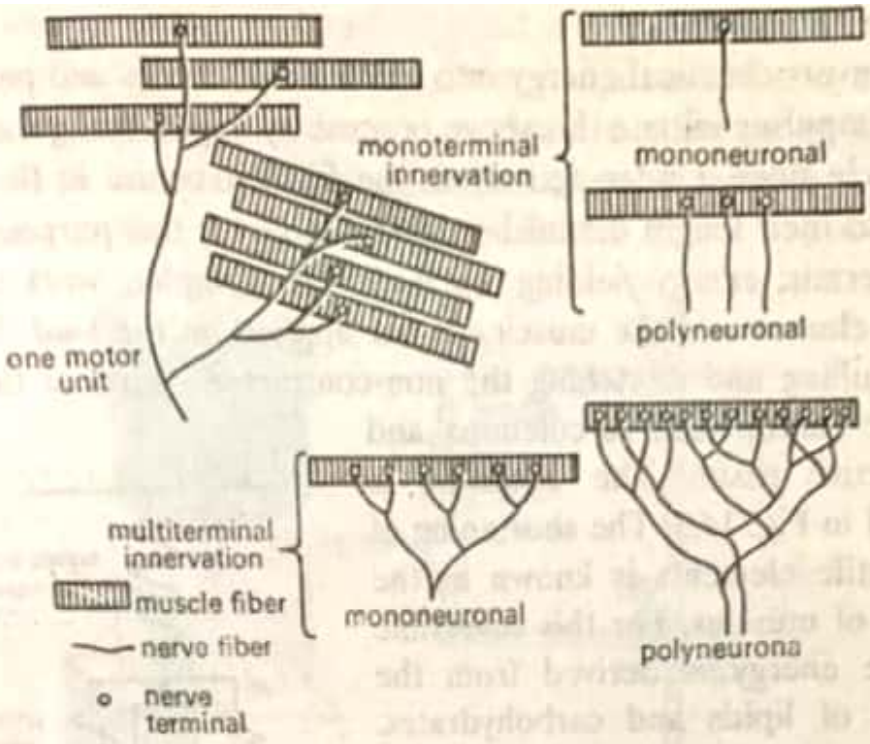


Fig. 14.4. Different types of innervation of muscle.

called its innervation. *Myoneural junction* or *neuromuscular junction* is the point where a synapse of an efferent nerve makes contact with the muscle fibre. The sensory nerve supply to the muscle is known as the *afferent* supply, while the motor supply is the *efferent* supply.

A single motor nerve may supply several fibres of muscle or fibres of different muscles. A *polyneural junction* (Hoyle 1957) is one in which muscle fibres receive innervation from two or more efferent axons. When a muscle fibre is innervated by several branches of one or more axons, the condition is called as *multiterminal innervation* (Fig. 14.4).

Motor unit

Motor nerves that initiates the contraction of muscles may supply any number of muscle cells. One motor nerve fibre, with all the muscle fibres it supplies constitute a *motor unit*. The number of muscle fibres in a motor unit may vary from as low as 3 to as high as 3000. In most fast muscles like those of hand (finger) and those concerned with eye motion (eye muscles) the muscle fibres are 3 to 6 per motor unit. On the other hand about 120-162 fibres per unit are known in cat leg muscles and even more upto 3000 fibres per motor unit are found in the leg and back (postural) muscles of man.

Infact the strength of a muscular contraction depends on the number of motor units activated at one time. If a stimulus is weak, muscle fibre of lesser motor units will contract resulting in a weak response. However, if the stimulus strength is increased, more motor units are stimulated resulting in a stronger contraction until all the motor units excited. After such a stage any further increase in stimulus strength will not increase further the strength of contraction.

Simple contraction or muscle twitch

The response of a muscle to a single brief stimulus, such as an electric shock, is known as *twitch* (Fig.14.8). A twitch can be divided into three portions or phases.

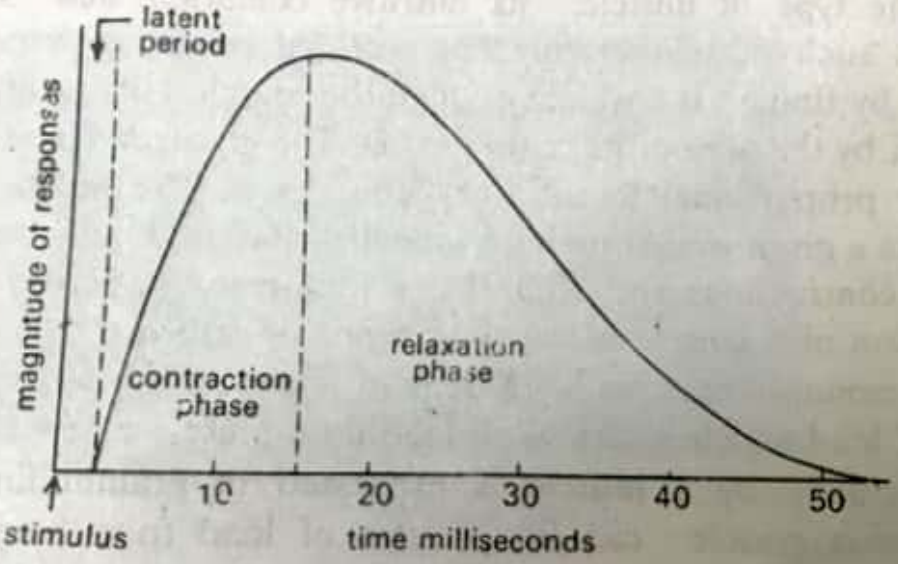


Fig. 14.8. Single contraction of skeletal muscle showing different components.

(1) A *latent period* in which the length of the muscle remains constant.
(2) A *contraction period* during which the muscle shortens, and
(3) A *relaxation period* in which the length of the muscle and its tension reach the normal level.

Latent period. By very sensitive recording methods, Roos (1932) found that the true latent period is of the order of 0.4 milliseconds. The duration

of the latent period varies with the species, type of muscle, temperature and internal conditions of muscle.

The latent period covers the time between the stimulation and the activation of the lever. This period covers two conditions over the muscle namely, the excitation wave due to the electrical disturbances, and the development of tension, which, according to Roos, spreads simultaneously with the electrical disturbance. Sandow (1944) has devised a method to convert the mechanical energy into electric potential and this is recorded by cathode ray oscillograph. It has been shown by this method that the isometric contraction of the frog sartorius muscle not only has a true latent period, but the muscle actually relaxes a little (latency relaxation) before developing tension. If the total latent period is 3.5 m. sec., the quiescent period is about 1.5 m sec. after direct excitation of the muscle during which no tension develops. This is followed by the latency relaxation period of about 1.5 m. sec. If the muscle is excited indirectly through nerves, the latent period increases. Thus, if the latent period is about 3.5 m. sec. for direct excitation of the muscle, it is 6.0 m. sec. for indirect excitation.

Contraction period. During this phase, in isotonic contraction shortening of the muscle filament takes place. The dark bands of the fibrils become shorter and wider. The light bands also decrease in length in isotonic contraction and may slightly increase in length during isometric contraction. During the phase of shortening of the muscle fibres, external work is done. The work done by a muscle is dependent on the size of the muscle, the type of muscle, its nutritive condition, and upon external conditions such as temperature.The working power of a muscle can be estimated by finding the weight or load the muscle fails to lift and dividing this weight by the area of its cross section. The glycogen content of a muscle is directly proportional to the work done by it. The height to which the muscle lifts a given weight under maximal excitation usually increases for the first 5-15 contractions and after that a long resting period follows. This phenomenon of a muscle is called as *treppe* or *staircase.*

The amount of external work done by a muscle is also dependent on the amount of load which it lifts. With load on a muscle external work is done. The work done by a muscle is expressed in grammillimeters. A frog gastrocnemius muscle can lift 20 gms of load to a height of 5mm by performing 100 g. mm of work. With increased amounts of load, if a single muscle is made to give a series of single contractions, the work done shows an optimum at a certain weight above and below this point the work done decreases gradually.

Relaxation period. Relaxation phase is the reversal of the contraction phase and involves activity which is dependent upon certain physicochemical changes within the muscle cells. Relaxation phase consumes more time than the contraction process. It is dependent upon certain conditions in the

muscle. Cold prolongs the relaxation process and during fatigue also this phase is prolonged very much. The failure of a muscle to relax is known as *contracture.*

Tetanus. If a muscle fibre is stimulated before it relaxes for a scond time, it can contract again. Therefore, a muscle can be maintained in a continuously contracted phase, if stimulated frequently within a given time. A continuous contraction of this type is known as "tetanus" (Fig. 14.9).

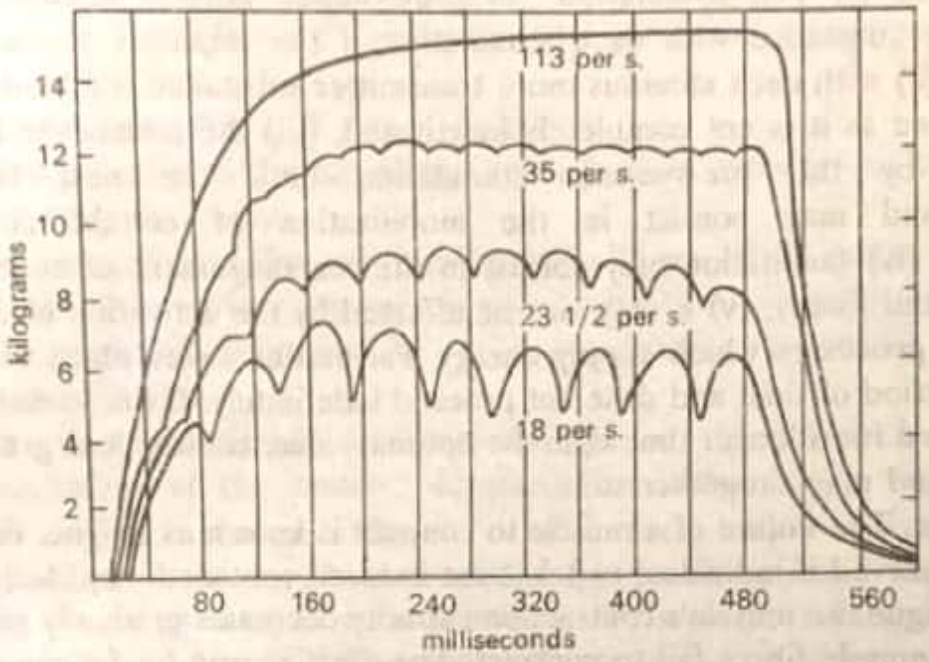


Fig. 14.9. Complete and incomplete tetanus due to stimuli on the gastrocnemius muscle.

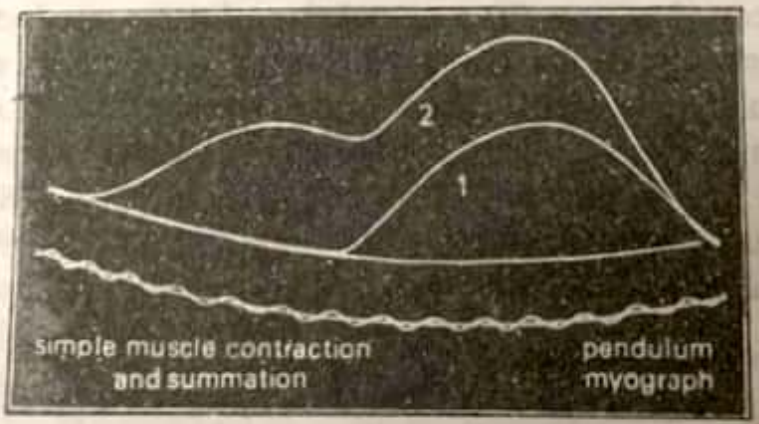


Fig. 14.10. Showing summation.

Summation. When the pre-synaptic portion of a nerve is stimulated more than two times, it tends to have an additive effect on the post synaptic portion. In the case of muscles, the effects may either be mechanical or electrical; accordingly summation or addition takes place in the electric behaviour of the membrane of the muscle fibre and in the contractile elements. However, as the electric membrane responses are of short duration, it is essential that the stimulations should be at short intervals. In muscles a series of stimuli causes contraction, which gradually increase and

the resulting final contraction is greater than a simple contraction, excited by a stimulus of greater intensity and excites all the fibres of a muscle. The additive effect of repeated contractions is known as summation (Fig. 14.10).

Facilitation. In contrast to summation, facilitation, refers to the series of contractions resulting from repeated stimulations. Facilitation is not a single phenomenon and various theories have been put forward to explain this process. (i) The stimulation of pre-synaptic portion releases more transmitter substance with each stimulation if the impulses are at close intervals, (ii) with each stimulus more transmitter substance is added to the previous one as it is not completely inactivated, (iii) the membrane is kept activated by the pre-synpatic stimulation until the next impulse arrives and may consist in the mobilization of certain chemical processes, (iv) facilitation may consist in the rearrangement of contractile filaments and finally, (v) it may also be affected by the activation of certain metabolic processes which supply energy. Facilitation takes place within a definite period of time and dose not proceed indefinitely. If the stimulations are rapeated for a longer time than the optimal value, contractions gradually decrease and may altogether stop.

Fatigue. The failure of a muscle to contract is known as fatigue. Fatigue may be observed if individual twitches are induced continually by electricity. During fatigue the muscle's contraction capacity decreases gradually and the individual muscle fibres fail to contract. The exact reason for fatigue is not known, various explanations have been made : (i) the expension of the metabolic sources of energy, (ii) accumulation of waste products, (iii) loss of potassium ions from the post-synaptic cell and accumulation of the same in the extracellular space, (iv) increase in the concentration of sodium ions in the post-synaptic cell, and (v) expension of the stored transmitter substance in the pre-synaptic cells. Experiments have shown that removal of the waste products, formed during contraction by repeatedly washing the muscle in a balanced salt solution, delays fatigue.

Physical changes during muscle contraction

During the contraction of muscle a number of changes take place. Important physical changes are given below :

1. Shortening. During isotonic contraction of the muscles cells get shortened and the shortening (degree of response) is proportional to the number of motor units involved and therefore, the strength of stimulus.

2. Viscosity. Muscle contraction causes densening of the sarcoplasm. In other words the viscosity of the cytoplasm is increased during muscle contraction.

3. Tone. Isometric contraction of the muscles is subjected to a considerable increase of tonicity. However, it does not change during isotonic contraction.

4. Production of heat. During muscle contraction heat is produced. In fact the high energy bonds of phosphagenes and ATP release energy which is used up in work done by the muscle. Considerable amount of this energy is converted into heat energy which is also helpful in maintaining body heat in homeotherms. That is why we feel hot after excercise and the body temperature shoots up after shivering in malaria.

Electrical changes. As recorded by two electrodes connected to an oscilloscope the following sequence wise electrical events occur during muscle contraction.

(1) Resting potential (-70 mv) is disturbed.
(2) The potential difference along two surfaces of sarcolemma comes to 0.0 mv (depolarization).
(3) The potential difference reaches to +35 mv which suggests that the inner surface of sarcolemma is positive by 35 mv (reverse polarization). A potential difference of −70 mv (resting potential) is set in repolarization.

As described already the functional unit of the muscle is sarcomere. On excitation of the muscle, depolarization followed by repolarization occurs. These changes liberate Ca^{++} into the sarcoplasm from T-sarcotubular system to L-sarcotubular system. These ions bring about the sliding (interdigitation) of actin filaments into myosin. During this process energy is supplied by ATP. The degree of interdigitation of actin and myosin is dependent on the amount of Ca^{++}. The amount of Ca^{++} liberated is dependent on electrical events and finally on the intensity of stimulus to a certain extent. Thus the strength of such contraction is dependent on the stimulus intensity to certain extent.

Histological changes. The following histological changes are seen during interdigitation of action and myosin.

(1) The two adjacent Z-lines come closer.
(2) I-bands disappear.
(3) There is no change in the A-band except that H zone disappears.

All these histological changes in a sarcomere are dependent on the strength of its contraction. In a strong contraction the two Z-lines come closest and H-zone disappears almost completely. Thus Ca^{++} and the ATP are very essential factors for muscle contraction.

Biochemistry of the contractile proteins

The bulk of the striated muscles is made up of three proteins : myosin 54%; actin 20.25%; and tropomyosin 11%. The properties of the contractile mechanism are dependent upon the characteristics and reactions of these proteins. Actin and myocin are extremely complex macromolecules.

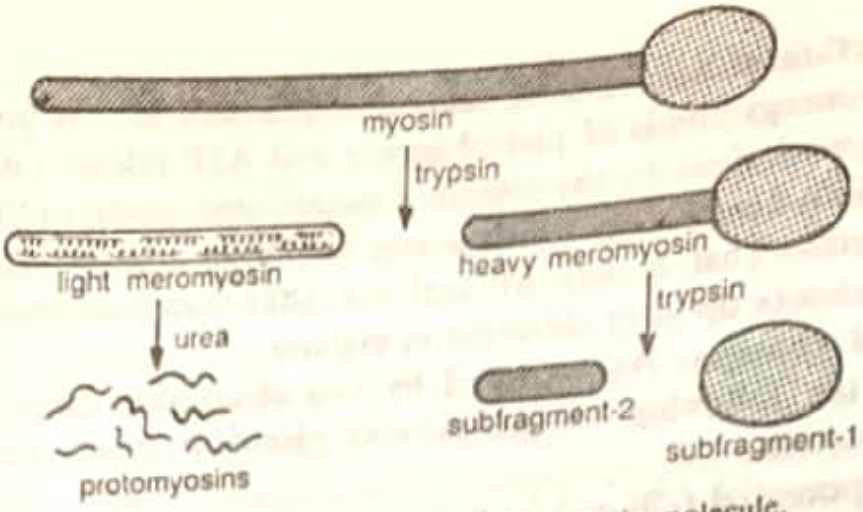


Fig. 14.11. Various subunits of the myosin molecule.

Myosin. Biochemical and physiological studies have shown the fibrous protein *myosin* to be the main constituent of the thick filaments. Myosin reacts with itself, other proteins, ATP and with divalent cations. Part of its biochemical properties are dependent on the strength of the ionic medium. Myosin possesses the properties of the enzyme Adenosine Triphosphotase and can combine with actin to form the complex protein **actomyosin.**

The molecular weight of myosin is 490,000. It can be hydrolyzed into a variety of subfragments whose characteristics are dependent upon the agents and conditions used to break bonds in the molecule. (Fig. 14.11). On digestion with trypsin myosin yields two major subfragments, the **light meromyosin** (LMM) and **heavy meromyosin** (HMM). Light meromyosin on treatment with alcohol leads to the formation of a soluble fraction LMM-I. Thus, LMM contains LMM-1 and other proteins. These subfragments are formed due to the breakage of peptide bonds and are not subunits of LMM from which a polymeric myosin is built up. Subunits of myosin are produced on treatment with urea due to breakage of hydrogen bonds. HMM molecule possesses a globular head and a tail region. The properties of the various fragments are shown in table 14.1 :

Table 14.1. **Physical properties of some contractile proteins.**

Protein	Molecular weight	Length (nm)	Width (nm)
Myosin (overall)	490,000	152	2
Myosin (tail)		15-25	4
Heavy meromyosin	350,000	50	–
Globular head		20	4-5
Tail		40	2
Light meromyosin	135,000	80	–
G-Actin	60,000		6

The shape of the myosin molecule is the result of the arrangement of the meromyosin molecules. There is one HMM molecule to one LMM in myosin. As HMM combines with actin, the bridges on the myosin molecule are the polypeptides of HMM subfragment. These bridges are used to combine actin and myosin during muscle contraction.

Actin. Actin occurs in two physical forms. *Fibrous actin (F-actin)* is a polymer of *Globular actin (G-actin)*. G-actin has a molecular weight of 60,000. The molecule is spherical with a diameter of 5.5 nm. G-actin polymerizes into F-actin in the presence of salts. 1 mole of ATP is bound per mole of G-actin and it is hydrolysed during the formation of the polymer, F-actin. Actin is soluble in 0.6 N KCl solution. It binds with calcium to form calcium actinate.

Actomyosin. Actin and myosin combine together to form actomyosin. This is accompanied by a great increase in viscocity. The combination rate is increased by Ca^{2+} or Mg^{2+} at ionic strength greater than 0.3M. The addition of ATP to actomyosin solution leads to the dissociation of the complex. At low ionic strengths the actomyosin is insoluble.

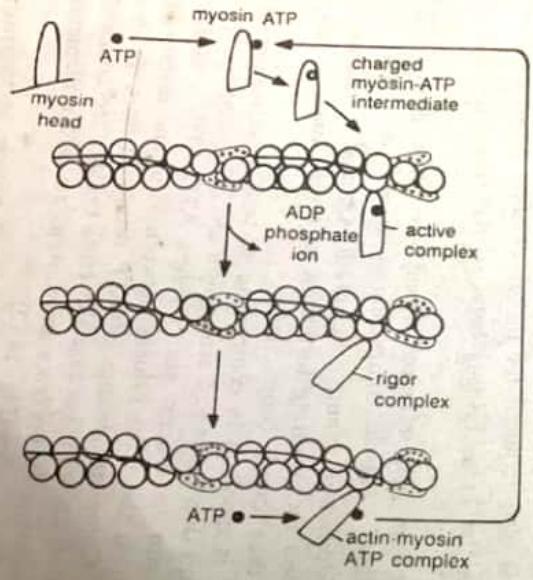
The processes of contraction and relaxation are controlled by a series of complex interactions of the contractile proteins, ATP, Ca^{2+}, Mg^{2+} and regulatory proteins. Pure myosin in the presence of Ca^{2+} and Mg^{2+} concentrations similar to active muscle, shows a low ATPase activity but in combination with actin, ATPase activity is much higher.

Mg^{2+} is required for actomyosin ATPase activity which is essential for muscle contraction. It is also required for the dissociation of actomyosin complex. Mg^{2+} breaks the cross bridges between the filaments briefly during contraction.

Under high magnesium concentration actomyosin dissociates, ATP hydrolysis is stopped and muscle contraction is prevented leading to relaxation of muscle. Addition of minute concentrations of Ca^{2+} overcomes inhibition of contraction by favouring formation of actomyosin complex, hydrolysis of ATP and thus muscle contraction.

Role of ATP. ATP is involved in contraction as well as relaxation of muscle, as formation and dissociation of actomyosin are influenced by ATP. Polymerization of G-actin to F-actin also involves ATP. ATP is also a plasticizer that can loosen the cross bridges between actin and myosin at higher concentrations. Complete absence of ATP results in *rigor*. In this state, stretching of muscles tears it because the cross bridges are attached and the formation of actomyosin prevents any movements of the filaments without destruction of the complex (Fig. 14.12).

Tonomyosin. Arterial wall is made up of smooth muscle. Actomyosin extracted from this source and uterine wall consists of a protein called *tonomyosin*. It is soluble in lower ionic strengths than actomyosin from



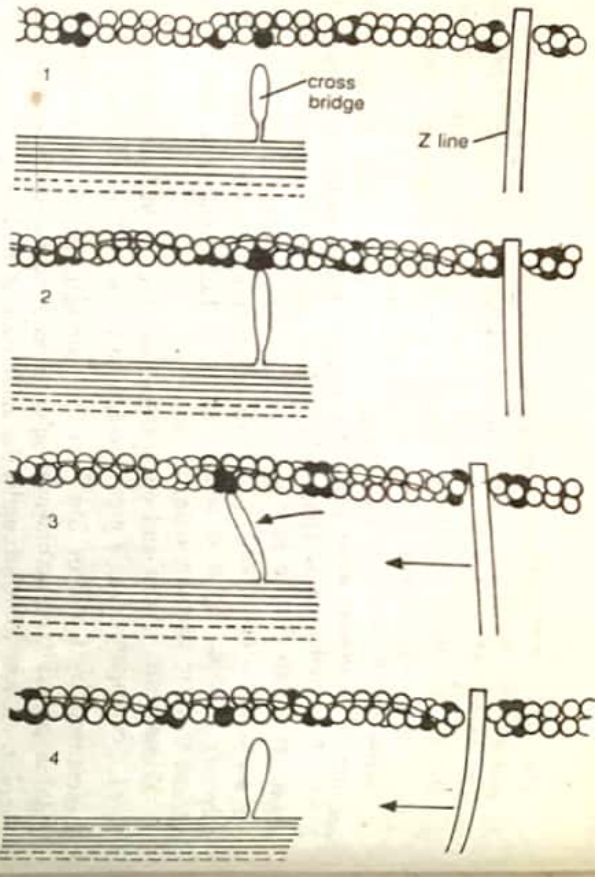


Fig. 14.12. (A) The chemical events of the contraction. The troponin molecule is fully occupied by calcium ions (small dots), and hence all of its inhibitory activity is supressed. Thus as soon as the charged myosin-ATP intermediate is formed, it combines with an actin molecule, and the ATP is split. Sufficient ATP is present to allow the rigor complex to be broken up and the myosin recycled through the process. (B) The events of the contraction cycle as they occur in intact muscle.

skeletal muscle. Tonomyosin requires ten times greater Ca^{2+} strength than actomyosin for activity.

Tropomyosin. *Tropomyosin* makes up about 5% of the dry weight of the muscle. It is a rod shaped protein molecule about 41nm long and 2nm is diameter. It has a molecular weight of about 70,000 and consists of two chains in an α-helical coiled coil arrangement . Tropomyosin is intimately associated with actin and lies in the grooves of actin double helix (Fig. 14.13) Tropomyosin A and Tropomyosin B are the two known forms of Tropomyosin. It has no ATPase activity.

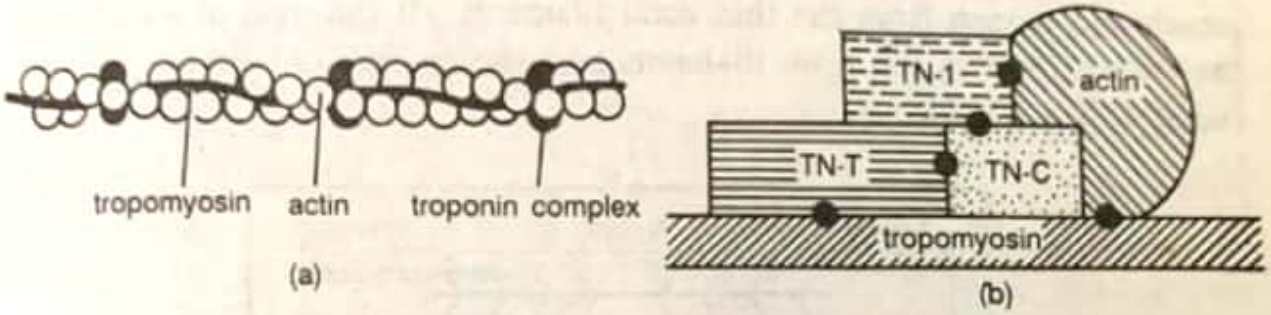


Fig. 14.13. (a) Model for the structure of the thin filament. The tropomyosin molecule lies in the groove of the double-helical actin. Two molecules of tropomyosin are present in each period of the actin helix, and two molecules of the troponin complex are also present in each period. (b) Hypothetical model showing proposed relationships among actin, tropomyosin, and components of the troponin complex. The solid circles represent probable sites of binding.

Troponin. *Troponin* is another protein associated with tropomyosin and actin. Its molecular weight is 80,000 and it is a complex of three proteins. Troponin-I or TN-I (mol. wt. 24,000), Troponin-C or TN-C (mol. wt. 18,000) and Troponin-T or TN-T (mol. wt. 37,000). TN-I is an inhibitory protein component that prevents the association of actin and myosin; TN-C binds specifically with Ca^{2+} and TN-T combines with tropomyosin. Although TN-C combines with only one calcium ion, when TN-C is complexed with TN-I, a second binding site appears and probably in muscle TN-I combines with two calcium ions (Fig. 14.13).

Troponin-tropomyosin complex prevents the formation of bridges between the actin and myosin filaments and the activity of actomyosin-ATPase, in the absence of calcium, when no contraction occurs. In the presence of calcium, TN-C binds with calcium and activates actomyosin resulting in contraction. Calcium produces conformational changes in the troponin complex, which affects the tropomyosin. Tropomyosin shifts its position in the actin helix grooves and exposes protein sites for interaction with the myosin molecule. Molluscan muscle is

controlled through the myosin and not through actin and tropomyosin troponin.

Tropomyosin-A or *Paramyosin* is found in the invertebrate slow or catch muscles of molluscs. Its molecular weight is 220,000 and size varies with species. Paramyosin is a dimer of two α-helixes packed side by side.

Mechanism of Muscle Contraction

Sliding filament model

Electron microscopic studies have shown that lateral extensions project outwards from the thick myosin filaments. These cross bridges alternately attach and detach from the thin actin filaments. At the time of attachment they move pulling the actin filaments past the myosin and deeper into the A-band (Fig. 14.14).

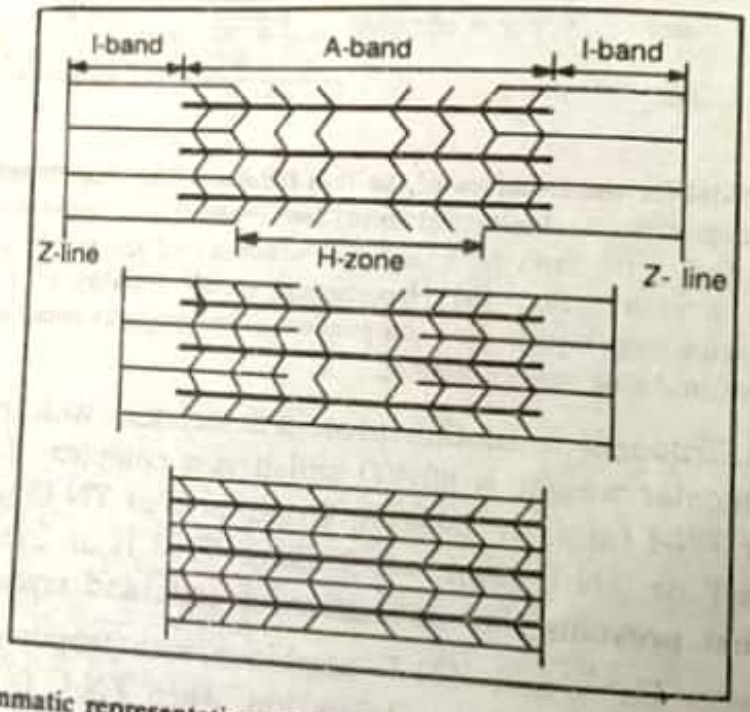


Fig. 14.14. Diagrammatic representation of the nature of contraction according to the sliding filament hypothesis. As shown in the middle and lower illustrations, shortening is the result of the inward movement of actin filaments into the A-band, drawing the Z-lines of each sarcomere inward.

Operation of the cross bridges. A space of about 13nm intervenes between the actin and myosin filaments. Cross bridges are visible in the H-zone of sarcomere in relaxed condition also, and in this zone actin filaments are absent, it is evident that the cross bridges arise from the myosin filament. The cross bridges are 4-5 nm wide and 12 nm long, and are considered to be formed of the HMM part of the myosin. Isolated HMM molecules possess a globular head region about 4-5 nm wide and 20 nm long, and a tail of about 40 nm long. The cross bridges consist of the

globular head region of the HMM and its tail region lies parallel to the thick filament. HMM possesses ATP and ATPase activity and also can combine with actin. Therefore, this region can form cross bridges which interact with the actin filament during contraction of muscle (Fig.14.15).

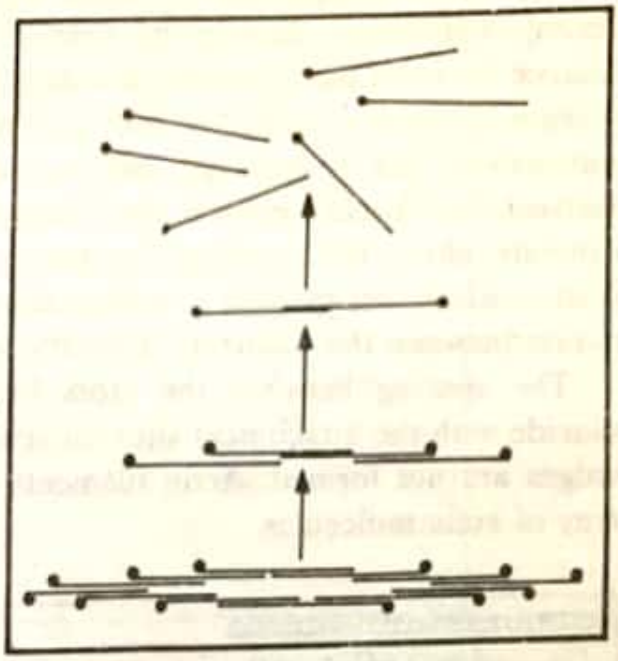


Fig. 14.15. Model for the arrangement of components in the myosin filament. Light meromyosin forms the backbone of the filament, and heavy meromyosin forms the movable cross bridge.

Regarding the mechanism of the sliding of the actin filaments, the cross bridges at some time during the process of contraction attach to the actin and a cyclic back and forth movement of the cross bridges brings about sliding movement. During this process, the cross bridges make contacts with the sites on actin filaments and break them and slide the thin filaments. Myosin molecules are arranged with a different orientation on either side of the center so that actin filaments from either side of the sarcomere can be moved inward during the process of contraction. The arrangement of the cross bridges on the myosin filaments is shown in (Fig. 14.16).

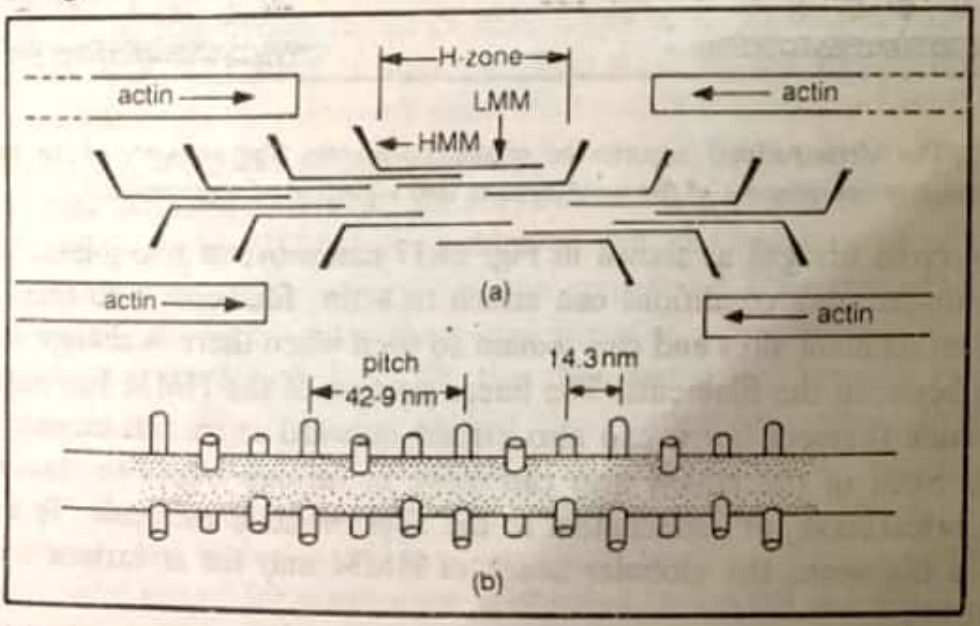


Fig. 14.16. (a) Showing the arrangement of myosin molecules in the thick filament. LMM forms the backbone of the thick filament, and HMM forms the cross bridge. The orientation of myosin molecules is different on each side of the center so that actin filaments can move from both sides of the sarcomere. (b) Model of the myosin filament showing the helical arrangement of cross bridges along the filament.

. The cross bridges are arranged helically over the myosin filament. During contraction, there is no change in the volume of muscle but the distance between the filaments increases by 18%. In the resting muscle the distance between actin and myosin is 21 nm which increases to 25 nm during contraction. The reason for this increase in spacing is the long range electrostatic forces between the filaments. Any overlap between the two filaments alters the spacing, for example contraction of muscle. These electrostatic forces provide a cushion along which the filaments slide, so that friction between the filaments is minimized.

The spacing between the cross bridges on myosin filament do not coincide with the attachment sites on actin filaments. Therefore, rigid cross bridges are not formed. Actin filaments are composed of a double helical array of actin molecules.

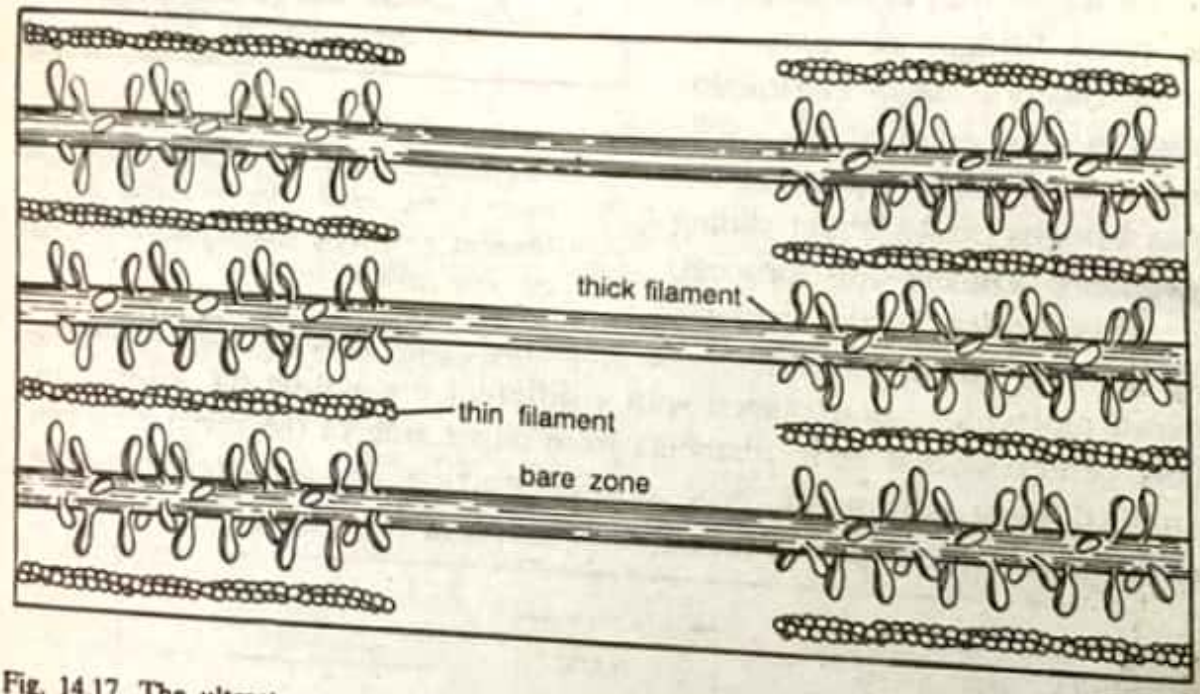


Fig. 14.17. The ultrastructural organization of skeletal muscle. The assembly of the various protein components of the myofilaments into a portion of a sarcomere.

The cross bridges as shown in Fig. 14.17 can move at two joints. Cross bridges under such conditions can attach to actin filaments with their non aligned attachment sites and can remain so even when there is change in the spacing between the filaments. The linear portion of the HMM lies parallel to the thick filament but it can also extend outward at various angles. The globular head of the HMM also can move in various angles to maintain proper orientation for attachment to the sites on actin molecule. To move the actin filaments, the globular heads of HMM may tilt at various angles (Fig. 14.18).

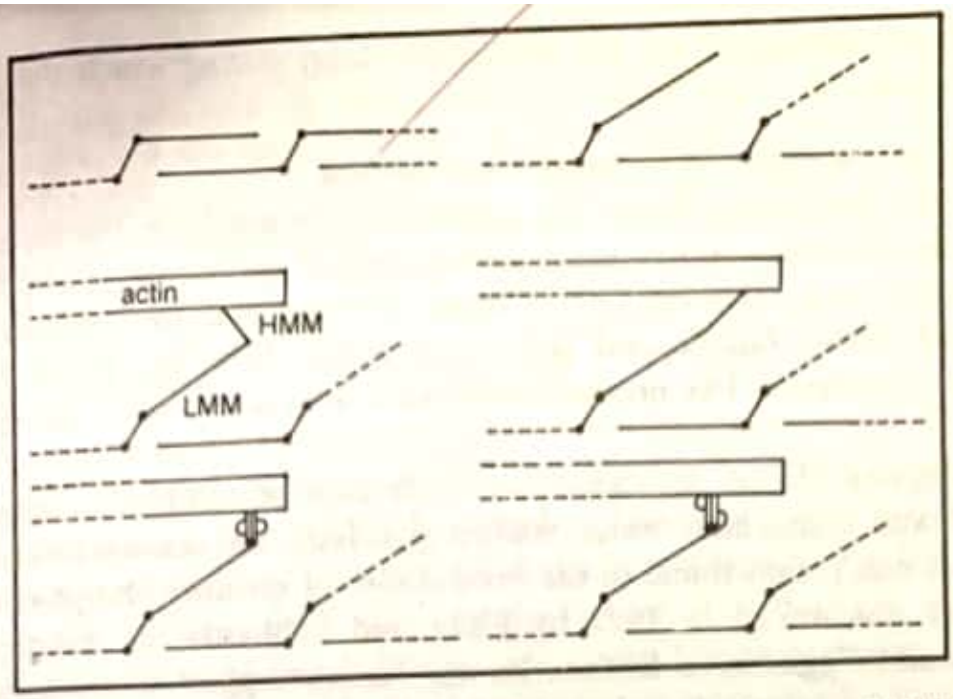


Fig. 14.18. Model for the arrangement of components in the myosin filament. LMM forms the back-bone of the filament.

Inorganic ions. The inorganic ions present in the muscles are potassium, sodium, calcium, magnesium, sulphate, bicarbonate and phosphate. The concentration of the potassium ions is higher in the muscles than in the extracellular fluid but the reverse is the condition with sodium ions. Chloride ions are present in much higher concentration in the outside fluid.

Immediate source of energy for contraction

Actin and myosin when mixed together form *actomyosin*. ATP when added to a solution of actomyosin in 0.6 N KCl, dissociates the actomyosin into actin and myosin. ATP itself is converted into ADP by the myosin formed. However, actomyosin is reformed when all the ATP is converted into ADP. Szent-Gyorgyi (1942) has demonstrated that muscle fibres, stored in cold 50% glycerol although no longer electrically excitable, could contract when placed in ATP solution. If inhibitors which prevent the break-down of ATP are added, no contraction occurs, but the contracted fibre extends to its original length. ATP acts under these conditions as a *plasticizer*. Thus, two actions can be attributed to ATP, the first being its breakdown and contraction of the fibre and the second the *plasticizing* action. In living muscle, actin and myosin are arranged in separate filaments, which slide along each other during interaction with ATP. The enzyme action of myosin during resting state is suppressed in a way which is not yet fully understood. The immediate energy for contraction is donated by the breakdown of ATP with the release of an activator liberated by a balance between the calcium and magneasium ions. The contraction of the muscle is effected by the interaction between the ATP, actin and myosin and subsequent closer interdigitation of the actin and myosin filaments. The plasticizing action of

the ATP is responsible for the relaxation stage, during which the muscle extends again.

Ultimate source of energy for muscle contraction

Though the immediate energy for contraction comes from the breakdown of ATP, the ultimate source is the metabolism of carbohydrate and fat.

Fletcher and Hopkins (1907) have demonstrated the production of lactic acid during fatigue and lactic acid results from the metabolism of glycogen (glycolysis). The production of lactic acid is less in the presence of oxygen.

Ludsgaard (1930) has shown the occurrence of contraction in muscles poisoned with iodoacetate which inhibits glycolysis. The tension produced in such cases was proportional to the breakdown of creatine phosphate which was first discovered in 1927 by Fiske and Subbarow in America and Eggleton and Eggleton in Britain. Its was further suggested by Ludsgaard that the energy released during glycolysis is utilized for the resynthesis of creatine phosphate. However, now it is well established that the energy released by the breakdown of creatine phosphate is used for the regeneration of ATP from ADP, under the influence of an enzyme creatine kinase. Some of the ATP formed reacts with creatine to regenerate creatine phosphate.

All the chemical processes involved in the muscular contraction can be summarized as follows :

(1) The immediate source of energy is from the breakdown of ATP.

$$ATP \xrightarrow{ATPase} ADP + iP$$

(2) ATP is regenerated by the transfer of its high energy phosphate bond from creatine phosphate to ATP. However, this reaction is of limited scope and serves only the immediate needs.

$$\text{Creatine phosphate} + ADP \xrightarrow[\text{kinase}]{\text{Creatine}} \text{Creatine} + ATP$$

(3) The next step is the metabolism of carbohydrate to yield pyruvate. The sources may be either glycogen in the muscle or glucose from the blood. These are converted into glucose 6-phosphate and finally into pyruvic acid and the process yields eight molecules of ATP for each glucose unit. In the lack of oxygen, pyruvate is converted into lactate. The complete process is anaerobic (Fig. 14.19).

(4) Pyruvate and lactate are oxidized to carbon dioxide and water (citric acid cycle). One unit of glucose results in the formation of about 30 molecules of ATP.

(5) Another source of energy for muscle contraction is the oxidation of fatty acids through the citric acid cycle.

As the initial stagse of contraction are anaerobic, the body is capable of sudden muscular contractions. The pyruvic acid formed during glycolysis is

at once oxidized into carbon dioxide and water. However, part of it is converted into glycogen by the reversal of glycolysis. Accumulation of pyruvate occurs if the rate of glycolysis is very rapid and when the oxygen supply is insufficient for its oxidation. Under these conditions, part of the pyruvate is converted into lactate, which is oxidized back into pyruvate when sufficient oxygen is available. When the lactic acid content of the muscle increases, a large portion of it diffuses into the blood and is converted into glycogen in the liver through *Cori's cycle.*

Actomyosin system

When actin and myosin are mixed together actomyosin forms and a shortening occurs in the molecular complex. This shortening is responsible for the contraction and mechanical work is performed by the muscle. Chemical energy is required for the formation of actomyosin and this is transformed into mechanical energy and heat. The shortening period is maintained by the paramyosin in the muscle, thereby avoiding continuous expenditure of energy.

The formation of actomyosin requires ATP and Ca^{++} ions together with other phosphagenes such as creatine phosphate. Relaxation of the muscles is aided by an organic compound, "the relaxing factor" in the presence of Mg^{++} ions.

Excitation-contraction coupling

The contraction of muscles poses some fundamental questions. How the chemical and electrical changes in the muscle fibre membrane are communicated to the muscle contractile filament? What is the exact mechanism by which excitation is transferred to the contractile elements?

Huxley and Taylor (1958) have studied excitation-contraction coupling based on combined optical and electro-physiological experiments and the movements of calcium ions.

If weak electric currents are applied to extremely small areas opposite the Z bands in frog muscle, and between A and I-bands in lizard and crab muscle fibres, contractions are produced. Experiments have revealed that these sites, where the tubular membranes of the endoplasmic reticulum are situated, tansfer excitation to the contractile elements. Another important factor is the role played by the Ca^{++} ions in the transfer of excitations. Introduction of calcium ions into the muscle fibres causes contraction and during the stimulation of a frog sartorius muscle, the influx of Ca^{++} ions increases 30 times more than the resting value (Bianchi 1961). Contraction does not take place if the Ca^{++} ions from the external medium are removed. An increase in the concentration of K^{+} ions in the external medium brings forward a long-lasting contraction and a simultaneous influx of Ca^{++} ions takes place. According to Shanes (1961), the duration of

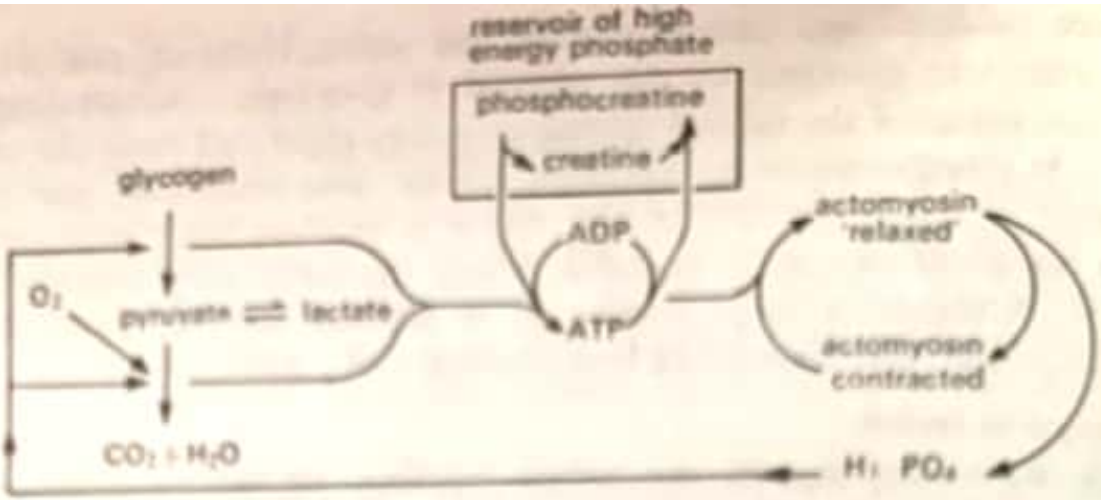
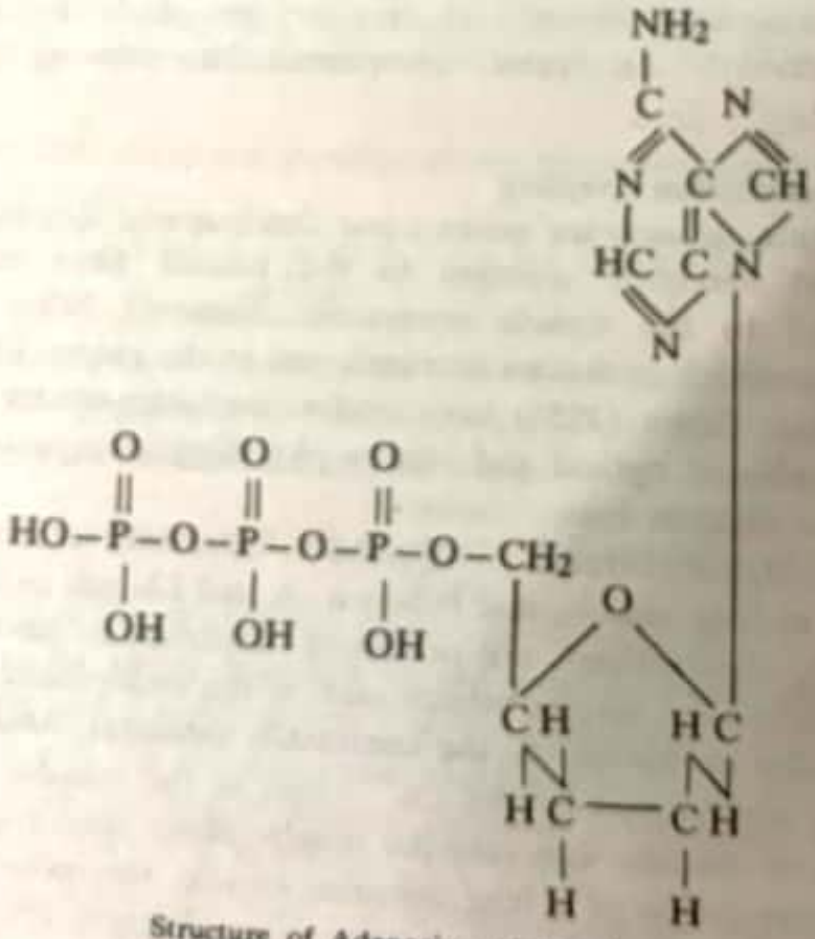


Fig. 14.19. Chemical basis of muscle contraction.

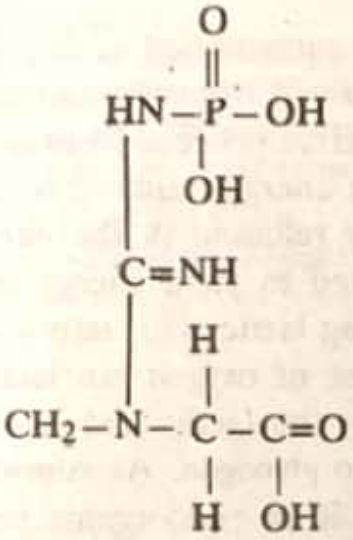
contraction produced by the increase in the K^+ ions in the external medium is proportional to the influx of Ca^{++} ions. It has been believed now that excitation of the membrane causes influx of Ca^{++} ions and this in turn activates the contraction mechanism.



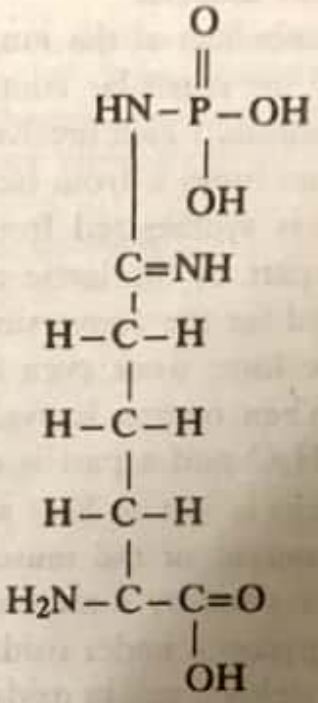
Structure of Adenosine triphosphate

Phosphagenes. According to Mommaerts, *phosphagen* is a substance which is not a transitory intermediate in a metabolic pathway but can transfer a phosphate into a nucleotide diphosphate by an enzyme present in the tissue (Mommaerts, Brady and Abbott, 1961). All the phosphagenes are

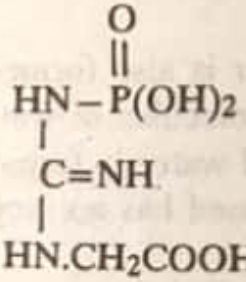
N-phosphorylated guanidine derivatives. *Creatine phosphate* is the predominant phosphagen in the vertebrates, while in invertebrates *arginine phosphate* either occurs instead of creatine phosphate or in combination with it. In annelids, *taurocyamine phosphate (Arenicola), glycocyamine phosphate (Nereis),* and *guanidyl-seryl phosphate (Lumbricus)* have been reported. Phosphagenes-act as storage for "high energy phosphate bonds." *Phosphokinase* enzymes help in the transfer of phosphate group to *Nucleotide diphosphate* (ADP) to form *Nucleotide triphosphate* ATP.



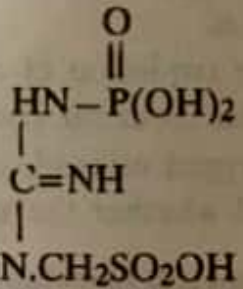
Creatine phospate



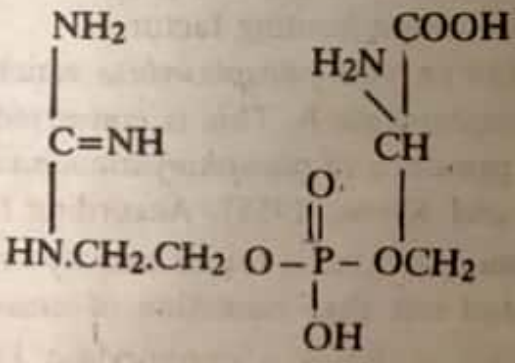
Arginine phosphate



Glycocyamine phosphate



Taurocyamine phosphate



Guanidyl-seryl phosphate

Structure of phosphagenes.

At some stage of contraction, energy is transferred from ATP to the contractile filaments. It is believed that, as oxidation cannot keep up the supply of ATP, phosphorylation of ATP by either creatine phosphate or arginine phosphate is essential. Another alternative is the donation of energy by creatine phosphate or arginine phosphate by dephosphorylation. Both these compounds are again activated by mitochondrial ATP, formed either oxidatively or in the glycolysis. The lactic acid produced is partly oxidized and partly it is converted into liver glycogen.

Metabolism of the muscles

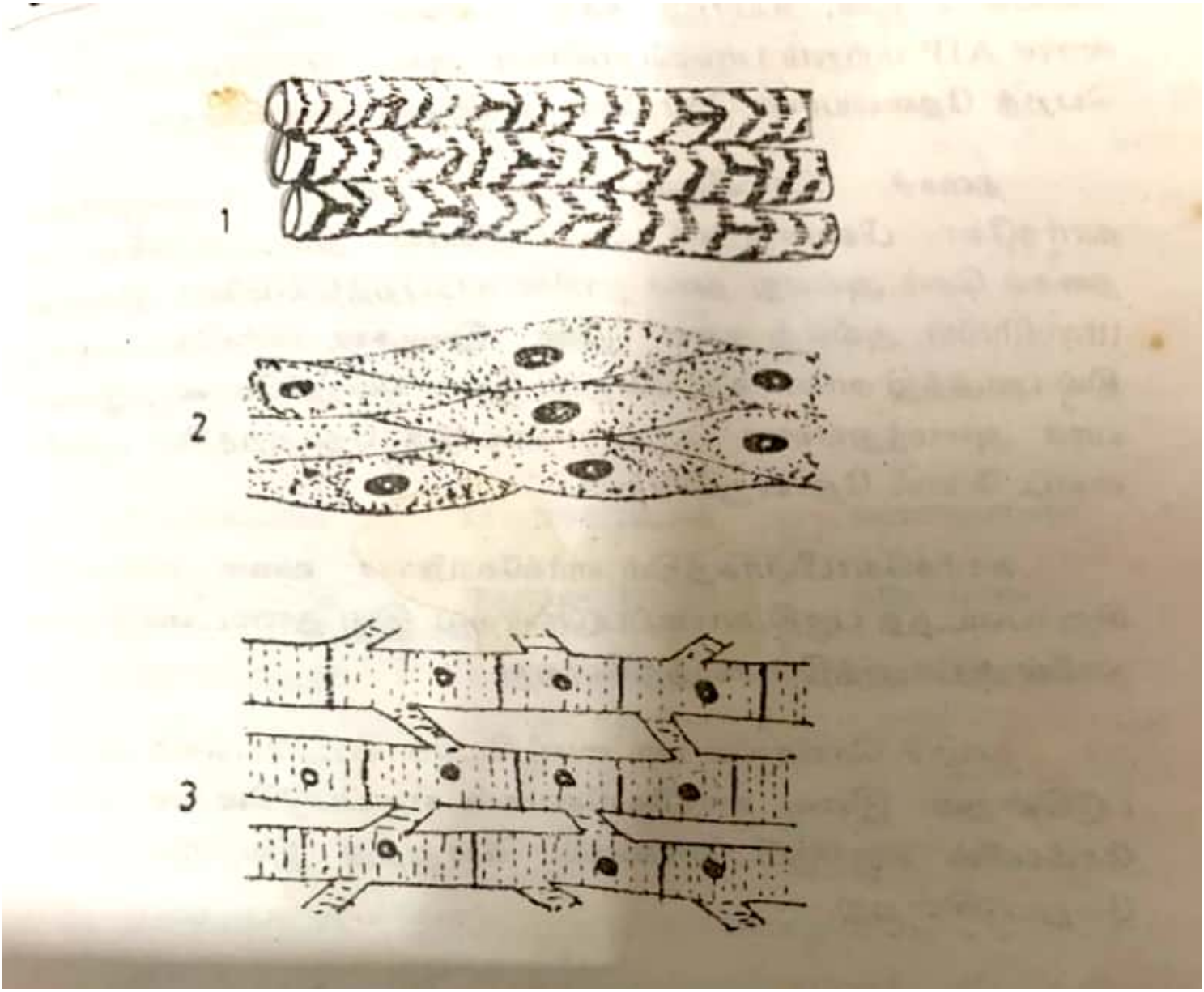
The overall metabolism of the muscles may be summarized as follows. The ultimate energy for muscular contraction is derived from the carbohydrate and lipid metabolism, which involve phosphorylation process. Muscles utilize glycogen and can build it from lactic acid. The energy required is donated by ATP, which is synthesized from the energy released in the citric acid cycle. Thus, a part of the lactic acid is oxidized to yield energy and this energy is utilized for the conversion of remaining lactic acid into glycogen. Muscles can perform work even in the absence of oxygen but lactic acid accumulates. When oxygen is available, part of the lactic acid is oxidized into CO_2 and H_2O and a part is converted into glycogen. As considerable amount of energy is lost as heat and glycolysis is an endoorganic reaction, the glycogen content of the muscle should be restored by nutrition. The amount of work done by a muscle is directly proportional to the glycogen used. 1 gm of glycogen under oxidation gives 4 kilocalories of energy, and 1 litre of oxygen yields 5 cal, in oxidative carbohydrate metabolism. Only 20% of the total energy is available as mechanical energy and the rest 80% is in the form of heat.

During the oxidation of carbohydrates, water is also formed. One unit of glucose when oxidized completely yields 6 molecules of water. For one molecule of oxygen utilized, one mole (19 ml) of water is formed. It is still not understood whether the metabolic water formed has got any function.

Regulation of muscle metabolism

In the oxidative metabolism of carbohydrates, formation of glucose phosphate appears to be a rate limiting factor.

Another factor is the enzyme *phosphorylase* which is in the form of an inactive proenzyme *phosphorylase b*. This is converted into the active form *phosphorylase a* in the presence of *phosphorylase kinase* and ATP (Cori and Green 1943 ; Fischer and Krebs, 1955). According to Krebs, Graves and Fischer (1959), phosphorylase kinase for its activity requires Ca^{++} ions. It has already been pointed out that excitation of muscle membrane causes Ca^{++} ions influx. This activates phosphorylase kinase and it in turn converts phosphorylase *b* into the active form, phosphorylase *a*. This enzyme phosphorylates the glucose unit and thus the first step in glycolysis is



initiated. Though the exact roles played by the K^+, Na^+ and Cl^- ions are not known, there is no doubt that the internal ionic concentration is a determining factor in muscle contraction.

Important Questions

1. Give the ultrastructure of skeletal muscle. Add a note on their innervation.
2. Give an account of different types of muscles found in animals.
3. Describe briefly the functions and properties of muscles.
4. Describe the physical and chemical change in the muscle during its contraction.
5. What are the structural proteins of the muscles ? Explain their role in muscular contraction.
6. Write short notes on : (i) Muscle fatigue, (ii) Isotonic and inometric contractions, (iii) Summation, (iv) Tetanus, (v) Treppy, (vi) Tonus, (vii) Excitation-contraction coupling, (viii) Sliding-filament theory, (ix) Absolute refractive period.
7. Distinguish each of the following levels of muscle organisation—Myofilaments, myofibrils, muscle fibres and muscle.
8. Draw and label the various components of the sarcomere.