COURSE CODE:	VBP301
COURSE TITLE:	Nerve, Muscle, Sensory, Motor, Autonomic and Integrative Nervous System
NUMBER OF UNITS:	3 Units
COURSE DURATION:	Three hours per week

COURSE DETAILS:

Course Coordinator: **Dr. Eyitayo Solomon Ajibola**, D.V.M, M.Sc.
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Office Location: Dept of Veterinary Physiology/ Pharmacology, COLVET, UNAAB
Other Lecturers: **Dr. O.E Adeleye**

COURSE CONTENT:

Physiology of nerve and muscle cell, functional classification of neurons, propagation and conduction of nervous impulses; classification of receptors; Ascending sensory pathways; physiology of the special senses; Pyramidal and Extra-pyramidal Motor system, Motor functions of the basal ganglia, the brain stem , Cerebellum, and the Cerebral cortex. The functions of the hypothalamus

COURSE REQUIREMENTS:

This is a compulsory course for all pre-clinical Veterinary Medical Students. In view of this students are expected to participate in all the course activities and have a minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. James G. Cunningham and Bradley G. Klein. Textbook of Veterinary physiology, 4thedition. Saunders Elsevier, Missouri, 2007.
2. Duke' Physiology of domestic Animals 10TH ed. Cornell University Press, London. 1984

LECTURE NOTES

NERVE AND SENSORY PHYSIOLOGY

SENSORY RECEPTORS

They are called selective transducers. They convert the stimulus energy into another form of energy. Sensory transduction converts stimuli into graded potential. Such changes in the receptor membrane potential are known as receptor potential. The stimulus opens ion channel in the receptor membrane. The complexity of sensory receptors ranges from free nerve endings to specialized nerve endings and receptor cells. Free nerve endings are simply branched endings of sensory neurons in the skin that function as mechanoreceptors, thermo receptors, and pain receptors. Encapsulated receptors are of several types

a. Meissner corpuscles adapt slowly to vibrations of low frequencies.
b. Ruffini endings are sensitive to steady touching and pressure and to temperature above 45 degrees Celsius.
c. The bulb of Krause is a themoreceptor that is sensitive to temperature below 20 degrees Celsius.
d. Pacinian corpuscles are located both in the dermis and near the joint; they are capable of detecting rapid pressure changes associated with touch and vibrations.

SENSORY REPRESENTATIONS

To create an accurate neural representation of sensory stimuli, the brain must distinguish four stimulus properties.

a. Stimulus modality
b. Stimulus location
c. Stimulus intensity
d. Stimulus duration.

STIMULUS MODALITY

Each receptor type is most sensitive to a particular type of stimulus. The brain thus associates a signal coming from a specific group of receptors with a specific modality. The direct association between a receptor and a sensation is called the labeled line coding. There are at least a dozen conscious sense modalities.

They are broadly classified as

a. Exteroceptors

i. Photoreceptors in the retina for vision. They respond to visible and ultraviolet light.

ii. Chemoreceptors for sensing of smell and taste.

iii. Mechanoreceptors for sensing touch, stretch, hearing, and equilibrium.

iv. Thermoreceptors detects radiant energy including infrared

b. Interoceptors

i. Chemorecepors in the carotid artery and aorta

ii. Mechanoreceptors in the labyrinth

iii. Osmoreceptors in the hypothalamus.

c. Proprioreceptors

i. Muscle spindles responding to changes in the body length

ii. Golgi tendon organ measuring muscle tension.

STIMULUS LOCATION

Each sensory receptor is most sensitive to stimulation of a specific area which defines the receptor's receptive field. When action potentials are elicited from a sensory neuron, the neuron's receptive field codes the stimulus location. Sensory receptive fields vary in size and frequently overlap. Convergence of inputs unto a single sensory neuron enhances that neuron's sensitivity but reduces spatial resolution. The size of neuronal receptive fields representing a given area determines the capacity to discriminate stimuli in an area. Sensory neuronal receptive fields are orderly organized in cortical sensory areas to form topographical maps.

Lateral inhibition; enhances the contrast between the stimulus and its surrounding, facilitating its perception and localization.

STIMULUS INTENSITY

Stimulus intensity is coded by;

a. the number of receptors activated(population coding) from low threshold receptors to high threshold ones
b. the frequency of action potentials(frequency coding) following not a linear but a power relationship

STIMULUS DURATION.

Stimulus duration can be coded by the spike train duration, but not all sensory receptors can sustain their responses. The neural code best reflects the change in stimulation, not the steady state.

SENSORY ADAPTATION

Process by which a sensory system becomes less sensitive to stimuli during prolonged or repeated stimulation. The result is a change in membrane potential as a receptor is subjected to a maintained stimulus.

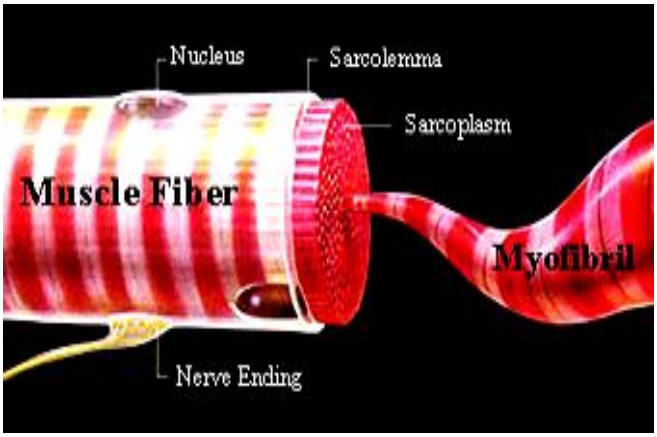
Phasic Adaptation

Receptors adapt rapidly. There is an initial burst of action potentials when the stimulus is applied followed by a reduced rate of firing. Examples: odour,touch, and temperature receptors.

Tonic Adaptation

Receptors do not adapt or adapt slowly. Continue to fire at a relatively constant rate as long as the stimulus is applied. Examples: Pain receptors

MUSCULOSKELETAL PHYSIOLOGY



PHYSIOLOGICAL ANATOMY OF SKELETAL MUSCLE

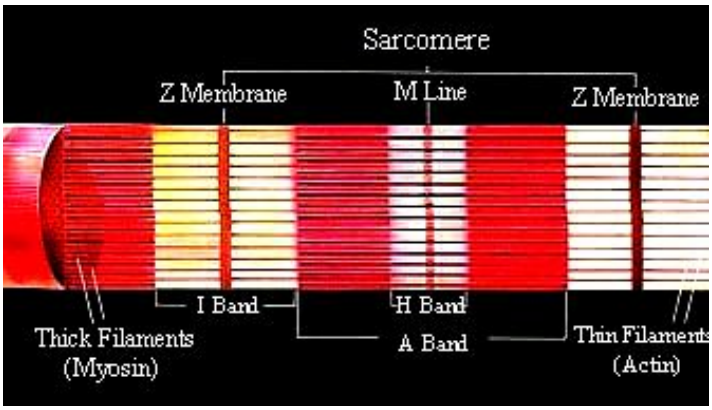
The muscle is made up of numerous **fibers** 10-80μm in diameter. In most muscles, about 98% of these fibers extend throughout the entire length of the muscle and each is innervated by only a simple nerve ending located normally in the middle of the nerve fiber. The cell membrane of a muscle fiber is called the **sarcolemma**. It consists of a true cell membrane called the plasma membrane and an outer coat made up of a thin layer of polysaccharide material that contains numerous collagen **fibrils**. At each end of the muscle fiber, the surface layer of sarcolemma fuses with a tendon fiber and the tendon fibers in turn collect into bundles to form the muscle tendons

that insert into the bone. Each muscle fiber is made up of several 100 to1,000s of **myofibrils** which are the organelles responsible for the contractile property of muscle cells. They are elongated protein threads 1-3µm in diameter in vertebrate skeletal muscle and lie with their axes parallel to the long axis of the muscle fiber.

The interior of muscle cells or fibers is literarily packed with myofibrils. It has been shown that 80-87% of the interior of skeletal muscle cells and approximately 50% of cardiac muscle cells are occupied by myofibrils. Each myofibril is made up of about 1,500 adjacent **myosin** filaments and 3000 **actin** filaments.

Actin filaments are large, polymerized protein molecules that are responsible for the actual muscle contraction and are the most abundant contractile protein in any muscle. In skeletal and cardiac muscle cells, adjacent myofibrils lie with their light and dark bands in register and this confers a cross-striated appearance on the entire cells, hence the name striated muscle. In the myofibrils, the thick filaments are the myosin filament, while the thin are actin filament.

Myosin and actin filament partially interdigitate such that, longitudinally, the myofibrils have alternate light and dark bands.



Striations in skeletal muscle as seen under the microscope

The light bands contain only actin filaments and because they are **isotropic to polarized light**, they are called "**I bands"**. Similarly, the dark bands which contain myosin filaments and sometimes the end of actin filament where they overlap are called "**A bands"**, because they are **anisotropic to polarized light**.

There are projections from the sides of the myosin filament called **cross-bridges**. They protrude from the surface of myosin filaments along the entire length except in the center. It is the

interaction between these cross-bridges (projections) and the actin filament that causes contraction of muscles.

The light I band is divided by a dark membrane called the **Z - disk or Z - line**. The filament extends on either side of the Z – disk or Z – line to interdigitate with the myosin filament. The Z - line passes from one myofibril to the other such that all the myofibrils are attached to one another all the way along the entire fiber, thus the entire muscle fiber has alternate **dark and light bands** and this is also true of individual myofibrils. These bands give the skeletal muscle its characteristic cross striations.

The portion of the myofibril that lies between two successive Z - lines is called a sarcomere. The length of a sarcomere in a muscle fiber that is at its normal fully stretched resting state length is about 2µm. At this state, the actin filament completely overlaps the myosin filament and is just beginning to overlap one another.

On closer examination, another region in the A band is seen where myosin filament do not overlap and only myosin filaments are present. This zone is called the **H – zone or H band**.

H band are rare in a normally functioning muscle, because normal sarcomere contraction occurs only when the length of the sarcomere at rest is between 1-2µ. Within this range, the ends of the actin filament not only overlap the myosin filament, but also overlap one another.

The myofibrils are suspended inside a muscle fiber in an intercellular matrix called the **sarcoplasm**. This comprises the usual intercellular components and its fluid also contains large amount of K^+, Mg^{++} and PO_4^{2-} and enzymes. Large number of mitochondria are located between and parallel to the myofibrils and this is a condition that indicates that the contracting myofibrils are in great need of large amount of energy (ATP) formed by the mitochondria.

Muscle cells also contain **sacrotubular system** and a series of **membranous tubules** that have a special structure and function.

The sarcoplasm contains an extensive endoplasmic reticulum called the **sarcoplasmic reticulum (SR)**. The more rapidly contracting muscle types are characterized by very extensive SR. The SR is composed of longitudinal tubules that lie parallel to the myofibrils and the two ends of each tubule ends in a bulbous structure called **lateral cisternae**.

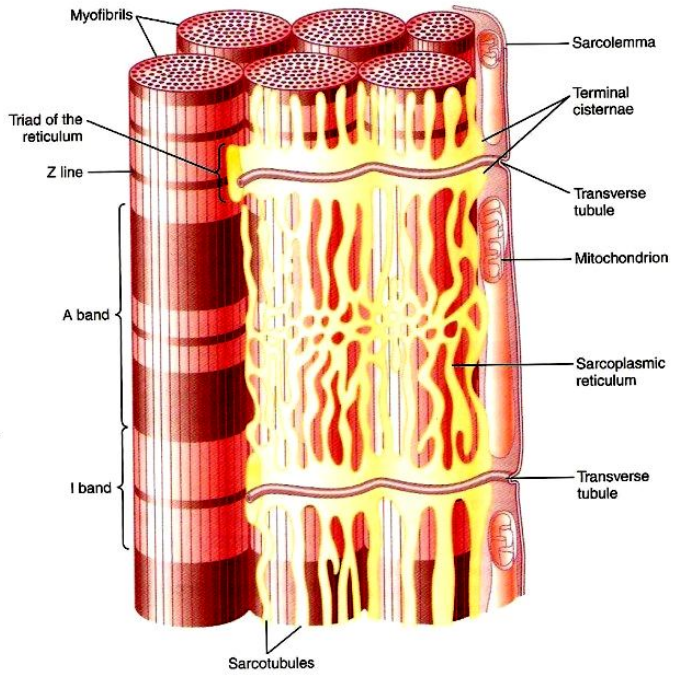


FIGURE 6-3 ■ Diagram of skeletal muscle showing the juxtaposition of myofibrils, transverse (T) tubules, and sarcoplasmic reticula. (From Guyton AC, Hall JE: *Textbook of medical physiology,* ed 11, Philadelphia, 2006, Saunders.)

Sacrotubular system

This system is divided into two sets of membranous tubules that do not open into one another but are able to transfer signals between themselves.

These two sets are called **Transverse or T system** and **Longitudinal or L system**. Longitudinal or L system is synonymous to the SR and structurally analogous (resembles) endoplasmic reticulum (ER) of other vertebrates.

Transverse System

The T system contains a set of tubules formed by invagination of the plasmalemma. They run parallel to the long axis of the muscle cell. The lumen of transverse tubules (T - tubules) opens to the extracellular space because they are invaginations of the plasmalemma.

T tubules occur at very regular intervals along the length of the muscle cell and in some muscles are found at the level of every Z disc i.e. there is one tubule for every sarcomere in such muscles. In most mammalian skeletal muscles however, T tubules are found at the level of every A - I junction giving two T tubules for every sarcomere and this type is usually found is fast acting muscles.

T tubules conduct signals from the sarcolemmal action potential (AP) to the interior of the muscle cell and in this way, myofibrils located in the center of the muscle all receive the signal to contract almost at the same time as those on the periphery.

L Tubule ≡ Sarcoplasmic Reticulum (SR)

Sarcoplasmic reticular tubules have the remarkable ability to accumulate Ca^{2+} against a concentration gradient. Most of the Ca^{2+} in resting muscle cells is localized in the lateral cisternae bound to a protein called **CALSEQUESTRIN**. A signal produced by the passage of an AP along the T tubule causes some of the Ca^{2+} in the lateral cisternae to be dislodged (disgorged) into the interior of the muscle cells and this results is free intracellular Ca^{2+} concentration rising momentarily. The increase in Ca^{2+} concentration triggers muscle contraction.

CHEMICAL COMPOSITION OF SKELETAL MUSCLES

Proximate Composition

Constituent	% by weight	Comments
Water	55-78	Fat-free muscle is 72-78%, water content varies inversely with lipid content.
Protein	15-23	Fat-free muscle is 20-23%protein, smooth muscle has a slightly lower protein content.
Lipid	1-20	About 1.0-1.5% is phospholipids, varies widely depending on neutral lipid content.
Carbohydrate	1-2	Mostly glycogen in living rested muscle, some lactate in exhausted or post mortem muscle. Also includes some mucopolysaccharide
Ash	1	Contains 100-120mM K^+, 60-80mM PO_4, 15-40mM Na^+, 5-10mM Cl,10-25mM Mg^{2+}
Nucleic Acid	<1	In porcine muscle, 25-30mg DNA/100g, 100mgRNA/100g.
Other soluble organic compounds	1	Contain 8-15mM ATP, 20mM phosphocreatine, 4-5mM creatine,350mg carmosine/100g, 140mg anserine / 100g.

Proximate composition of mammalian Skeletal Muscle

Muscle is almost entirely protein in aqueous salt solution. Small part is made up of lipid (1-1.5% of total muscle weight). The lipid is either phospholipid or cholesterol and is found in membranes of the plasmalemma, sacrotubular system and other membranous sub cellular organelles.
Muscle cells contain relatively high concentration of K^+ and $P0_4^{2-}$ and relatively low concentration of Na^+ and Cl^- ions.

Protein classification

This is based on the solubility of the protein in aqueous solution.

i. Sarcoplasmic protein
ii. Myofibrillar protein (contractile protein)
iii. Stroma protein

Sarcoplasmic protein is the most soluble of all the 3 classes and generally includes the protein found in the cytoplasm of the muscle cells. It contains most of the enzymes associated with carbohydrate, lipid and amino acid metabolism as well as those of the synthesis of cell constituents. During development and growth, when expressed as a percentage of the total muscle weight(TMW), the sarcoplasmic protein content increases during pre-natal development and also post-natally until the animals is half matured.
As a percentage of total muscle protein (TMP), sarcoplasmic protein is highest early in prenatal period (approximately 70%) and decreases during both prenatal and postnatal period.
Myofibrillar protein is the contractile protein and is one of the largest fractions of protein in muscle cells. They are insoluble in water but soluble in dilute salt solution. They become soluble in water once they have been extracted from the myofibril. Myofibrillar proteins increase during both pre- and postnatal development when expressed as TMW or TMP.
Stroma proteins are the least soluble class of muscle proteins and they contain a large number of different proteins. Most of the muscle fractions are collagen and elastin. Most stroma proteins are extracellular in origin because they originate from the epimysial, endomysial and perimysial connective tissue layers. They decrease during both pre- and postnatal development in both ways i.e. TMW and TMP.

Summary

Protein class	Properties
Sarcoplasmic Protein	-Soluble at ionic strength of 0.1 or less at neutral pH -Constitutes 30-35% of total protein in skeletal muscles and slightly more in cardiac muscles. -Contains at least 200-300 different protein and is sometimes called **Mycogen**
Myofibrillar Protein	-Constitutes the myofibril -Makes up 52-56% total protein in skeletal muscles & 45-50% in cardiac muscles. -Ionic strengths above 0.3 are generally required to disrupt the myofibril, but many of the Myofibrillar protein are soluble in water once they've are been extracted from the myofibril.
Stroma Protein	-insoluble in neutral and aqueous solvents -Constitutes 10-15% of total protein in skeletal muscles and slightly more in cardiac ms -Includes lipoproteins and mucoproteins from cell membrane and surfaces as well as connective tissue protein. -Exact percentages vary widely, but collagen generally makes up 40-60% while elastin makes up 10-20% of total stroma protein.

<u>Protein Composition of Mammalian Muscle (Whole muscle)</u>

Myofibrillar Protein

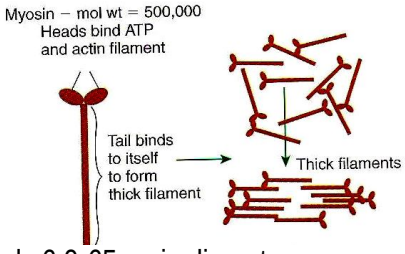
Myofibril is the contractile organelle in skeletal muscles is composed of approximately 12-14 proteins (myosin, actin, tropomyosin, troponin, C-protein, α-actinin, β-actinin, M-protein, creatine kinase, filomin, desmin, titin, nebulin, paramyosin etc.

Myosin and actin are both necessary and most important for muscle contraction. The other myofibrillar proteins either,

1. Regulate the myosin-actin interaction so that contraction can be initiated or stopped in the presence of ATP e.g. tropomyosin, troponin and possibly α-actinin.

2. They assist in the assembly of the myofibril into the proper 3 dimensional structure. Those involved are C-protein, α-actinin, β-actinin, M-protein, creatine kinase, filomin, desmin, tiitn, nebulin and paramyosin.

Myosin



It is a very large protein molecule that contains 6 different polypeptide chains. The myosin molecule consists of a long rod with 2 pear shaped or ellipsoidal heads at one end. The entire molecule is 170-175nm long and the rod portion being 155-160nm long and approximately 1.5nm in diameter. The 2 heads are approximately 6.0-65nm in diameter 18-19nm long.

Physiological properties of myosin include:

1. **The enzymatic ability to split ATP and release energy ►** in the absence of actin, myosin adenosine triphosphatase (ATPase) activity is inhibited by Mg^{2+}. But when myosin is combined with actin, its ATPase activity is activated by Mg^{2+} ions.
2. **Myosin binds strongly to actin ►** the actin-myosin complex is specifically dissociated by ATP, pyrophosphate and a few other poly anions when Mg^{2+} is present.
3. **Myosin aggregates spontaneously to form dimers ►** this aggregation of myosin form thick filaments.

Proteolytic enzymes like chymotrypsin and trypsin will split myosin molecule to:

a. Light meromyosin (LMM)

b. Heavy meromyosin (HMM)

Light Meromyosin (LMM)

- It forms the tail part of the myosin molecule
- has no ATPase activity
- does not bind to actin
- forms thick filaments with no cross bridges

Heavy meromyosin (HMM)

- Contains the head and part of the tail portion of the original myosin molecule
- has ATPase activity
- binds to actin but does not form filament

With longer incubation time, trypsin splits HMM to **sub-fragment 1 (HMM-S1)** and **sub-fragment 2 (HMM-S2)**

HMM-S1

- contains the 2 pear shaped heads
- has ATPase activity
- binds to actin but does not form filaments
- contains 2 polypeptide chains of the 6 found in the myosin molecule

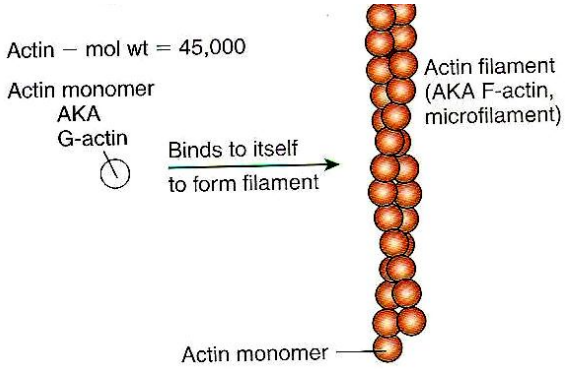
HMM-S2

- The tail portion of the HMM (Short stab of myosin tail)
- does not have ATPase activity
- does not bind to actiOn
- does not form filaments.

Note: The active site for myosin ATPase activity and sites for actin-myosin binding are both located in the 2 myosin heads.

Actin

Actin is much smaller than myosin and contains only 1 polypeptide chain or molecule. Every molecule of actin contains 1 molecule of ATP and 1 molecule Ca^{2+} (function is unknown). Conditions like 100mM KCl or 1-5mM Mg^{2+} causes aggregation of actin to form a double stranded helix or helical filament



During or immediately following aggregation, the ATP associated with actin is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate (P*i*). The ADP remains associated with the actin aggregate but the P*i* does not.

Actin exists almost entirely in the aggregated filamentous form in muscle cells because vertebrate muscle cells contain at least 100mM KCl & 5mM Mg^{2+}.

MOLECULAR ANATOMY OF THICK AND THIN FILAMENTS

Two **myosin** molecules spontaneously aggregate head-to-tail to form dimers. These dimers then aggregate tail-to-tail to produce a short filament with a smooth central region flanked on either sides by projections. The tail-to-tail aggregation of the dimers may occur with the involvement of M-protein and creatine kinase.

The projections are the double heads of the myosin and these represents the cross bridges observed in striated muscles. Therefore, each cross bridge is formed from a single myosin molecule with 2 heads. The active binding site of myosin is in the cross bridges. Additional growth of the thick filament then occurs by head-to-tail addition of the myosin dimers to the nucleated filament.

Thick filaments are 1.5 – 1.6μm long and one thick filament contains about 300 myosin molecules. Thick filaments have a very specific geometrical structure. C-protein is located in bands that completely encircle the thick filament, like staves around a barrel. Each thick filament contains 14 bands of C-protein, 7 on each side of the M line with each band containing 2-4 molecules of C-protein.

Actin molecule is seen as a double-stranded filament with an axial helical repeat distance of 37.5nm. One stand contains 13 actin monomers per strand per turn and makes a complete turn every 75nm. Actin monomers are bilobular in shape with two lobes or globules connected by a short bridge. Thin filaments are 1μm long and contain 340-380 actin monomers.

Note: Molar concentration of actin in muscle is about 4 times the molar concentration of myosin

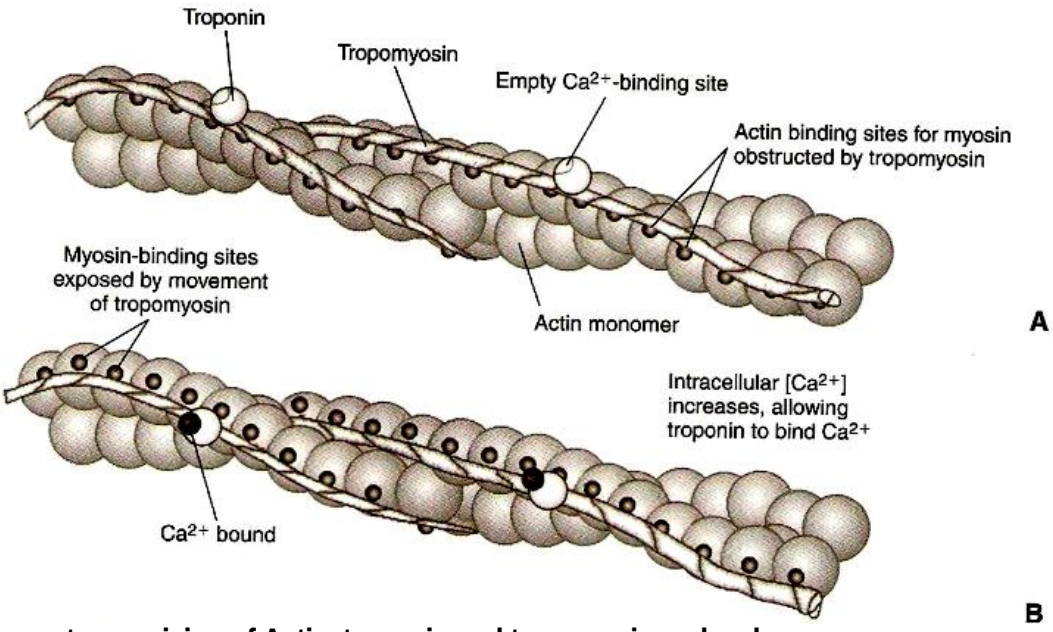
Tropomyosin molecule is rod shaped and 42.3nm long. It lies in the two grooves of the double stranded actin filament and aggregate end-to-end to produce two stands of tropomyosin running the entire length of the thin filament.

Troponin is an ellipsoidal or more globular molecule that binds to tropomyosin at particular sites on the tropomyosin molecule. Troponin is located at periodic intervals of 38.5nm apart along the thin filaments on binding sites of tropomyosin.

Troponin is the switch for muscle contraction: It contains a complex protein molecule that has 3 different subunit polypeptide chains:

1. Troponin T ► it contains the principal binding site that attaches troponin complex to tropomyosin. T stands for tropomyosin.

2. Troponin I ► binds to troponin T and troponin C and also to actin in the absence of Ca^{2+}. It inhibits the actin activated ATPase activity of myosin (i.e. that activated by Mg in the presence of actin). I stands for inhibition.
3. Troponin C ► has four high affinity binding sites for divalent cations or molecules 2 of this site bind Ca^{2+} reversibly during contraction & relaxation. C stands for Ca^{2+}.



Thin filament comprising of Actin, troponin and tropmyosin molecules

MUSCLE CONTRACTION

There are two types, viz:

Twitch / propagating ≡ phasic (fast speed of contraction) seen in skeletal muscles

Non-twitch /non propagating ≡ tonic

For twitch the time for contraction is less than 80-100ms. Response is very fast and sharp. Twitch fibers are usually innervated by one or sometimes two motor neurons. Twitch also propagates an action potential.

Neuromuscular space – junction between muscle and neuron. They do not touch but are brought into contact by neurotransmitter.

Non-twitch is seen in amphibians, fish and reptiles and anterior latissimus dorsi muscle of chickens.

Motor End Plate Region (MEP)

This is an area of the twitch muscle where the motor neuron collateral impinges on a muscle fiber at a particular area. The motor end plate of a twitch muscle is called *en plague*. It is formed by 1or 2 major neurons impinging on the muscle while that of a non-twitch muscle is called *en grappe* formed by small neurons impinging on several points on the muscle.

Motor Unit and Motor End Plate Depolarization

Motor unit is defined as the **motor neuron**, its **axon**, the **axon collaterals** emanating from that axon and all the **muscle cells innervated by the axon collaterals**. One nerve serves a number of different motor units in a single muscle because that particular nerve contains many neurons and their axons.

Innervation Ratio

This is the number of muscle fibers / cells innervated by the motor neuron. Each axon collateral generally innervates one muscle cell. So the innervation ratio is equal to the number of axon collaterals emanating from that motor neuron. Muscles where delicate control is required e.g. extrinsic muscles of the eye may have innervation ratio of as low 3-6. Muscles where fine control is not necessary e.g. limbs have as high a 1000 innervation ratio.

NB: the perineural epithelium of the finger like projection and the infolded plasmalemma are separated by a 40-60nm space. Nerve impulse, propagated along the motor neuron and its axon collateral reaches the terminus of the axon collateral at the neuromuscular junction. The terminus of the axon collateral contains pre synaptic vesicles that contain chemical compounds {acetylcholine (ACH), dopamine, serotonin, nor epinephrine}. Propagation of action potential along the neuron causes the release of the chemical compounds (neurotransmitters) from the synaptic vesicle which causes the depolarization of the motor end plate region. Motor end plate does not propagate an AP on its own, but if the depolarization of the motor end plate is sufficiently extensive, it depolarizes the adjacent plasmalemmal membrane. Muscle plasmalemma resembles neuronal membrane in its ability to propagate an AP.

At rest, the inner surface of the muscle plasmalemma is approximately -90mV with respect to the outer surface. For typical mammalian muscle, conduction of an impulse along a motor neuron requires approximately 2 milliseconds between leaving the spinal cord and entering the muscle, 2ms between entering the muscle, passing along axon collateral and reaching the neuromuscular junction and 1ms to traverse the neuromuscular junction.

N.B. the rate of propagation of AP decreases as it leaves the motor neuron (the rate of propagation at this point is 80 ms^{-1} or 80 m/s) and passes along the axon collateral.

The rate of propagation of AP along muscle plasmalemma is slower i.e. 5m/s. Then plasmalemma AP spreads to the many openings of the T - tubules which exists in skeletal muscle cell membranes and is propagated down the T - tubules to the lateral cisternae.

Time between the polarization of motor end plate and arrival of AP at the lateral cisternae is approximately 0.5-1ms. The total time required for an impulse leaving he spinal cord to generate an AP in the T - tubule is between 5.5-6.0 ms.

Excitation-Contraction Coupling

This is the mechanism by which an AP propagated along the muscle plasma membrane eventually initiates contraction. This mechanism involves only muscle cells and includes those events that occur between passage of an AP along the T – tubule and shortening of the sarcomere.

N.B. It has been noticed that T- tubules can also conduct a propagated AP.

Lateral cisternae contains up to 90% Ca^{2+} in the resting cell. The Ca^{2+} is bound to **CALSEQUESTRIN** which is located along the inner walls of the lateral cisternae. 1 mole of calsequestrin (about 44000g) binds approximately 45moles of Ca^{2+}.

When T - tubules signal the lateral cisternae, Ca^{2+} is released into the medium immediately surrounding the myofibrils. Free Ca^{2+} concentration inside muscle cells is from 10^{-8}M to 10^{-6}M to 10^{-5}M. This increase in concentration initiates muscle contraction.

MOLECULAR MECHANISM OF MUSCLE CONTRACTION

Contraction is accomplished by the sliding together /telescoping of the interdigitating thick and thin filaments without any detectable shortening of the filament themselves. Muscle contraction causes a narrowing and eventual disappearance of the H – zone as thin filaments slide into this area and a narrowing of I - band as the thick filament pass into I - band region. Shortening does not proceed beyond the stage of loss of H - zone in living vertebrates, but can exceed beyond it experimentally. The force causing the thick and thin filament to slide past each other is generated by the cross bridges or myosin head that project outwards from the surface of the thick filament.

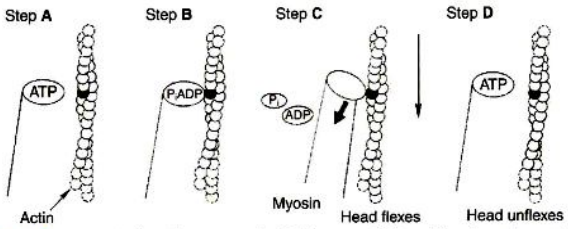


FIGURE 1-4 ■ Power stroke of actomyosin. **A,** The myosin head has bound to adenosine triphosphate *(ATP)*. In this conformation, myosin has little affinity to bind to actin. **B,** ATP is partially hydrolyzed to adenosine diphosphate *(ADP)* and inorganic phosphate *(P_i)*; the hydrolysis is partial because the products remain bound to the myosin head. The change in what is bound to the myosin (ADP and P_i, not ATP) has the conformation of myosin so that it binds to actin with high affinity. **C,** Hydrolysis is complete; myosin releases ADP and P_i. This change in what is bound at the myosin head causes an allosteric change in the head; it flexes. Because the myosin head is still bound to the thin filament, the flexion causes the thin filament to slide past the thick filament. **D,** New ATP molecule binds to the myosin head; as for step *A*, myosin had little affinity for actin in this state, and the head releases from the thin filament and unflexes.

In contraction, the cross bridges attach to actin in the thin filament. Actin-myosin head interaction causes the myosin head to swivel / angle with respect to the shaft of the myosin filament. The swiveling pushes the actin filament towards the center of the sarcomere and causes contraction. Force for muscle contraction is generated when myosin head interacts with actin. The events that occur can be divided into:

1. Those occurring within the thick filament,
2. Those occurring within the thin filament.

In the thin filament: events here are concerned with turning muscle contraction on and off (like a switch). Troponin-C (TnC) binds to Ca^{2+} when free intracellular Ca^{2+} concentration rises to 10^{-6}-10^{-5} M. This binding causes the conformation of TnC subunit to change and this change triggers a series of other changes in the binding of the troponin subunits to one another.
In the absence of Ca^{2+}(when muscle is resting), the intracellular Ca^{2+} concentration will be 10^{-8} M. Troponin-T (TnT) binds strongly to tropomyosin (Tm), TnC binds loosely to Troponin-I (TnI) & TnT, TnI binds loosely to TnT but firmly to actin. Here, Tm inhibits binding.

In the presence of Ca^{2+}, intracellular Ca^{2+} concentration increases to 10^{-6} – 10^{-5}M or higher (there is Ca^{2+} on TnC). TnT binds strongly to Tm, TnC binds strongly to TnI and TnT, TnI binds to TnT but looses affinity for actin.

N.B.: The most important change here is that binding of Ca^{2+} to TnC causes TnI to loose its affinity for actin.

Location of Tm strand in resting muscle is out of the groove of the double stranded actin helix in a position where it might partly block the binding site for myosin on actin.

Binding of TnI and actin enables TnI to act as a prop that holds Tm strand in this blocking position.

When TnC binds Ca^{2+}, the linkage between TnI and actin is weakened and Tm moves back into the groove of the double stranded helix and the myosin binding site on actin is exposed. Myosin then binds to actin and contraction occurs and continues till Ca^{2+} is removed from TnC and the muscle goes back to its resting stage.

Although TnI does not contact TnT in this diagram, there is experimental evidence that they come in contact when there is Ca^{2+} influx.

In the thick filament: Events here principally involve those in the cross bridges. During a single muscle twitch, each myosin cross bridge may perform many cycles of attaching to actin, swiveling and then dissociating from the actin filament. Only 5-20% of the total cross bridges in a single sarcomere are attached to the thin filament at any given instant during contraction.

The thick and thin filament slide past each other at a uniform rate rather than with a jerky, ratchet like motion because of the asynchronous interaction of the cross bridges with actin. The range of movement of 1 individual cross bridge is about 10-15nm and time required for a cross bridge cycle in contracting muscle is approximately 0.1ms.

N.B.: ATP hydrolysis provides energy for muscle contraction; ATP prevents the actin-myosin interaction and even dissociates the actin-myosin complex necessary for contraction.

In living resting muscle, almost every myosin cross bridge is energized and contains 1molecule each of ADP and inorganic phosphate **P*i*** (hydrolysis product of ATP). In this resting state, actin filament is turned off i.e. TnI binds strongly with actin and Tm is preventing actin-myosin binding. Ca^{2+} released by sarcoplasmic reticulum turns on the thin filament.

Myosin cross bridges interact with actin immediately after the actin in unblocked in the switching-on process. This interaction triggers the swiveling/rotating of the myosin cross bridges so that the actin filament is pushed towards the center of the myosin filament. Once the myosin cross bridges has swiveled, ADP and P*i* are quickly released. Now ATP can then bind to the myosin head and this binding immediately dissociates the actin-myosin complex and this reorientate back to the resting state and ATP is hydrolysed to ADP and P*i* by myosin while it is dissociated from actin.
Hydrolysis of ATP is the energy required for the reorientation process, yielding ADP and P*i*.
This series of events continues until Ca^{2+} is rebound by sarcoplasmic reticulum and thin filament is turned off so that actin is no longer available to bind myosin cross bridges or until ATP is available to bind to the spent cross bridge and dissociates it from actin.
At death, all myosin cross bridges stop, attached to actin at an angled position (rigor mortis).

Ionic Phenomena

The time course of tension development following electrical stimulation of a muscle cell can be divided into 3 phrases:

1. Latent period- brief period between the stimulus and initiation of tension. This phase is characterised by invisible muscle contraction that does not involve all muscle fiber simultanously. During this period, the total muscle lenght remains unchanged. It is about 3 – 10 ms in vertebrate skeletal muscle.

2. Contraction period - period of rising tension. The muscle apparently shortens at this period. Approximately 15 – 100 ms.

3. Relaxation period – period of gradually declining tension. This is the period where muscle returns to it's normal resting position from the excited state. Approximately 15 – 100 ms.

All – or – nothing Law

Electrical stimulation of motor neuron or muscle will not elicit any response until the stimulus exceeds a certain level called the threshold level. After attaining this level, further increase in strength of stimulation has no effect on the AP or response elicited. This phenomenon of having to exceed a certain minimal level to evoke a response and having the threshold stimulus evoking a maximal response is called all-or-nothing (all –or – none) effect.
Single muscle fiber responds to single AP with maximal contraction.

ISOTONIC CONTRACTION

Contraction is said to be isotonic when the muscle shortens but the tension on the muscle remains the same e.g. when we pick up a light weight, the muscle shortens and move the skeleton. This can be measured by firmly attaching the muscle at one end and hanging a constant load at the other end. As the muscle raises the constant load, the length is recorded.

ISOMETRIC CONTRACTION

Contraction is said to be isometric when the length of the muscle does not shorten during contraction. This can be measured by clamping both of the muscle so that they cannot move and by incorporating a force meter at one end which does not vary in length under load. A change in force measured while muscle remains constant is called isometric contraction i.e tension drops. e.g of isometric control is when we attempt to pick up a weight that is too heavy: the muscle tenses but does not shorten. When muscle under an isometric contraction, the contractile unit shortens (actin and myosin filaments slide past each other) but other passive parts of the cell attached to the contractile unit i.e. the tendon and connective tissue are stretched so there is no nett movement. The passive parts that are stretched by the contractile unit are called series elastic component (SEC) of the muscle (tendons, connective tissue and hinged arm of the cross bridges.

SKELETAL SYSTEM

3 types of bone cells - osteoblast - precursor cells

- osteocytes
- osteoclasts

Summary of function of Bone & Bone cells

1. They function to help maintain, thru homeostatic regulation, a constant ionic environment, within the organism e.g. release of calcium.
2. They support & protect soft tissues and organs including the bone marrow.
3. Together with the aid of muscle and tendons, bone aids the animal in movement.

BONE CELLS

Osteoblast

They are columnar cells that vary greatly in size from 15-150μm in length, although most fall within a range of 20-30cm. These cells cover the surfaces of newly forming bone. Osteoblasts are polarized in that the nucleus is located farthest from the bone surface.

The cytoplasm contains abundant endoplasmic reticulum with some having dense granules (ribosomes) on their outer surface. Mitochondria often lie in close opposition to the granular endoplasmic reticulum, Golgi apparatus is also present.

Histologically osteoblasts are characterised by their strong cytoplasmic basophylia that ranges directly with cell activities. The basophilia is due to the presence of ribonucleic acid (RNA). These granules (RNA granules) are decreased in number when the osteoblast is in the resting stage. Alkaline phosphatase is confined to the external surface of the osteoblast plasma membrane. Acid phosphatase is present in the osteoblast lysosome.

Osteoblasts participate in the ossification process and are readily observed when new bones are being formed.

Osteocytes

They are osteoblasts that become entrapped and embedded in growing bone. They are situated within the flat oval lacunae and have cytoplasmic processes extending thru apertures into the canaliculae of bone to connect directly with other bone cells.

The cytoplasm is slightly basophilic with few mitochondria and a small Golgi network. It also contains fat globules and glycogen. Acid phosphatase and other lysosomal enzymes are present. Osteocytes participate in osteolytic osteolysis and bone formation.

Osteoclasts

They are usually multinucleated giant cells observed at site of bone resorption and in eroded cavities of bone called **Howship's lacunae**. They are polarized like osteoblast. They contain numerous mitochondria. Lysosomes are found in abundance in regions where bone is being resorbed (bone resorption, dissolution, removal of mineral and intercellular matrix. osteoclasts are highly motile and capable of migrating along surfaces of resorbing bones and also of entering the blood streams.

Osteoclasts arise from precursor cells in bone marrow or spleen.

MEMBRANES

Anatomical evidence shows that in the normal state, osteons and trabeculae are separated from the vascular compartment by a membrane composed of osteoblast, osteocytes (and their filaments).

This membrane is thought to serve as a barrier to the free flow of ion and other substances between body fluids and the crystalline surfaces of bone. It may also regulate or facilitate the transfer of nutrients to and from sites of bone formation and resorption.

INTERCELLULAR MATRIX

Composition - osteogenic cells (osteocytes and osteoblasts) synthesize and release organic components of the intercellular matrix which subsequently calcify and form bone.

1. **Bone mineral**
2. **Collagen** (a fibrillar protein) constitutes 90-95% of total
3. **Amorphous ground substance** composed mainly of MPS mucopolysaccharide (Chondroitin sulfate), fatty acid, phospholipids and other substances.
4. **Water**

Adult bone contains approximately 25% water, 30% organic matter, 45% ash (37% calcium, 18.5% phosphorus).

On dry weight basis, the mineral content is 65-70% while the organic fraction is 30-35% (95-99% is collagen which upon heating is converted to aqueous **Gelatin**.

COLLAGEN

This is a fibrous protein synthesized by fibroblast and related cells such as osteoblasts of bone cells and chondroblast of cartilage. It is found in all tissues of the body. Collagen formation occurs both intra- and extracellularly.

Intracellular pathway for collagen formation

- Synthesis of protocollagen molecules
- hydroxylation of some proline and lysine residues
- glycoxylation of hydrolysine residues.
- formation of procollagen monomers
- extrusion of procollagen trimers in helical configuration

Extracellular formation of collagen

There is limited proteolytic hydrolysis of procollagen to form tropocollagen which subsequently forms the matrix fibrils.

The amino acid component of collagen contains $^1/_3$ glycine, $^1/_3$ proline and hydroxyproline, while the remaining is lysine hydroxylysine.

Collagen is considered to be crystalline in nature due to the ordered aggregation of the collagen macromolecules and to the high degree of structural regularity of the collagen fibrils.

GROUND SUBSTANCE

This is the extracellular and interfibrillar amorphous component of all connective tissue. It is interspersed between collagen fibers and connective tissues. It is continuous with the interstitial fluid and exhibits varying degrees of condensation. It consists mainly of protein polysaccharide (chondroitin sulphate), glycoproteins, non-structural protein, electrolytes and water.

BONE MINERAL (BONE SALTS)

These are deposited within the interstitial substances and are calcium, phosphates, OH^-, carbonate, citrate and water. Others are Na, Mg, K, Cl^- and F^-.

It is generally accepted that the crystalline structure of bone minerals is that of the apatite series and is approximated by the formula of hydroxyapatite i.e. $Ca_{10}\ (P0_4)_6\ (OH)_2$

CALCIFICATION MECHANISM

Osteoblasts synthesize and secrete organic components into the intercellular space and these components are required for bone mineralization.

Note: Proper mineralization requires that an adequate supply of inorganic ions is available and that the synthesis and elaboration of appropriate organic constituents by the osteoblasts have occurred.

Calcium and Phosphorus

Ca is present in the body mainly in the form of calcium phosphate ($Ca_3(PO_4)_2$). The concentration of Ca and PO_4 ions in the ECF are considerably greater than those required to cause precipitation of hydroxyapatite. However inhibitors are present in almost all tissues of the body as well as in plasma to prevent such precipitation. One of such inhibitors is **PYROPHOSPHATE.** Therefore

hydroxyapatite crystals fail to precipitate in normal tissues except in bones despite the state of super saturation of the ions.

Mechanism

The initial stage is the **secretion of collagen** molecules (collagen monomers) and ground substance by osteoblasts. Then the **collagen monomers polymerize** rapidly to form collagen fibers and an **osteoid is formed**. (An osteoid is a cartilage-like material differing from cartilage in that salts readily precipitate in it). Some of the **osteoblasts then become entrapped** in the **osteoid** as it is formed and they **become quiescent and are called osteocytes**.

Ca salts now begin to precipitate on the surfaces of the collagen fibers within a few days after osteoid formation.

This Ca precipitation first appear at intervals along each collagen fiber forming minute nidi to rapidly multiply and grow over a period of days and weeks into the finished product i.e. hydroxyapatite crystals.

Note: Initial Ca salts deposits are not hydroxyapatite crystals but amorphous compound (non crystalline) i.e. a mixture of salts such as $CaHPO_4.2H_20$, $Ca_3(PO_4)_2.3H_20$and others.

These salts are over a period of weeks or months converted into hydroxyapatite by a process of substitution and addition of atoms, or reabsorption and reprecipitation.

<u>Deposition and Absorption of Bone</u>

Bone Deposition by Osteoblasts

Bone is continually being deposited by osteoblast and continually being absorbed where osteoclasts are active. Osteoclasts are found on the outer surfaces of the bone in the bone cavity. A small amount of osteoblastic activity occurs continually in all living bone (on about 4% of all surfaces of bone at any given time in an adult) so that some new bone is being formed constantly.

Bone absorption

This is a function of the osteoclasts bone is continually absorbed in the presence of these osteoclasts. The osteoclasts are normally active on <1% of the bone surfaces of an adult. Parathyroid hormone (PTH) controls the bone absorptive activity of osteoclasts. Bone absorption occurs immediately adjacent to the osteoclast.

Bone resorption

This is the removal by degradation and dissolution of the entire complicated structure of bone. The components of the organic matrix are degraded and released and bone salts are solubilised.

Mechanism of Resorption (Osteoclastic osteolysis)

Osteoclast sends out villus-like projections towards the bone, forming a ruffle border adjacent to the bone.

The villus secretes 2 types of substances:

1. A proteolytic enzyme released from the lysosomes of the osteoclasts e.g. collagenase.
2. Several acids e.g lactic and citric acid released from the mitochondria and secretory vessicle.

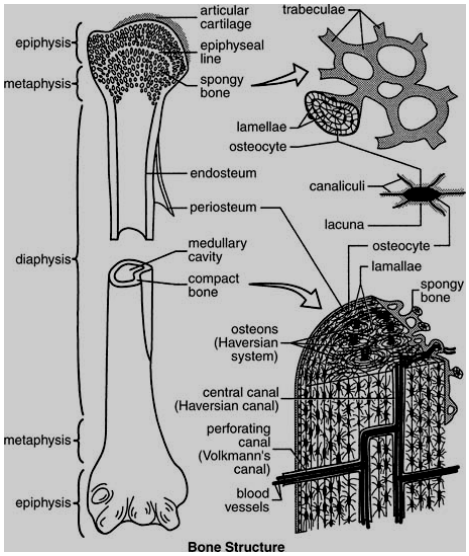
These enzymes digest and dissolve the organic matrix of the bone and the acid cause dissolution of the bone salts.

Osteoclastic cells also imbibe by phagocytosis, minute particles of bone matrix and crystals, eventually also dissoluting these and releasing the products into the blood.

Note: normally (except in growing bones), rate of bone deposition and absorption are equal to each other so that the total mass of bone remains constant.

Osteoclastic activity in about 3 weeks creates a tunnel of about 0.2-1mm or several mms long. The tunnel is then invaded by osteoblasts and new bones start to develop. This continues for several months. New bone is laid down in successive layers of concentric circles lamellae on the inner surface of the cavity until the tunnel is

filled. Bone deposition ceases when the bone begins to encroach on blood vessels supplying the area (blood vessels are in Haversian canal) and each new area of bone deposited in this way is called an **OSTEON**.



Bone Structure

Osteocytic Osteolysis

This occurs as a result of the ability of osteocytes to resorb bones from the surfaces of the lacunae which they reside. Replacement of the resorbed lacunar bone is also carried out by the osteocyte and **these 2 osteocytic processes i.e. bone removal and replacements are responsible for bone remodelling.**

Osteoclastic and osteoytic osteolysis are both stimulated by PTH. Relative contribution of these 2 processes to bone removal under stress of Ca deficiency, have not been resolved satisfactorily.

REGULATION OF BONE METABOLISM AND HOMEOSTASIS

Several input factor influence the rate of bone turnover and renewal, bone growth, bone resorption, Ca and Phosphorus concentration in blood, the degree of intestinal Ca and P absorption, and the reabsorption of these elements by the kidney tubules.

Of significance among these factors are the gonadal hormones (estrogen and androgens), thyroid hormone, hydrocortisone, growth hormone (GH), vitamin A and ascorbic acid. Most of these are prominent in the normal growth and maturation of bone tissues, and their deficiency or excess can exert profound effects e.g.

- ascorbic acid deficiency will lead to impaired collagen synthesis.
- excess vitamin A in the body will lead to production of excessive bone resorption because there will be a release of lysosomal digestive enzyme.
- deficiency of vitamin A leads to inhibition of osteoclastic activity resulting in abnormal bone resorption pattern.
- GH defifiency will lead to dwarfism while excess will lead to gigantism in growing individuals or acromegaly in adults.

Maintenance of blood Ca level is very important because the bone represents an available reservoir. Abnormaly low or high blood Ca concentration will lead to acute or chronic pathological states. The prominent factors for homeostatic regulation of Ca and Phosphorus metabolism are Parathyroid hormone (PTH), Calcitonin and vitamin D.

PTH AND CALCITONIN

Parathyroid gland secretes PTH that influence blood Ca levels. Parathyroidectomy in most animal species results in a rapid decline of blood Ca.

Parafollicular C- cells of the thyroid gland in mammals' produce calcitonin which also has a central role in Ca homeostasis. The action of calcitonin is the opposite of PTH, functioning in the same way as the insulin-glucagon system in the control of blood sugar.

When blood Ca levels are high, calcitonin is secreted and apparently acts by inhibiting bone resorption to cause a rapid decrease in blood Ca level presumably to normal.

PTH affects bone resorption by stimulating osteoclastic and osteocytic osteolysis, thereby increasing the number of osteoclasts on the bone surface. PTH has a phosphaturic effect i.e. it inhibits reabsorption of phosphate by the kidneys.

Effects of PTH

1. It directly stimulates the membrane-bound enzyme adenylate cyclase which catalyses the formation of 3,5 adenosyl monophosphate (cyclicAMP or cAMP).
2. PTH also increases Ca movement into responsive cells and these cellular Ca in conjunction with cAMP may be the immediate effector of PTH action on osteocytes, osteoblasts and osteoclasts.

Note: there is no direct effect of PTH by the stimulation of osteoclasts. Osteoblast responds directly to PTH by the stimulation of adenylate cyclase activity and inhibition of collagen synthesis. PTH causes shrinkage of the osteoblast that covers the bone surface thereby exposing bone surface to osteoclastic action.

Calcitonin (anti-hypercalcemic hormone), inhibits osteoclastic bone resorption and this action is mediated by the production of cAMP.

Vitamin D

Deficiency leads to rickets in young animals and osteomalacia in adults. This is characterised by the depressed mineralization of the skeleton while the synthesis of uncalcified matrix, the osteon and osteoid continues. It results in bone with low ash or mineral content in relation to its wet weight, dry weight or total nitrogen content. Other causes of vitamin D deficiency are renal tubular defects, steatorrhoa (maladsorption by intestine) chronic uremia.

Biochemically, in rickets and osteomalacia, blood Ca is normal or low while blood phosphate is low and alkaline phosphatase is either high or normal. Vitamin D which is derived from diet or ultra-violet irradiation of skin is first hydroxylated in the liver to 25-hydoxylcholecalciferol form and subsequently to 1,25-dihydroxylcholecaciferol [1,25-$(OH)_2D_3$]in the kidney. 1,25-$(OH)_2D_3$ is

considered to be the hormonal form of vitamin D (it meets the usual criteria for hormone i.e. produced at one site and causes a response elsewhere and its production is feedback regulated). It is produced in the kidney and elicits its effect in the intestine, bone and other tissues. Its rate of formation is related to the Ca, phosphate and/or PTH levels in the blood and of Ca and Phosphorus needs of the animal.

The major site of action of 1,25-$(OH)_2D_3$ is the intestines where it increases the absorption of Ca. The mechanism appears to involve the stimulation of the synthesis of macromolecules that constitute essential parts of the Ca transport system e.g. of the macromolecule is vitamin D-dependent calcium-binding protein (CaBP)

Vitamin D and its metabolites have a direct effect on the kidney, increasing Ca and phosphate reabsorption. A vitamin CaBP is present in the distal tubule. Receptors for 1,25-$(OH)_2D_3$ have been identified in the bone, pancreas, kidneys, intestine, parathyroid gland and the shell gland of laying hens.

Vitamin D and 1,25-$(OH)_2D_3$ have a direct effect on the intestinal absorption of phosphate.