UNIT-1

Syllabus

Cell Degeneration, repair and neoplasia-Cell injury and Necrosis, Apoptosis, Intracellular accumulations, Pathological calcification, cellular adaptations of growth and differentiation, Inflammation and Repair including fracture healing, Neoplasia, Classification, Benign and Malignant tumours, carcinogenesis, spread of tumours.

Introduction to Pathology and Microbiology:

Pathology is the study and diagnosis of disease through examination of organs, tissues, bodily fluids, and whole bodies (autopsies). Pathology involves examining the cause of illness, how it develops, the effect of the illness on cells and the outcome of the illness. The aspects of illness that may be studied include cellular pathology, cell necrosis or cell death, wound healing, cancer formation and inflammation.

Microbiology is the study of microscopic organisms, such as bacteria, viruses, archaea, fungi and protozoa.

Cell Degeneration:

It is defined as deterioration (death) of live cells following injury, but with a possibility of the injured cells to reverse to normal when the injury is removed. In other words, it is the gradual deterioration of specific tissues, cells with impairment or loss of function, caused by injury, disease, or aging. Deterioration of cells is evaluated in terms of morphological changes that occur inside or outside the cells.

Degeneration Process:

You are DYING every day! More accurately, the degeneration process is slowly killing you, day by day. We have so many cells, and every day we lose more and more. Our bodies' completely regenerate every year and a half. That is, the oldest cell in your body is 1 year and 6 months old. That's it. There are no cells older than that. So, you ask, how come we don't look younger and keep our looks from when we were kids?

The fact is, as we grow older we have less and less proper nutrition, and we are far more affected by free radicals (stress). Our cells have less and less fuel to work with, and when they do multiply they are reborn with a poorer structure. (This is the degeneration process.) All this is to say that the prematurely aging body is caused by a lack of nutrition and the rise in free radicals.

All the cells in our bodies require the right form of nutrients (the materials), the complete formula (the tools) and the proper proportion to create balance (the builder/worker). Let's stay

with this construction terminology to help you understand.

The only time your body will regenerate is at night, because during the day our body fights back all the aggression of the free radicals, and, of course, reacts to your daily needs. Night is when the little workers come in to fix the body. In order for the worker to do his job, he need his materials and his tools. If you are missing any of one of the three (worker, materials and tools) the job will not be complete.

In other words, if you don't have the proper nutrients, your body will not be able to regenerate itself to its maximum. The body however must get the job done while you sleep.

Every year and a half, your body is reproducing cells missing something, and this continues over a 60 year cycle.

Can you imagine what the before and after cells would be like after 60 years? That's why we age and we seem to be more sick, getting degenerative disease and lots more. But even worse, what took 60 years before, now, because of all the stress and lack of nutrition, we can accomplish in less than 10 years.

Notice that a malnourished person often looks years older than they really are, and they are more susceptible to get a degenerative disease.

Basically the degeneration process can be summed up in the degeneration of the cells over a period of time changing the structure and the functionality of the cells.

Cell repair (Regeneration):

When a cell is damaged the body will try to repair or replace the cell to continue normal functions. If a cell dies the body will remove it and replace it with another functioning cell, or fill the gap with connective tissue to provide structural support for the remaining cells. The motto of the repair process is to fill a gap caused by the damaged cells to regain structural continuity. Normal cells try to regenerate the damaged cells but this cannot always happen. Asexual reproduction is what repairs cells.

How our cells repair their damaged DNA

New research shows that some previously overlooked molecules in the body's cells play a key role in the repair of damaged DNA.

The neglected molecule is called histone 1 (H1), and has so far mainly been described as a molecule that helps to organise DNA within cells. But a new study suggests that H1 also plays an important role in DNA repair.

Cancer is characterised as a disease that causes damage to the DNA. Therefore H1 evidently plays an important role in the defence against cancer, as it's pivotal in the recruitment of repair proteins that repair this damage, that H1 plays such a central role in such an important mechanism.

In every cell of the body, DNA suffers damage between 50,000 and 100,000 times a day. This happens when DNA building blocks are swapped or changed around, or where one or both strands of DNA is torn.

When damage occurs, the cell sends repair proteins to the spot to quickly resolve it. In the process of repairing itself, it may be destroyed or converted to a cancer cell.

Scientists have known for some time that the protein ubiquitin plays an important role in the recruitment of repair proteins. But until now they didn't know how ubiquitin actually repaired damaged DNA or how the repair system was regulated.

The new research discovered that ubiquitin sits within the H1 molecule, close to the damaged DNA. When needed, H1 is nearby to help recruit repair proteins directly to the damaged spot.

Two main results**:**

First, the new results are an important piece of the puzzle when it comes to understanding the cellular mechanisms that explain how the body repairs damaged DNA and how cancer arises in the first place. Eventually, this could lead to preventative treatments that are targeted at this repair process.

Second, H1 may have many other undiscovered functions. The recruitment of repair proteins is potentially only one of many.

Cell Regeneration: A Matter of Life and Death

The human body completely regenerates itself every 7-10 years – replacing all of the old, worn out cells with brand spanking new ones. Like many popular myths, this one is only partially true. Many systems of the body (but not all of them) indeed function on regenerative cycles that fall within this time span. This means that, for the most part, we really *do* get entirely new bodies every decade or so.

This important process is called cellular or cell regeneration. It functions as the means by which our bodies stay alive and continue forging on. It helps us to **grow and develop in our younger years, and avoid premature aging and death in our older years**.

Cell Regeneration Timeline: How Long It Takes for Body Parts to Regenerate

Cellular regeneration is evident in the fact that, throughout our lives, we get:

- a new heart every 20 years
- new bones every decade
- new hair every 3-6 years
- new nails every 6-10 months
- new red blood cells every 4 months
- a new liver every 5 months
- a new outer layer of skin every month
- new lungs every three weeks
- new taste buds every two weeks
- new stomach lining every 2-3 days

Cell injury/Cell Damage:

Cell injury is a variety or changes of stress that a cell suffers due to external as well internal environmental changes, is also known as Cell Injury. Among other causes, this can be due to physical, chemical, infectious, biological, nutritional or immunological factors. Cell damage can be reversible or irreversible. Depending on the extent of injury, the cellular response may be adaptive and where possible, homeostasis is restored. Cell death occurs when the severity of the injury exceeds the cell's ability to repair itself. Cell death is relative to both the length of exposure to a harmful stimulus and the severity of the damage caused. Cell death may occur by necrosis or apoptosis.

Causes

- Physical agents capable of causing cell injury include mechanical trauma, extremes of temperature (burns and deep cold), sudden changes in atmospheric pressure, radiation, and electric shock.
- Impaired nutrient supply, such as lack of oxygen or glucose, or impaired production of adenosine triphosphate (ATP) may deprive the cell of essential materials needed to survive.
- Oxygen Deprivation- Hypoxia is a deficiency of oxygen, which causes cell injury by reducing aerobic oxidative respiration. Hypoxia is an extremely important and common cause of cell injury and cell death.
- Causes of hypoxia include reduced blood flow (celled ischemia), inadequate oxygenation of the blood due to cardiorespiratory failure, and decreased oxygen-carrying capacity of the blood, as in anemia or carbon monoxide poisoning (producing a stable carbon monoxyhemoglobin that blocks oxygen carriage) or after severe blood loss.
- Depending on the severity of the hypoxic state, cells may adapt, undergo injury, or die. For example, if an artery is narrowed, the tissue supplied by that vessel may initially shrink in size (atrophy), whereas more severe or sudden hypoxia induces injury and cell death.

- Chemical Agents and Drugs: Simple chemicals such as glucose or salt in hypertonic concentrations may cause cell injury directly or by deranging electrolyte balance in cells. Even oxygen at high concentrations is toxic.
- Trace amounts of poisons, such as arsenic, cyanide, or mercuric salts, may destroy sufficient numbers of cells within minutes or hours to cause death.
- Other potentially injurious substances are our daily companions: environmental and air pollutants, insecticides, and herbicides; industrial and occupational hazards, such as carbon monoxide and asbestos; recreational drugs such as alcohol; and the ever-increasing variety of therapeutic drugs.
- Infectious Agents- These agents range from the submicroscopic viruses to the large tapeworms. In between are the rickettsiae, bacteria, fungi, and higher forms of parasites. The ways by which these biologic agents cause injury are diverse.
- Immunologic Reactions- The immune system serves an essential function in defense against infectious pathogens, but immune reactions may also cause cell injury. Injurious reactions to endogenous self-antigens are responsible for several autoimmune diseases.
- Immune reactions to many external agents, such as microbes and environmental substances, are also important causes of cell and tissue injury
- Genetic Derangements- genetic abnormalities may result in a defect as severe as the congenital malformations associated with Down syndrome, caused by a chromosomal anomaly, or as subtle as the decreased life span of red blood cells caused by a single amino acid substitution in hemoglobin in sickle cell anemia.
- Genetic defects may cause cell injury because of deficiency of functional proteins, such as enzyme defects in inborn errors of metabolism, or accumulation of damaged DNA or misfolded proteins, both of which trigger cell death when they are beyond repair
- Variations in the genetic makeup can also influence the susceptibility of cells to injury by chemicals and other environmental insults.
- Nutritional Imbalances • Nutritional imbalances continue to be major causes of cell injury. Protein-calorie deficiencies cause an appalling number of deaths, chiefly among underprivileged populations. Deficiencies of specific vitamins are found throughout the world
- Nutritional problems can be self-imposed, as in anorexia nervosa (self-induced starvation). Ironically, nutritional excesses have also become important causes of cell injury. Excess of cholesterol predisposes to atherosclerosis; obesity is associated with increased incidence of several important diseases, such as diabetes and cancer.

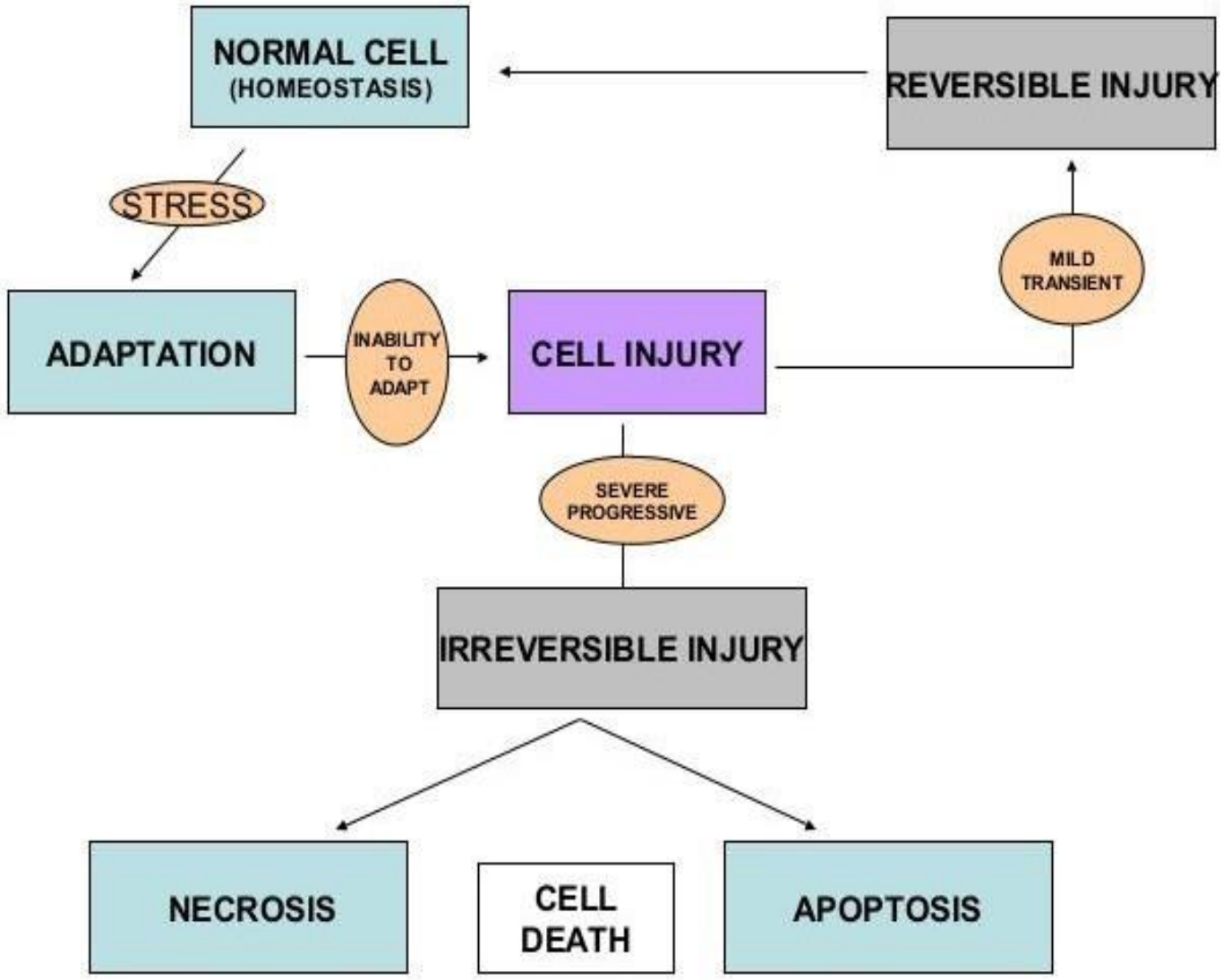
Replacement

When a cell cannot be regenerated the body will replace it with stromal connective tissue to maintain tissue/organ function. Stromal cells are the cells that support the parenchymal cells in any organ. Fibroblasts, immune cells, pericytes, and inflammatory cells are the most common types of stromal cells.

Targets

The most notable components of the cell that are targets of cell damage are the DNA and the cell membrane.

- **DNA damage:** In human cells, both normal metabolic activities and environmental factors such as ultraviolet light and other radiations can cause DNA damage, resulting in as many as one million individual molecular lesions per cell per day.
- **Membrane damage:** damage to the cell membrane disturbs the state of cell electrolytes, e.g. calcium, which when constantly increased, induces apoptosis.



Necrosis

Structural changes of cells undergoing necrosis and apoptosis

Necrosis (from the Greek word "death, the stage of dying, the act of killing") is a form of cell injury which results in the premature death of cells in living tissue by autolysis.

Necrosis is caused by factors external to the cell or tissue, such as infection, toxins, or trauma which result in the unregulated digestion of cell components. It is the death of living cells or tissues. Necrosis can be due, for example, to ischemia (lack of blood flow).

It is a form of premature tissue death, as opposed to the spontaneous natural death or wearing out of tissue, which is known as necrobiosis. Necrosis is further distinguished from apoptosis, or programmed cell death, which is internally regulated by cells, plays a critical role in embryonic development, and serves as a protective mechanism against disease and other factors.

Necrosis may follow a wide variety of injuries, both physical and biological in nature. Examples of physical injuries include cuts, burns, bruises, oxygen deprivation (anoxia), and hyperthermia. Biological injuries can include immunological attack and the effects of disease-causing agents. Notable conditions involving necrotic tissue death include avascular necrosis and gangrene, which result from a lack of blood supply to the affected area; necrotizing fasciitis, which is caused by a rapidly spreading bacterial infection; and loxoscelism, in which venom in a bite from a recluse spider (*Loxosceles*) produces a gangrenous wound. Such injuries and diseases inhibit crucial intracellular metabolic processes, in which intracellular enzymes become activated upon injury and destroy damaged cells. Lesions caused by necrosis often are of diagnostic value.

Early cellular signs of necrosis include swelling of the mitochondria, a process that impairs intracellular oxidative metabolism. Later, localized densities appear, with condensation of genetic material. Cytoplasmic organelles are disrupted, and affected cells separate from neighbouring cells. The dissolution of lysosomes, which normally house hydrolytic enzymes, leads to intracellular acidosis. The nucleus swells and darkens (pyknosis) and eventually ruptures (karyolysis). The outer membrane of the cell also ruptures, resulting in a loss of ion-pumping capacity and a rapid flow of sodium and calcium ions into the intracellular environment, resulting in osmotic shock (a sudden shift in intracellular and extracellular solute concentrations).

Classification

Structural signs that indicate irreversible cell injury and the progression of necrosis include dense clumping and progressive disruption of genetic material, and disruption to membranes of cells and organelles.

Morphological patterns

There are six distinctive morphological patterns of necrosis:

1. **Coagulative necrosis:** It is characterized by the formation of a gelatinous (gel-like) substance in dead tissues in which the architecture of the tissue is maintained, and can be observed by light microscopy. Coagulation occurs as a result of protein denaturation, causing albumin to transform into a firm and opaque state. This pattern of necrosis is typically seen in hypoxic (low-oxygen) environments, such as infarction. Coagulative necrosis occurs primarily in tissues such as the kidney, heart and adrenal glands. Severe ischemia most commonly causes necrosis of this form.
2. **Liquefactive necrosis (or colliquative necrosis)**, in contrast to coagulative necrosis, is characterized by the digestion of dead cells to form a viscous liquid mass. This is typical of bacterial, or sometimes fungal, infections because of their ability to stimulate an inflammatory response. The necrotic liquid mass is frequently creamy yellow due to the presence of dead leukocytes and is commonly known as pus. Hypoxic infarcts in the brain presents as this type of necrosis, because the brain contains little connective tissue but high amounts of digestive enzymes and lipids, and cells therefore can be readily digested by their own enzymes.
3. **Gangrenous necrosis**: It can be considered a type of coagulative necrosis that resembles mummified tissue. It is characteristic of ischemia of lower limb and the gastrointestinal tracts. If superimposed infection of dead tissues occurs, then liquefactive necrosis ensues (wet gangrene)
4. **Caseous necrosis:** It can be considered a combination of coagulative and liquefactive necrosis, typically caused by mycobacteria (e.g. tuberculosis), fungi and some foreign substances. The necrotic tissue appears as white and friable, like clumped cheese. Dead cells disintegrate but are not completely digested, leaving granular particles. Microscopic examination shows amorphous granular debris enclosed within a distinctive inflammatory border. Granuloma has this characteristic.
5. **Fat necrosis:** It is specialized necrosis of fat tissue, resulting from the action of activated lipases on fatty tissues such as the pancreas. In the pancreas it leads to acute pancreatitis, a condition where the pancreatic enzymes leak out into the peritoneal cavity, and liquefy the membrane by splitting the triglyceride esters into fatty acids through fat saponification. Calcium, magnesium or sodium may bind to these lesions to produce a chalky-white substance. The calcium deposits are microscopically distinctive and may be large enough to be visible on radiographic examinations. To the naked eye, calcium deposits appear as gritty white flecks.
6. **Fibrinoid necrosis:** It is a special form of necrosis usually caused by immune-mediated vascular damage. It is marked by complexes of antigen and antibodies, sometimes referred to as "immune complexes" deposited within arterial walls together with fibrin.

Causes

Necrosis may occur due to external or internal factors.

External factors may involve mechanical trauma (physical damage to the body which causes cellular breakdown), damage to blood vessels (which may disrupt blood supply to associated tissue), and ischemia. Thermal effects (extremely high or low temperature) can result in necrosis due to the disruption of cells.

In frostbite, crystals form, increasing the pressure of remaining tissue and fluid causing the cells to burst. Under extreme conditions tissues and cells die through an unregulated process of destruction of membranes and cytosol.

Internal factors causing necrosis include: trophoneurotic disorders; injury and paralysis of nerve cells. Pancreatic enzymes (lipases) are the major cause of fat necrosis.

Pathogenesis

Until recently, necrosis was thought to be an unregulated process. There are two broad pathways in which necrosis may occur in an organism.

The first of these two pathways initially involves oncosis, where swelling of the cells occur. The cell then proceeds to blebbing, and this is followed by pyknosis, in which nuclear shrinkage transpires. In the final step of this pathway the nucleus is dissolved into the cytoplasm, which is referred to as karyolysis.

The second pathway is a secondary form of necrosis that is shown to occur after apoptosis and budding. Cellular changes of necrosis occur in this secondary form of apoptosis, where the nucleus breaks into fragments, which is known as karyorrhexis.

Cellular changes

The nucleus changes in necrosis, and characteristics of this change are determined by manner in which its DNA breaks down:

- **Karyolysis**: the chromatin of the nucleus fades due to the loss of the DNA by degradation.
- **Pyknosis**: the nucleus shrinks and the chromatin condenses.
- **Karyorrhexis**: the shrunken nucleus fragments to complete dispersal.

Plasma alterations are also seen in necrosis. Plasma membranes appear discontinuous when viewed with an electron microscope. This discontinuous membrane is caused by cell blebbing and the loss of microvilli.

Treatment

There are many causes of necrosis, and as such treatment is based upon how the necrosis came about. Treatment of necrosis typically involves two distinct processes: Usually, the underlying cause of the necrosis must be treated before the dead tissue itself can be dealt with.

- Debridement, referring to the removal of dead tissue by surgical or non-surgical means, is the standard therapy for necrosis. Depending on the severity of the necrosis, this may range from removal of small patches of skin to complete amputation of affected limbs or organs. Chemical removal of necrotic tissue is another option in which enzymatic debriding agents, categorised as proteolytic, fibrinolytic or collagenases, are used to target the various components of dead tissue. In select cases, special maggot therapyusing *Lucilia sericata* larvae has been employed to remove necrotic tissue and infection.
- In the case of ischemia, which includes myocardial infarction, the restriction of blood supply to tissues causes hypoxia and the creation of reactive oxygen species (ROS) that react with, and damage proteins and membranes. Antioxidant treatments can be applied to scavenge the ROS.
- Wounds caused by physical agents, including physical trauma and chemical burns, can be treated with antibiotics and anti-inflammatory drugs to prevent bacterial infection and inflammation. Keeping the wound clean from infection also prevents necrosis.
- Chemical and toxic agents (e.g. pharmaceutical drugs, acids, bases) react with the skin leading to skin loss and eventually necrosis. Treatment involves identification and discontinuation of the harmful agent, followed by treatment of the wound, including prevention of infection and possibly the use of immunosuppressive therapies such as anti-inflammatory drugs or immunosuppressants. In the example of a snake bite, the use of anti-venom halts the spread of toxins whilst receiving antibiotics to impede infection.

Even after the initial cause of the necrosis has been halted, the necrotic tissue will remain in the body. The body's immune response to apoptosis, which involves the automatic breaking down and recycling of cellular material, is not triggered by necrotic cell death due to the apoptotic pathway being disabled.

Apoptosis

Apoptosis is a process that occurs in multicellular when a cell intentionally “decides” to die. This often occurs for the greater good of the whole organism, such as when the cell’s DNA has become damaged and it may become cancerous.

Apoptosis is referred to as **“Programmed Cell Death (PCD)”** because it happens due to biochemical instructions in the cell’s DNA; this is opposed to the process of “necrosis,” when a cell dies due to outside trauma or deprivation. Like many other complex cellular processes, apoptosis is triggered by signal molecules that tell the cell it’s time to commit cellular “suicide.”

The two major types of apoptosis pathways are “intrinsic pathways,” where a cell receives a signal to destroy itself from one of its own genes or proteins due to detection of DNA damage; and “extrinsic pathways,” where a cell receives a signal to start apoptosis from other cells in the

organism. The extrinsic pathway may be triggered when the organism recognizes that a cell has outlived its usefulness or is no longer a good investment for the organism to support. Apoptosis plays a role in causing and preventing some important medical processes. In humans, apoptosis plays a major role in preventing cancer by causing cells with damaged DNA to commit "suicide" before they can become cancerous. It also plays a role in the atrophy of muscles, where the body decides that it's no longer a good idea to spend calories on maintaining muscle cells if the cells are not being regularly used. Because apoptosis can prevent cancer, and because problems with apoptosis can lead to some diseases, apoptosis has been studied intensely by scientists since the 1990s.

Apoptosis and Cancer

One primary function of apoptosis is to destroy cells that are dangerous to the rest of the organism. A common reason for apoptosis is when a cell recognizes that its DNA has been badly damaged. In these cases, the DNA damage triggers apoptosis pathways, ensuring that the cell cannot become a malignant cancer.

However, clearly this process sometimes fails. All instances of cancer are presumably instances where a damaged cell did not commit apoptosis, but instead went on to make more of itself.

Apoptosis may be unable to occur if essential genes required for it are among those that are damaged. However, some doctors and scientists have been studying apoptosis intensely in hopes that they may be able to learn to trigger it specifically in cancer cells using new medications or other therapies.

As with all drugs designed to kill cancer cells, the challenge with drugs designed to induce apoptosis is to ensure that these drugs *only* effect cancer cells. A medication that causes healthy cells as well as cancerous ones to commit programmed cell death could be very dangerous.

Apoptosis Pathway

There are two major types of apoptosis pathways, each of which illustrates an important point about how apoptosis is triggered and why it is useful. The steps are discussed in more detail in the following lists:

Extrinsic Pathway

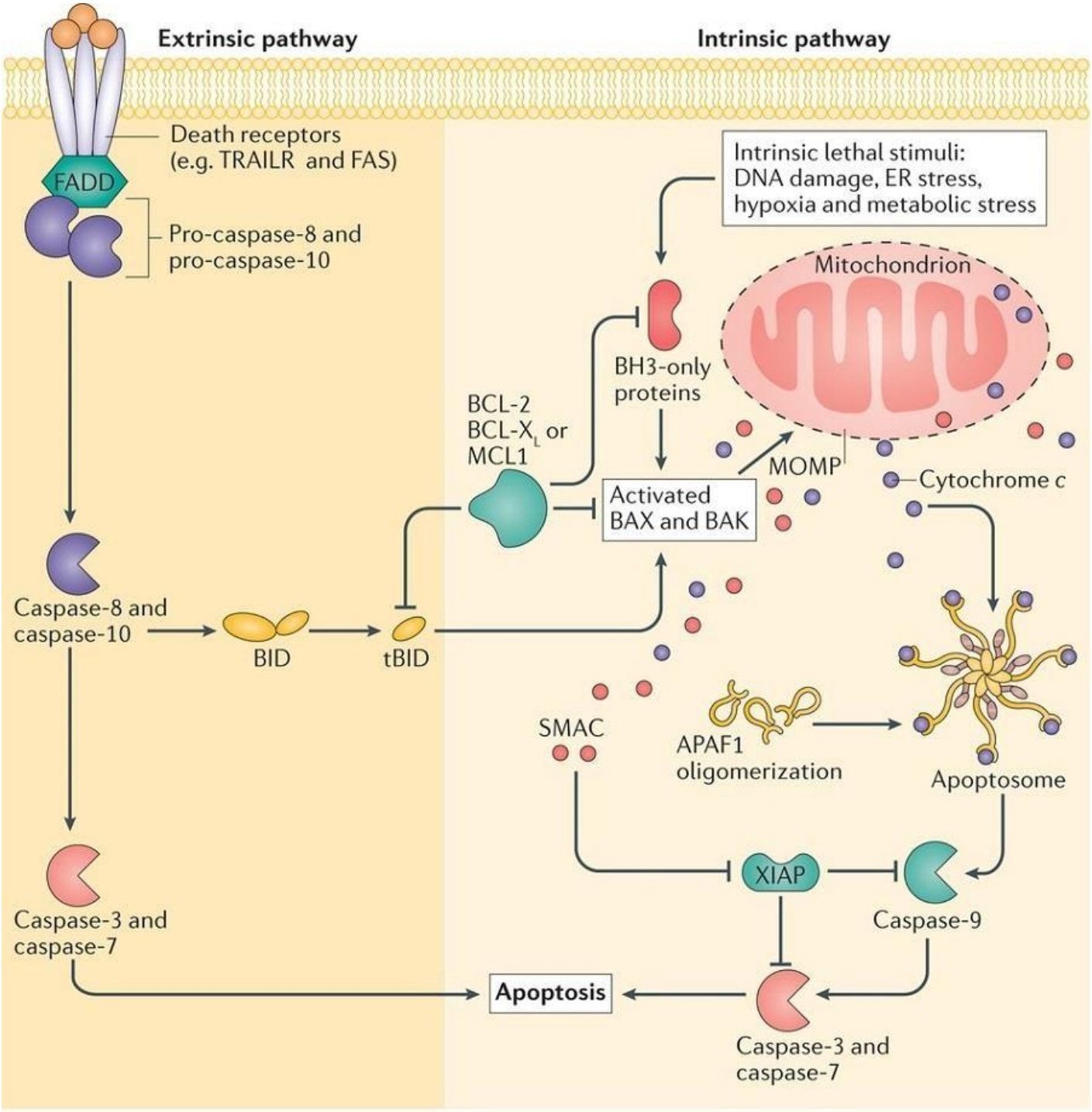
In the "extrinsic" pathway to apoptosis, a signal is received from outside the cell instructing it to commit programmed cell death. This may occur if the cell is no longer needed, or if it is diseased.

Like many pathways for bringing about complex changes in a cell, the extrinsic pathway to apoptosis involves many steps, each of which can be "upregulated" or "downregulated" by gene expression or by other molecules:

Step 1:
Like most signaling between cells, the extrinsic pathway of apoptosis starts with a signal molecule binding to a receptor on the outside of the cell membrane. Two common types of chemical messengers that trigger the extrinsic pathway to apoptosis are FAS and TRAIL. These molecules may be excreted by neighboring cells if a cell is damaged or no longer needed. The receptors that bind to FAS and TRAIL are called "FASR" for "FAS Receptor" or "TRAILR" for "TRAIL Receptor." As with most receptor proteins, when FASR and TRAILR encounter to their signal molecule – sometimes called a "ligand" – they bind to it. The binding process causes changes to the receptor's intracellular domain.

Step 2:
In response to the changes in the intracellular domain of TRAILR or FASR, a protein inside the cell called FADD also changes. FADD's name is either amusing or terrifying: it stands for "FAS-Associated Death Domain" protein. Once FADD has been activated by changes to the receptor, it interacts with two additional proteins, which go on to start the process of cell death.

Step 3:
Pro-caspase-8 and pro-caspase-10 are inactive proteins until they interact with an activated FADD. But if two of these molecules encounter an activated FADD, the parts of the proteins that keep them inactive are "cleaved" or "cut" away. The pro-caspases then become caspase-8 and caspase-10 – which have been romantically referred to by scientists as "the beginning of the end" due to their role in starting apoptosis. Caspases-8 and -10 disperse through the cytoplasm and trigger changes to several other molecules throughout the cell, including messengers that start the breakdown of DNA after being activated by the caspases.



Step 4:

Another inactive molecule called BID is transformed into tBID when the activated caspases cleave off the part of BID that keeps the molecule inactive. After BID is transformed into tBID, tBID moves to the mitochondria. tBID activates the molecules BAX and BAK. The activation of

BAX and BAK are the first steps shared by both the extrinsic and intrinsic pathways to apoptosis.

Steps 1-4 listed here are unique to the extrinsic pathway. But after BAX and BAK are activated, the subsequent steps are the same between both pathways. As such, steps 3-7 of the intrinsic pathway, listed below, are also steps 5-9 of the extrinsic pathway!

Intrinsic Pathway

Step 1:
The intrinsic pathway to apoptosis is triggered by stress or damage to the cell. Types of stress and damage that can lead the cell to apoptosis include damage to its DNA, oxygen deprivation, and other stresses that impair a cell's ability to function. In response to these damages or stresses, the cell "decides" that its continued existence might be dangerous or costly to the organism as a whole. It then activates a set of proteins called "BH3-only proteins."

Step 2:
BH3-only proteins are a class of proteins including several pro- and anti-apoptosis proteins. Apoptosis can be encouraged or discouraged, depending on which BH3-only proteins are activated or expressed. Pro-apoptotic BH3-only proteins activate BAX and BAK – the same proteins that are activated by tBID after it is created through the extrinsic pathway to apoptosis.

Step 3:
Activated BAX and BAK cause a condition known as "MOMP." MOMP stands for "mitochondrial outer membrane permeability." MOMP is considered the "point of no return" for apoptosis. The steps leading up to MOMP can be stopped in their tracks by inhibitor molecules, but once MOMP has been achieved, the cell will complete the death process. MOMP plays its key role in apoptosis by allowing the release of cytochrome C into the cytoplasm.

Step 4:
Under normal circumstances, cytochrome C plays a key role in the mitochondrial electron transport chain. During MOMP, however, cytochrome C can escape the mitochondria and act as a signaling molecule in the cell cytoplasm. Cytochrome-C in the cell cytoplasm prompts the formation of the ominous-sounding "apoptosome" – a complex of proteins that performs the final step to beginning cellular breakdown.

Step 5:
The apoptosome, once it is formed, turns pro-caspase-9 into caspase-9. Just as with the activation of caspases-8 and -10 in the extrinsic pathway to apoptosis, caspase-9 is able to trigger further changes throughout the cell.

Step 6:
Caspase-9 performs several functions to promote apoptosis. Among the most important is the activation of caspases-3 and -7.

Step 7:
Once activated, caspases-3 and -7 begin the breakdown of cellular materials. Caspase-3 condenses and breaks down the cell's DNA.

When Does Apoptosis Occur?

Apoptosis occurs when a cell's existence is no longer useful to the organism. This can occur for a few reasons. If a cell has become badly stressed or damaged, it may commit apoptosis to prevent itself from becoming dangerous to the organism as a whole. Cells with DNA damage, for example, may become cancerous, so it is better for them to commit apoptosis before that can happen.

Other cellular stresses, such as oxygen deprivation, can also cause a cell to "decide" that it is dangerous or costly to the host. Cells that can't function properly may initiate apoptosis, just like cells that have experienced DNA damage.

In a third scenario, cells may commit apoptosis because the organism doesn't need them anymore due to its natural development.

Intracellular accumulations

DEFINITION: Accumulation of abnormal amounts of various substances due to manifestations of metabolic derangements in the cell.

One of the manifestations of metabolic derangements in cells is the intracellular accumulation of abnormal amounts of various substances.
The stockpiled substances fall into three categories:

- (1) a normal cellular constituent accumulated in excess, such as water, lipids, proteins, and carbohydrates;
- (2) an abnormal substance, either exogenous, such as a mineral or products of infectious agents, or endogenous, such as a product of abnormal synthesis or metabolism;
- (3) a pigment.

These substances may accumulate either transiently or permanently, and they may be harmless to the cells, but on occasion they are severely toxic. The substance may be located in either the cytoplasm (frequently within phagolysosomes) or the nucleus.
In some instances, the cell may be producing the abnormal substance, and in others it may be merely storing products of pathologic processes occurring elsewhere in the body.
Many processes result in abnormal intracellular accumulations, but most accumulations are attributable to three types of abnormalities.

A normal endogenous substance is produced at a normal or increased rate, but the rate of metabolism is inadequate to remove it. An example of this type of process is fatty change in the liver because of intracellular accumulation of triglycerides.

Another is the appearance of reabsorption protein droplets in renal tubules because of increased leakage of protein from the glomerulus. A normal or abnormal endogenous substance accumulates because of genetic or acquired defects in the metabolism, packaging, transport, or secretion of these substances. One example is the group of conditions caused by genetic defects of specific enzymes involved in the metabolism of lipid and carbohydrates resulting in intracellular deposition of these substances, largely in lysosomes.

Another is alpha1-antitrypsin deficiency, in which a single amino acid substitution in the enzyme results in defects in protein folding and accumulation of the enzyme in the endoplasmic reticulum of the liver in the form of globular eosinophilic inclusions.
An abnormal exogenous substance is deposited and accumulates because the cell has neither the enzymatic machinery to degrade the substance nor the ability to transport it to other sites. Accumulations of carbon particles and such nonmetabolizable chemicals as silica particles are examples of this type of alteration. Whatever the nature and origin of the intracellular accumulation, it implies the storage of some product by individual cells.

If the overload is due to a systemic derangement and can be brought under control, the accumulation is reversible. In genetic storage diseases, accumulation is progressive, and the cells may become so overloaded as to cause secondary injury, leading in some instances to death of the tissue and the patient.

Intracellular accumulations include the following:

1. Water (hydropic change)
2. Fatty change: fats may accumulate in the liver as fatty change
3. Cholesterol & esters: sphingolipidoses and other lipid accumulations
4. Proteins: abnormal protein accumulation is often irreversible.
5. Glycogen: glycogen storage diseases
6. Complex carbohydrates: mucopolysaccharidoses and other complex carbohydrate diseases.
7. Minerals: iron, as hemosiderin, or carbon, as anthracotic pigment
8. Pigments
9. Calcium
10. Amyloid

Causes

Intracellular accumulations may take place by:

1. Increased production of normal products due to inadequate functioning
2. Defective mechanism of removal of normal/abnormal substance which has accumulated
3. Increased exogenous substance with no removal mechanism.

Fatty Change

Accumulation of excessive lipid in cells is known as fatty change. It may occur in liver, heart or the kidneys.

Hepatic lipid accumulation is characterized by intracellular accumulation of triglycerides, and due to the failure of metabolic removal. Defects in fat metabolism are often induced by alcohol consumption, and also associated with diabetes, obesity, and toxins. Fatty change is most often seen in the liver (and heart), and is generally reversible.

Fatty acids enter hepatocytes, triglycerides combine with apoproteins and exit the liver. Defect in any of these above steps may lead to fatty change.

Causes of Fatty Change

Causes of fatty change include:

1. Toxins, alcohol
2. Protein malnutrition
3. Diabetes mellitus
4. Hypoxia ,anemia, ischemia
5. Drugs, pregnancy & obesity

Morphology of Fatty change

Gross morphology in liver depends on severity. Increased size is observed and liver becomes yellow and greasy when severe.

Histology

Microvesicular cytoplasm or macrovsicular cytoplasm is seen.

Microscopy

Fat vacuoles coalesce and displace the nucleus to the periphery of the cell. Vacuoles appear clear, with well-defined edges. Lipid accumulations must be distinguished from accumulations of water or glycogen, using special preparation and stain – Oil Red-O.

Cholesterol and Esters

Cholesterol & esters accumulate in macrophages (foam cells) and foreign body giant cells, resulting in:

a. Atherosclerosis
b. Hereditary & aquired hyperlipidemias with xanthoma formation

Pigments

Pigments may be endogenous or exogenous:

1. Endogenous

– Hb derived iron, bilirubin
– Non Hb derived melanin, lipofusion
– Melanin -Brown pigment synthesized in melanocytes which protects nuclei of basal epidermal cells from UV light.
– Malignant melanoma of eye, rectum

2. Exogenous

– Anthracosis -Accumulation of carbon, black pigment
– Tatooing

Bile pigment (bilirubin)

Bilirubin is derived from heme of Hb from destroyed RBCs in reticuloendothelial system. It is conjugated in hepatocytes with glucuronic acid & excreted as bile. Increased bilirubin may occur due to:

a. Hyperbilirubinemia, jaundice
b. Hemolysis, liver disease, obstruction to outflow of bile.

Excess iron accumulation

Total body iron is 2-4 gms. In functional pool, it is present in Hb, myoglobin, cytochrome and catalase. In the storage pool, it is present in macrophages of reticuloendothelial cells as Fe3+, ferritin or hemosiderin. It gives Purssian blue reaction.

Iron overload

Iron overload may occur due to:

- **Localized increase in iron in tissue**

a. Hematoma
b. Chronic venous congestion lung, heart

- **Systemic increase in iron**

a. Hemosiderosis, Fe in RES without damage
b. Hemolytic disease
c. Multiple blood transfusions
d. I/V Fe administration

Idiopathic hemochromatosis

Lack of regulation of iron absorbtion & defects in monocyte macrophage system leads to hemochromatosis. Iron gets deposited in liver, pancrease & RES, resulting in fibrosis, secondary diabetes mellitus, liver cirrhosis & cancer.

Haemosiderin

Haemosiderin is derived from hemoglobin. It is a golden yellow, granular or crystalline pigment and is the storage form of iron. It forms in response to local or systemic excess of iron. Ferritin forms hemosiderin granules.

- local excess: from gross or minute hemorrhage (eg. bruise)
- systemic excess: from increased absorption of dietary iron, impaired use of iron, hemolytic anemia, transfusions

Lipofuscin

Lipofuscin is the brown pigment in cytoplasm, which is oxidized lipid, derived from digested membrane organelles. It is part of aging process and atrophy in which lipid peroxidation occurs. It is harmless to cells and increased amount is seen in brown atrophic organs.
It is also known as "wear & tear" pigment derived through lipid peroxidation of polyunsaturated lipids of subcellular membranes. It accumulates in tissues undergoing slow, regressive changes – common in liver and heart of aging patients or patients with severe malnutrition and cancer cachexia.
It appears as a yellow-brown, finely granular, intracytoplasmic (or perinuclear) pigment.

Nevi

There are many, many adjectives and classifications of nevi. The MAIN things to differentiate these from melanomas. They may be:

- Junctional (more pigmented, more closely associated with melanoma)
- Intradermal
- Compound (both)

Malignant Melanoma

Malignant melanomas are malignant proliferations of melanocytes. The incidence of malignant melanoma is rising. It is related to sun like all skin cancers. It is the only primary skin cancer that

can kill (except for the rare Merkel cell tumor). It quickly metastasizes, having both vertical and horizontal growth phase.
It is difficult to differentiate from nevus clinically and often microscopically.

Pathological Calcification:

Calcification is the accumulation of calcium salts in a body tissue. It normally occurs in the formation of bone, but calcium can be deposited abnormally in soft tissue, causing it to harden. Calcifications may be classified on whether there is mineral balance or not, and the location of the calcification. It is the Deposition of calcium salts in tissues other than osteoid or enamel is called pathologic or heterotopic calcification.

Classification:

Pathological calcification is of two types:

1. DYSTROPHIC CALCIFICATION:

Macroscopic deposition of Ca salts in dead or degenerated tissue(injured tissues). It represents extracellular deposition of calcium from circulation or interstitial fluid. Its often visible to naked eye and ranges from gritty sand like grains to firm rock hard material. It requires persistence of necrotic tissue. Caseous necrosis in tuberculosis is the most commen site and has no functional consequences. Dystrophic calcification may also occur in crucial locations such as in mitral or aortic valves after rheumatic fever. In such instances calcification leads to impeded blood flow because it produces inflexible valve leaflets and narrowed valve orifices (mitral and aortic stenosis). Dystrophic calcification in artherosclerosis leads to narrowing of vessels. It also plays a role in diagnostic radiography. Mammography is based on detection of calcification in breast cancer. Diagnosis of congenital toxoplasmosis, an infection involving the CNS is suggested by visualization of calcification in infant brain.

2. METASTATIC CALCIFICATION:

It reflects deranged calcium metabolism, a change associated with an increased serum calcium concentration. Any disorder that increases the serum calcium level can lead to calcification in inappropriate locations. Its seen in various disorders like chronic renal failure, vitamin D intoxication etc.

CAUSES OF METASTATIC CALCIFICATION

1.Increased secretion of parathyroid hormone
2.Destruction of bone tissue as in tumors of bone marrow
3.Vit D related causes –vit D intoxication.
4.Renal failure

OTHER FORMS OF CALCIFICATION: Formation of stones containing calcium carbonate in sites such as gall bladder, renal pelvis and pancreatic duct. Under certain conditions the mineral salts precipitate from solution and crystallize around foci of organic material.

Types of calcification

Calcifications can form in many places throughout your body, including:

- small and large arteries
- heart valves
- brain, where it's known as cranial calcification
- joints and tendons, such as knee joints and rotator cuff tendons
- soft tissues like breasts, muscles, and fat
- kidney, bladder, and gall bladder

Some calcium buildup is harmless. These deposits are believed to be the body's response to inflammation, injury, or certain biological processes. However, some calcifications can disrupt organ function and affect blood vessels.

Causes of calcification

Many factors play a role in calcification. These include:

- Infections.
- Calcium metabolism disorders that cause hypercalcemia (too much calcium in the blood).
- Genetic or autoimmune disorders affecting the skeletal system and connective tissues.
- Persistent inflammation.

Diagnosing calcification

Calcifications are usually found via X-rays. X-ray tests use electromagnetic radiation to take pictures of your internal organs and usually cause no discomfort. Your doctor will likely detect any calcification issues right away with X-rays.

Your doctor may also order blood tests. For example, if you have kidney stones, these tests can determine your overall kidney function.

Sometimes calcium deposits are found in areas of cancer. A calcification is usually tested to rule out cancer as a cause. Your doctor will order a biopsy (often through a fine needle) to collect a tissue sample. The sample is then sent to a laboratory for testing. If there aren't any cancer cells detected, your doctor will label the calcification as benign.

Treating calcification

Calcification treatment depends on several factors:

- Where do the calcium deposits occur?
- What is their underlying cause?
- What, if any, complications arise?

Your doctor will require regular follow-up appointments to check for potential complications once calcifications have been found. Minor artery calcifications aren't considered dangerous. Heart valves can also develop calcifications. In this case, you may need surgery to open or replace the valve if the calcium buildup is severe enough to affect the valve's function.

Kidney stone treatments help break down calcium buildup in the kidneys. Your doctor may prescribe a diuretic called thiazide to help prevent future calcium kidney stones. This diuretic signals the kidneys to release urine while holding on to more calcium.

Calcium deposits in your joints and tendons don't always cause painful symptoms, but they can affect range of motion and cause discomfort. Treatments may include taking anti-inflammatory medicines and applying ice packs. If the pain doesn't go away, your doctor may recommend surgery.

Adaptations of Cellular Growth and Differentiation:

Adaptations are reversible changes in the size, number, phenotype, metabolic activity, or functions of cells in response to changes in their environment. Such adaptations may take several distinct forms.

HYPERTROPHY

Hypertrophy refers to an increase in the size of cells, resulting in an increase in the size of the organ. The hypertrophied organ has no new cells, just larger cells. The increased size of the cells is due to the synthesis of more structural components of the cells. Cells capable of division may respond to stress by undergoing both hyperplasia (described below) and hypertrophy, whereas in nondividing cells (e.g., myocardial fibers) increased tissue mass is due to hypertrophy.

In many organs hypertrophy and hyperplasia may coexist and contribute to increased size. Hypertrophy can be physiologic or pathologic and is caused by increased functional demand or by stimulation by hormones and growth factors. The striated muscle cells in the heart and skeletal muscles have only a limited capacity for division, and respond to increased metabolic demands mainly by undergoing hypertrophy.

The most common stimulus for hypertrophy of muscle is increased workload. For example, the bulging muscles of bodybuilders engaged in “pumping iron” result from an increase in size of the individual muscle fibers in response to increased demand. In the heart, the stimulus for hypertrophy is usually chronic hemodynamic overload, resulting from either hypertension or faulty valves. In both tissue types the muscle cells synthesize more proteins and the number of myofilaments increases. This increases the amount of force each myocyte can generate, and thus increases the strength and work capacity of the muscle as a whole.

Mechanisms of Hypertrophy

Hypertrophy is the result of increased production of cellular proteins. Much of our understanding of hypertrophy is based on studies of the heart. Hypertrophy can be induced by the linked actions of mechanical sensors (that are triggered by increased work load), growth factors (including TGF-β, insulin-like growth factor-1 [IGF-1], fibroblast growth factor), and vasoactive agents (such as α-adrenergic agonists, endothelin-1, and angiotensin II). Indeed, mechanical sensors themselves induce production of growth factors and agonists. These stimuli work coordinately to increase the synthesis of muscle proteins that are responsible for the hypertrophy.

The two main biochemical pathways involved in muscle hypertrophy seem to be the phosphoinositide 3-kinase/Akt pathway (postulated to be most important in physiologic, e.g., exercise-induced, hypertrophy) and signaling downstream of G protein-coupled receptors (induced by many growth factors and vasoactive agents, and thought to be more important in pathologic hypertrophy).

Hypertrophy may also be associated with a switch of contractile proteins from adult to fetal or neonatal forms. For example, during muscle hypertrophy the α isoform of myosin heavy chain is replaced by the β isoform, which has a slower, more energetically economical contraction. In addition, some genes that are expressed only during early development are reexpressed in hypertrophic cells, and the products of these genes participate in the cellular response to stress.

For example, the gene for atrial natriuretic factor (ANF) is expressed in both the atrium and the ventricle in the embryonic heart, but it is down-regulated after birth. Cardiac hypertrophy, however, is associated with reinduction of ANF gene expression. ANF is a peptide hormone that causes salt secretion by the kidney, decreases blood volume and pressure, and therefore serves to reduce hemodynamic load.

HYPERPLASIA

Hyperplasia is an increase in the number of cells in an organ or tissue, usually resulting in increased mass of the organ or tissue. Although hyperplasia and hypertrophy are distinct processes, frequently they occur together, and they may be triggered by the same external stimulus. Hyperplasia takes place if the cell population is capable of dividing, and thus increasing the number of cells.

Hyperplasia can be physiologic or pathologic.

Physiologic Hyperplasia:

Physiologic hyperplasia can be divided into:

(1) hormonal hyperplasia, which increases the functional capacity of a tissue when needed, and
(2) compensatory hyperplasia, which increases tissue mass after damage or partial resection.

Hormonal hyperplasia is well illustrated by the proliferation of the glandular epithelium of the female breast at puberty and during pregnancy, usually accompanied by enlargement

(hypertrophy) of the glandular epithelial cells. The classical illustration of compensatory hyperplasia comes from the myth of Prometheus, which shows that the ancient Greeks recognized the capacity of the liver to regenerate.

In individuals who donate one lobe of the liver for transplantation, the remaining cells proliferate so that the organ soon grows back to its original size. Experimental models of partial hepatectomy have been very useful for defining the mechanisms that stimulate regeneration of the liver.

Pathologic Hyperplasia:

Most forms of pathologic hyperplasia are caused by excesses of hormones or growth factors acting on target cells. Endometrial hyperplasia is an example of abnormal hormone-induced hyperplasia. Normally, after a menstrual period there is a rapid burst of proliferative activity in the epithelium that is stimulated by pituitary hormones and ovarian estrogen. It is brought to a halt by the rising levels of progesterone, usually about 10 to 14 days before the end of the menstrual period.

In some instances, however, the balance between estrogen and progesterone is disturbed. This results in absolute or relative increases in the amount of estrogen, with consequent hyperplasia of the endometrial glands. This form of pathologic hyperplasia is a common cause of abnormal menstrual bleeding. Benign prostatic hyperplasia is another common example of pathologic hyperplasia induced by responses to hormones, in this case, androgens.

Although these forms of hyperplasia are abnormal, the process remains controlled because there are no mutations in genes that regulate cell division, and the hyperplasia regresses if the hormonal stimulation is eliminated. In cancer the growth control mechanisms become dysregulated or ineffective because of genetic aberrations, resulting in unrestrained proliferation. Thus, hyperplasia is distinct from cancer, but pathologic hyperplasia constitutes a fertile soil in which cancerous proliferation may eventually arise. For instance, patients with hyperplasia of the endometrium are at increased risk for developing endometrial cancer.

Hyperplasia is a characteristic response to certain viral infections, such as papillomaviruses, which cause skin warts and several mucosal lesions composed of masses of hyperplastic epithelium. Here, growth factors produced by viral genes or by infected cells may stimulate cellular proliferation.

Mechanisms of Hyperplasia:

Hyperplasia is the result of growth factor–driven proliferation of mature cells and, in some cases, by increased output of new cells from tissue stem cells. For instance, after partial hepatectomy growth factors are produced in the liver that engage receptors on the surviving cells and activate signaling pathways that stimulate cell proliferation. But if the proliferative capacity of the liver cells is compromised, as in some forms of hepatitis causing cell injury, hepatocytes can instead regenerate from intrahepatic stem cells.

ATROPHY

Atrophy is reduced size of an organ or tissue resulting from a decrease in cell size and number. Atrophy can be physiologic or pathologic.

Physiologic atrophy is common during normal development. Some embryonic structures, such as the notochord and thyroglossal duct, undergo atrophy during fetal development. The uterus decreases in size shortly after parturition, and this is a form of physiologic atrophy.

Pathologic atrophy depends on the underlying cause and can be local or generalized. The common causes of atrophy are the following:

1. Decreased workload (atrophy of disuse): When a fractured bone is immobilized in a plaster cast or when a patient is restricted to complete bedrest, skeletal muscle atrophy rapidly ensues. The initial decrease in cell size is reversible once activity is resumed. With more prolonged disuse, skeletal muscle fibers decrease in number (due to apoptosis) as well as in size; this atrophy can be accompanied by increased bone resorption, leading to osteoporosis of disuse.

2. Loss of innervation (denervation atrophy): The normal metabolism and function of skeletal muscle are dependent on its nerve supply. Damage to the nerves leads to atrophy of the muscle fibers supplied by those nerves

3. Diminished blood supply: A decrease in blood supply (ischemia) to a tissue as a result of slowly developing arterial occlusive disease results in atrophy of the tissue. In late adult life, the brain may undergo progressive atrophy, mainly because of reduced blood supply as a result of atherosclerosis. This is called senile atrophy; it also affects the heart.

4. Inadequate nutrition: Profound protein-calorie malnutrition (marasmus) is associated with the use of skeletal muscle as a source of energy after other reserves such as adipose stores have been depleted. This results in marked muscle wasting (cachexia). Cachexia is also seen in patients with chronic inflammatory diseases. In the former, chronic overproduction of the inflammatory cytokine tumor necrosis factor (TNF) is thought to be responsible for appetite suppression and lipid depletion, culminating in muscle atrophy.

5. Loss of endocrine stimulation: Many hormone-responsive tissues, such as the breast and reproductive organs, are dependent on endocrine stimulation for normal metabolism and function. The loss of estrogen stimulation after menopause results in physiologic atrophy of the endometrium, vaginal epithelium, and breast.

6. Pressure: Tissue compression for any length of time can cause atrophy. An enlarging benign tumor can cause atrophy in the surrounding uninvolved tissues. Atrophy in this setting is probably the result of ischemic changes caused by compromise of the blood supply by the pressure exerted by the expanding mass.

Mechanisms of Atrophy:

Atrophy results from decreased protein synthesis and increased protein degradation in cells. Protein synthesis decreases because of reduced metabolic activity. The degradation of cellular proteins occurs mainly by the ubiquitin-proteasome pathway. Nutrient deficiency and disuse may activate ubiquitin ligases, which attach the small peptide ubiquitin to cellular proteins and target these proteins for degradation in proteasomes. This pathway is also thought to be responsible for the accelerated proteolysis seen in a variety of catabolic conditions, including cancer cachexia.

In many situations, atrophy is also accompanied by increased autophagy, with resulting increases in the number of autophagic vacuoles. Autophagy (“self eating”) is the process in which the starved cell eats its own components in an attempt to find nutrients and survive. Autophagic vacuoles are membrane-bound vacuoles that contain fragments of cell components. The vacuoles ultimately fuse with lysosomes, and their contents are digested by lysosomal enzymes. Some of the cell debris within the autophagic vacuoles may resist digestion and persist as membrane-bound residual bodies that may remain as a sarcophagus in the cytoplasm. When present in sufficient amounts, they impart a brown discoloration to the tissue (brown atrophy).

METAPLASIA:

Metaplasia is a reversible change in which one differentiated cell type (epithelial or mesenchymal) is replaced by another cell type. It may represent an adaptive substitution of cells that are sensitive to stress by cell types better able to withstand the adverse environment. The most common epithelial metaplasia is columnar to squamous, as occurs in the respiratory tract in response to chronic irritation.

In the habitual cigarette smoker, the normal ciliated columnar epithelial cells of the trachea and bronchi are often replaced by stratified squamous epithelial cells. Stones in the excretory ducts of the salivary glands, pancreas, or bile ducts may also cause replacement of the normal secretory columnar epithelium by stratified squamous epithelium. A deficiency of vitamin A (retinoic acid) induces squamous metaplasia in the respiratory epithelium. In all these instances the more rugged stratified squamous epithelium is able to survive under circumstances in which the more fragile specialized columnar epithelium might have succumbed. However, the change to metaplastic squamous cells comes with a price.

In the respiratory tract, for example, although the epithelial lining becomes tough, important mechanisms of protection against infection—mucus secretion and the ciliary action of the columnar epithelium—are lost. Thus, epithelial metaplasia is a double-edged sword and, in most circumstances, represents an undesirable change. Moreover, the influences that predispose to metaplasia, if persistent, may initiate malignant transformation in metaplastic epithelium. Thus, a common form of cancer in the respiratory tract is composed of squamous cells, which arise in areas of metaplasia of the normal columnar epithelium into squamous epithelium.

Mechanisms of Metaplasia:

Metaplasia does not result from a change in the phenotype of an already differentiated cell type; instead it is the result of a reprogramming of stem cells that are known to exist in normal tissues, or of undifferentiated mesenchymal cells present in connective tissue. In a metaplastic change, these precursor cells differentiate along a new pathway. The differentiation of stem cells to a particular lineage is brought about by signals generated by cytokines, growth factors, and extracellular matrix components in the cells' environment. These external stimuli promote the expression of genes that drive cells toward a specific differentiation pathway.

A deficiency or excess, it is known that retinoic acid regulates gene transcription directly through nuclear retinoid receptors, which can influence the differentiation of progenitors derived from

tissue stem cells. How other external stimuli cause metaplasia is unknown, but it is clear that they too somehow alter the activity of transcription factors that regulate differentiation.

Inflammation and repair:

Inflammation is the body's mechanism for coping with agents that could damage it. In other words, inflammation is a protective response to rid the body of the cause of cell injury and the resultant necrotic cells that cell injury produces.

Definition: - Inflammation is the response of living tissue to injury. It involves a well-organized cascade of fluid and cellular changes within living tissue.

Cardinal features: – Rubor (redness); Tumor (swelling); Calor (heat); Dolor (pain); Functiolaesa (loss of function)

CAUSES – Etiologic agents – viruses, bacteria, fungi, parasites – Hypersensitivity – body reacts against itself, there are four types of reactions – Physical and chemical agents - trauma, sunburn, acid – Necrosis - anoxia, trauma.

What is inflammation?

Inflammation is the response of living tissue to injury. It involves a well-organized cascade of fluidic and cellular changes. It is recognizable grossly and histologically and has both beneficial and detrimental effects locally and systemically.

Some general characteristics of inflammation are as follows:

1. The inflammatory process is redundant and complex. This makes it a challenging subject to study. You will see that many mediators of inflammation have the same functions and many mediators have multiple functions. Also, the same mediator may have different effects on different tissues.

2. The process is continuous over a period of time. Peracute, acute, subacute, and chronic are terms used to describe different stages of inflammation.

3. Inflammation is caused by a stimulus and removal of the stimulus should result in abatement of inflammation. If it doesn't get fixed in the acute period, it becomes chronic.

4. Blood is the primary delivery system for inflammatory components.

5. Inflammation is on a continuum with the healing process.

The four principal effects of inflammation are (rubor, tumor, calor et dolor) were described nearly 2,000 years ago by the Roman Aulus Cornelius Celsus, more commonly known as Celsus.

Redness (rubor): An acutely inflamed tissue appears red, due to dilatation of small blood vessels within the damaged area (hyperemia).

Swelling (tumor): Swelling results from edema, the accumulation of fluid in the extravascular space as part of the inflammatory fluid exudate, and to a much lesser extent, from the physical mass of the inflammatory cells migrating into the area.

Heat (calor): Increase in temperature is readily detected in the skin. It is due to increased blood flow (hyperemia) through the region, resulting in vascular dilation and the delivery of warm blood to the area.

Pain (dolor): Pain results partly from the stretching and distortion of tissues due to inflammatory edema and, in part from some of the chemical mediators of acute inflammation, especially bradykinin and some of the prostaglandins.

Causes of Inflammation

Microbial infections:

One of the most common causes of inflammation is microbial infection. Microbes include viruses, bacteria, protozoa, fungi and various parasites. Viruses lead to death of individual cells by intracellular multiplication, and either cause the cell to stop functioning and die, or cause explosion of the cell (cytolytic), in which case it also dies. Bacteria release specific toxins – either exotoxins or endotoxins.

What's the difference?

Exotoxins are produced specifically for export (like anthrax toxins or tetanus toxins) whereas endotoxins are just part of the cell walls of Gram negative bacteria and they do terrible things to the body too but they aren't as specific in their actions as the exotoxins.

Hypersensitivity reactions: A hypersensitivity reaction occurs when an altered state of immunologic responsiveness causes an inappropriate or excessive immune reaction that damages the tissues. The types of reaction will be discussed in more detail later (In the lesson on Immune Mediated Inflammation).

Physical agents, irritant and corrosive chemicals Tissue damage leading to inflammation may occur through physical trauma, ultraviolet or other ionizing radiation, burns or excessive cooling ('frostbite').

Corrosive chemicals (acids, alkalis, oxidizing agents) provoke inflammation through direct tissue damage. These chemical irritants cause tissue damage that leads directly to inflammation. Tissue necrosis Death of tissues from lack of oxygen or nutrients resulting from inadequate blood flow (infarction) is a potent inflammatory stimulus. The edge of a recent infarct often shows an acute inflammatory response.

ACUTE INFLAMMATION

In the early stages of inflammation, the affected tissue becomes reddened, due to increased blood flow, and swollen, due to edema fluid. These changes are the result of vascular response to inflammation. The vascular events of the acute inflammatory response involve three main processes:

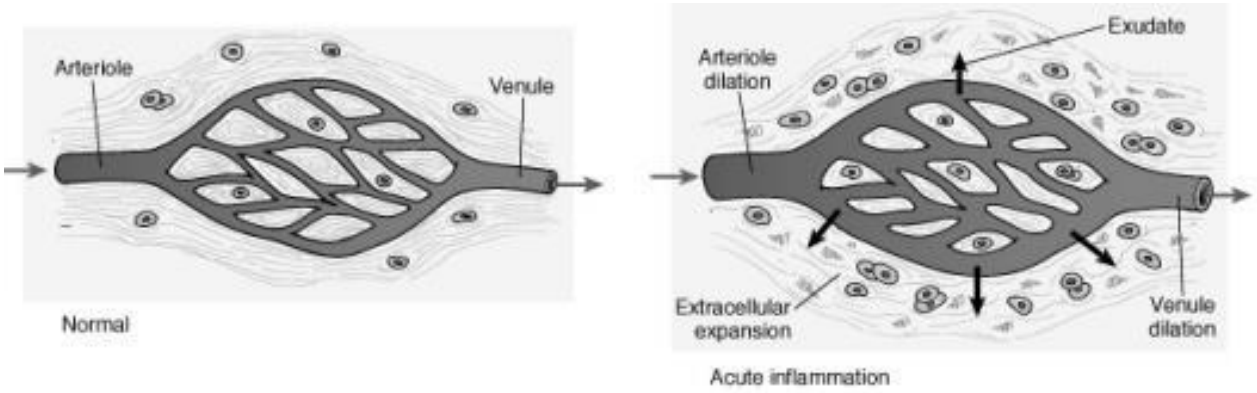
1. changes in vessel caliber and, consequently, blood flow (hemodynamics)
2. increased vascular permeability and
3. formation of the fluid exudate

1. Changes in Vessel Caliber:

The microcirculation consists of the network of small capillaries lying between arterioles, which have a thick muscular wall, and thin-walled venules. Capillaries have no smooth muscle in their walls to control their caliber, and are so narrow that red blood cells must pass through them in single file. The smooth muscle of arteriolar walls forms pre-capillary sphincters that regulate blood flow through the capillary bed. Flow through the capillaries is intermittent, and some form

preferential channels for flow while others are usually shut down. In other words, there is not blood flowing through all capillaries all the time. They take turns. When inflammation happens, none of them gets to take their scheduled tea break. They are all open. Experimental evidence indicates that blood flow to the injured area may increase up to ten-fold as vessels dilate.

What causes this to happen?
MEDIATORS - including nitric oxide, histamine and prostaglandins (PGI2) and LTB4.



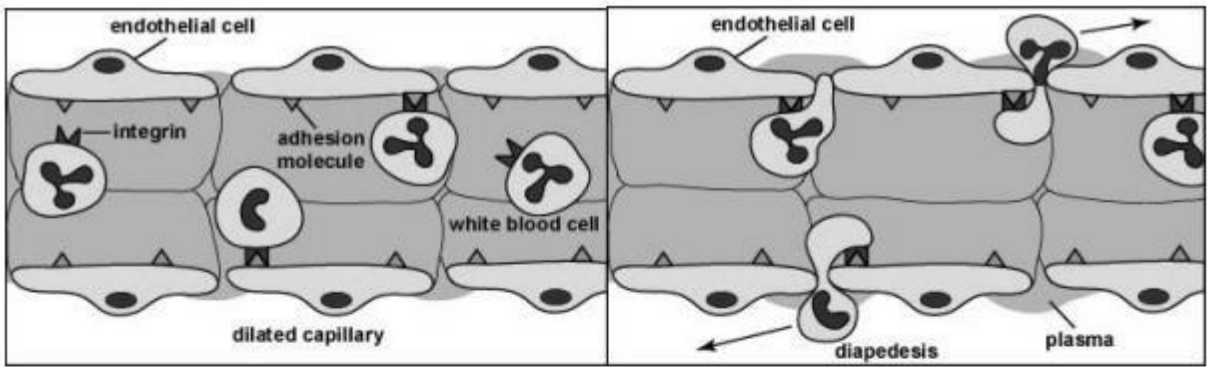
2. Increased vascular permeability:

In acute inflammation, the capillary hydrostatic pressure increases, and there is also escape of plasma proteins into the extravascular space due to increased vascular permeability (endothelial contraction allowing proteins to escape between cells).
Consequently, much more fluid leaves the vessels than is returned to them. The net escape of protein-rich fluid is called exudation; hence, the fluid is called an exudate.

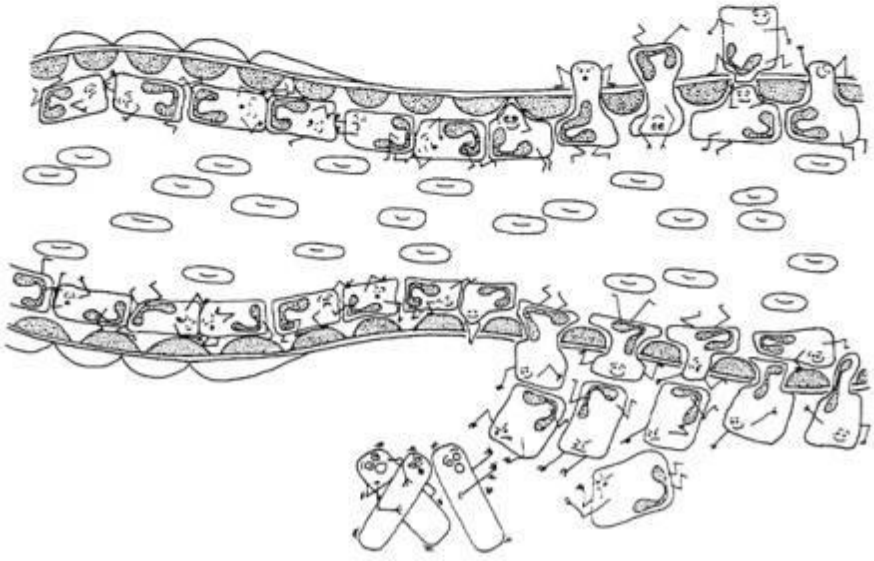
What causes the increase in vascular permeability in acute inflammation?
There are two mechanisms –

- Chemical mediators of acute inflammation may cause retraction of endothelial cells, leaving intercellular gaps (chemical mediated vascular leakage).
- Toxins and physical agents may cause necrosis of vascular endothelium, leading to abnormal leakage (injury induced vascular leakage).

3. Formation of the Cellular Exudate:
How do white blood cells get out of the circulation and into the area where they are needed?



Cells are called out to the area of inflammation in a process called CHEMOTAXIS.

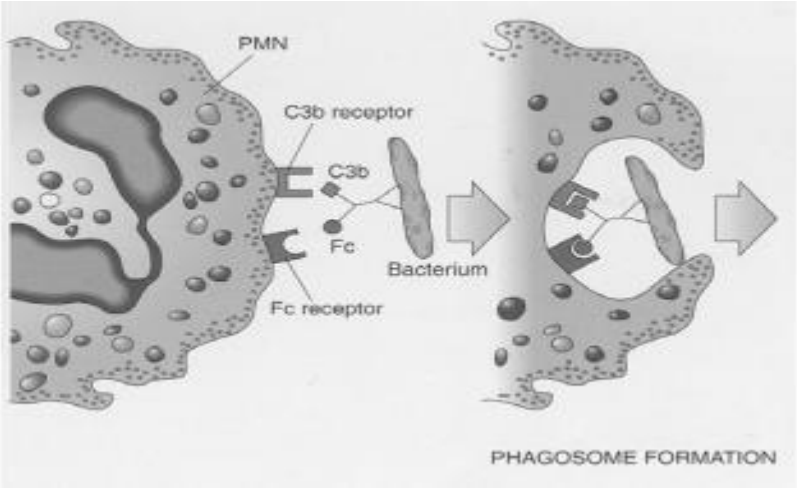


Cells are called out to the area of inflammation in a process called CHEMOTAXIS. Chemotaxis of leukocytes The movement of leukocytes from the vessel lumen in a directional fashion to the site of tissue damage is called chemotaxis. All granulocytes and monocytes respond to chemotactic factors and move along a concentration gradient (from an area of lesser concentration of the factor to an area of greater concentration of the factor). Important neutrophil chemotactic factors Bacteria are strongly chemotactic for neutrophils C5a, C3a Fibrin, fibrinopeptides Leukotriene B4 (LTB4) IL-8 (a chemokine, from macrophages) Important eosinophil chemotactic factors Histamine IL-5 (also known as eotaxin, a chemokine, from mast cells) Important monocyte chemotactic factors Complement factors Fibrinopeptides.

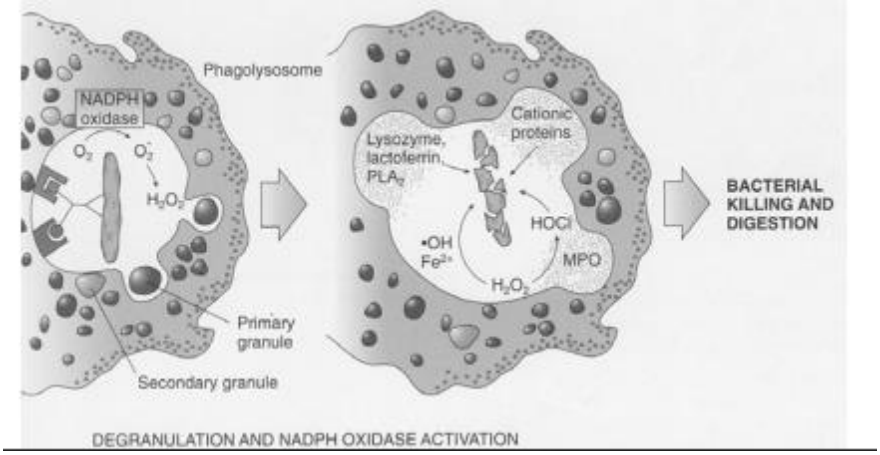
Leukocytes play a very important role in microbial killing. In any inflammatory response, leukocyte activation is a prerequisite to their full participation in the process. Leukocytes become

activated during inflammation. Leukocytes and phagocytosis The process whereby cells ingest solid particles is termed phagocytosis.

The first step in phagocytosis is adhesion of the particle to be phagocytosed to the cell surface. The phagocyte ingests the attached particle by sending out pseudopodia around it. These meet and fuse so that the particle lies in a phagocytic vacuole (also called a phagosome) bounded by cell membrane. Lysosomes, membrane-bound packets containing the toxic compounds, then fuse with phagosomes to form phagolysosomes. It is within these that intracellular killing of microorganisms occurs.



Intracellular killing of micro-organisms by leukocytes Neutrophils and macrophages are specialized cells, containing noxious antimicrobial agents. Neutrophils produce hydrogen peroxide (bactericidal by itself) which reacts with myeloperoxidase in the cytoplasmic granules to create oxygen radicals which are wickedly damaging. Antibacterial cationic proteins, lysozyme, and defensins all affect bacterial permeability so the bacteria leak to death.



- Release of lysosomal products from the cell damages local tissues and can kill microorganisms outside of the cell. Enzymes such as elastase and collagenase will chew through tissue. Some of the compounds are pyrogens, producing fever by acting on the hypothalamus. Acid hydrolases degrade tissue matrixes.

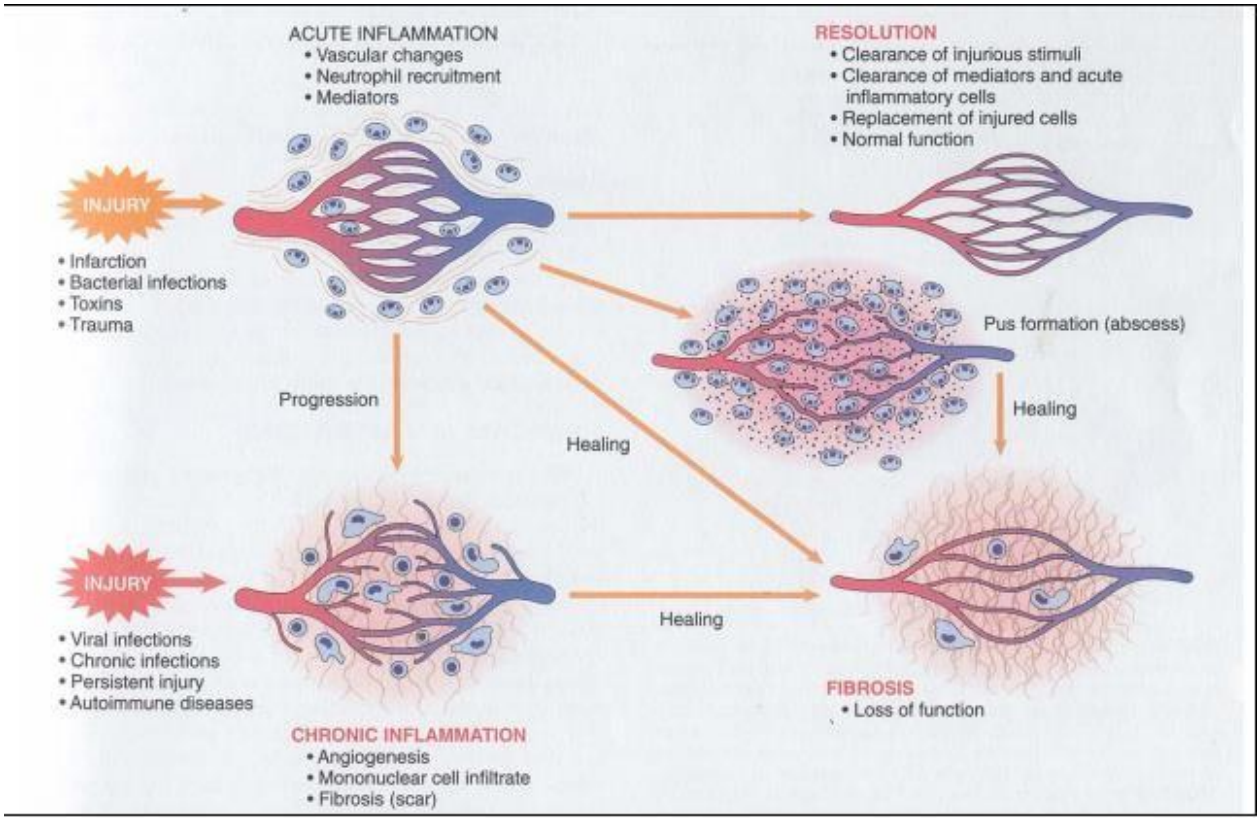
Chronic Inflammation

Chronic inflammation, like its acute cousin, is a host response to an inciting stimulus. There are, however, some distinct differences. First and foremost is the time factor. Chronic inflammation is considered to be inflammation of prolonged duration - weeks to months. Second, rather than being just exudative, chronic inflammation usually is productive or proliferative. Chronic inflammation is rarely gooey.

Cells in the chronic inflammatory process tend to produce substances that add new tissue, such as collagen and new blood vessels. Many of these changes also represent the repair process and there is a blurry continuum between chronic inflammation and the whole repair process. In general, chronic inflammation is characterized by inflammation, tissue destruction, and attempts at repair all happening at once. Grossly, chronic inflammation does not have as much rubor (redness) or calor (heat) as in the acute reaction. Also, exudates aren't so grossly apparent as they are in acute inflammation. Because of the fibroplasia and neovascularization, areas affected by chronic inflammation tend to be slightly swollen and firm. If fibrosis is extensive the lesions can be large and disfiguring. Fibrosis (granulation tissue) is the best indicator that the inflammatory response is chronic.

Chronic inflammation tends to occur under the following conditions: Infections by organisms which are resistant to killing and clearing by the body tend to cause chronic inflammation. Such persistent organisms include some of the higher bacteria (including mycobacteria), fungi, and quite a few metazoan parasites. Repeated bouts of acute inflammation can result in a chronic reaction. Prolonged exposure to toxins can cause chronic inflammation.

Chronic inflammation is a common component in many of the autoimmune diseases. Because the reaction is against a host epitope, which is always present, the inflammation is by definition chronic and persistent. Because chronic inflammation doesn't ooze, rather its exudates tends to be kind of solid and white or grayish and it looks the same no matter what the cell types, the only way to add an exudative moniker is to see the histology.



Here are the cell types:

1. The simplest type of chronic inflammation has mostly lymphocytes with lesser numbers of macrophages. This will occur mostly in viral infections where the virus survives longer than the acute phase. This is called “lymphohistiocytic”.

2. Chronic active inflammation is the same but in this one there are still some neutrophils present, so there are acute things going on inside of the chronicity. This happens in many bacterial infections that are not due to very pus-producing bacteria.

3. Next is granulomatous - here the cell types are almost all macrophages. Good examples here are fungal infections or mycobacteria.

4. Some people use a term pyogranulomatous - which means granulomatous but within the macrophages are pockets of neutrophils. The most common disease causing this is FIP.

5. Granulomas occur when the inciting cause stimulates macrophages but the agents are distributed discretely within an organ. Think TB. Think Blastomyces. Think foreign body.

But with all of these types, there is evidence of fibroblasts moving in and some new blood vessels. If plasma cells are present in the inflammation, you know two things. First, it is a chronic problem because it takes a good 2-4 weeks to generate plasma cells. Second, there must be some persistent antigen in there, because plasma cells indicate the body needs plenty of antibody at the site.

Healing and Repair

Inflammation and repair can be viewed as two parts of a single vital function, the physiologic response to tissue injury, the objective of which is restoration of normal structure and function. Up until now we have focused on the inflammation portion but it is good to remember that repair starts pretty soon after inflammation does and then continues during and beyond the inflammatory phase.

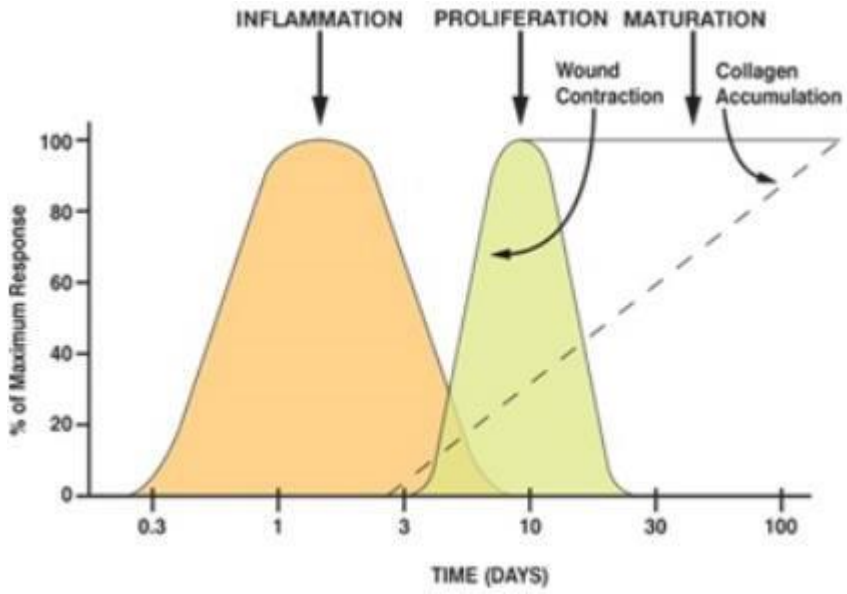
Perfect restoration of function is dependent upon the regeneration of lost cells by similar cells (repair by regeneration), and the orderly arrangement of these new cells in relation to preexisting cells so that tissue functions are restored. If the original cells cannot be replaced by their own kind then they are replaced by other cell types (repair by replacement), usually by fibrous connective tissue. If necrosis is extensive, even tissues that are capable of regeneration are replaced by fibrous connective tissue.

There are three cell types based on ability to regenerate: permanent cells (almost never divide) - nerve cell bodies, cardiac myocytes, cells of the lens stable cells (will divide if stimulated) - fibroblasts, osteoblasts, parenchyma of liver, kidney, pancreas and endocrine glands, smooth muscle, vascular endothelium labile cells (multiply through life) - epithelial cells of surfaces or linings of ducts and hollow visci, lymphoid and hematopoietic cells

Granulation tissue (= vascular fibrous connective tissue) The term “granulation tissue” is derived from its pink soft granular appearance on the surface of wounds. Granulation tissue is recognized histologically by the presence of newly formed fibrous tissue and numerous small blood vessels. The fibrous connective tissue eventually may come to have the maturity of the loose fibrillar connective tissue of normal histology, but in the formative stages the fibroblasts are plump and only a few collagen fibrils have been produced.

Contraction occurs as a result of the action of myofibroblasts. These cells have features intermediate between those of fibroblasts and smooth muscle cells. They appear in the wound area 2 or 3 days after injury. Their origin is not entirely clear, but they probably derive either

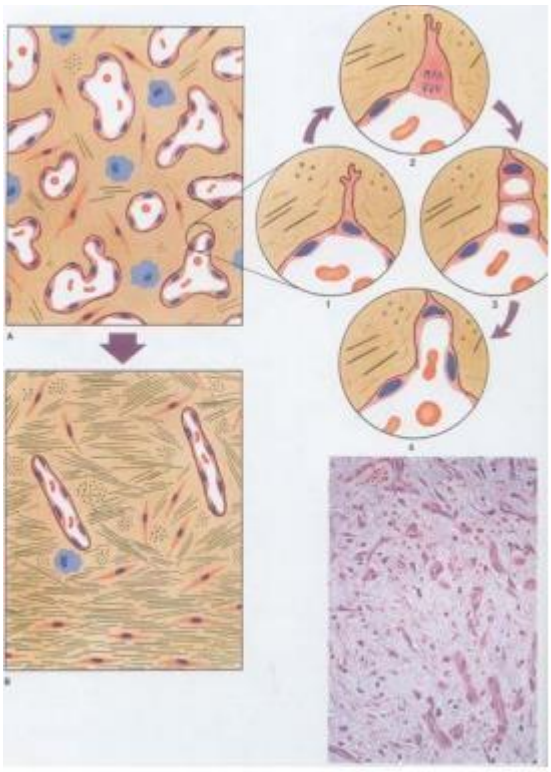
from perivascular cells or from other mesenchymal cells. Contraction may reduce the original defect by as much as 70% and greatly facilitate healing.



Fibroplasia begins early after injury, with existing fibroblasts adjacent to the wound being the source of new fibroblasts. The immature fibroblasts are characterized by their plumpness, basophilia, rich complement of rough endoplasmic reticulum, and prominent nucleoli, all evidence of active synthesis. They are active in synthesizing glycosaminoglycans and collagen fibers.

The new capillaries most commonly run in a perpendicular direction to the outer surface of the wound since their function is to carry supplies to the free surface, while fibroblasts tend to grow in from the side of the defect to bridge the gap. This often produces a histological picture of capillaries running at right angles to the direction of the fibroblasts. The formation of new capillaries is termed angiogenesis or neovascularization.

Remember, granulation tissue is part of the repair process and consists of inflamed, proliferating fibrous tissue and granulomatous refers to inflammatory infiltrates characterized by macrophages. And sometimes granulation tissue doesn't know when to quit growing and start turning into the permanent scar it is supposed to form. Think "exuberant granulation tissue" in horses, also known as "proud flesh." A healing process that goes too far and too long. All this healing takes energy and if an animal is in a compromised state, the repair will take a whole lot longer.



Repair of bone

Repair of bone is a specialized category of healing and repair and deserves individual attention. Immediately following fracture of bone, blood flows out of the broken vessels into the gap between the broken ends of bone and displaced or disrupted periosteum, and into the surrounding soft tissue. A big callus forms with fibrovascular tissue. The osteoprogenitor cells eventually invade into this callus and slowly new bone is formed and then remodeled. Repair of Nervous Tissue Repair in the central nervous system (CNS) is very limited because mature neurons do not divide. When damage occurs in the CNS, neurons and their processes are lost forever; they cannot be regenerated. Take care of your neurons! In the peripheral nervous system (PNS), injury to the nerves may be followed by regeneration if the nerve cell body remains alive. Repair of the Myocardium Myocardial cells do not have any regenerative capacity. When they die, as in a heart attack, they are gone forever and repair can only take place by fibrosis. This replacement by fibrosis decreases myocardial contractility.

FRACTURE HEALING

Bone healing, or **fracture healing**, is a proliferative physiological process in which the body facilitates the repair of a bone fracture.

Generally bone fracture treatment consists of a doctor reducing (pushing) displaced bones back into place via relocation with or without anaesthetic, stabilizing their position to aid union, and then waiting for the bone's natural healing process to occur.

Adequate nutrient intake has been found to significantly affect the integrity of the fracture repair. Age, Bone type, drug therapy and pre existing bone pathology are factors which affect healing. The role of bone heasling is to produce new bone without a scar as seen in other tissues which would be a structural weakness or deformity.

The process of the entire regeneration of the bone can depend on the angle of dislocation or fracture. While the bone formation usually spans the entire duration of the healing process, in some instances, bone marrow within the fracture has healed two or fewer weeks before the final remodeling phase.

While immobilization and surgery may facilitate healing, a fracture ultimately heals through physiological processes. The healing process is mainly determined by the periosteum (the connective tissue membrane covering the bone). The periosteum is one source of precursor cells which develop into chondroblasts and osteoblasts that are essential to the healing of bone. Other sources of precursor cells are the bone marrow (when present), endosteum, small blood vessels, and fibroblasts.

Primary healing

Primary healing (also known as direct healing) requires a correct anatomical reduction which is stable, without any gap formation. Such healing requires only the remodeling of lamellar bone, the Haversian canals and the blood vessels without callus formation. This process may take a few months to a few years.

Contact healing

When the gap between the bone ends is less than 0.01 mm, and interfragmentary strain is less than 2%, contact healing can occur. In this case, cutting cones, which consists of osteoclasts, form across the fracture lines, generating cavities at a rate of 50–100 μm/day. Osteoblasts fill up the cavities with the Haversian system. This caused the formation of lamellar bone that oriented longitudinally along the long axis of the bone. Blood vessels form that penetrate the Harversian system. Remodelling of lamellar bone results in healing without callus formation.

Gap healing

If the fracture gap is 800 μm to 1 mm, the fracture is filled by osteoclasts and then by lamellar bone oriented perpendicular to the axis of the bone. This orientation of lamellar bone is weak, thus a secondary osteonal reconstruction is required to re-orient the lamellar bone longitudinally. This process takes three to eight weeks.

Secondary healing

Secondary healing (also known as indirect fracture healing) is the most common form of bone healing. It usually consists of only endochondral ossification. Sometimes, intramembranous ossification occurs together with endochondral ossification. Intramembranous ossification, mediated by perisoteal layer of bone, occurs without formation of callus. For endochondral ossification, deposition of bone only occurs after the mineralised cartilage. This process of healing occurs when the fracture is treated conservatively using orthopaedic cast or immobilisation, external fixation, or internal fixation.

There are three major phases of fracture healing, two of which can be further sub-divided to make a total of five phases:

1. **Reaction**
    - i. Inflammation
    - ii. Granulation tissue formation
2. **Repair**
    - iii. Cartilage callus formation
    - iv. Lamellar bone deposition
3. **Remodeling**
    - v. Remodeling to original bone contour

Reaction

After fracture, blood cells accumulate adjacent to the injury site. Soon after fracture, blood vessels constrict, stopping further bleeding. Within a few hours, the extravascular blood cells form a clot called a hematoma that acts as a template for callus formation. These cells, including macrophages, release inflammatory mediators such as cytokines (tumor necrosis factor alpha (TNFα), interleukin-1 family (IL-1), interleukin 6 (IL-6), 11 (IL-11), and 18 (IL-18)) and increase blood capillary permeability. Inflammation peaks by 24 hours and completes by seven days. Through tumor necrosis factor receptor 1 (TNFR1) and tumor necrosis factor receptor 2, TNFα mediates the differentiation of mesenchymal stem cell(originated from the bone marrow) into osteoblast and chondrocytes.

Stromal cell-derived factor 1 (SDF-1) and CXCR4 mediate recruitment of mesenchymal stem cells. IL-1 and IL-6 are the most important cytokines for bone healing. IL-1 promotes formation of callus and of blood vessels. IL-6 promotes differentiation of osteoblasts and osteoclasts. All cells within the blood clot degenerate and die. Within this area, the fibroblasts replicate. Within 7-14 days, they form a loose aggregate of cells, interspersed with small blood vessels, known as granulation tissue.[5] Osteoclasts move in to reabsorb dead bone ends, and other necrotic tissue is removed.

Repair

Seven to nine days after fracture, the cells of the periosteum replicate and transform. The periosteal cells proximal to (on the near side of) the fracture gap develop into chondroblasts, which form hyaline cartilage. The periosteal cells distal to (at the far end of) the fracture gap develop into osteoblasts, which form woven bone through bone resorption of calcified cartilage and recruitment of bone cells and osteoclasts. The fibroblasts within the granulation tissue develop into chondroblasts which also form hyaline cartilage. These two new tissues grow in size until they unite with each other. These processes culminate in a new mass of heterogeneous tissue known as a fracture callus. Callus formation peaks at day 14 of fracture. Eventually, the fracture gap is bridged.

The next phase is the replacement of the hyaline cartilage and woven bone with lamellar bone. The replacement process is known as endochondral ossification with respect to the hyaline cartilage and bony substitution with respect to the woven bone. Substitution of woven bone happens before substitution of hyaline cartilage. The lamellar bone begins forming soon after the collagen matrix of either tissue becomes mineralized. At this stage, the process is induced by IL-1 and TNFα.

The mineralized matrix is penetrated by microvessel and numerous osteoblasts. The osteoblasts form new lamellar bone upon the recently exposed surface of the mineralized matrix. This new lamellar bone is in the form of trabecular bone. Eventually, all of the woven bone and cartilage of the original fracture callus is replaced by trabecular bone, restoring most of the bone's original strength.

Remodelling

Remodeling begins as early as three to four weeks after fracture and may take 3 to 5 years to complete. The process substitutes the trabecular bone with compact bone. The trabecular bone is first resorbed by osteoclasts, creating a shallow resorption pit known as a "Howship's lacuna". Then osteoblasts deposit compact bone within the resorption pit. Eventually, the fracture callus is remodelled into a new shape which closely duplicates the bone's original shape and strength. This process can be achieved by the formation of electrical polarity during partial weight bearing a long bone; where electropositive convex surface and electronegative concave surface activates osteoclasts and osteoblasts respectively. This process can be enhanced by certain synthetic injectable biomaterials, such as cerament, which are osteoconductive and actively promote bone healing.

Obstructions

1. Poor blood supply which leads to the death of the osteocytes. Bone cell death also depends on degree of fracture and disruption to the Haversian system.
2. Condition of the soft tissues. Soft tissue between bone ends restricts healing.

3. Nutrition and drug therapy. Poor general health reduces healing rate. Drugs that impair the inflammatory response impede healing also.
4. Infection. Diverts the inflammatory response away from healing towards fighting off the infection.
5. Age. Young bone unites more rapidly than adult bone.
6. Pre-existing bone malignancy.
7. Mechanical factors such as the bone not being aligned, and too much or too little movement. Excess mobility can disrupt the bridging callus, interfering with union; but slight biomechanical motion is seen to improve callus formation.[6]

Complications

Complications of fracture healing include:

1. Infection: this is the most common complication of fractures and predominantly occurs in open fractures. Post-traumatic wound infection is the most common cause of chronic osteomyelitis in patients. Osteomyelitis can also occur following surgical fixation of a fracture.[8]
2. Non-union: no progression of healing within six months of a fracture occurring. The fracture pieces remain separated and can be caused by infection and/or lack of blood supply (Ischaemia) to the bone.[9] There are two types of non-union, atrophic and hypertrophic. Hypertrophic involves the formation of excess callus leading to bone ends appearing sclerotic causing a radiological "Elephants Foot" appearance[6] due to excessive fracture ends mobility but adequate blood supply.[4] Atrophic non-union results in re-absorption and rounding of bone ends[6] due to inadequate blood supply and excessive mobility of the bone ends.[4]
3. Mal-union: healing occurs but the healed bone has 'angular deformity, translation, or rotational alignment that requires surgical correction'. This is most common in long bones such as the femur.[10]
4. Delayed union: healing times vary depending on the location of a fracture and the age of a patient. Delayed union is characterised by 'persistence of the fracture line and a scarcity or absence of callus formation' on x-ray. Healing is still occurring but at a much slower rate than normal.[9]

Neoplasia

Neoplasia is new, uncontrolled growth of cells that is not under physiologic control. A "tumor" or "mass lesion" is simply a "growth" or "enlargement" which may not be neoplastic (such as a granuloma). The term "cancer" implies malignancy, but neoplasms can be subclassified as either benign or malignant.

There is no single mechanism by which a neoplasm arises. Many different mechanisms give rise to neoplasms, and that is what makes diagnosis and treatment so challenging.

Nomenclature of Neoplasia

Based upon origin:

- Malignant neoplasms arising from tissue embryologically derived from ectoderm or endoderm are usually carcinomas. Examples include:
  - Squamous cell carcinoma of cervix
  - Adenocarcinoma of stomach
  - Hepatocellular carcinoma
  - Renal cell carcinoma
- Malignancies arising from mesoderm (connective tissues) are usually sarcomas. Examples include:
  - Leiomyosarcoma
  - Chondrosarcoma
  - Osteosarcoma
  - Liposarcoma
- Neoplasms with more than one cell type but arising from only one germ layer are called "mixed tumors". The best example is the benign mixed tumor (also called pleomorphic adenoma) of salivary gland.
- Neoplasms with more than one cell type and arising from more than one germ layer are called teratomas. Such neoplasms are common in the ovary.
- Neoplasms ending in "-blastoma" resemble primitive embryonic tissues, which are often pediatric neoplasms. Examples include:
  - Retinoblastoma
  - Neuroblastoma
  - Hepatoblastoma
  - Medulloblastoma
- Not all malignant neoplasms have benign counterparts:
  - Hematopoietic and lymphoid cells (as in bone marrow and lymph node) give rise to leukemias and lymphomas. They have no benign counterpart.
  - Gliomas (astrocytomas, oligodengrogliomas, glioblastoma, etc) arise from glial cells in the CNS. They have no benign counterpart.

Carcinomas

Carcinomas arise from epithelial surfaces (in gastrointestinal tract, in respiratory tract, in urogenital tract, in biliary tract, in skin) and in organs with epithelial-lined ducts (breast, pancreas, salivary gland, liver, etc). Endocrine glands, including testis and ovary, may

also give rise to carcinomas. In general, carcinomas are composed of polygonal-shaped cells.

- Carcinomas that form glandular configurations are called adenocarcinomas.
- Carcinomas that form solid nests of cells with distinct borders, intercellular bridges, and pink keratinized cytoplasm are called squamous cell carcinomas.

Sarcomas

Sarcomas arise from soft tissues (connective tissues such as cartilage, bone, or fascia, smooth or skeletal muscle, blood vessels, lymph vessels, coverings of organs such as mesothelium). In general, sarcomas are composed of very pleomorphic spindle-shaped cells. Sarcomas are generally big and bad.

Causes of Neoplasia

The origin for many neoplasms is obscure. However, there are several theories of origin:

Environmental causes:

- **Chemicals:** including those that are man-made (such as aniline dyes and bladder cancer), drugs (cigarette smoke and lung cancer), and natural compounds (aflatoxins and liver cancer) which are carcinogenic.
- **Oncogenic viruses:** such as human papillomavirus (HPV) implicated in most squamous cell carcinomas of cervix and anogenital squamous papillomas, Epstein-Barr virus (EBV) implicated in African Burkitt's lymphoma, and hepatitis B virus (HBV) implicated in development of hepatocellular carcinomas.
- **Radiation:** including ultraviolet light that induces pyrimidine dimers in DNA and promotes skin cancers. Ionizing radiation (such as gamma radiation) induces mutations in DNA and promotes malignancies such as leukemia, thyroid, lung, colon, and breast cancers.

Chemical carcinogenesis

- There are two steps: initiation and promotion
- An initiating carcinogenic agent irreversibly damages cell DNA (it is mutagenic) to start the process. Examples of carcinogenic initiators include: alkylating agents like cyclophosphamide, polycyclic aromatic hydrocarbons like epoxides found in smoked foods, aromatic amines or azo dyes used in food coloring, aflatoxins in moldy peanuts, nitrosamines in pickled foods.
- A promoting agent (which may be the same as the carcinogen) then acts (reversibly) to cause proliferation of a neoplastic cell clone, but there appears to

be a "dose-threshold" concentration of promoter below which neoplasia will not occur. Examples of promoters include: hormones such as estrogen, drugs such as diethylstilbesterol, and chemicals.

- An example of chemical carcinogenesis involves grilled meats. Meats exposed to high temperatures (above 162°C, or 325°F), either in an oven or over an open flame (grilling), undergo changes in proteins that form compounds called heterocyclic amines and polycyclic aromatic hydrocarbons. These compounds can be carcinogenic. Following ingestion of these compounds, not only directly exposed tissues such as stomach and colon have an increased risk for cancer, but also sites elsewhere, including pancreas, breast, and prostate. A char on the meat indicates risk.

Hereditary causes:

- Chromosomes which have absent or defective anti-oncogenes that control growth (retinoblastoma results from defective chromosome 13)
- Obscure defects: racial predilections (American women have breast cancer more often than Japanese women; Japanese men have stomach cancer far more often than American men).

Age: older persons have a greater propensity to develop neoplasms from lack of effective control mechanisms.

Altered DNA:

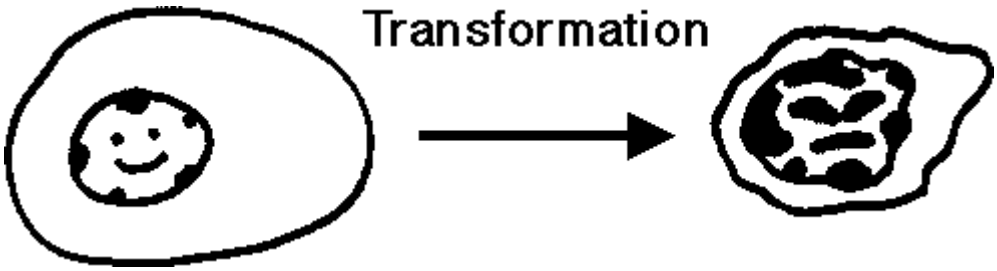
- All of the above are probably mediated by the cause, whatever it is, producing a mutation in, or damage to, cell DNA
- There can be mutations involving tumor suppressor genes (such as p53), which then fail to exert a controlling influence upon growth activation. The majority of human neoplasms probably arise via this mechanism.
- In some cases these mutations are probably mediated by proto-oncogenes (genes which control cellular growth) that undergo mutation to oncogenes which give rise to neoplasia. Proto-oncogenes can be activated by point mutations, translocations, and by gene amplification.
    - An example of this is chronic myelogenous leukemia (CML) which is a neoplastic proliferation of white blood cells. All cases of CML have the "Philadelphia chromosome" which is a translocation between chromosomes 9 and 22. This translocation juxtaposes the proto-oncogene ABL with the breakpoint cluster region (BCR) on chromosome 22. The chimeric ABL-BCR gene leads to production of a mutant protein with

enhanced tyrosine kinase activity. This protein may play a role in regulation of cell growth in CML.

- About 15 to 20% of human cancers have been linked to oncogenic activity. The *ras* oncogene is the transforming gene found most frequently in human cancers.
- Oncogenic viruses may bring oncogenes with them, so-called viral oncogenes (typical of RNA containing "retroviruses" such as human T-lymphotropic viruses (HTLV's).
- DNA repair mechanisms may be affected. There are DNA excision repair genes that can be mutated, introducing genomic instability and a greater likelihood that mutations in other genes will occur to drive oncogenesis. Examples include:
    - DNA mismatch repair genes: defective nucleotide "spell checker" introducing "microsatellite instability" of tandem repeat sequences in DNA. Seen in hereditary non-polyposis colon cancer (HNPCC)
    - Nucleotide excision repair genes: defective function in xeroderma pigmentosa, allowing DNA damage from pyrimidine dimer formation induced by ultraviolet light
- Growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and colony-stimulating factor-1 (CSF-1) assist oncogene activity. Transforming growth factor (TGF-alpha) also promotes tumor growth.

Cellular Transformation

- Some factor, as discussed above, causes a cell to be transformed to a neoplastic cell that is not controlled by normal body processes. Probably most transformed cells die because they are too abnormal to function or are abnormal enough for the body's immune system to destroy them. However, if the factors promoting neoplasia persist, a transformed cell may some day give rise to a clone that does continue to grow.



- Malignant neoplasms do not tend to arise from benign neoplasms (e.g., malignant melanomas do not come from benign nevi). However, in some cases such as adenomas of the colon, the appearance of the benign neoplasm is a step toward possible malignancy,

because oncogenic forces are at work producing additonal abnormalities in DNA in existing lesions.

- There are "pre-cancerous" conditions in which malignant neoplasia is more likely to occur (but not in every case): liver cirrhosis, chronic ulcerative colitis, atrophic gastritis, epidermal actinic keratosis, and oral leukoplakia. In these cases, there is ongoing cellular proliferation for repair of damaged tissue, often from ongoing inflammation. Abnormal cell proliferation leads to a greater likelihood for mutation to occur.

Clonality

- Neoplastic cells tend to be monoclonal, or similar in genetic makeup, indicating origin from a transformed cell. Non-neoplastic proliferations (such as reactions to inflammation) have cells that are polyclonal in origin.
- The concept of "tumor progression" holds that subclones may arise over time from the original malignant clone. These subclones may differ from the original clone in characteristics such as invasiveness, metastatic potential, and response to therapy. The subclones may arise from acquisition of additional mutations.

Tumor Genetics

Neoplasms have a greater tendency to demonstrate karyotypic abnormalities such as translocations, deletions, and gene amplifications (which are also activators of proto-oncogenes). Leukemias and lymphomas are famous with chronic myelogenous leukemia, and the t(8:14) translocation in Burkitt lymphoma.

Tumor growth

- In general, the less differentiated a neoplasm, the faster it grows. The cell cycle of neoplastic cells is not shortened, rather the growth fraction of cells proliferating is increased. This is offset by neoplastic cell death. Tumor growth is expressed as a "doubling time" or the time to increase twice in volume (e.g., from 1 to 1.3 cm diameter). An aggressive malignant neoplasm doubles in 1 to 3 months, while benign neoplasms double in years.
- Some neoplastic growth is influenced by host factors. Estrogenic hormones aid growth of breast fibroadenomas or carcinomas and uterine leiomyomas because the tumor cells have hormone receptors.
- Growth is also dependent upon the ability of the tumor to develop a blood supply. Factors secreted by neoplastic cells promote angiogenesis and fibroblast proliferation.

Characteristics of Transformed (Neoplastic) Cells

- Neoplastic cell growth is not inhibited by contact with surrounding cells and is not dependent on anchorage to a solid surface.
- They are discohesive and transplantable--favoring invasion and metastasis.
- Tumor cells can bind to laminin and fibronectin in connective tissues, then secrete collagenases or proteases, and then invade.
- Neoplastic cells may attain "immortality" or the ability to keep dividing indefinitely.

Tissue evidence of carcinogenic factors at work

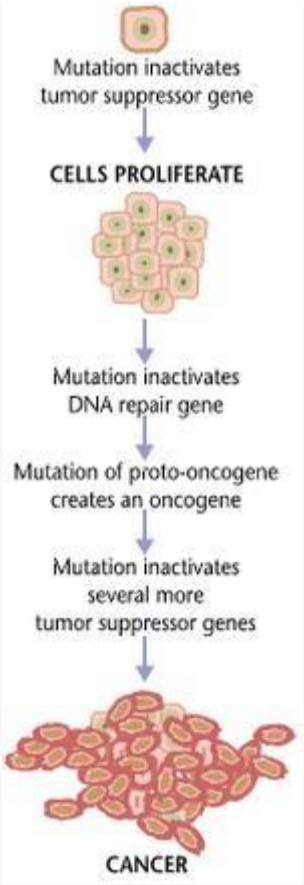
- **Metaplasia:** an initial change from normal cells to a different cell type (such as chronic irritation of cigarette smoke causing ciliated pseudostratified epithelium to be replaced by squamous epithelium more able to withstand the insult).
- **Dysplasia:** an increasing degree of disordered growth or maturation of the tissue (often thought to precede neoplasia) such as cervical dysplasia as a result of human papillomavirus infection. Dysplasia is still a reversible process. However, once the transformation to neoplasia has been made, the process is not reversible.
- Thus, there is a natural history from metaplasia to dysplasia to neoplasia. This is best evidenced in development of uterine cervix and respiratory tract neoplasms.

***(FOR BENIGN AND MALIGNANT TUMOUR, REFER PATHOLOGY MICROBIOLOGY LAB MANUAL)**

Carcinogenesis

Carcinogenesis, also called oncogenesis or tumorigenesis, is the formation of a cancer, whereby normal cells are transformed into cancer cells. The process is characterized by changes at the cellular, genetic, and epigenetic levels and abnormal cell division. Cell division is a physiological process that occurs in almost all tissues and under a variety of circumstances. Normally the balance between proliferation and programmed cell death, in the form of apoptosis, is maintained to ensure the integrity of tissues and organs.

According to the prevailing accepted theory of carcinogenesis, the somatic mutation theory, mutations in DNA and epimutations that lead to cancer disrupt these orderly processes by disrupting the programming regulating the processes, upsetting the normal balance between proliferation and cell death. This results in uncontrolled cell division and the evolution of those cells by natural selection in the body. Only certain mutations lead to cancer whereas the majority of mutations do not.



Cancers and tumors are caused by a series of mutations. Each mutation alters the behavior of the cell somewhat.

Variants of inherited genes may predispose individuals to cancer. In addition, environmental factors such as carcinogens and radiation cause mutations that may contribute to the development of cancer. Finally random mistakes in normal DNA replication may result in cancer causing mutations. A series of several mutations to certain classes of genes is usually required before a normal cell will transform into a cancer cell. On average, for example, 15 "driver mutations" and 60 "passenger" mutations are found in colon cancers.[2] Mutations in genes that regulate cell division, apoptosis (cell death), and DNA repair may result in uncontrolled cell proliferation and cancer.

Cancer is fundamentally a disease of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, genes that regulate cell growth and differentiation must be altered. Genetic and epigenetic changes can occur at many levels, from gain or loss of entire chromosomes, to a mutation affecting a single DNA nucleotide, or to silencing or activating a microRNA that controls expression of 100 to 500 genes.

There are two broad categories of genes that are affected by these changes. Oncogenes may be normal genes that are expressed at inappropriately high levels, or altered genes that have novel

properties. In either case, expression of these genes promotes the malignant phenotype of cancer cells. Tumor suppressor genesare genes that inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Finally Oncovirinae, viruses that contain an oncogene, are categorized as oncogenic because they trigger the growth of tumorous tissues in the host. This process is also referred to as viral transformation.

Types of carcinogens and how they cause cancer

There are three types of chemicals, known as carcinogens, that can cause cancer:

- Procarcinogens, which cause cancer due to being changed during metabolism.
- Cocarcinogens, which cause cancer by acting with another chemical.
- Direct acting carcinogens, which can cause cancer as is.
- Cancer can be caused if a carcinogen damages a person's DNA cells to the point of being irreparable. But cancer can also be caused by inherited genetic mutations. Moreover, it can take a long time for cancer to be detected. When you take these factors into account, you can understand why it is difficult to determine the true cause of cancer after it has been discovered.
- And this means that if you suspect that you developed cancer due to your working conditions and want to receive compensation for medical care and other damages, you may have to put forth a great effort to prove your illness is work-related. As you may expect, companies and insurance agencies are not anxious to foot the medical bills for cancer treatment. Therefore, because so much is on the line, you may want to have an experienced workers' compensation attorney help you seek an appropriate settlement.

How cancer can spread

Primary and secondary cancer

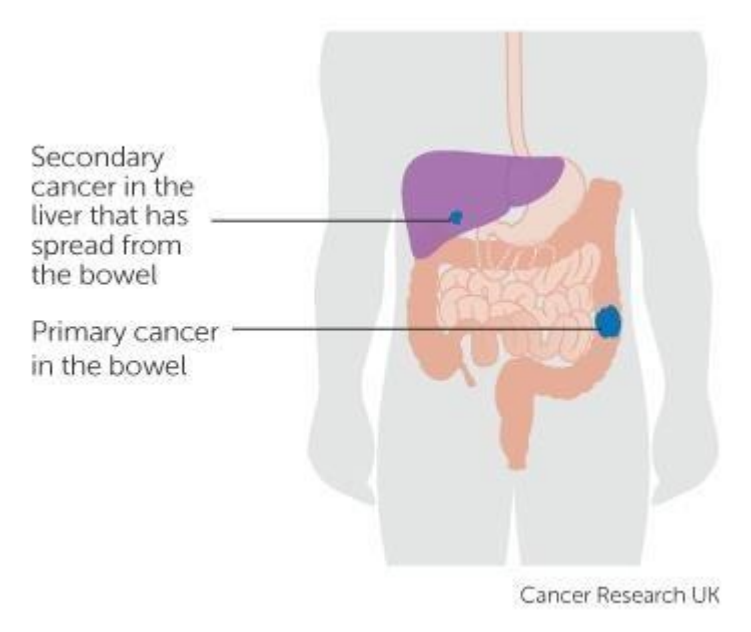
The place where a cancer starts in the body is called the primary cancer or primary site. Cells from the primary site may break away and spread to other parts of the body. These escaped cells can then grow and form other tumours, which are known as secondary cancers or metastases.

How cancer can spread to other areas of the body

Cancer cells can spread to other parts of the body through the bloodstream or lymphatic system. There they can start to grow into new tumours.

Cancers are named according to where they first started developing. For example, if you have bowel cancer that has spread to the liver, it's called bowel cancer with liver metastases or secondaries. It is not called liver cancer. This is because the cancerous cells in the liver are actually cancerous bowel cells. They are not liver cells that have become cancerous.

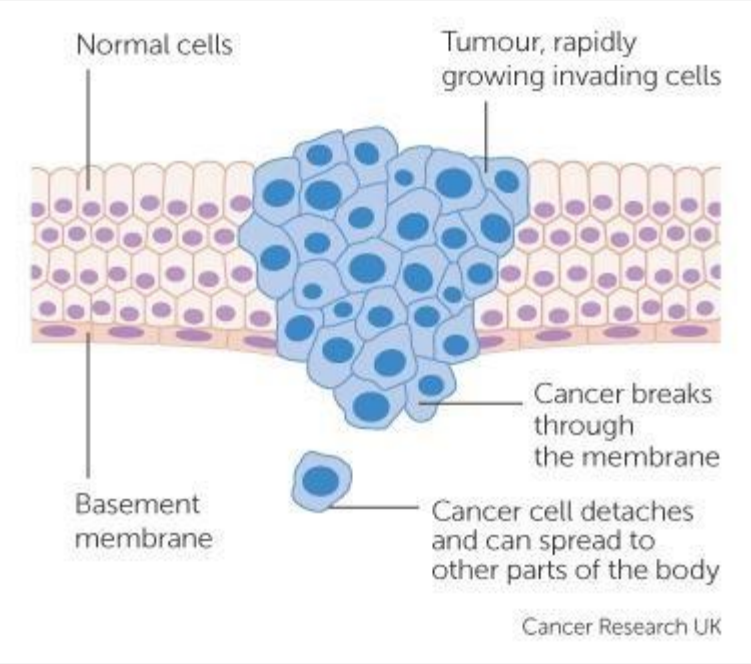
diagram showing a primary and secondary cancer.



In order to spread, some cells from the primary cancer must break away, travel to another part of the body and start growing there. Cancer cells don't stick together as well as normal cells do. They may also produce substances that stimulate them to move.

The diagram below shows a tumour in the cells lining a body structure such as the bowel wall. The tumour grows through the layer holding the cells in place (the basement membrane).

diagram showing a malignant tumour.



Some cells can break away and go into small lymph vessels or blood vessels called capillaries in the area.

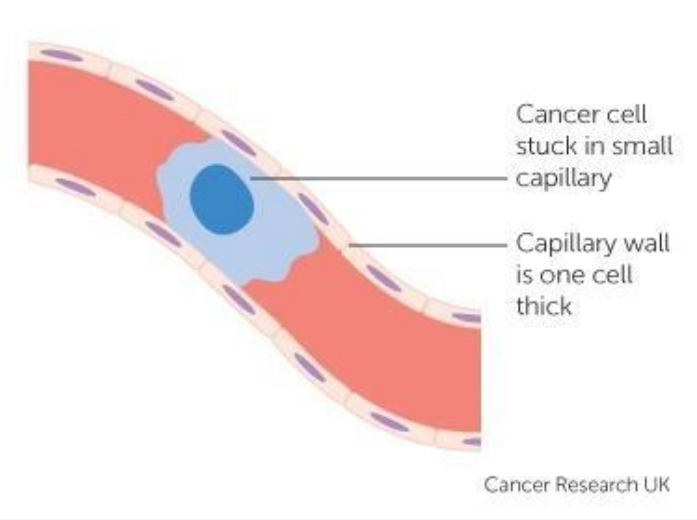
Spread through the blood circulation

When the cancer cells go into small blood vessels they can then get into the bloodstream. They are called circulating tumour cells (or CTCs).

Researchers are currently looking at using circulating tumour cells to diagnose cancer and avoid the need for tests such as biopsies. They are also looking at whether they can test circulating cancer cells to predict which treatments will work better.

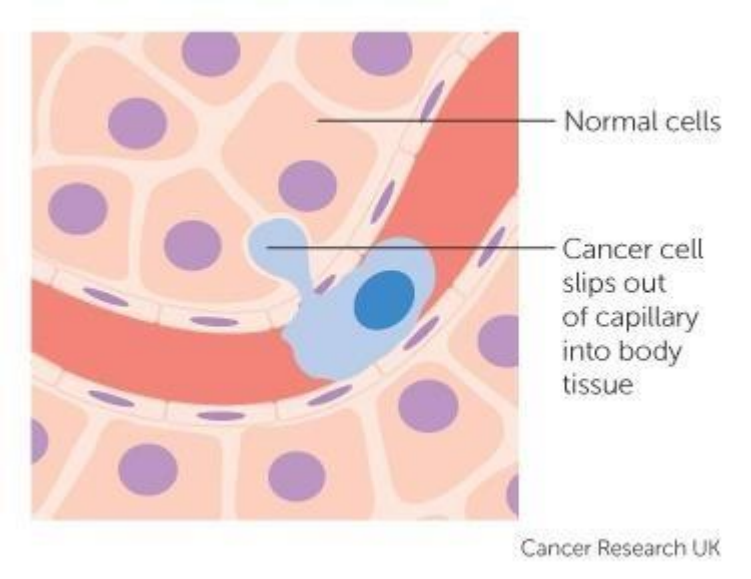
The circulating blood sweeps the cancer cells along until they get stuck somewhere. Usually they get stuck in a very small blood vessel such as a capillary.

diagram showing a cancer cell stuck in a small blood vessel capillary.



Then the cancer cell must move through the wall of the capillary and into the tissue of the organ close by. The cell can multiply to form a new tumour if the conditions are right for it to grow and it has the nutrients that it needs.

diagram showing how cancer cells get into the bloodstream.



This is quite a complicated process and most cancer cells don't survive it. Probably, out of many thousands of cancer cells that reach the bloodstream, only a few will survive to form a secondary cancer.

Some cancer cells are probably killed off by the white blood cells in our immune system. Others cancer cells may die because they get battered around by the fast flowing blood.

Cancer cells in the circulation may try to stick to platelets to form clumps to give themselves some protection. Platelets are blood cells that help the blood to clot. This may also help the cancer cells to move into the surrounding tissues.

Spread through the lymphatic system

The lymphatic system is a network of tubes and glands in the body that filters body fluid and fights infection. It also traps damaged or harmful cells such as cancer cells.

Cancer cells can go into the small lymph vessels close to the primary tumour and travel into nearby lymph glands. In the lymph glands, the cancer cells may be destroyed but some may survive and grow to form tumours in one or more lymph nodes. Doctors call this lymph node spread.

Micrometastases

Micrometastases are areas of cancer spread (metastases) that are too small to see. Some areas of cancer are too small to show up on any type of scan.

For a few types of cancer, blood tests can detect certain proteins released by the cancer cells. These may give a sign that there are metastases in the body that are too small to show up on a scan. But for most cancers, there is no blood test that can say whether a cancer has spread or not.

For most cancers, doctors can only say whether it is likely or not that a cancer has spread. Doctors base this on a number of factors:

- previous experience – doctors collect and publish this information to help each other
- whether there are cancer cells in the blood vessels in the tumour removed during surgery – if cancer cells are found then the cancer is more likely to have spread to other parts of the body
- the grade of the cancer (how abnormal the cells are) – the higher the grade, the more quickly the cancer grows and the more likely for cells to spread
- whether lymph nodes removed during an operation contained cancer cells – if the lymph nodes contained cancer cells this shows that cancer cells have broken away from the original cancer (but there is no way of knowing whether the cells have spread to any other areas of the body)

This information is important in treating cancer. Doctors may offer extra treatment, such as chemotherapy, radiotherapy, biological therapy or hormone therapy if they suspect of micrometastases. The extra treatments may increase the chance of curing the cancer.

UNIT II

Fluid and hemodynamic derangements:

The cardiovascular disease which were responsible for 35% to 40% of deaths Included in this category are diseases the primarily affect one of the major component of cardiovascular system: the heart, the blood vessels, and the blood. And they composed of water , salts and a wide variety of proteins, elements that regulate the clotting mechanisms. Here we focus on disorders of haemodynamic (edema,congestion,and shock) and hemostasis (hemorrahage and thrombosis) as well as various forms of embolism.

Edema:

The Greek word *oidema* means swelling. *Oedema may be defined as abnormal and excessive accumulation of "free fluid" in the interstitial tissue spaces and serous cavities*. The presence of abnormal collection of fluid within the cell is sometimes called intracellular oedema.

Free fluid in body cavities*:* Depending upon the body cavity in which the fluid accumulates, it is correspondingly known as ascites (if in the peritoneal cavity), hydrothorax or pleural effusion (if in the pleural cavity), and hydropericardium or pericardial effusion (if in the pericardial cavity).

Free fluid in interstitial space: The oedema fluid lies free in the interstitial space between the cells and can be displaced from one place to another. In the case of oedema in the subcutaneous tissues, momentary pressure of finger produces a depression known as pitting oedema. The other variety is non-pitting or solid oedemain which no pitting is produced on pressure e.g. in myxoedema, elephantiasis.

The oedema may be of 2 main types:

1. **Localised** when limited to an organ or limb e.g. lymphatic oedema, inflammatory oedema, allergic oedema.

2. **Generalised** (anasarca or dropsy)when it is systemic in distribution, particularly noticeable in the subcutaneous tissues e.g. renal oedema, cardiac oedema, nutritional oedema.

Depending upon fluid composition, oedema fluid may be:

Transudate: It is the fluid pushed through the capillary tube due to high pressure within the capillary.

Exudate: The fluid that leak around the cells of capillaries caused due to inflammation.

PATHOGENESIS OF OEDEMA

Edema is caused by mechanisms that interfere with normal fluid balance of plasma, interstitial fluid and lymph flow. The following mechanisms may be operating singly or in combination to produce edema.

1. Decreased plasma oncotic pressure
2. Increased capillary hydrostatic pressure
3. Lymphatic obstruction
4. Tissue factors (increased oncotic pressure of interstitial fluid, and decreased tissue tension)
5. Increased capillary permeability
6. Sodium and water retention

DECREASED PLASMA ONCOTIC PRESSURE:

The oncotic pressure is a kind of osmotic pressure exerted by the plasma protein present in the body which tends to draw fluid into the vessels normally.

A fall in the total plasma protein level (hypoproteinaemia), results in lowering of plasma oncotic pressure in a way that it can no longer counteract the effect of hydrostatic pressure of blood. This results in increased outward movement of fluid from the capillary wall and decreased inward movement of fluid from the interstitial space causing oedema.

INCREASED CAPILLARY HYDROSTATIC PRESSURE:

The hydrostatic pressure of the capillary is the force that normally tends to drive fluid through the capillary wall into the interstitial space by counteracting the force of plasma oncotic pressure. A rise in the hydrostatic pressure at the venular end of the capillary which is normally low (average 12 mmHg) to a level more

than the plasma oncotic pressure results in minimal or no reabsorption of fluid at the venular end, consequently leading to oedema.

LYMPHATIC OBSTRUCTION:

Normally, the interstitial fluid in the tissue spaces escapes by way of lymphatics. Obstruction to outflow of these channels causes localised oedema, known as lymphoedema.

TISSUE FACTORS:

The two forces acting in the interstitial space—oncotic pressure of the interstitial space and tissue tension, are normally quite small and insignificant to counteract the effects of plasma oncotic pressure and capillary hydrostatic pressure respectively. However, in some situations, the tissue factors in combination with other mechanisms play a role in causation of oedema.

INCREASED CAPILLARY PERMEABILITY:

An intact capillary endothelium is a semipermeable membrane which permits the free flow of water and crystalloids but allows minimal passage of plasma proteins normally. However, when the capillary endothelium is injured by various 'capillary poisons' such as toxins and their products, histamine, anoxia, venoms, certain drugs and chemicals, the capillary permeability to plasma proteins is enhanced due to development of gaps between the endothelial cells, leading to leakage of plasma proteins into interstitial fluid. This, in turn, causes reduced plasma oncotic pressure and elevated oncotic pressure of interstitial fluid which consequently produces oedema.

SODIUM AND WATER RETENTION:

The possible factors responsible for causation of oedema by excessive retention of sodium and water in the extravascular compartment via stimulation of intrinsic renal and extra-renal mechanisms as well as via release of ADH are as under:

i) Reduced glomerular filtration rate in response to hypovolaemia.

ii) Enhanced tubular reabsorption of sodium and consequently its decreased renal excretion.

iii) Increased filtration factor i.e. increased filtration of plasma from the glomerulus.

ORGAN SPECIFIC EDEMA:

1) Cerebral edema: the interstitial fluid collection in the brain tissues.
2) Pulmonary edema: fluid collection in lungs.
3) Periorbital edema: eye puffiness.
4) Lymph edema: mainly due to obstruction in the lymph channel lead to edema in the lower extremities.

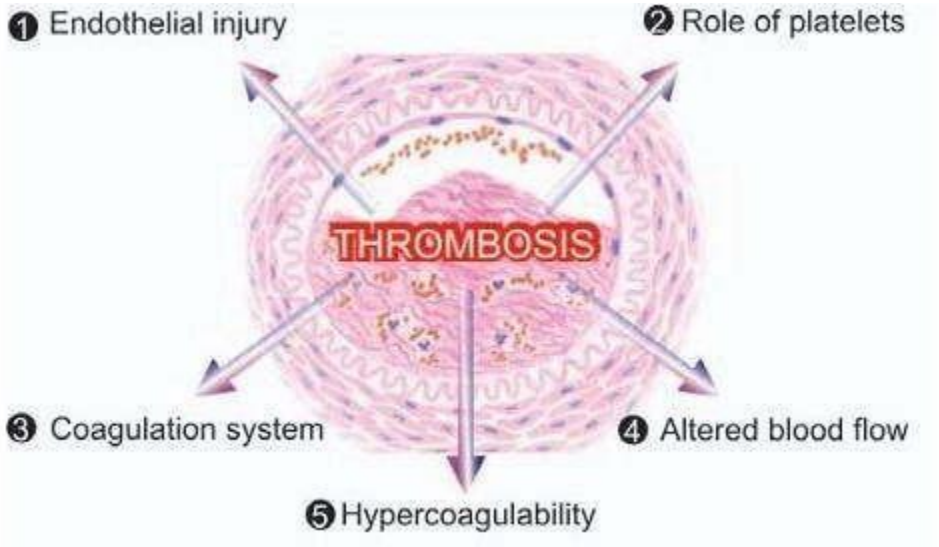
THROMBOSIS:

Thrombosis is the process of formation of solid mass in circulation from the constituents of flowing blood; the mass itself is called a thrombus. In contrast, a blood clot is the mass of coagulated blood formed in vitroe.g. in a test tube. Haematomais the extravascular accumulation of blood clot e.g. into the tissues. Haemostatic plugsare the blood clots formed in healthy individuals at the site of bleeding e.g. in injury to the blood vessel. Haemostatic plugs are useful as they stop the escape of blood and plasma, whereas thrombi developing in the unruptured cardiovascular system may be life-threatening by causing one of the following harmful effects:

1. **Ischaemic injury:**Thrombi may decrease or stop the blood supply to part of an organ or tissue and cause ischaemia which may subsequently result ininfarction.
2. **Thromboembolism**.The thrombus or its part may get dislodged and be carried along in the bloodstream as embolus to lodge in a distant vessel.

PATHOPHYSIOLOGY:

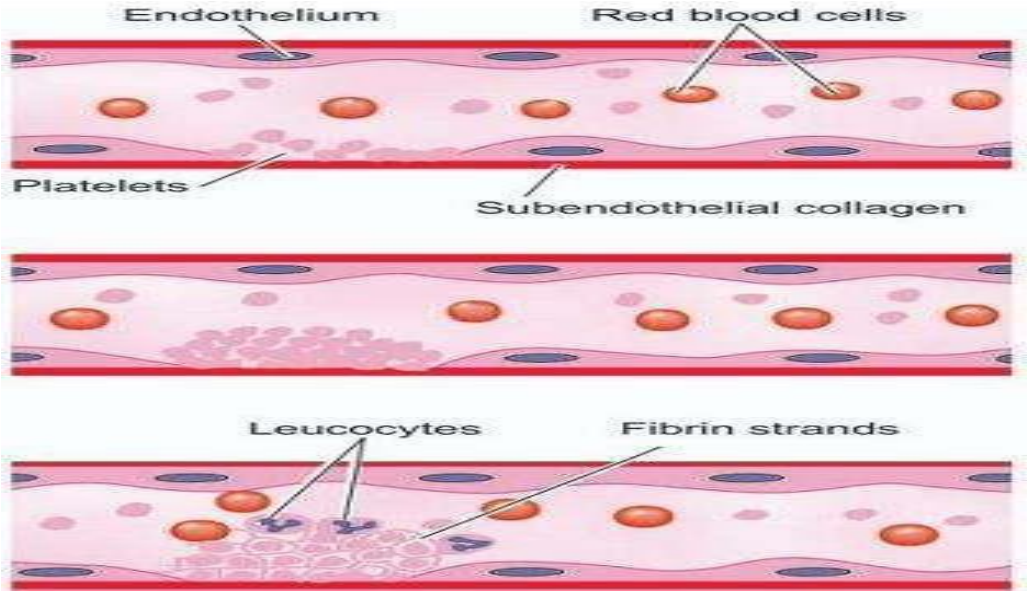
Since the protective haemostatic plug formed as a result of normal haemostasis is an example of thrombosis. Virchow described three primary events which predispose to thrombus formation (Virchow's triad): endothelial injury, altered blood flow, and hypercoagulability of blood.



ENDOTHELIAL INJURY:

The integrity of blood vessel wall is important for maintaining normal blood flow. Vascular injury exposes the subendothelial connective tissue (e.g. collagen, elastin, fibronectin, laminin) which are thrombogenic and thus plays important role in initiating haemostasis as well as thrombosis. Injury to vessel wall also causes vasoconstriction of small blood vessels briefly so as to reduce the blood loss.

Endothelial injury is of major significance in the formation of arterial thrombi and thrombi of the heart, especially of the left ventricle.

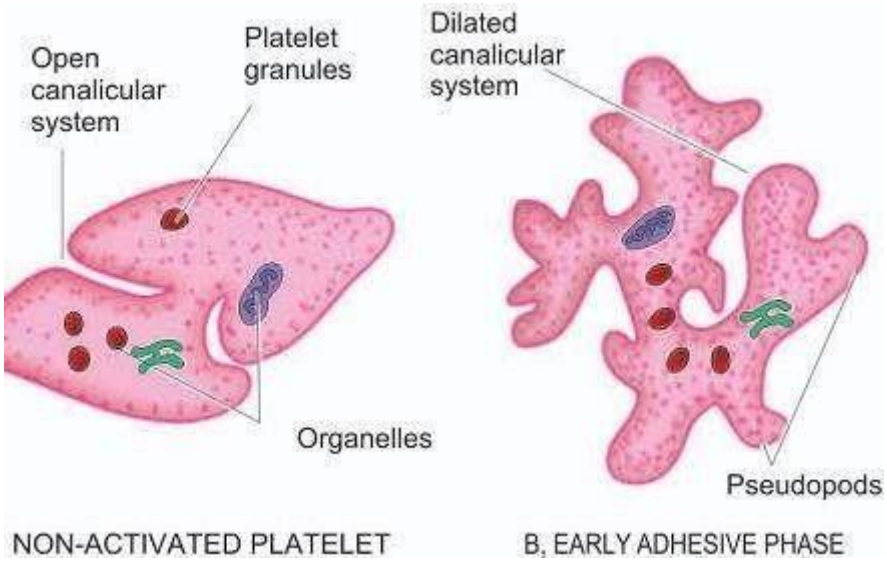


ROLE OF PLATELETS:

Following endothelial cell injury, platelets come to play a central role in normal haemostasis as well as in thrombosis. The sequence of events is as under

i) Platelet adhesion:

The platelets in circulation recognize the site of endothelial injury and adhere to exposed subendothelial collagen (primary aggregation); von Willebrand's factor is required for such adhesion between platelets and collagen. Normal non-activated platelets have open canalicular system with cytoplasmic organelles (granules, mitochondria, endoplasmic reticulum) dispersed throughout the cytoplasm. During the early adhesion process, there is dilatation of canalicular system with formation of pseudopods and the cytoplasmic organelles shift to the centre of the cell.

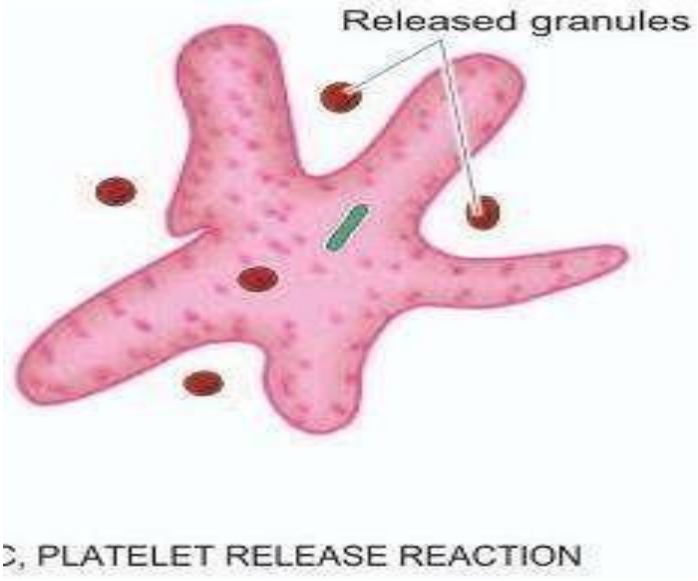


ii) Platelet release reaction:

The activated platelets then undergo release reaction by which the platelet granules are released to the exterior. Two main types of platelet granules are released:

a) Alpha granules: containing fibrinogen, fibronectin, plateletderived growth factor, platelet factor 4 (an antiheparin) and cationic proteins.

b) Dense bodies: containing ADP (adenosine diphosphate), ionic calcium, 5-HT (serotonin), histamine and epinephrine. As a sequel to platelet activation and release reaction, the phospholipid complex-platelet factor 3 gets activated which plays important role in the intrinsic pathway of coagulation.



C, PLATELET RELEASE REACTION

iii) Platelet aggregation:

Following release of ADP, a potent platelet aggregating agent, aggregation of additional platelets takes place (secondary aggregation). This results in formation of temporary haemostatic plug. However, stable haemostatic plug is formed by the action of fibrin, thrombin and thromboxane A2.

D, PLATELET AGGREGATION

3. ROLE OF COAGULATION SYSTEM:

Coagulation mechanism is the conversion of the plasma fibrinogen into solid mass of fibrin. The coagulation system is involved in both haemostatic process and thrombus formation is by intrinsic (blood) pathway, the extrinsic (tissue) pathway, and the common pathway leading to formation of fibrin polymers.

i) **In the intrinsic pathway**,contact with abnormal surface leads to activation of factor XII and the sequential interactions

of factors XI, IX, VIII and finally factor X, alongwith calcium ions (factor IV) and platelet factor 3.

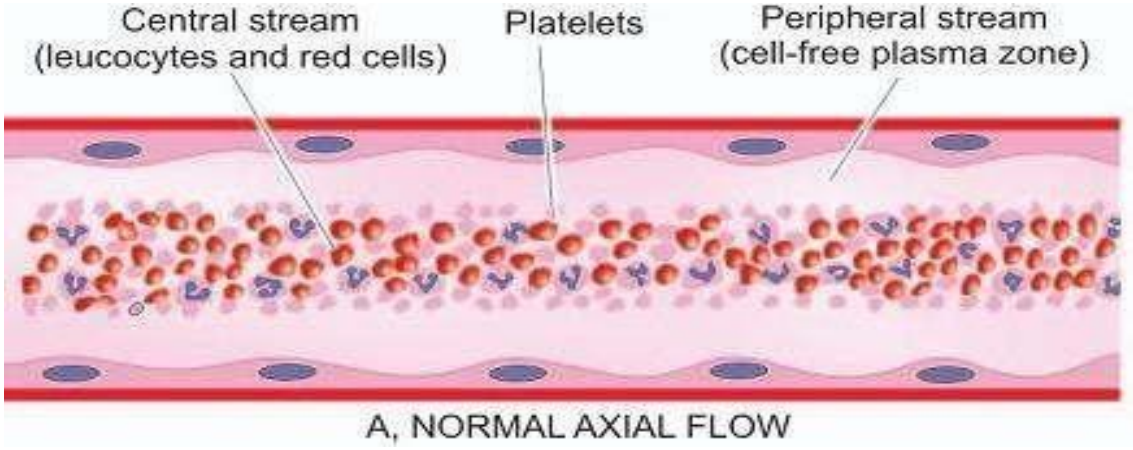
ii) **In the extrinsic pathway**,tissue damage results in the release of tissue factor or thromboplastin. Tissue factor on interaction with factor VII activates factor X.

iii)The common pathwaybegins where both intrinsic and extrinsic pathways converge to activate factor X which forms a complex with factor Va and platelet factor 3, in the presence of calcium ions. This complex activates prothrombin (factor II) to thrombin (factor IIa) which, in turn, converts fibrinogen to fibrin. Initial monomeric fibrin is polymerised to form insoluble fibrin by activation of factor XIII.

4. ALTERATION OF BLOOD FLOW:

Turbulence means unequal flow while stasis means slowing.

i) Normally, there is axial flow of blood in which the most rapidly-moving central stream consists of leucocytes and red cells. The platelets are present in the slow-moving laminar stream adjacent to the central stream while the peripheral stream consists of most slow-moving cell-free plasma closeto endothelial layer.



A, NORMAL AXIAL FLOW

ii) Turbulence and stasis occur in thrombosis in which the normal axial flow of blood is disturbed. When blood slows down, the blood cells including platelets marginate to the periphery and form a kind of pavement close to endothelium (margination and pavementing) . While stasis allows a higher release of oxygen from the blood, turbulence may actually injure the endothelium resulting in deposition of platelets and fibrin.

B, MARGINATION AND PAVEMENTING

5. HYPERCOAGULABILITY OF BLOOD:

The occurrence of thrombosis in some conditions such as in nephritic syndrome, advanced cancers, extensive trauma, and burns. Hypercoagulability may occur by the following changes in the composition of blood:

i) Increase in coagulation factorse.g. fibrinogen, prothrombin, factor VIIa, VIIIa and Xa.

ii) Increase in platelet countand their adhesiveness.

iii) Decreased levels of coagulation inhibitorse.g. antithrombin III, fibrin split products.

DISSEMINATED INTRAVASCULAR COAGULATION (DIC):

Disseminated intravascular coagulation (DIC), also termed defibrination syndrome or consumption coagulopathy, is a complex thrombo-haemorrhagic disorder (intravascular coagulation and haemorrhage) occurring as a secondary complication in some systemic diseases.

ETIOLOGY:

Although there are numerous conditions associated with DIC, most frequent causes are listed below:

1. Massive tissue injury:in obstetrical syndromes (e.g. abruptio placentae, amniotic fluid embolism, retained dead foetus), massive trauma, metastatic malignancies, surgery.

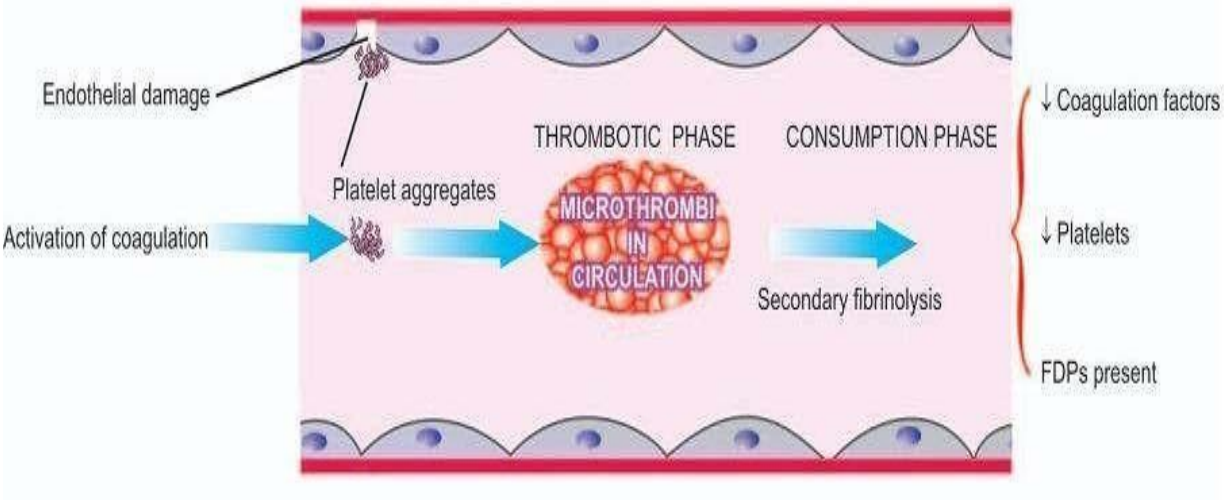
2. Infections:especially endotoxaemia, gram-negative and meningococcal septicaemia, certain viral infections, malaria, aspergillosis.

3. Widespread endothelial damage:in aortic aneurysm, haemolytic-uraemic syndrome, severe burns, acute glomerulonephritis.

4. Miscellaneous:snake bite, shock, acute intravascularhaemolysis, heat stroke.

PATHOGENESIS:

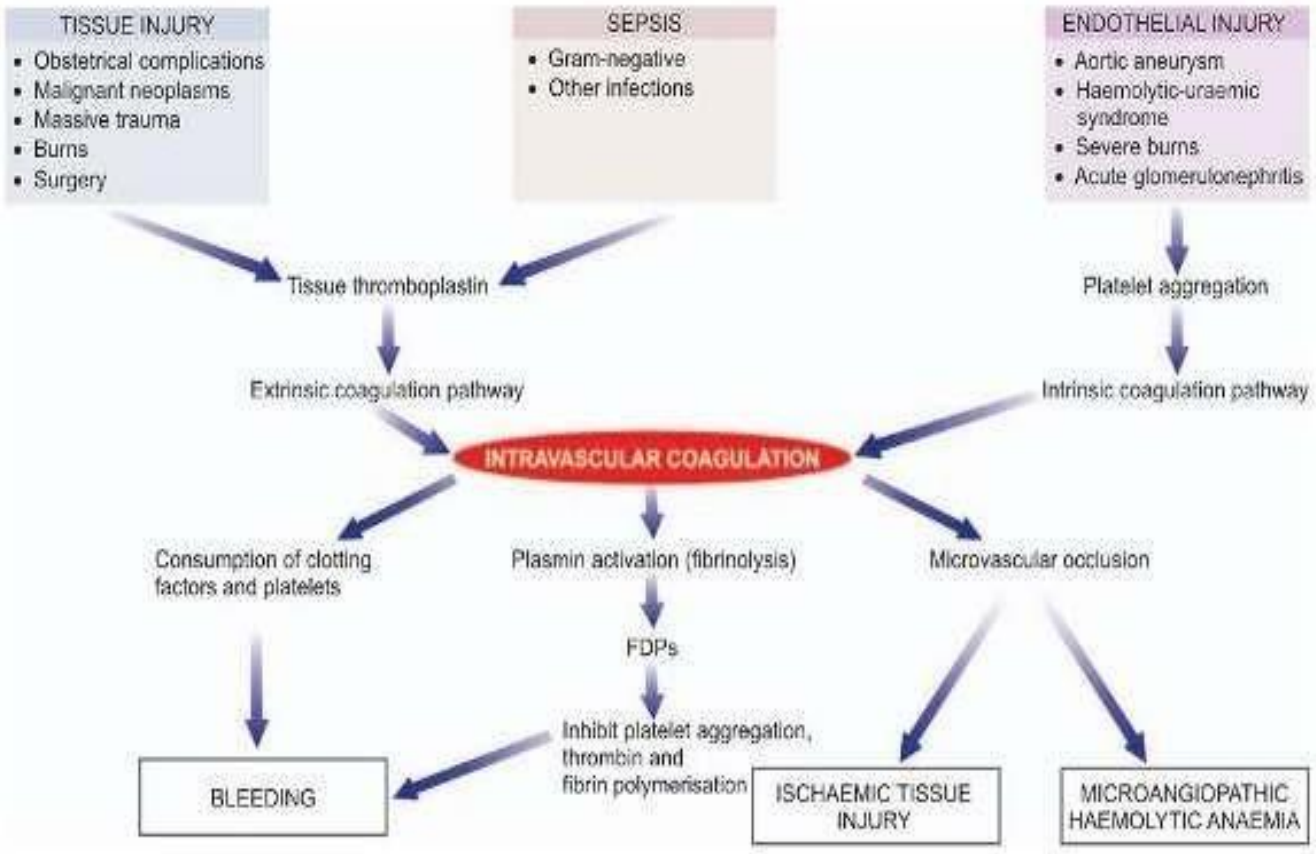
1. Activation of coagulation.The etiologic factors listed above initiate widespread activation of coagulation pathway by release of tissue factor.
2. Thrombotic phase.Endothelial damage from the various thrombogenic stimuli causes generalised platelet aggregation and adhesion with resultant deposition of small thrombi and emboli throughout the microvasculature.
3. Consumption phase.The early thrombotic phase is followed by a phase of consumption of coagulation factors and platelets.
4. Secondary fibrinolysis.As a protective mechanism, fibrinolytic system is secondarily activated at the site of intravascular coagulation. Secondary fibrinolysis causes breakdown of fibrin resulting in formation of FDPs(fibrin degraded products) in the circulation.



CLINICAL FEATURES:

There are 2 main features of DIC— bleeding as the most common manifestation, and organ damage due to ischaemia caused by the effect of widespread intravascular thrombosis such as in the kidney and brain. Less common manifestations include: microangiopathic haemolytic anaemia and thrombosis in larger arteries and veins.

PATHOPHYSIOLOGY OF DIC:



EMBOLISM:

Embolismis the process of partial or complete obstruction of some part of the cardiovascular system by any mass carried in the circulation; the transported intravascular mass detached from its site of origin is called an embolus. Most usual forms of emboli (90%) are thromboemboli i.e. originating from thrombi or their parts detached from the vessel wall.

Emboli may be of various types:

1) Depending upon the matter in the emboli:

i) Solide.g. detached thrombi (thromboemboli), atheromatous material, tumour cell clumps, tissue fragments, parasites, bacterial clumps, foreign bodies.

ii) Liquide.g. fat globules, amniotic fluid, bone marrow.

iii) Gaseouse.g. air, other gases.

2) Depending upon whether infected or not:

i) Bland, when sterile.

ii) Septic, when infected.

3) Depending upon the source of the emboli:

i) Cardiac emboli from left side of the heart e.g. emboli originating from atrium and atrial appendages, infarct in the left ventricle, vegetations of endocarditis.

ii) Arterial embolie.g. in systemic arteries in the brain, spleen, kidney, intestine.

iii) Venous embolie.g. in pulmonary arteries.

iv) Lymphatic emboli can also occur.

4) Depending upon the flow of blood,two special types of emboli are mentioned:

i) Paradoxical embolus: An embolus which is carried from the venous side of circulation to the arterial side or vice versa is called paradoxical or crossed embolus.

ii) Retrograde embolus. An embolus which travels against the flow of blood is called retrograde embolus.

TYPES OF EMBOLISM:

THROMBOEMBOLISM:

A detached thrombus or part of thrombus constitutes the most common type of embolism. These may arise in the arterial or venous circulation :

Arterial (systemic) thromboembolism: Arterial emboli may be derived from the following sources:

A. Causes within the heart(80-85%): These are mural thrombi in the left atrium or left ventricle, vegetations on the mitral or aortic valves, prosthetic heart valves and cardiomyopathy.

B. Causes within the arteries: These include emboli developing in relation to atherosclerotic plaques, aortic aneurysms, pulmonary veins.

Venous thromboembolism: Venous emboli may arise from the following sources:

i) Thrombi in the veins of the lower legs are the most common cause of venous emboli.

ii) Thrombi in the pelvic veins.

iii) Thrombi in the veins of the upper limbs.

iv) Thrombosis in cavernous sinus of the brain.

v) Thrombi in the right side of heart

PULMONARY THROMBOEMBOLISM:

Pulmonary embolism is the most common and fatal form of venous thromboembolism in which there is occlusion of pulmonary arterial tree by thromboemboli. Pulmonary thrombosis as such is uncommon and may occur in pulmonary atherosclerosis and pulmonary hypertension.

FAT EMBOLISM:

Obstruction of arterioles and capillaries by fat globules constitutes fat embolism. If the obstruction in the circulation is by fragments of adipose tissue, it is called fat-tissue embolism.

ETIOLOGY. Following are the important causes of fat embolism:

i) Traumatic causes:

Trauma to bones is the most common cause of fat embolism e.g. in fractures of long bones leading to passage of fatty marrow in circulation, concussions of bones, after orthopaedic surgical procedures etc.

Trauma to soft tissuee.g. laceration of adipose tissue and in puerperium due to injury to pelvic fatty tissue.

ii) Non-traumatic causes:

Extensive burns
Diabetes mellitus
Fatty liver

GAS EMBOLISM:

Air, nitrogen and other gases can produce bubbles within the circulation and obstruct the blood vessels causing damage to tissue. Two main forms of gas embolism—air embolism and decompression sickness.

Air Embolism

Air embolism occurs when air is introduced into venous or arterial circulation.

Venous Air Embolism:

Air may be sucked into systemic veins under the following circumstances:

i) Operations on head and neck, and trauma.

ii) Obstetrical operations and trauma.

iii)Intravenous infusion of blood and fluid.
iv) Angiography.

Arterial Air Embolism:

Entry of air into pulmonary vein or its tributaries may occur in the following conditions:

i) Cardiothoracic surgery and trauma.
ii) Paradoxical air embolism.
iii)Arteriography.

Decompression Sickness

This is a specialised form of gas embolism known by various names such as caisson's disease, divers' palsy or aeroembolism.

PATHOGENESIS:

Decompression sickness is produced when the individual decompresses suddenly, either from high atmospheric pressure to normal level, or from normal pressure to low atmospheric pressure. In divers, workers in caissons (diving-bells), offshore drilling and tunnels, who descendto high atmospheric
pressure, increased amount of atmospheric gases (mainly nitrogen; others are O2, CO2) are dissolved in blood and tissue fluids. When such an individual ascends too rapidly i.e. comes to normal level suddenly from high atmospheric pressure, the gases come out of the solution as minute bubbles, particularly in fatty tissues which have affinity for nitrogen. These bubbles may coalesce together to form large emboli.

AMNIOTIC FLUID EMBOLISM:

This is the most serious, unpredictable and unpreventable cause of maternal mortality. During labour and in the immediate postpartum period, the contents of amniotic fluid may enter the uterine veins and reach right side of the heart resulting in fatal complications. The amniotic fluid components which may be found in uterine veins, pulmonary artery and vessels of other organs.

TUMOUR EMBOLISM:

Malignant tumour cells invade the local blood vessels and may form tumour emboli to be lodged elsewhere, producing metastatic tumour deposits. Notable examples are clear cell carcinoma of kidney, carcinoma of the lung.

MISCELLANEOUS EMBOLI:

Various other endogenous and exogenous substances may act as emboli. These are:

i) Fragments of tissue
ii) Placental fragments
iii) Red cell aggregates (sludging)
iv) Bacteria
v) Parasites

INFARCTION:

Infarction is the process of tissue necrosis resulting from some form of circulatory insufficiency; the localised area of necrosis so developed is called an infarct.

ETIOLOGY:

Most commonly, infarcts are caused by interruption in arterial blood supply, called ischaemic necrosis. Less commonly, venous obstruction can produce infarcts termed stagnant hypoxia. Generally, sudden, complete, and continuous occlusion (e.g. thrombosis or embolism) produces infarcts. Infarcts may be produced by non occlusive circulatory insufficiencyas well e.g. incomplete atherosclerotic narrowing of coronary arteries may produce myocardial infarction due to acute coronary insufficiency.

TYPES OF INFARCTS:

Infarcts are classified depending upon different features:

1. According to their colour:

Pale or anaemic,due to arterial occlusion and are seen in compact organs e.g. in the kidneys, heart, spleen.

Red or haemorrhagic,seen in soft loose tissues and are caused either by pulmonary arterial obstruction (e.g. in the lungs) or by arterial or venous occlusion (e.g. in the intestines).

2. According to their age:

Recent or fresh
Old or healed

3. According to presence or absence of infection:

Bland, when free of bacterial contamination

Septic, when infected.

PATHOGENESIS:

The process of infarction takes place as follows:

i) Localised hyperaemiadue to local anoxaemia occurs immediately after obstruction of the blood supply.

ii) Within a few hours, the affected part becomes swollen due to oedema and haemorrhage. The amount of haemorrhage is variable, being more marked in the lungs and spleen, and less extensive in the kidneys and heart.

iii) Cellular changessuch as cloudy swelling and degeneration appear early, while death of the cells (i.e. necrosis) occurs in 12-48 hours.

iv) There is progressive proteolysisof the necrotic tissue and there is lysis of the red cells.

v) An acute inflammatory reaction and hyperaemia appear at the same time in the surrounding tissues in response to products of proteolysis.

vi) Blood pigments, haematoidin and haemosiderin, liberated by lysis of RBCs are deposited in the infarct. At this stage, most infarcts become pale-grey due to loss of red cells.

vii) Following this, there is progressive ingrowth of granulation tissue from the margin of the infarct so that eventually the infarct is replaced by a fibrous scar. Dystrophic calcification may occur sometimes.

INFARCTS OF DIFFERENT ORGANS:

A few representative examples of infarction of some organs (lungs, kidney, liver and spleen).

SHOCK:

Shock is a life-threatening clinical syndrome of cardiovascular collapse characterised by:

1) an acute reduction of effective circulating blood volume (hypotension);
2) an inadequate perfusion of cells and tissues (hypoperfusion).

TRUE SHOCK:

The term“true (or secondary) shock”is a circulatory imbalance between oxygen supply and oxygen requirements at the cellular level, and is also called as circulatory shock.

INITIAL SHOCK:

The term "initial (or primary) shock"is used for transient and usually a benign vasovagal attack resulting from sudden reduction of venous return to the heart.

CLASSIFICATION AND ETIOLOGY:

A simple etiologic classification of shock syndrome divides it into following 3 major types and a few other variants.

1. Hypovolaemic shock:

This form of shock results from inadequate circulatory blood volume by various etiologic factors that may be eitherfrom the loss of red cell mass and plasma from haemorrhage, or from the loss of plasma volume alone.

2. Cardiogenic shock:

Acute circulatory failure with sudden fall in cardiac output from acute diseases of the heart without actual reduction of blood volume results in cardiogenic shock.

3. Septic (Toxaemic) shock:

Severe bacterial infections or septicaemia induce septic shock. It may be the result of Gramnegative septicaemia (endotoxic shock) which is more common, or Gram-positive septicaemia (exotoxic shock).

4. Other types:

These include following types:

i) Traumatic shock:

Shock resulting from trauma is initially due to hypovolaemia, but even after haemorrhage has been controlled, these patients continue to suffer loss of plasma volume into the interstitium of injured tissue and hence is considered separately in some descriptions.

ii) Neurogenic shock:

Neurogenic shock results from causes of interruption of sympathetic vasomotor supply.

iii) Hypoadrenal shock:

Hypoadrenal shock occurs from unknown adrenal insufficiency in which the patient fails to respond normally to the stress of trauma, surgery or illness.

PATHOGENESIS:

In general, all forms of shock involve following 3 derangements:

1) Reduced effective circulating blood volume.

2) Reduced supply of oxygen to the cells and tissues with resultant anoxia.
3) Inflammatory mediators and toxins released from shockinduced cellular injury.

1. Reduced effective circulating blood volume:

It may result by either of the following mechanisms:

i) by actual loss of blood volume as occurs in hypovolaemic shock; or

ii) by decreased cardiac output without actual loss of blood (normovolaemia) as occurs in cardiogenic shock and septic shock.

2. Impaired tissue oxygenation:

Following reduction in the effective circulating blood volume from either of the above two mechanisms and from any of the etiologic agents, there is decreased venous return to the heart resulting in decreased
cardiac output. This consequently causes reduced supply of oxygen to the organs and tissues and hence tissue anoxia, which sets in cellular injury.

3. Release of inflammatory mediators:

In response to cellular injury, innate immunity of the body gets activated as a body defense mechanism and release inflammatory mediators but eventually these agents themselves become the cause of cell injury.

PATHOGENESIS OF HYPOVOLAEMIC SHOCK:

Hypovolaemic shock occurs from inadequate circulating blood volume due to various causes. The major effects of hypovolaemic shock are due to decreased cardiac output and low intracardiac pressure. The severity of clinical features depends upon degree of blood volume lost, haemorrhagic shock is divided into 4 types:

< 1000 ml: Compensated
1000-1500 ml: Mild
1500-2000 ml: Moderate
>2000 ml: Severe

Accordingly, clinical features are increased heart rate (tachycardia), low blood pressure (hypotension).

PATHOGENESIS OF CARDIOGENIC SHOCK:

Cardiogenic shock results from a severe left ventricular dysfunction from various causes. The resultant decreased cardiac output has its effects in the form of

decreased tissue perfusion and movement of fluid from pulmonary vascular bed into pulmonary interstitial space initially (interstitial pulmonary oedema) and later into alveolar spaces (alveolar pulmonary oedema).

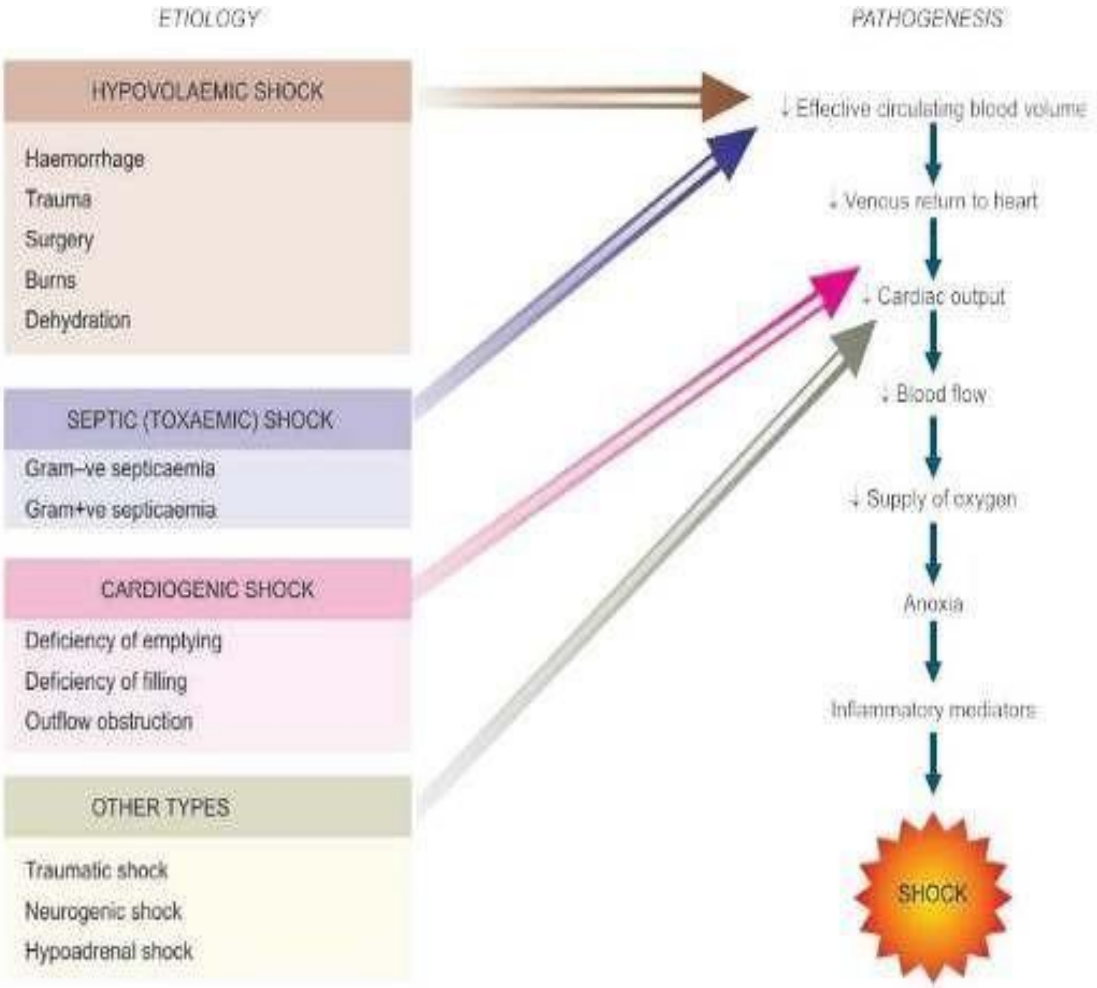
PATHOGENESIS OF SEPTIC SHOCK:

Septic shock results most often from Gram-negative bacteria entering the body from genitourinary tract, alimentary tract, respiratory tract or skin, and less often from Gram-positive bacteria. In septic shock, there is immune system activation and severe systemic inflammatory response to infection as follows:

i) Activation of macrophage-monocytes.

ii) Activation of other inflammatory responses.

PATHOGENESIS OF CIRCULATORY SHOCK:



PATHOPHYSIOLOGY (STAGES OF SHOCK):

Shock has been divided arbitrarily into 3 stages

1. Compensated (non-progressive, initial, reversible) shock.
2. Progressive decompensated shock.
3. Irreversible decompensated shock.

COMPENSATED (NON-PROGRESSIVE, INITIAL, REVERSIBLE) SHOCK:

In the early stage of shock, an attempt is made to maintain adequate cerebral and coronary blood supply by redistribution of blood so that the vital organs (brain and heart) are adequately perfused and oxygenated. This is achieved by activation of various neurohormonal mechanisms causing widespread vasoconstriction and by fluid conservation by the kidney.

PROGRESSIVE DECOMPENSATED SHOCK:

This is a stage when the patient suffers from some other stress or risk factors (e.g. pre-existing cardiovascular and lung disease) besides persistence of the shock so that there is progressive deterioration.

IRREVERSIBLE DECOMPENSATED SHOCK:

When the shock is so severe that in spite of compensatory mechanisms and despite therapy and control of etiologic agent which caused the shock, no recovery takes place, it is called decompensated or irreversible shock.

HEMATOLOGIC DISEASE

Hematologic disorders involve the blood and include problems with red blood cells, white blood cells, platelets, bone marrow, lymph nodes, and spleen. Children can experience a variety of disorders, some are genetic while others are acquired.

Hematologic disorders can affect your child's spleen, lymphatic system, or blood vessels.

- **Spleen problems** can have many causes. Sometimes the spleen becomes very large or stops working properly, which can cause low platelet (thrombycytopenia) and low blood counts (anemia).
- **Lymphatic conditions**, such as a lymphatic malformation, can cause a variety of symptoms. Lymphatic malformations are benign masses that result from improperly-developed lymph channels. If left untreated, they can increase in size and cause repeated infections.
- **Abnormally formed blood vessels** can cause vascular malformations and hemangiomas, which are non-cancerous tumors.
- **Abnormally-formed blood cells** can result in a variety of conditions, such as idiopathic thrombocytopenic purpura (ITP), sickle cell disease and hereditary

spherocytosis. These conditions can result in low blood counts (anemia), low platelet counts (thrombocytopenia) and cause many symptoms, including shortness of breath, rapid heart rate, paleness, lack of energy and easy bruising.

Laboratory Blood Tests

- Complete Blood Count
- Reticulocyte Count
- Special Tests of Blood Cells
- Clotting Tests
- Proteins and Other Substances
- Other Blood Tests

Haematological disorders

- Acute Lymphoblastic Leukemia
- Acute Myeloid Leukemia
- **Anemia**
- Antiphospholipid Syndrome
- Aplastic Anemia & Fanconi Anemia
- Deep Vein Thrombosis
- Essential or Primary Thrombocythemia
- Waldenström Macroglobulinemia
- Hemochromatosis
- Hemolytic Anemia
- Idiopathic Myelofibrosis
- Iron-Deficiency Anemia
- Leukopenia
- **Leukemia**
- Myelodysplasia
- Neutropenia
- Pancytopenia
- Pernicious Anemia
- Polycythemia Vera
- Sickle Cell Anemia
- Thalassemias

- TTP Thrombotic Thrombocytopenic Purpura

LYMPHOMAS

- The term lymphoma identifies a heterogeneous group of biologically and clinically distinct neoplasms that originate from cells in the lymphoid tissue.
- They have been historically divided into 2 distinct categories : **Hodgkin's and Non- Hodgkin's Lymphoma.**
- 85% of lymphomas originate from mature B cell.
- 10% to 15% derive from the T-cell lineage.

Hodgkin Lymphoma (Hodgkin's Disease)

- It is distinguished by the presence of large abnormal tumor cells called Hodgkin Reed-Sternberg cells.
- Hodgkin lymphoma has two main subtypes: classical Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma.

Non-Hodgkin Lymphoma

- (NHL) is the most common type of lymphoma.
- There many types of NHL, all of which are divided into two major groups: B cell lymphoma and T cell lymphoma.
- B cell lymphoma is much more common. It accounts for about 80 percent of all NHL cases.
- T cell lymphoma is less common

Causes

- Have a weak immune system or because you were born with an immune disease
- Have an immune system disease such as rheumatoid arthritis.
- Have been infected with a virus such as Epstein-Barr, hepatitis C, or human T-cell leukemia/lymphoma (HTLV-1)
- Have a close relative who had lymphoma
- Were exposed to benzene
- Were treated for Hodgkin or non-Hodgkin lymphoma in the past
- Were treated for cancer with radiation

Symptoms

Warning signs of lymphoma include:

- Swollen glands (lymph nodes), often in the neck, armpit, or groin that are

painless

- Cough
- Shortness of breath
- Fever
- Night sweats
- Fatigue
- Weight loss
- Itching

Diagnosis

- Lymph node biopsy to check for cancer cells.
- Bone marrow aspiration or biopsy.
- Chest X-ray.
- MRI.
- PET scan.
- Blood tests.

Treatment

The treatment you get depends on what type of lymphoma you have and its stage.

The main **treatments for non-Hodgkin lymphoma** are:

- Chemotherapy, which uses drugs to kill cancer cells
- Radiation therapy, which uses high-energy rays to destroy cancer cells
- Immunotherapy, which uses your body's immune system to attack cancer cells
- Targeted therapy that targets aspects of lymphoma cells to curb their growth

The main **treatments for Hodgkin lymphoma** are:

- Chemotherapy
- Radiation therapy
- Immunotherapy

If these treatments don't work, you might have a stem cell transplant.

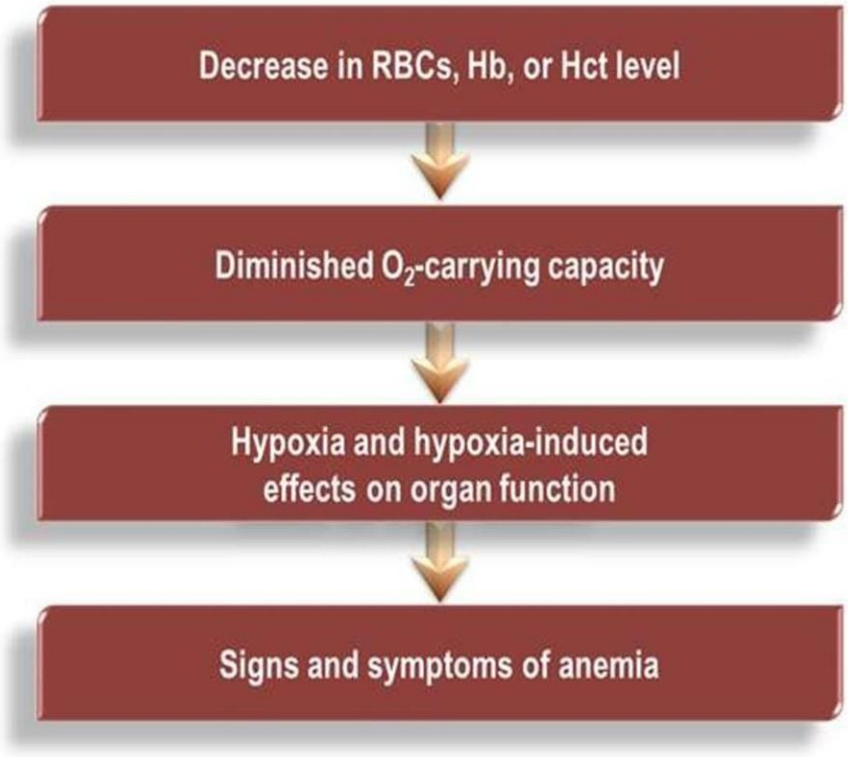
Two types of stem cell transplants can be done:

- An autologous transplant uses your own stem cells.
- An allogeneic transplant uses stem cells taken from a donor.

ANAEMIA

- Anemia affects both adults and children of both sexes, although pregnant women and adolescent girls are most susceptible and most affected by this disease.
- Anemia (An-without,emia- blood) is a decrease in the RBC count, hemoglobin and/or Hematocrit values resulting in a lower ability for the blood to carry oxygen to body tissues .

PATHOPHYSIOLOGY



NORMAL VALUES- Hb COUNT

Category Values	Reference
Men	13.5 to 17.5 g/dl
Women	12 to 15.5 g/dl
Infants from 2 to 6 months	9.5 to 13 g/dl
Newborn	14 to 24 g/dl

TYPES OF ANEMIA

Based on clinical picture-

- Iron deficiency anemia.
- Megaloblastic anemia.
- Pernicious anemia.
- Hemorrhagic anemia.
- Hemolytic anemia.

-Thalassemia anemia

-Sickle cell anemia

- Aplastic anemia

- **Iron deficiency anemia**

Excessive loss of iron .

Women are at risk.

- **Megaloblastic anemia**

Less intake of vitamin B 12 and folic acid.

Red bone marrow produces abnormal RBC.

- **Pernicious anemia**

Inability of stomach to absorb vitamin B 12 in small intestine.

- **Hemorrhagic anemia**

Excessive loss of RBC through bleeding, stomach ulcers, menstruation

- **Hemolytic anemia. 2 types: Inherited, acquired**

RBC plasma membrane ruptures.

may be due to parasites,toxins,antibodies.

- **Thalassemmia**

Less synthesis of hemoglobin .

- **Sickle cell anemia**

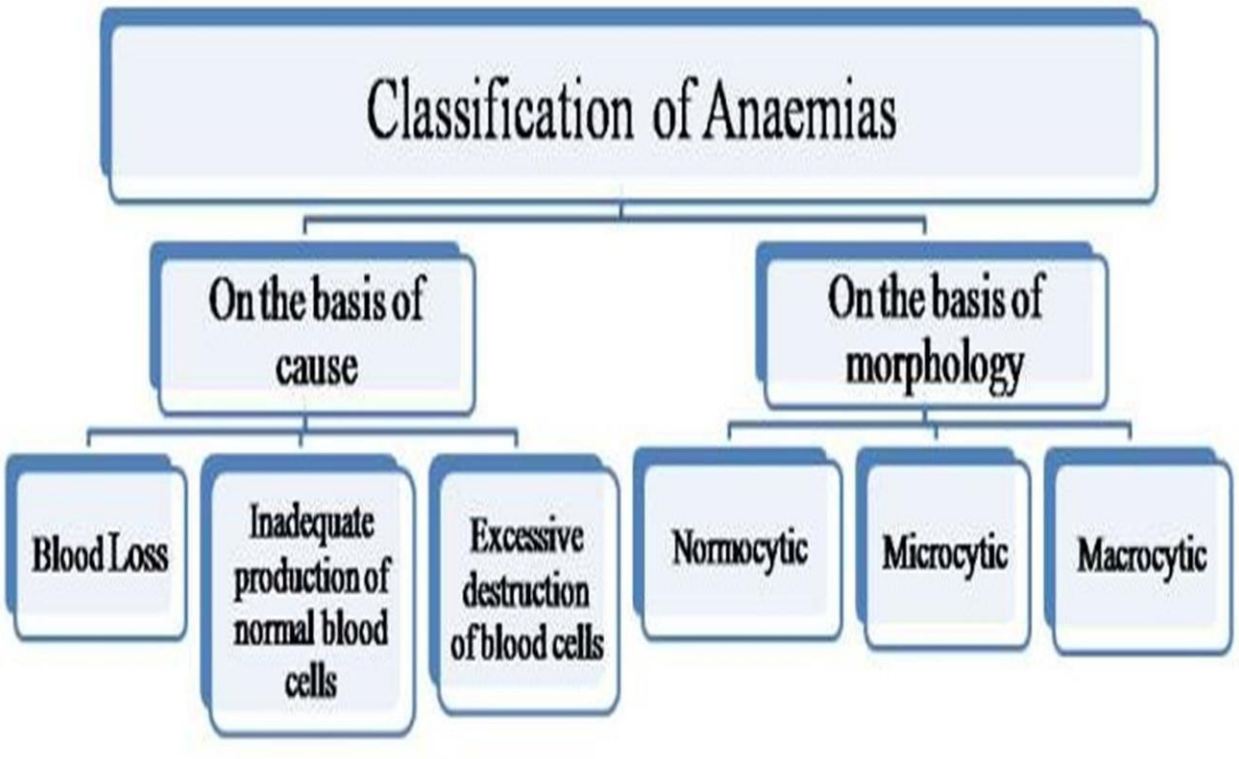
Hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape.

- **Aplastic anemia**

destruction of red bone marrow .

caused by toxins,gamma radiation.

CLASSIFICATION



CAUSES

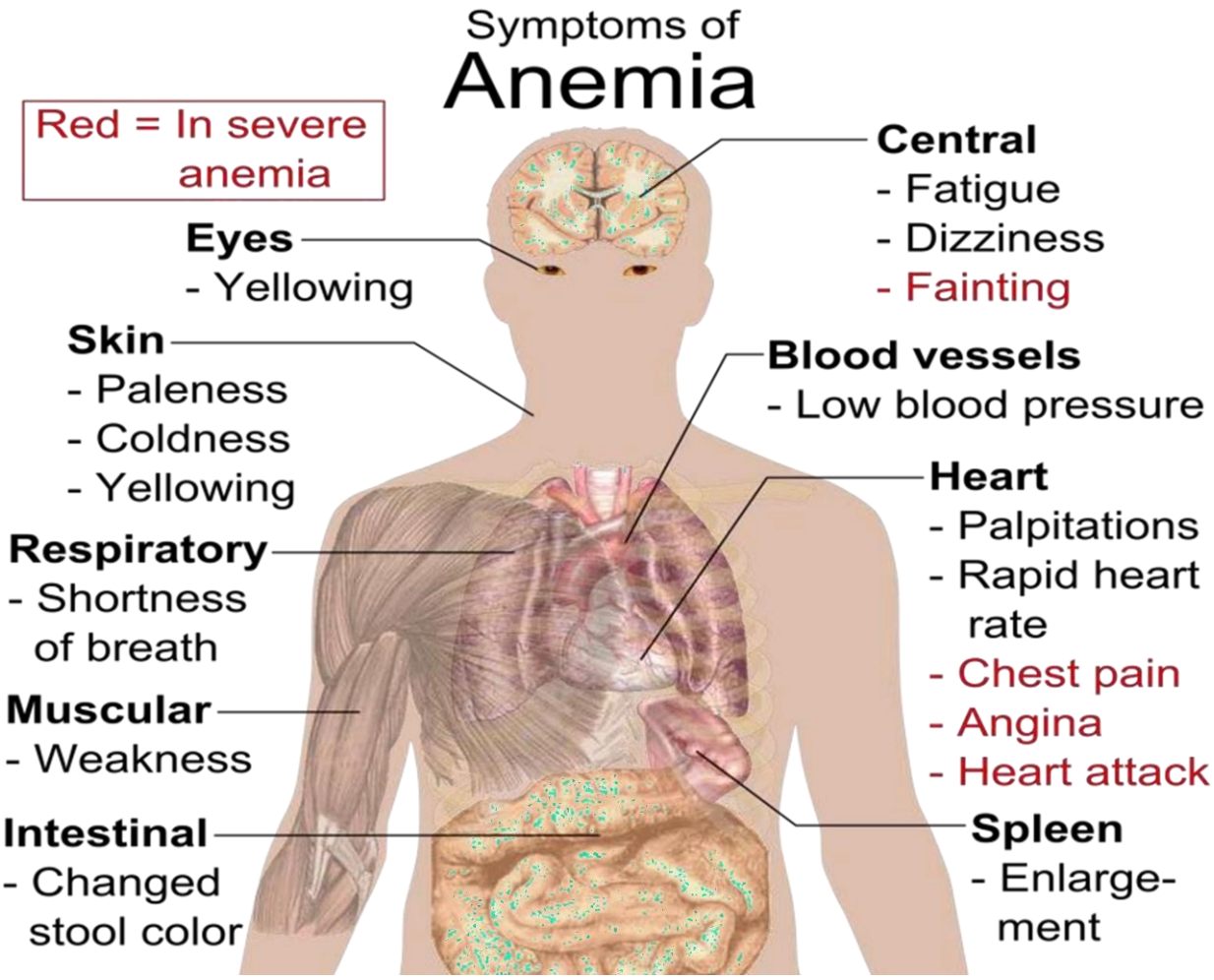
- Diets lacking iron, Vit B12, folic acid
- Inherited blood disorders
- Hemolytic [RBC break down too fast]
- Hypo, hyperthyroidism
- Advanced kidney disease
- Bone marrow disease

SYMPTOMS

- Easy fatigue and loss of energy
- Unusually rapid heart beat, particularly with exercise
- Shortness of breath and headache, particularly with exercise
- Difficulty concentrating
- Dizziness
- Pale skin
- Leg cramps
- Insomnia

SIGNS

- Brittle nails
- Koilonychia (spoon shaped nails)
- Atrophy of the papillae of the tongue [Vit B12 deficiency with smooth, glossy tongue]
- Angular stomatitis [Inflammation in corners of mouth]
- Brittle hair
- Dysphagia[difficult to swallow] and Glossitis[Inflammation in tongue]



DIAGNOSIS

- Complete blood count (CBC).
- A test to determine the size and shape of your red blood cells.
- Occasionally, it can be necessary to study a sample of your bone marrow to diagnose anemia.

LEUKEMIA

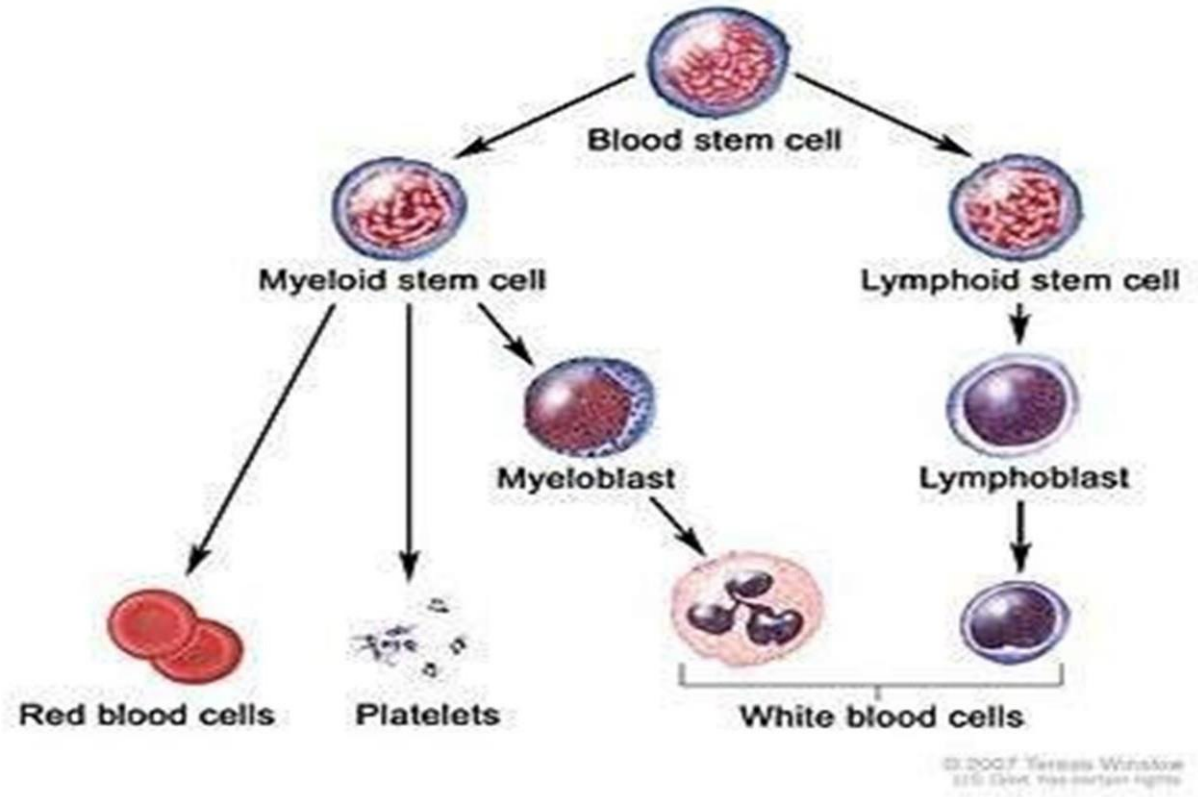
INTRODUCTION

- The word Leukemia comes from the Greek *leukos* which means "white" and *aima* which means "blood".
- Type of Blood cancer that begins in bone marrow.
- WBC[leukocytes] are used by body to fight infections.
- Leukemia leads to uncontrolled rise in WBC.
- The cancer cells spread to bloodstream an lymph nodes.

ETIOLOGY

- No one knows exactly what causes leukemia.
- Smoke
- Are exposed to a lot of radiation or certain chemicals
- Had radiation therapy or chemotherapy to treat cancer
- Have a family history of leukemia
- Have a genetic disorder

PATHOPHYSIOLOGY



SYMPTOMS

- Weakness or fatigue
- Bruising or bleeding easily
- Fever or chills
- Infections that are severe or keep coming back
- Pain in your bones or joints
- Headaches&Vomiting
- Seizures
- Weight loss
- Night sweats
- Shortness of breath

TYPES OF LEUKEMIA

- It may be acute or chronic
- Acute leukemia gets worse very fast and may make feel sick right away.
- Chronic leukemia gets worse slowly and may not cause symptoms for years.
- **CLASSIFICATION OF LEUKAEMIA**
- **Acute lymphocytic leukemia (ALL).** This is the most common form of childhood leukemia. It can spread to your lymph nodes and central nervous system.
- **Acute myelogenous leukemia (AML).** This is the second most common form of childhood leukemia and one of the most common forms for adults.
- **Chronic lymphocytic leukemia (CLL).** This is the other most common form of adult leukemia. Some kinds of CLL will be stable for years and won't need treatment. But with others, your body isn't able to create normal blood cells, and you'll need treatment.
- **Chronic myelogenous leukemia(CML).** With this form, you might not have noticeable symptoms. You might not be diagnosed with it until you have a routine blood test. People 65 and older have a higher risk of this type.

DIAGNOSIS

- Blood tests
- Bone marrow biopsy
- Spinal tap

- Imaging tests

TREATMENT

- Chemotherapy
- Targeted Therapy
- Radiation Therapy and
- Stem Cell Transplant.

UNIT-III

MICROSCOPE

- A microscope ("to look" or "see") is an instrument used to see objects that are too small to be seen by the naked eye. Microscopy is the science of investigating small objects and structures using such an instrument. Microscopic means invisible to the eye unless aided by a microscope.
- Total magnification= Magnifying power of the objective lens by that of eye piece lens.

Why do we need microscopes?

We used to ignore things we couldn't see. But thanks to modern science, we know there's a whole lot happening on the microscopic scale that can help us to live our lives more effectively. Scientists have known since the 17th century that the insides of living things are made up of tiny functioning factories called cells; understanding how they work helps us to tackle sickness and disease. More recently, during the 20th century, scientists figured out how materials are made of atoms and how atoms themselves are built from smaller "subatomic" particles; understanding atomic structure paved the way for all kinds of amazing inventions, from electronic transistors to nuclear power.

Principle of Microscopes

A general biological microscope mainly consists of an objective lens, eyepiece lens, lens tube, stage, and reflector. An object placed on the stage is magnified through the objective lens. When the target is focused, a magnified image can be observed through the eyepiece lens. A microscope is designed to emit light onto or through objects and magnify the transmitted or reflected light with the objective and eyepiece lenses.

Types of Microscope

- Optical / Light Microscope
- Dark Field Microscope
- Phase Contrast Microscope
- Fluorescent Microscope
- Electron Microscope

1. Optical/Light microscope

- It is a type of microscope which uses visible light and a system of lenses to magnify images of small samples.

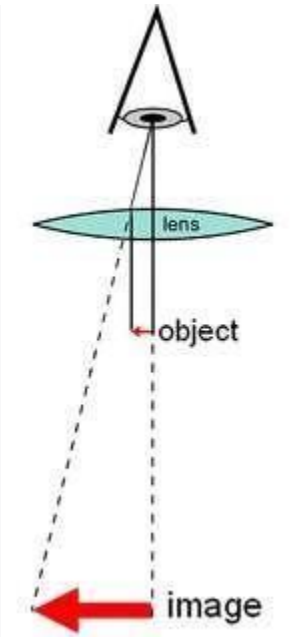
- The image from an optical microscope can be captured by normal light-sensitive cameras to generate a micrograph. Originally images were captured by photographic film but modern developments in cameras allow the capture of

Types

There are two basic types of optical microscopes: **Simple Microscopes and Compound Microscopes.** A simple microscope is one which uses a single lens for magnification, such as a magnifying glass. A compound microscope uses several lenses to enhance the magnification of an object. The vast majority of modern research microscopes are compound microscopes while some cheaper commercial digital microscopes are simple single lens microscopes. Compound microscopes can be further divided into a variety of other types of microscopes which differ in their optical configurations, cost, and intended purposes.

Simple microscope

A simple microscope uses a lens or set of lenses to enlarge an object through angular magnification alone, giving the viewer an erect enlarged virtual image. The use of a single convex lens or groups of lenses are still found in simple



Ray Diagram of a simple microscope

magnification devices such as the magnifying glass, loupes, and eyepieces for telescopes and microscopes.

Compound Microscope

A compound microscope is an optical instrument consisting of two convex lenses of short focal lengths which is used for observing the highly magnified images of tiny objects. The compound microscope can magnify the image of a tiny object up to 1000.

Principle of Compound Microscope

A compound microscope works on the principle that when a tiny object to be magnified is placed just beyond the focus of its objective lens, a virtual, inverted and highly magnified image of the object is formed at the least distance of distinct vision from the eye held close to the eye piece.

Construction

The compound microscope is most commonly used in clinical and educational laboratories. It has a combination of lenses that enhances both magnifying power as well as the resolving power. The specimen or object, to be examined is usually mounted on a transparent glass slide and positioned on the specimen stage between the condenser lens and objective lens.

A beam of visible light from the base is focused by a condenser lens onto the specimen. The objective lens picks up the light transmitted by the specimen and create a magnified image of the specimen called primary image inside the body tube. This image is again magnified by the ocular lens or eye piece.

When higher magnification is required, the nose piece is rotated after low power focusing to bring the objective of higher power (generally 45X) in line with the illuminated part of the slide. The objective lens comes very near the cover slip but it does not touch the same. Only fine adjustment it moved for proper focusing. More light may be required. After observation under high power, the nose piece is rotated to bring back the slide under low power.

Occasionally very high magnification it required (e.g. for observing bacterial cell). In that case, oil immersion objective lens (usually 100X) is employed. After

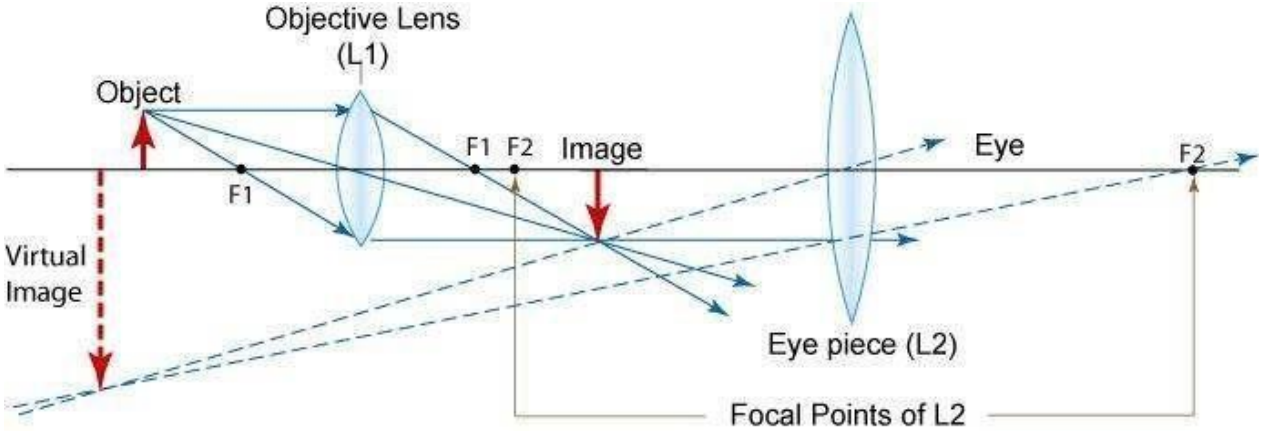
focusing under low power a drop of immersion oil (e.g. cedar oil, olive oil) placed over the illuminated part of the cover-slip.

The eye piece is rotated to bring the oil immersion lens in line with die specimen. It comes in contact with the oil. By using fine adjustment only, the specimen is brought under focus. Immersion oil increases the sharpness of the image. Soon after observation, both the lens and the slide are cleared of the oil by fine cotton cloth or lens paper.

Working Principle:

The most commonly used microscope for general purposes is the standard compound microscope. It magnifies the size of the object by a complex system of lens arrangement.

It has a series of two lenses; (i) the objective lens close to the object to be observed and (ii) the ocular lens or eyepiece lens, through which the image is viewed by eye. Light from a light source (mirror or electric lamp) passes through a thin transparent object.



The objective lens produces a magnified 'real image' first image) of the object. This image is again magnified by the ocular lens (eyepiece) to obtain a magnified 'virtual image' (final image), which can be seen by eye through the eyepiece. As light passes directly from the source to the eye through the two lenses, the field of vision is brightly illuminated. That is why; it is a bright- field microscope.

Applications:

-> Blood analysis
-> Human cells examination
-> Plant cell

Sl. No	Simple Microscope	Compound Microscope
1	There is single lens in simple microscope.	There are 3 to 5 objective lenses in a compound which helps in magnifying algae, fungi and bacterium.
2	Has only one lens for magnifying objects.	Has two sets of lenses for magnifying objects: eyepiece lens and objective lenses
3	Condenser lens is absent.	Condenser lens is present which is used to adjust the intensity of light for magnification of object.
4	Light source is natural	Illuminator is a source of light which is helpful when small, minutest pieces needed to be seen.
5	Total magnification is 10x	Total magnification is (400-1000)x

DARK FIELD MICROSCOPE:

A microscope that has a special condenser and objective with a diaphragm or stop that scatters light from the objectobserved, with the result that the object appears bright on a dark backgroud. It is arranged so that the light source is blocked off, causing light to scatter as it hits the specimen.

Principle

To view a specimen in dark field, an opaque disc is placed underneath the condenser lens, so that only light that is scattered by objects on the slide can reach the eye. Instead of coming up through the specimen, the light is reflected by particles on the slide. Everything is visible regardless of color, usually bright white against a dark background. Pigmented objects are often seen in "false colors," that

is, the reflected light is of a color different than the color of the object. Better resolution can be obtained using dark field as opposed to bright field viewing.

When to use dark field illumination

Dark field illumination is most readily set up at low magnifications (up to 100x), although it can be used with any dry objective lens. Any time you wish to view everything in a liquid sample, debris and all, dark field is best. Even tiny dust particles are obvious. Dark field is especially useful for finding cells in suspension. Dark field makes it easy to obtain the correct focal plane at low magnification for small, low contrast specimens. Use dark field for

- Initial examination of suspensions of cells such as yeast, bacteria, or cell and tissue fractions including cheek epithelial cells, chloroplasts, mitochondria, even blood cells (small diameter of pigmented cells makes it tricky to find them sometimes despite the color).
- Initial survey and observation at low powers of pond water samples, hay or soil infusions, or metazoan cultures.
- Examination of lightly stained prepared slides. Initial location of any specimen of very small size for later viewing at higher power.
- Determination of motility in cultures

Working

In a dark-field microscope, the object is brilliantly illuminated against a dark background. This is accomplished by equipping a light microscope with a special kind of condenser.

It is a condenser with a dark-field stop, which is an opaque disc obstructing the path of light from the light source centrally, but allowing a peripheral ring of light.

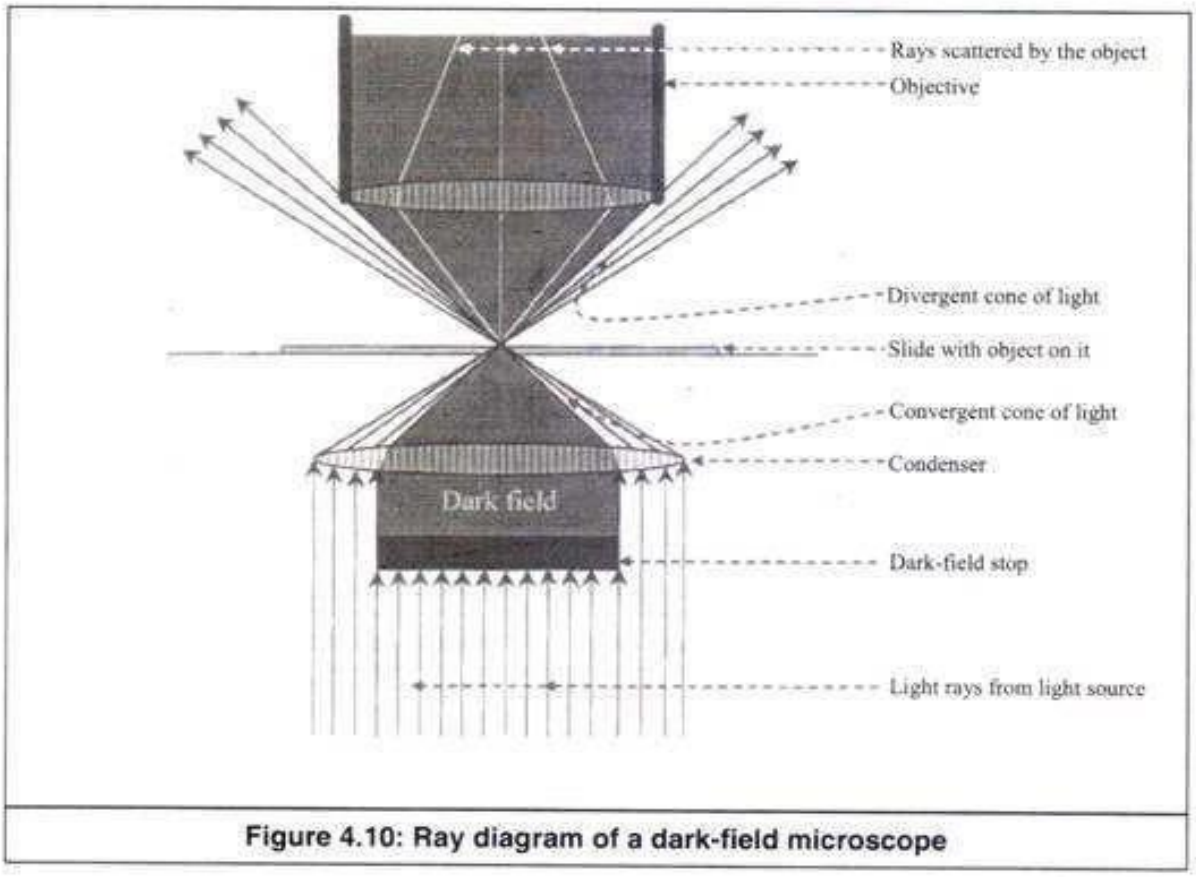


Figure 4.10: Ray diagram of a dark-field microscope

Thus, the condenser transmits a hollow cone of light from the light source. This cone of light converges on the object and diverges from there again as an inverted hollow cone. Thus, no light enters into the objective, as it remains in the dark cone and the field essentially appears dark in absence of any object.

However, if some objects such as microbial cells are present, some of the light rays are scattered (diffracted) by them. These diffracted rays enter into the objective and reach the eye. Thus, the object (microbial cells) appears bright in an otherwise dark microscopic field.

Applications:

- Used to study marine organisms such as algae and plankton, diatoms, insects, fibers, hairs, yeast and protozoa as well as some minerals and crystals, thin polymers and some ceramics.
- Used to view the blood cells.

PHASE CONTRAST MICROSCOPY

Phase contrast microscopy, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and sub cellular particles (including nuclei and other organelles).

In effect, the phase contrast technique employs an optical mechanism to translate minute variations in phase into corresponding changes in amplitude, which can be visualized as differences in image contrast. One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without previously being killed, fixed, and stained. As a result, the dynamics of ongoing biological processes can be observed and recorded in high contrast with sharp clarity of minute specimen detail.

Working Principle

Light passing from one object into another object of a slightly different refractive index or thickness undergoes a change in phase.

In a phase-contrast microscope, this difference in phase is translated into variation in brightness of the image and hence is detectable by eye. With a phase-contrast microscope, the differences among various cells with different refractive indices or thickness can be seen in unstained condition.

Microscopic objects can be seen in unstained condition, due to the difference in the refractive index of the object and its surrounding medium. Unstained structures within cells, not discernible by other microscopic methods can also be observed due to the slight differences in their refractive indices or thickness.

A phase-contrast microscope is a compound microscope fitted with a phase-contrast condenser and a phase-contrast objective. An annular aperture in the diaphragm placed in the focal plane of the sub-stage condenser controls the illumination of the object.

The image of the aperture is formed at the rear focal plane of the objective. In this

plane, there is a phase-shifting element or phase- plate. The phase plate also has an annular ring of phase altering pattern, which can increase the wavelength of light passing through it.

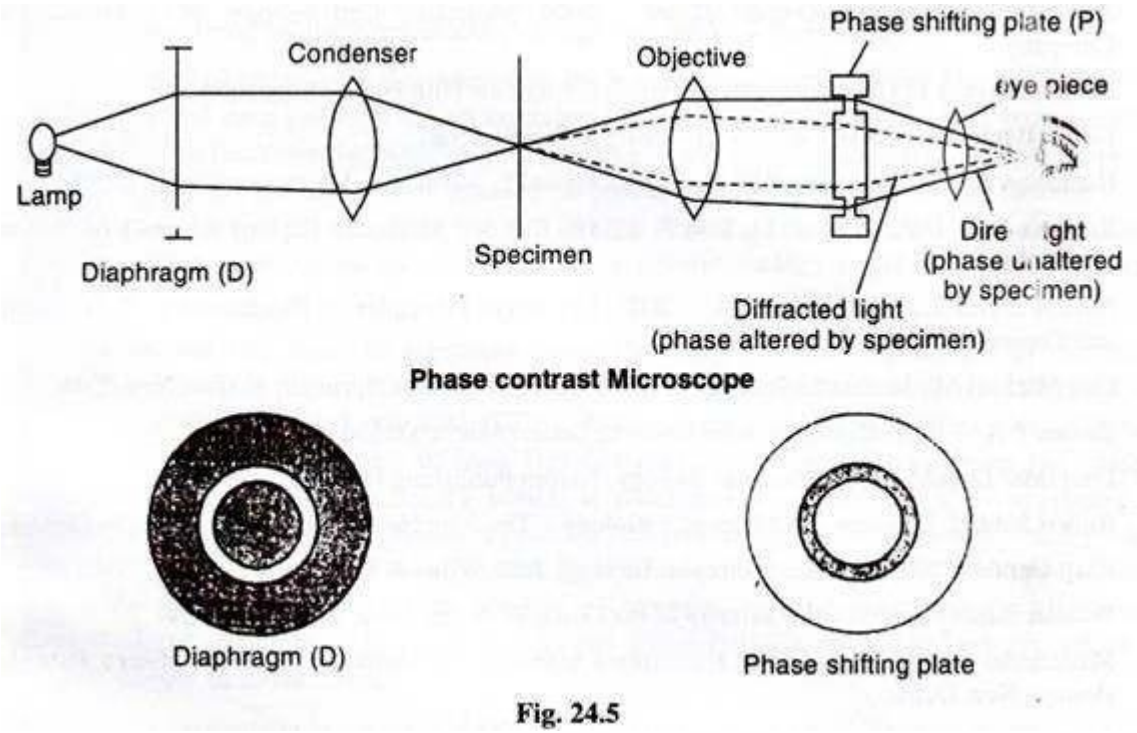


Fig. 24.5

Light coming through the annular aperture of condenser passes through the object. Those rays, which are not deviated by the object (solid lines in figure), pass through the phase-altering pattern of the phase plate and acquire longer wavelength.

Those rays, which are deviated by the object structures (broken lines in figure), due to different refractive index, pass through the phase-plate not covered by the phase altering pattern. Thus, their wavelength remains unchanged. The difference in phase (wavelength) gives the contrast for clear visibility of the object.

Applications:

- Small unstained specimens such as a living cell can be seen.
- It makes Highly Transparent objects more visible.
- Examining Intracellular components of living cells at relatively high resolution. eg: The dynamic motility of Mitochondria, mitotic chromosomes & vacuoles.

- It made it possible for Biologists to study living cells and how they proliferate through cell division.

FLUORESCENT MICROSCOPE

A fluorescence microscope is much the same as a conventional light microscope with added features to enhance its capabilities.

- The conventional microscope uses visible light (400-700 nanometers) to illuminate and produce a magnified image of a sample.
- A fluorescence microscope, on the other hand, uses a much higher intensity light source which excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source.

Fluorescent microscopy is often used to image specific features of small specimens such as microbes. It is also used to visually enhance 3-D features at small scales. This can be accomplished by attaching fluorescent tags to anti-bodies that in turn attach to targeted features, or by staining in a less specific manner. When the reflected light and background fluorescence is filtered in this type of microscopy the targeted parts of a given sample can be imaged. This gives an investigator the ability to visualize desired organelles or unique surface features of a sample of interest. Confocal fluorescent microscopy is most often used to accentuate the 3-D nature of samples. This is achieved by using powerful light sources, such as lasers, that can be focused to a pinpoint. This focusing is done repeatedly throughout one level of a specimen after another. Most often an image reconstruction program pieces the multi level image data together into a 3-D reconstruction of the targeted sample.

- A microscope fitted with a source of ultraviolet radiation to aid inthe detection and exam ination of fluorescent specimens.
- A microscope equipped to examine material that fluoresces under ultraviolet light.
- Fluorescence microscopy is based on the principle that fluorescent materials emit visible light when they are irradiated with ultraviolet rays or with violet-blue visible rays.

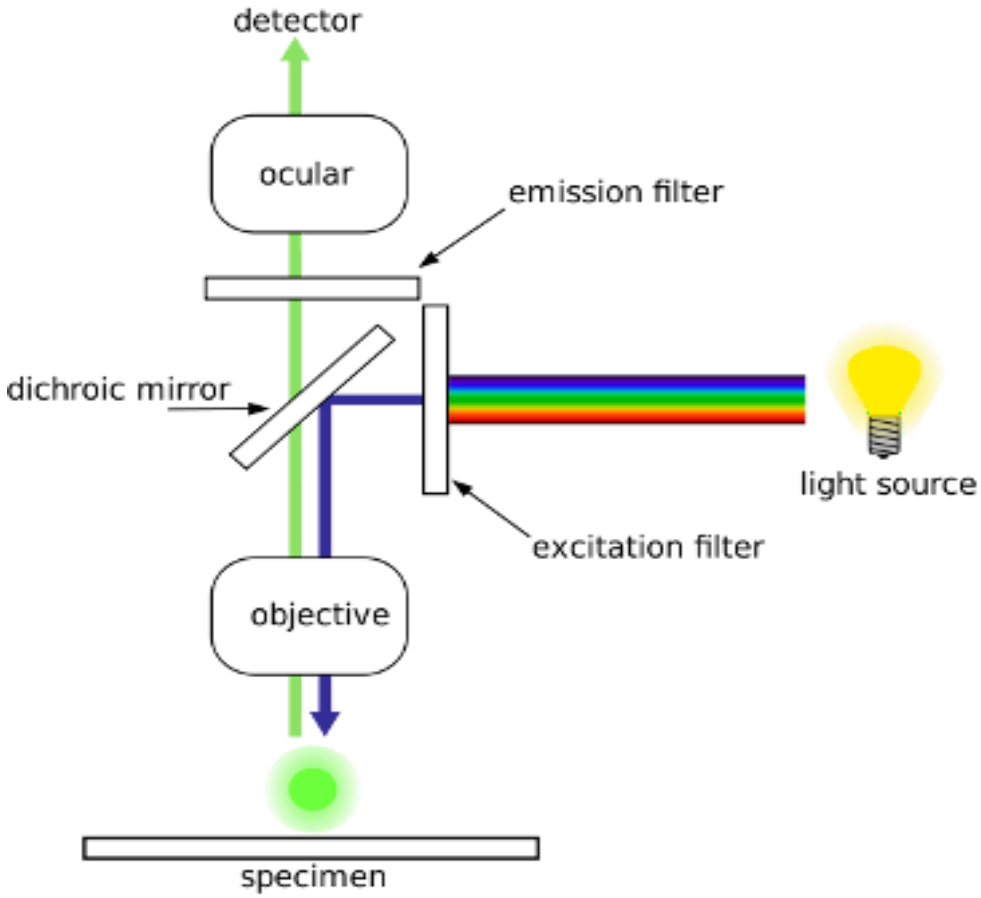
Fluorescence is the emission of light by a substance that has absorbed

light or other electromagnetic radiation. It is a form of luminescence. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation. The most striking example of fluorescence occurs when the absorbed radiation is in the ultraviolet region of the spectrum, and thus invisible to the human eye, while the emitted light is in the visible region, which gives the fluorescent substance a distinct color that can only be seen when exposed to UV light.

Working:

- 2 filters are used: an excitation filter, an emission filter.
- Each fluorophore has a specific absorption or excitation wavelength band, the excitation filter will transmit only that specific range of wavelengths.
- The fluorophore, once excited, will emit a different range of wavelengths.
- The emission filter transmits only the emission wavelengths.
- A dichroic mirror that is specifically designed to reflect the emission wavelengths and transmit the excitation wavelengths is used to separate the excitation and emission channels.

In most cases the sample of interest is labeled with a fluorescent substance known as a fluorophore and then illuminated through the lens with the higher energy source. The illumination light is absorbed by the fluorophores (now attached to the sample) and causes them to emit a longer lower energy wavelength light. This fluorescent light can be separated from the surrounding radiation with filters designed for that specific wavelength allowing the viewer to see only that which is fluorescing.



The basic task of the fluorescence microscope is to let excitation light radiate the specimen and then sort out the much weaker emitted light from the image. First, the microscope has a filter that only lets through radiation with the specific wavelength that matches your fluorescing material. The radiation collides with the atoms in your specimen and electrons are excited to a higher energy level. When they relax to a lower level, they emit light. To become detectable (visible to the human eye) the fluorescence emitted from the sample is separated from the much brighter excitation light in a second filter. This works because the emitted light is of lower energy and has a longer wavelength than the light that is used for illumination.

Most of the fluorescence microscopes used in biology today are epi-fluorescence microscopes, meaning that both the excitation and the observation of the fluorescence occur above the sample. Most use a Xenon or Mercury arc-discharge lamp for the more intense light source.

Applications:

- Imaging structural components of small specimens, such as cells
- Conducting viability studies on cell populations (are they alive or dead?)
- Imaging the genetic material within a cell (DNA and RNA)
- Viewing specific cells within a larger population with techniques such as FISH

ELECTRON MICROSCOPE

A microscope with high magnification and resolution, employing electron beams in place of light and using electron lenses.

- An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination. As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects.

Electron microscopes are used to investigate the ultrastructure of a wide range of biological microorganisms, cells, large molecules, biopsy samples, metals, and crystals. Industrially, electron microscopes are often used for quality control and failure analysis. Modern electron microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the image.

- An electron-optical instrument that utilizes a beam of electrons, rather than light, to focus on cell surfaces of a very thin specimen to produce an enlarged image on a fluorescent screen or photographic plate.
- Electron microscopes use a beam of electrons rather than visible light to illuminate the sample.
- They focus the electron beam using electromagnetic coils instead of glass lenses because electrons can't pass through glass.
- It can't be used to look directly at living things because of the special preparation that samples must undergo before they are visualised.

- **2 Types.**

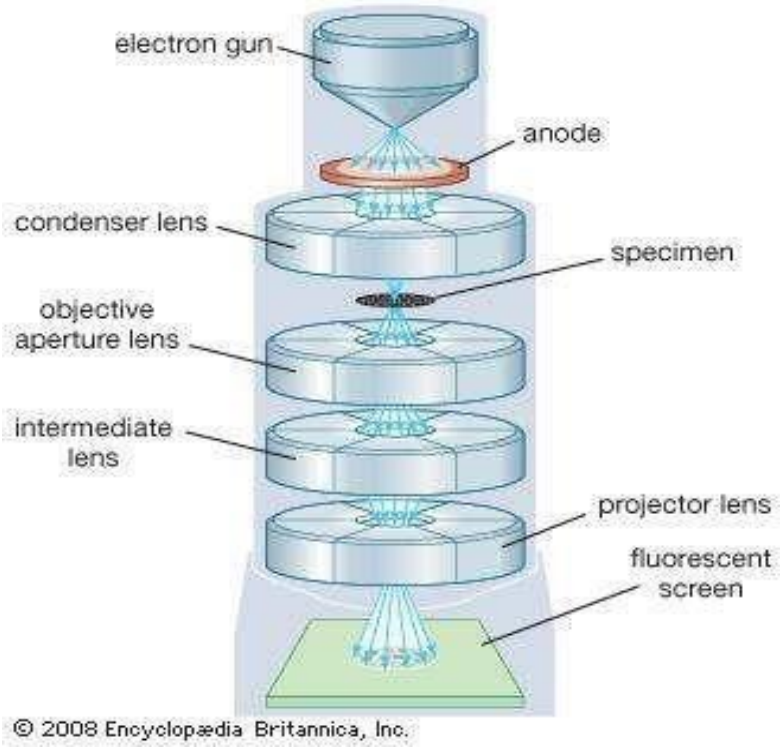
1. ***Transmission Electron Microscope(TEM)***
2. ***Scanning Electron Microscope(SEM)***

TEM

- It is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image.
- It is capable of imaging at a significantly higher resolution.

Three essential systems:

- An electron gun, which produces the electron beam, and the condenser system, which focuses the beam onto the object
- The image-producing system, consisting of the objective lens, movable specimen stage, and intermediate and projector lenses, which focus the electrons passing through the specimen to form a real, highly magnified image.
- The image-recording system, which converts the electron image into some form perceptible to the human eye.



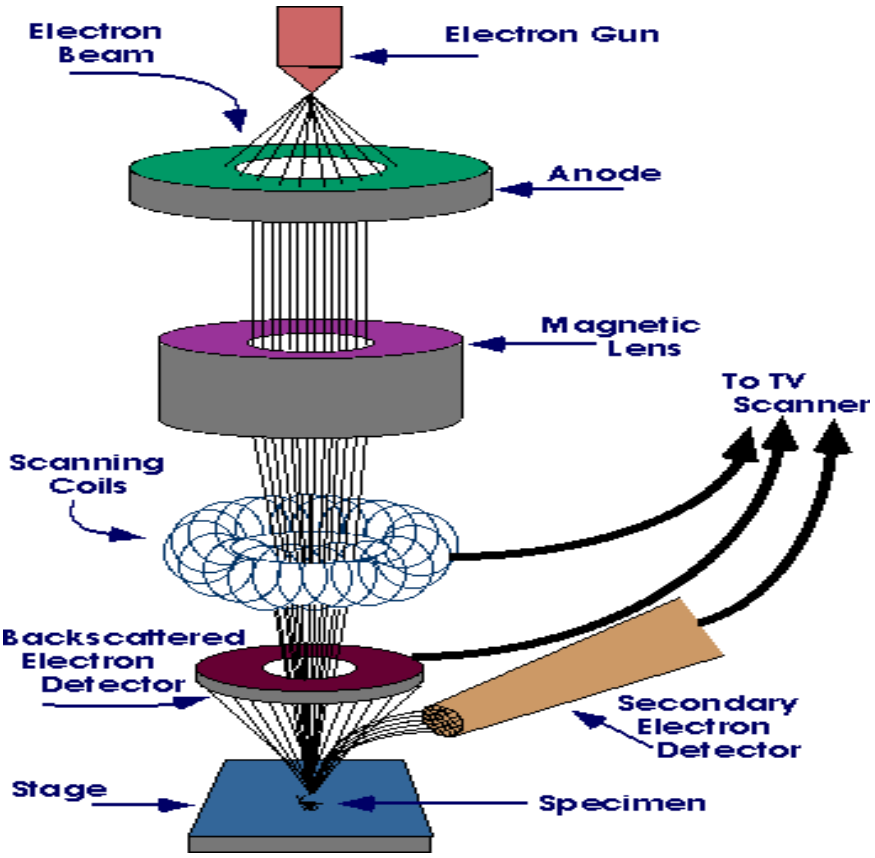
Uses a high voltage electron beam to illuminate the specimen and create an image. The electron beam is produced by an electron gun, commonly fitted with a tungsten filament cathode as the electron source. The electron beam is accelerated by an anode typically at +100 keV (40 to 400 keV) with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lenssystem of the microscope. The spatial variation in this information (the "image") may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide. Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fibre optic light-guide to the sensor of a digital camera. The image detected by the digital camera may be displayed on a monitor or computer.

One major disadvantage of the transmission electron microscope is the need for extremely thin sections of the specimens, typically about 100 nanometers.

SEM

- It produces images of a sample by scanning the surface with a focused beam of electrons.
- An electron microscope that produces a high magnification image of the surface of a metal-coated specimen by scanning an electron beam
  and building an image from the electrons reflected at each point.
- A beam of electrons is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscope, which is held within a vacuum.
- The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample.
- Once the beam hits the sample, electrons and X-rays are ejected from the sample.
- Detectors collect these X-rays, backscattered electrons, and secondary

electrons and convert them into a signal that is sent to a screen similar to a television screen. This produces the final image.



- SEM produces images by detecting secondary electrons that are emitted from the surface due to excitation from a primary electron beam. In more detail, SEM works by rapidly scanning your sample with a focused electron beam. This causes electrons to be knocked off the surface of your sample. These secondary electrons provide signals carrying information about the properties of the specimen surface, such as its topography and composition. And it is these secondary or backscattered electrons that are picked up by a detector and are used to produce your image.

Bacteria Cell

Bacterial cells may be gram positive or gram negative. Both types of bacterial cells have cell walls, but these have different compositions. Bacteria are prokaryotic, unicellular microorganisms, which lack chlorophyll pigments. The average diameter of spherical bacteria is 0.5-2.0µ. For rod-shaped or filamentous bacteria, length is 1-10µ and diameter is 0.25-1 .0µ. Gram positive

bacterial cells contain thick peptidoglycan walls that contain high concentrations of peptides. Gram negative bacterial walls contain thin peptidoglycan walls and exhibit enzymes that help them with mobility. Gram negative bacteria also contain lipid A, which is an endotoxin that can protect the bacterial cell.

Shape and Arrangement of Bacteria:

1. Spherical Bacteria:

Bacteria, which are spherical or ovoid in shape, are called 'coccus' (plural: cocci). Based on the
arrangement of the cells they are of the following types.

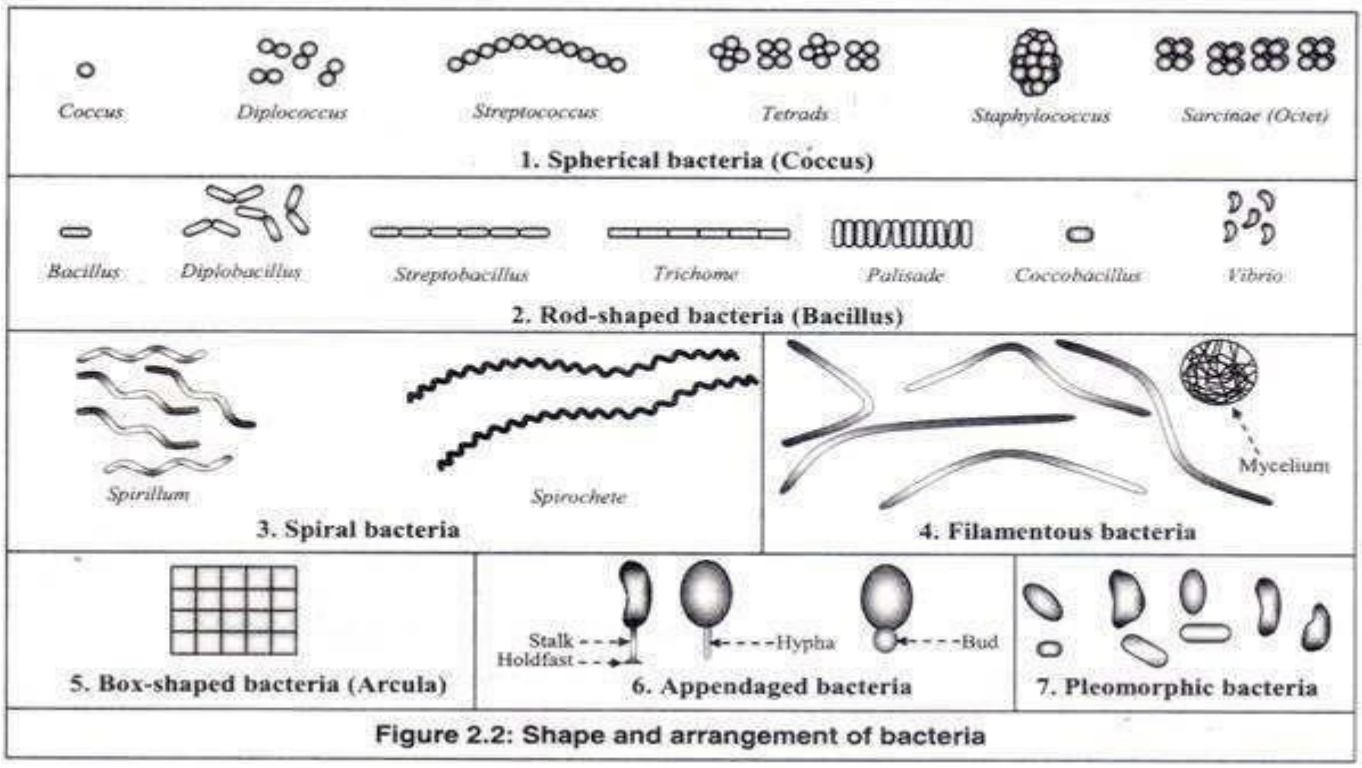


Figure 2.2: Shape and arrangement of bacteria

(a) Coccus:

The spherical bacteria cells, called cocci, are present as single individuals.

(b) Diplococcus:

The cocci are arranged in pairs.

(c) Streptococcus:

The cocci are arranged in chains, as the cells divide in one plane. **(d) Tetrads:**

The cocci are arranged in packets of four cells, as the cells divide in two plains.

(e) Staphylococcus:
The cocci are arranged in grape-like clusters formed by irregular cell divisions in three plains.

(f) Sarcinae (Octet):
The cocci are arranged in a cuboidal manner, as the cells are formed by regular cell divisions in three planes.

2. Rod-shaped Bacteria:
The cylindrical or rod-shaped bacteria are called 'bacillus' (plural: bacilli).

They are of three shapes as follows:
(a) Bacillus:
They are rod-shaped bacteria. Based on arrangement they are of the following types.

(i) Bacillus:
The rod-shaped bacteria cells, called bacilli, are present as single individuals.

(ii) Diplobacillus:
The bacilli are arranged in pairs.

(iii) Streptobacillus:
The bacilli are arranged in chains, as the cells divide in one plane.

(iv) Trichomes:
The bacilli are arranged in chains with larger area of end-to-end contact between the cells.

(v) Palisades:
The bacilli bend at the points of division following the cell divisions, resulting in a palisade arrangement resembling a picket fence and angular patterns that

look like Chinese letters.

(b) Coccobacillus:
These are so short and stumpy that they appear ovoid. They look like coccus and bacillus.

(c) Vibrios:
They are comma-shaped bacteria with less than one complete turn or twist in the cell.

3. Spiral Bacteria:
Unlike the vibrios, which have less than one complete turn or twist in the cell, the spiral bacteria are rod-shaped bacteria, which have more than one twist in the cell. They usually occur singly.

They are of two types as follows:
(a) Spirillum:
They have rigid spiral structure. Spirillum with many turns can superficially resemble spirochetes. They do not have outer sheath and endoflagella, but have typical bacterial flagella.

(b) Spirochetes:
They are flexible and can twist and contort their shape. They have outer sheath and endoflagella, but lack typical bacterial flagella.

4. Filamentous Bacteria:
They are very long thin filament-shaped bacteria. Some of them form branching filaments resulting in a network of filaments called 'mycelium'.

5. Box-shaped or Square-shaped Bacteria (Arcula):
They are flat, box-shaped bacteria with perfectly straight edges and sharp 90° angles at the corners. Smaller cells are usually perfectly squares (2X2µ), while larger cells are rectangular; about twice as long as they are wide (4X2µ).

Each bacterium is a thin flexible sheet with smooth surface. After cell divisions, the cells remain attached to each other, producing large sheets of squares. It was first discovered in 1980 in natural salt ponds.

6. Appendaged Bacteria:

They possess extension of their cells, as long tubes in the form of stalk or hypha, or as buds.

7. Pleomorphic Bacteria:
These bacteria do not have any characteristic shape unlike all others described above. They can change their shape. In pure cultures, they can be observed to have different shapes.

Nutrition in Bacteria

Bacteria exhibit different modes of nutrition. On this basis, broadly two types of bacteria can be recognised **autotrophic bacteria** and **heterotrophic bacteria**.

Autotrophs are of two types:

1. **Photoautotrophs:** The bacteria that use light energy, CO_2 as their carbon source and an inorganic electron source (**Examples**: H_2, H_2S) are called Photoautotrophs
2. **Chemoautotrophs:** The bacteria that obtain energy by oxidizing inorganic compounds and use CO_2 as their sole source of carbon are called Chemoautotrophs. These include nitrifying bacteria, iron bacteria and sulphur bacteria.

Heterotrophs are of two types:

1. **Photo heterotrophs**: Bacteria that use light energy and organic electron donors as well as simple organic molecules rather than CO_2 as the source of carbon are called as Photo heterotrophs. These include Purple and Green Bacteria
2. **Chemo heterotrophs:** Bacteria which use organic compounds as source of energy, electrons, hydrogen and carbon for biosynthesis are called Chemo heterotrophs.

They are as unrelated to human beings as living things can be, but bacteria are essential to human life and life on planet Earth. Although they are notorious for their role in causing human diseases, from tooth decay to the Black Plague,

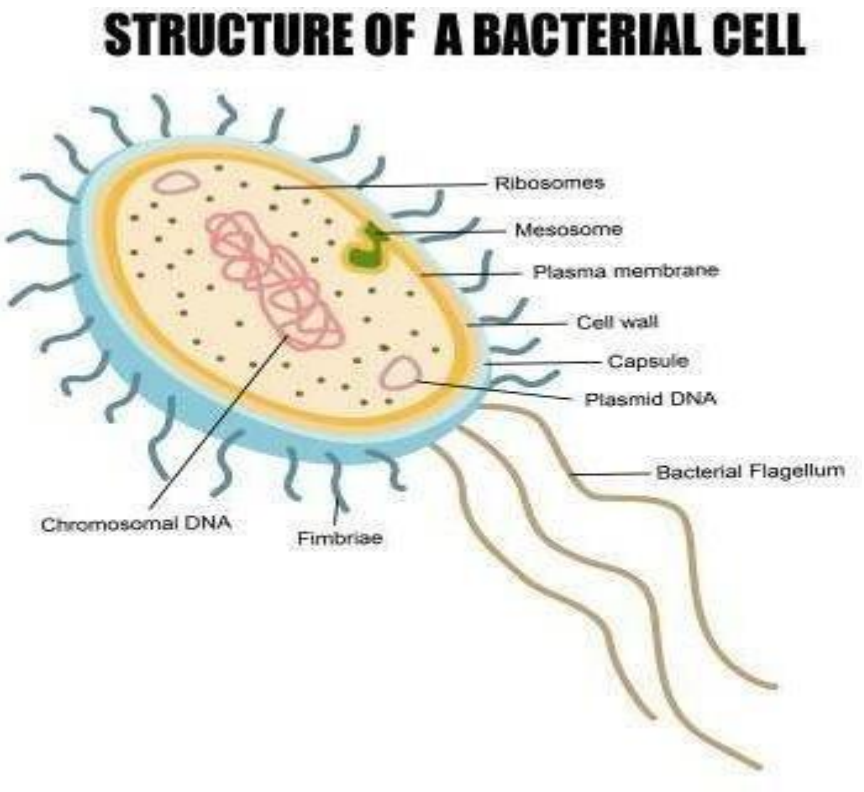
there are beneficial species that are essential to good health. For example, one species that lives symbiotically in the large intestine manufactures vitamin K, an essential blood clotting factor. Other species are beneficial indirectly. Bacteria give yogurt its tangy flavor and sour dough breads its sour taste. They make it possible for ruminant animals (cows, sheep, and goats) to digest plant cellulose and for some plants, (soybean, peas, alfalfa) to convert nitrogen to a more usable form.

Bacteria are prokaryotes, lacking well-defined nuclei and membrane-bound organelles, and with chromosomes composed of a single closed DNA circle. They come in many shapes and sizes, from minute spheres, cylinders and spiral threads, to flagellated rods, and filamentous chains. In the late 1600s, Anton van Leeuwenhoek became the first to study bacteria under the microscope. During the nineteenth century, the French scientist Louis Pasteur and the German physician Robert Koch demonstrated the role of bacteria as pathogens (causing disease).

There are two different ways of grouping bacteria. They can be divided into three types based on their response to gaseous oxygen. Aerobic bacteria require oxygen for their health and existence and will die without it. Anaerobic bacteria can't tolerate gaseous oxygen at all and die when exposed to it.

STRUCTURE OF BACTERIAL CELL

- **Capsule** - Some species of bacteria have a protective covering, a capsule made up of polysaccharides (complex carbohydrates). Capsules play a number of roles, but the most important are to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms. The capsule is a major virulence factor in the major disease-causing bacteria, such as ***Escherichia coli*** and ***Streptococcus pneumoniae***. Nonencapsulated mutants of these organisms are avirulent, i.e. they don't cause disease.



- **Cell Wall** - Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.
- **Mesosomes -** are folded invaginations in the plasma membrane of bacteria that are produced by the chemical fixation techniques used to prepare samples for electron microscopy. It increases the surface area of the plasma membrane. This drastic increase in the surface area of the membrane mainly helps the cell to carry out cellular respiration more efficiently.
- **Cytoplasm** - The cytoplasm, or protoplasm, of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and

gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components. Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus. The chromosome, a single, continuous strand of DNA, is localized, but not contained, in a region of the cell called the nucleoid. All the other cellular components are scattered throughout the cytoplasm.

One of those components, plasmids, are small, extrachromosomal genetic structures carried by many strains of bacteria. Like the chromosome, plasmids are made of a circular piece of DNA. Unlike the chromosome, they are not involved in reproduction. Only the chromosome has the genetic instructions for initiating and carrying out cell division, or binary fission, the primary means of reproduction in bacteria. Plasmids replicate independently of the chromosome and, while not essential for survival, appear to give bacteria a selective advantage.

Plasmids are passed on to other bacteria through two means. For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission. Other types of plasmids, however, form a tubelike structure at the surface called a pilus that passes copies of the plasmid to other bacteria during conjugation, a process by which bacteria exchange genetic information. Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts. The ability to insert specific genes into plasmids have made them extremely useful tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering.

- **Cytoplasmic Membrane** - A layer of phospholipids and proteins, called the cytoplasmic membrane, encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structural trait bacteria share with all other living cells; a barrier that allows them to selectively interact with their environment. Membranes are highly

organized and asymmetric having two sides, each side with a different surface and different functions. Membranes are also dynamic, constantly adapting to different conditions.

- **Nucleoid** - The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for replication, although a few species do have two or more. Smaller circular auxiliary DNA strands, called plasmids, arealso found in the cytoplasm.
- **Ribosomes** - Ribosomes are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids—the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm. There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.
- **Fimbriae:** *Most Gram-negative bacteria have fimbriae; hair-like projections, external to the cell wall, that allow bacteria to stick to the cells they infect.* Long, thin flagella are used by some bacteria to move about. Sex pili allow bacteria to share genes. Shorter extensions, called fimbriae (singular fimbria), enable bacteria to adhere to surfaces and potentially infect the cells of their host.

VIRAL CELL:

A virus is a small parasite that cannot reproduce by itself. Once it infects a susceptible cell, however, a virus can direct the cell machinery to produce more viruses. Most viruses have either RNA or DNA as their genetic material.

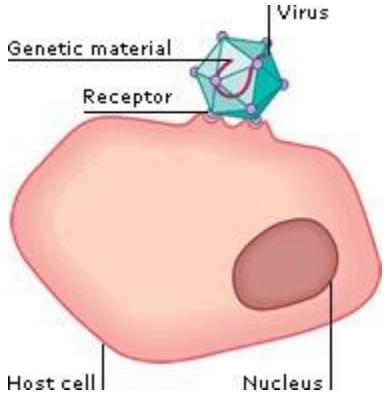
The entire infectious virus particle, called a virion, consists of the nucleic acid and an outer shell of protein. The simplest viruses contain only enough RNA or DNA to encode four proteins. The most complex can encode 100 – 200 proteins.

Viruses are the smallest infectious organisms, and they are so tiny that millions of them could fit inside a single human cell. Viruses are only capable of reproduction inside a living cell, called a host cell that they invade. A virus consists of little more than a single or double strand of genetic material surrounded by a protein shell. However, some types of virus also have a protective outer envelope.

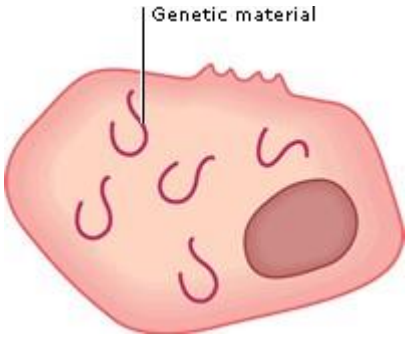
How viruses reproduce

To survive, viruses must reproduce inside living cells. The genetic material from an infecting virus takes over the functions of the host cell to make millions of new virus particles. The new viruses leave the host cell by bursting out of the cell or by budding out from the cell surface.

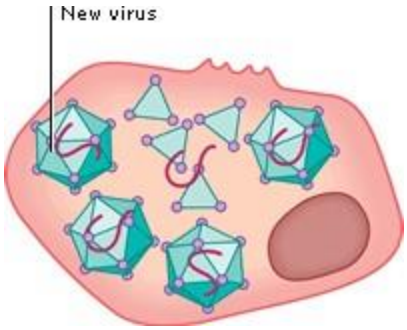
Proteins on the virus attach to specific receptors on the surface of a host cell. The virus may enter the cell by being engulfed by the cell membrane or by fusing into the cell membrane.



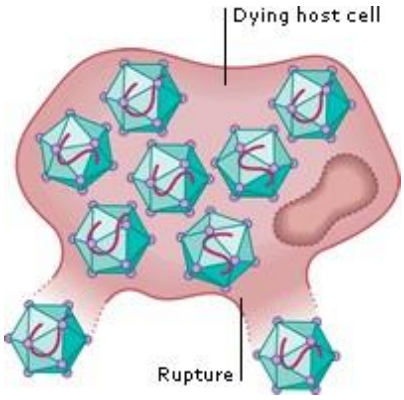
When inside the cell, the virus sheds its protein shell. The genetic material of the virus reproduces, using substances from inside the cell.

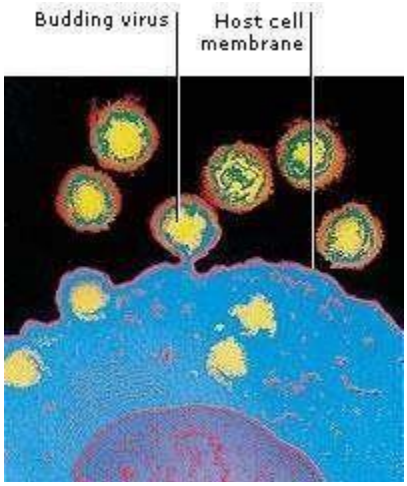


Each copy of the genetic material programmes the formation of a new protein shell. Once the shells have formed, the new viruses are complete.



The viruses leave the cell either by suddenly rupturing the cell membrane, which destroys the host cell, or by slowly budding out from the surface of the cell membrane.





Budding viruses

When certain viruses bud out from their host cell, they envelop themselves in host cell surface membrane.

Structure:

Without a host cell, viruses cannot carry out their life-sustaining functions or reproduce. They cannot synthesize proteins, because they lack ribosomes and must use the ribosomes of their host cells to translate viral messenger RNA into viral proteins. Viruses cannot generate or store energy in the form of adenosine triphosphate (ATP), but have to derive their energy, and all other metabolic functions, from the host cell. They also parasitize the cell for basic building materials, such as amino acids, nucleotides, and lipids (fats). Although viruses have been speculated as being a form of protolife, their inability to survive without living organisms makes it highly unlikely that they preceded cellular life during the Earth's early evolution. Some scientists speculate that viruses started as rogue segments of genetic code that adapted to a parasitic existence.

All viruses contain nucleic acid, either DNA or RNA (but not both), and a protein coat, which encases the nucleic acid. Some viruses are also enclosed by an envelope of fat and protein molecules. In its infective form, outside the cell, a virus particle is called a virion. Each virion contains at least one unique protein synthesized by specific genes in its nucleic acid. Viroids (meaning "viruslike") are disease-causing organisms that contain only nucleic acid and have no structural proteins. Other viruslike particles called prions are composed primarily of a protein tightly integrated with a small nucleic acid molecule.

Viruses are generally classified by the organisms they infect, animals, plants, or bacteria. Since viruses cannot penetrate plant cell walls, virtually all plant viruses are transmitted by insects or other organisms that feed on plants. Certain bacterial viruses, such as the T4 bacteriophage, have evolved an elaborate process of infection. The virus has a "tail" which it attaches to the bacterium surface by means of proteinaceous "pins." The tail contracts and the tail plug penetrates the cell wall and underlying membrane, injecting the viral nucleic acids into the cell.

Viruses are further classified into families and genera based on three structural considerations:
1) the type and size of their nucleic acid, 2) the size and shape of the capsid, and 3) whether they have a lipid envelope surrounding the nucleocapsid (the capsid enclosed nucleic acid).

There are predominantly two kinds of shapes found amongst viruses: rods, or filaments, and spheres. The rod shape is due to the linear array of the nucleic acid and the protein subunits making up the capsid. The sphere shape is actually a 20-sided polygon (icosahedron).

The nature of viruses wasn't understood until the twentieth century, but their effects had been observed for centuries. British physician Edward Jenner even discovered the principle of inoculation in the late eighteenth century, after he observed that people who contracted the mild cowpox disease were generally immune to the deadlier smallpox disease. By the late nineteenth century,

scientists knew that some agent was causing a disease of tobacco plants, but would not grow on an artificial medium (like bacteria) and was too small to be seen through a light microscope. Advances in live cell culture and microscopy in the twentieth century eventually allowed scientists to identify viruses. Advances in genetics dramatically improved the identification process.

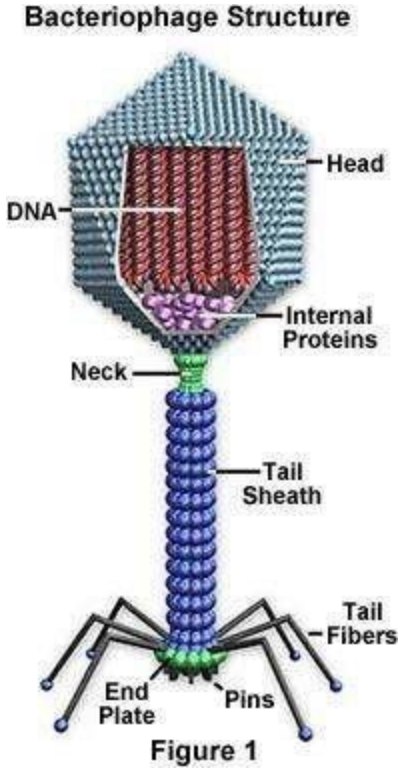


Figure 1

- **Capsid** - The capsid is the protein shell that encloses the nucleic acid; with its enclosed nucleic acid, it is called the nucleocapsid. This shell is composed of protein organized in subunits known as capsomers. They are closely associated with the nucleic acid and reflect its configuration, either a rod-shaped helix or a polygon-shaped sphere. The capsid has three functions: 1) it protects the nucleic acid from digestion by enzymes, 2) contains special sites on its surface that allow the virion to attach to a host cell, and 3) provides proteins that enable the virion to penetrate the host cell membrane and, in some cases, to inject the infectious nucleic acid into the cell's cytoplasm. Under the right conditions, viral RNA in a liquid suspension of protein molecules will self-assemble a capsid to become a functional and infectious virus.
- **Envelope** - Many types of virus have a glycoprotein envelope

surrounding the nucleocapsid. The envelope is composed of two lipid layers interspersed with protein molecules (lipoprotein bilayer) and may contain material from the membrane of a host cell as well as that of viral origin. The virus obtains the lipid molecules from the cell membrane during the viral budding process. However, the virus replaces the proteins in the cell membrane with its own proteins, creating a hybrid structure of cell-derived lipids and virus-derived proteins. Many viruses also develop spikes made of glycoprotein on their envelopes that help them to attach to specificcell surfaces.

- **Nucleic Acid** - Just as in cells, the nucleic acid of each virus encodes the genetic information for the synthesis of all proteins. While the double-stranded DNA is responsible for this in prokaryotic and eukaryotic cells, only a few groups of viruses use DNA. Most viruses maintain all their genetic information with the single-stranded RNA. There are two types of RNA-based viruses. In most, the genomic RNA is termed a plus strand because it acts as messenger RNA for direct synthesis (translation) of viral protein. A few, however, have negative strands of RNA. In these cases, the virion has an enzyme, called RNA-dependent RNA polymerase (transcriptase), which must first catalyze the production of complementary messenger RNA from the virion genomic RNA before viral protein synthesis can occur.

The Influenza (Flu) Virus - Next to the common cold, influenza or "the flu" is perhaps the most familiar respiratory infection in the world. In the United States alone, approximately 25 to 50 million people contract influenza each year. The symptoms of the flu are similar to those of the common cold, but tend to be more severe. Fever, headache, fatigue, muscle weakness and pain, sore throat, dry cough, and a runny or stuffy nose are common and may develop rapidly. Gastrointestinal symptoms associated with influenza are sometimes experienced by children, but for most adults, illnesses that manifest in diarrhea, nausea, and vomiting are not caused by the influenza virus though they are often inaccurately referred to as the "stomach flu." A number of complications, such as the onset of bronchitis and pneumonia, can also occur in association with influenza and are especially common among the elderly,

young children, and anyone with a suppressed immune system.

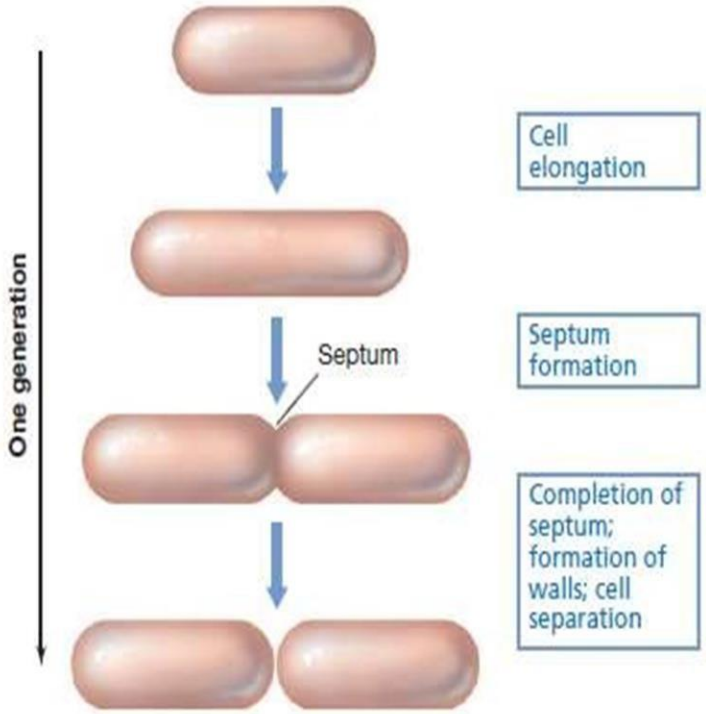
The Human Immunodeficiency Virus (HIV) - The virus responsible for HIV was first isolated in 1983 by Robert Gallo of the United States and French scientist Luc Montagnier. Since that time, a tremendous amount of research focusing upon the causative agent of AIDS has been carried out and much has been learned about the structure of the virus and its typical course of action. HIV is one of a group of atypical viruses called retroviruses that maintain their genetic information in the form of ribonucleic acid (**RNA**). Through the use of an enzyme known as reverse transcriptase, HIV and other retroviruses are capable of producing deoxyribonucleic acid (DNA) from RNA, whereas most cells carry out the opposite process, transcribing the genetic material of DNA into RNA. The activity of the enzyme enables the genetic information of HIV to become integrated permanently into the genome (chromosomes) of a host cell.

BACTERIAL GROWTH

- **Bacterial growth** is the asexual reproduction, or cell division, of a bacterium into two daughter cells, in a process called binary fission.
- Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell.
- Hence, "local doubling" of the **bacterial** population occurs.

Cell Growth and Binary Fission

- In microbiology, growth is defined as an increase in the number of cells. Microbial cells have a finite life span, and a species is maintained only as a result of continued growth of its population.
- Knowledge of how microbial populations can rapidly expand is useful for designing methods to control microbial growth, whether the methods are used to treat a life-threatening infectious disease or simply to disinfect a surface. Knowledge of the events surrounding bacterial growth also allows us to see how these processes are related to cell division in higher organisms.
- In a growing culture of a rod-shaped bacterium such as Escherichia coli, cells elongate to approximately twice their original length and then form a partition that constricts the cell into two daughter cells.



Binary fission in a rod-shaped prokaryote.

- This partition is called a septum and results from the inward growth of the cytoplasmic membrane and cell wall from opposing directions; septum formation continues until the two daughter cells are pinched off.
- There are variations in this general pattern. In some bacteria, such as Bacillus subtilis, a septum forms without cell wall constriction, while in the budding bacterium Caulobacter, constriction occurs but no septum is formed.
- But in all cases, when one cell eventually separates to form two cells, we say that one generation has occurred, and the time required for this process is called the **generation time.**

GROWTH PHASE:

1. Lag Phase

- When a microbial culture is inoculated into a fresh medium, growth usually begins only after a period of time called the lag phase.
- This interval may be brief or extended, depending on the history of the inoculum and the growth conditions.
- If an exponentially growing culture is transferred into the same medium under the same conditions of growth (temperature, aeration, and the like), there is no lag and exponential growth begins immediately.

- However, if the inoculum is taken from an old (stationary phase) culture and transferred into the same medium, there is usually a lag even if all the cells in the inoculum are alive. This is because the cells are depleted of various essential constituents and time is required for their biosynthesis.
- A lag also ensues when the inoculum consists of cells that have been damaged (but not killed) by significant temperature shifts, radiation, or toxic chemicals because of the time required for the cells to repair the damage.
- A lag is also observed when a microbial population is transferred from a rich culture medium to a poorer one; for example, from a complex medium to a defined medium. To grow in any culture medium the cells must have a complete complement of enzymes for synthesis of the essential metabolites not present in that medium.
- Hence, upon transfer to a medium where essential metabolites must be biosynthesized, time is needed for production of the new enzymes that will carry out these reactions.

2. Exponential Phase

- During the exponential phase of growth each cell divides to form two cells, each of which also divides to form two more cells, and so on, for a brief or extended period, depending on the available resources and other factors.
- Cells in exponential growth are typically in their healthiest state and hence are most desirable for studies of their enzymes or other cell components.
- Rates of exponential growth vary greatly. The rate of exponential growth is influenced by environmental conditions (temperature, composition of the culture medium), as well as by genetic characteristics of the organism itself.
- In general, prokaryotes grow faster than eukaryotic microorganisms, and small eukaryotes grow faster than large ones.
- Small cells have an increased capacity for nutrient and waste exchange compared with larger cells, and this metabolic advantage can greatly affect their growth and other properties.

3. Stationary Phase

- In a batch culture (tube, flask bottle, Petri dish), exponential growth is limited. Consider the fact that a single cell of a bacterium with a 20-min

generation time would produce, if allowed to grow exponentially in a batch culture for 48 h, a population of cells that weighed 4000 times the weight of Earth.

- This is particularly impressive when it is considered that a single bacterial cell weighs only about one-trillionth (10-12) of a gram.
- Obviously, this scenario is impossible. Something must happen to limit the growth of the population. Typically, either one or both of two situations limit growth: (1) an essential nutrient of the culture medium is used up, or (2) a waste product of the organism accumulates in the medium and inhibits growth. Either way, exponential growth ceases or the population reaches the stationary phase.
- In the stationary phase, there is no net increase or decrease in cell number and thus the growth rate of the population is zero.
- Although the population may not grow during the stationary phase, many cell functions can continue, including energy metabolism and biosynthetic processes.
- Some cells may even divide during the stationary phase but no net increase in cell number occurs.
- This is because some cells in the population grow, whereas others die, the two processes balancing each other out. This is a phenomenon called cryptic growth.

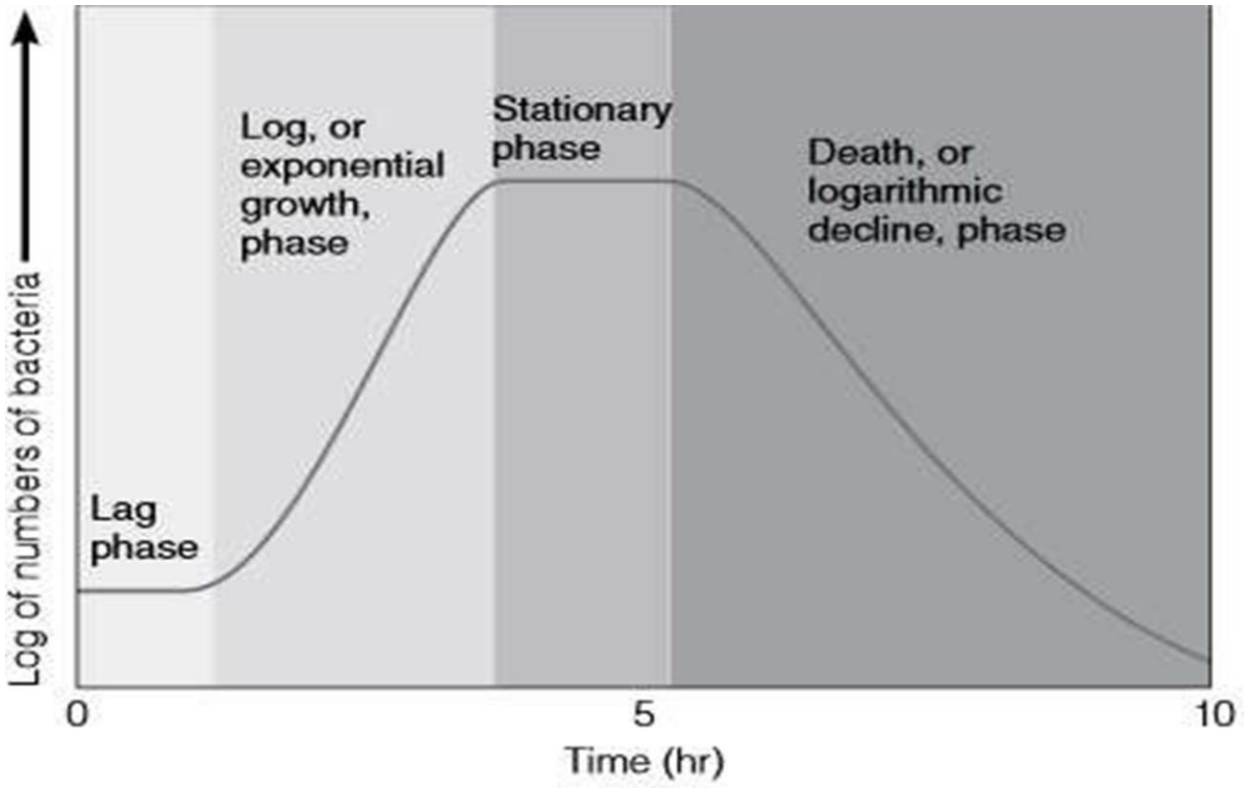
4. **Death Phase**

- If incubation continues after a population reaches the stationary phase, the cells may remain alive and continue to metabolize, but they will eventually die.
- When this occurs, the population enters the death phase of the growth cycle. In some cases death is accompanied by actual cell lysis. Figure indicates that the death phase of the growth cycle is also an exponential function.
- Typically, however, the rate of cell death is much slower than the rate of exponential growth.
- The phases of bacterial growth are reflections of the events in a population of cells, not in individual cells. Thus the terms lag phase, exponential phase, stationary phase, and death phase have no meaning with respect to

individual cells but only to cell populations.

- Growth of an individual cell is a necessary prerequisite for population growth. But it is population growth that is most relevant to the ecology of microorganisms, because measurable microbial activities require microbial populations, not just an individual microbial cell.

Growth curve



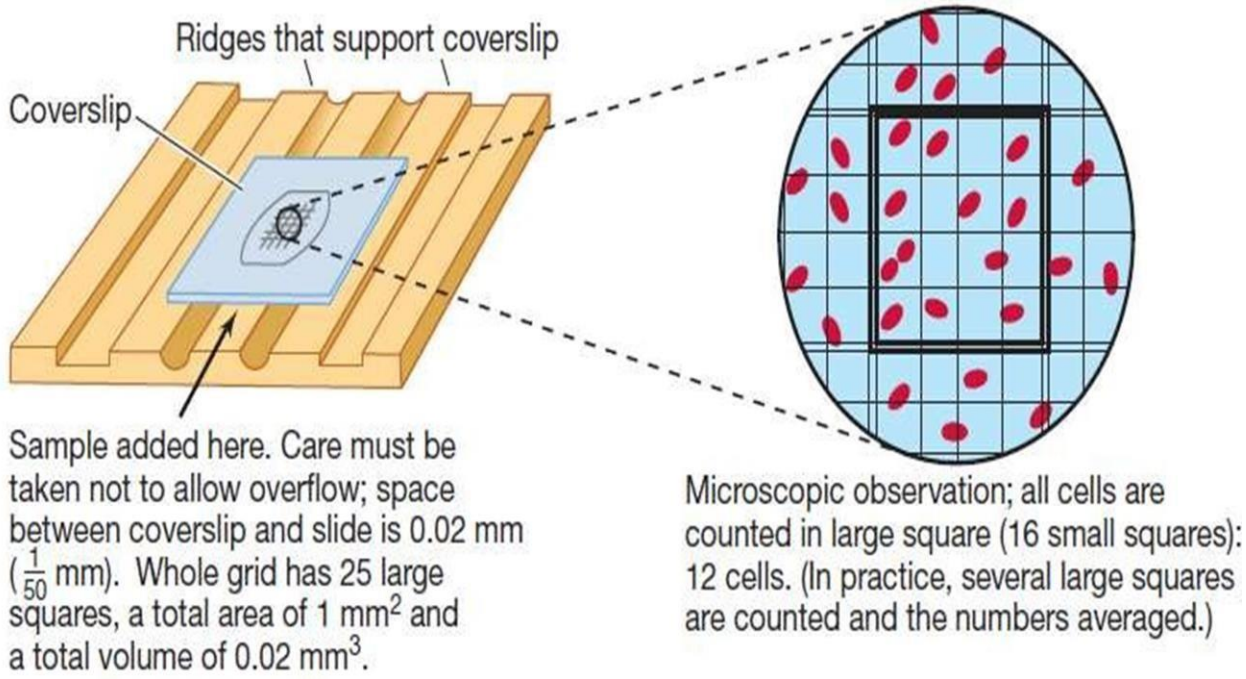
Measurement of Microbial growth

Microscopic Counts

- A total count of microbial numbers can be achieved using a microscope to observe and enumerate the cells present in a culture or natural sample. The method is simple, but the results can be unreliable.
- The most common total count method is the microscopic cell count. Microscopic counts can be done on either samples dried on slides or on samples in liquid. Dried samples can be stained to increase contrast between cells and their background. With liquid samples, specially designed counting chambers are used. In such a counting chamber, a grid with squares of known area is marked on the surface of a glass slide.
- When the cover slip is placed on the chamber, each square on the grid has a

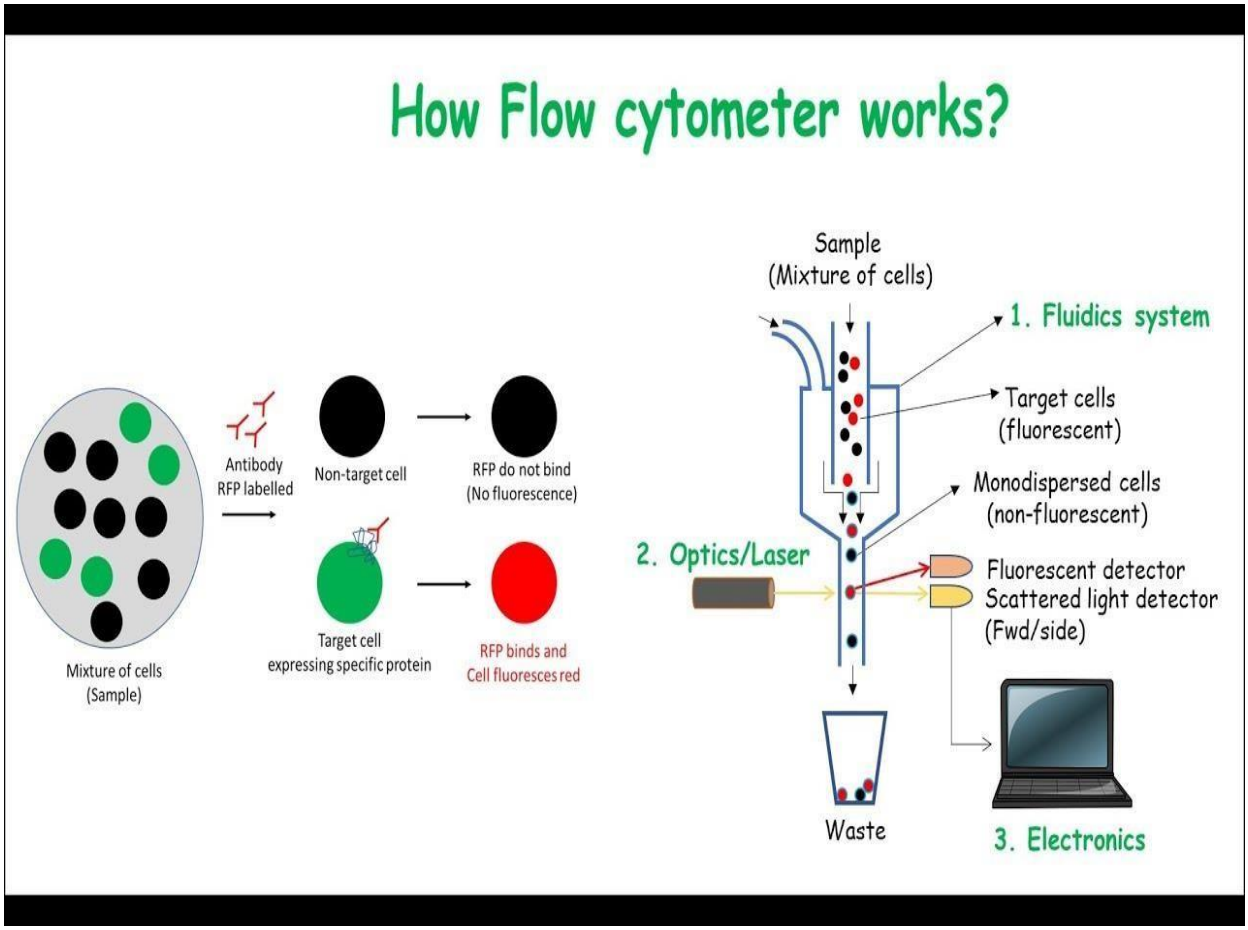
precisely measured volume. The number of cells per unit area of grid can be counted under the microscope, giving a measure of the number of cells per small chamber volume.

- The number of cells per milliliter of suspension is calculated by employing a conversion factor based on the volume of the chamber sample.



Direct microscopic counting procedure using the Petroff–Hausser counting chamber.

- A second method of enumerating cells in liquid samples is with a flow cytometer. This is a machine that employs a laser beam and complex electronics to count individual cells.
- Flow cytometry is rarely used for the routine counting of microbial cells, but has applications in the medical field for counting and differentiating blood cells and other cell types from clinical samples.
- It has also been used in microbial ecology to separate different types of cells for isolation purposes.



Viable Counts

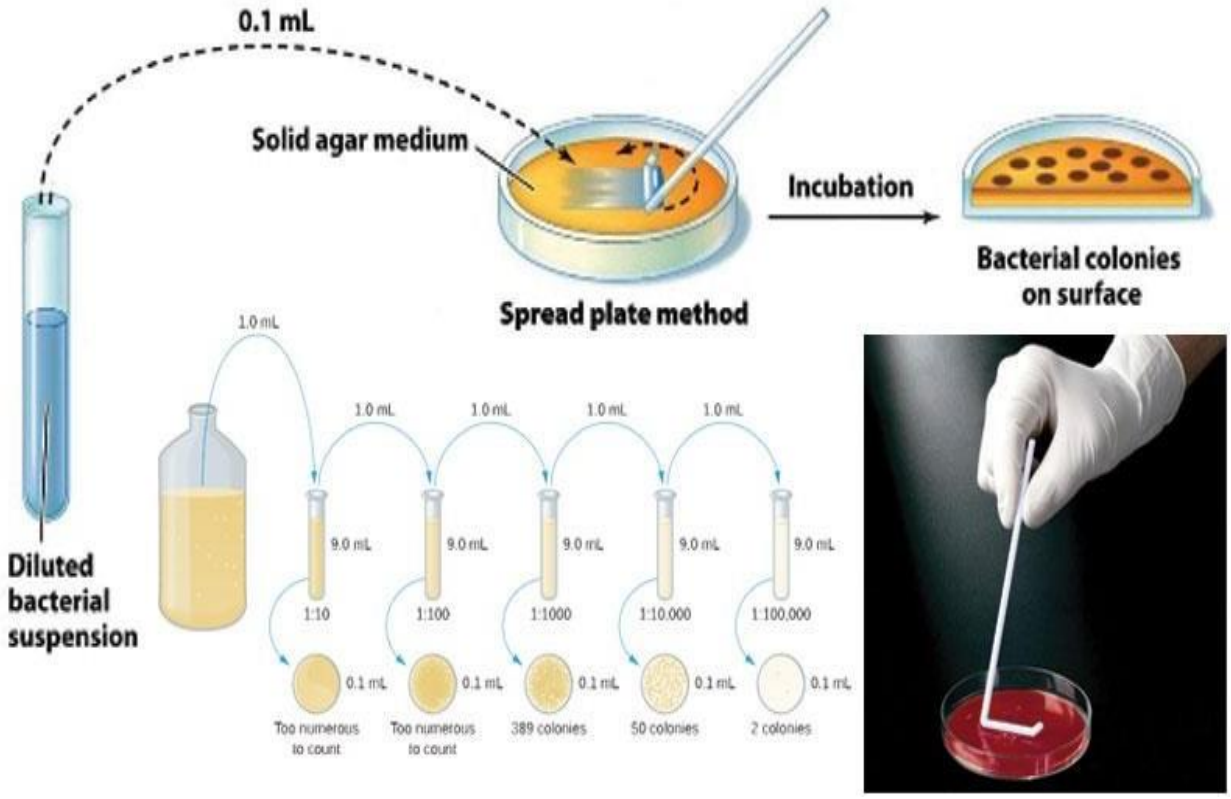
- A **viable** cell is one that is able to divide and form offspring, and in most cell-counting situations, these are the cells we are most interested in.
- For these purposes, we can use a viable counting method.
- To do this, we typically determine the number of cells in a sample capable of forming colonies on a suitable agar medium.
- For this reason, the viable count is also called a **plate count**.
- The assumption made in the viable counting procedure is that each viable cell can grow and divide to yield one colony.
- Thus, colony numbers are a reflection of cell numbers.
- There are at least two ways of performing a plate count: the spread-plate method and the pour-plate method.

Spread-plate method

- In the spread-plate method, a volume (usually 0.1 ml or less) of an appropriately diluted culture is spread over the surface of an agar plate using

a sterile glass spreader.

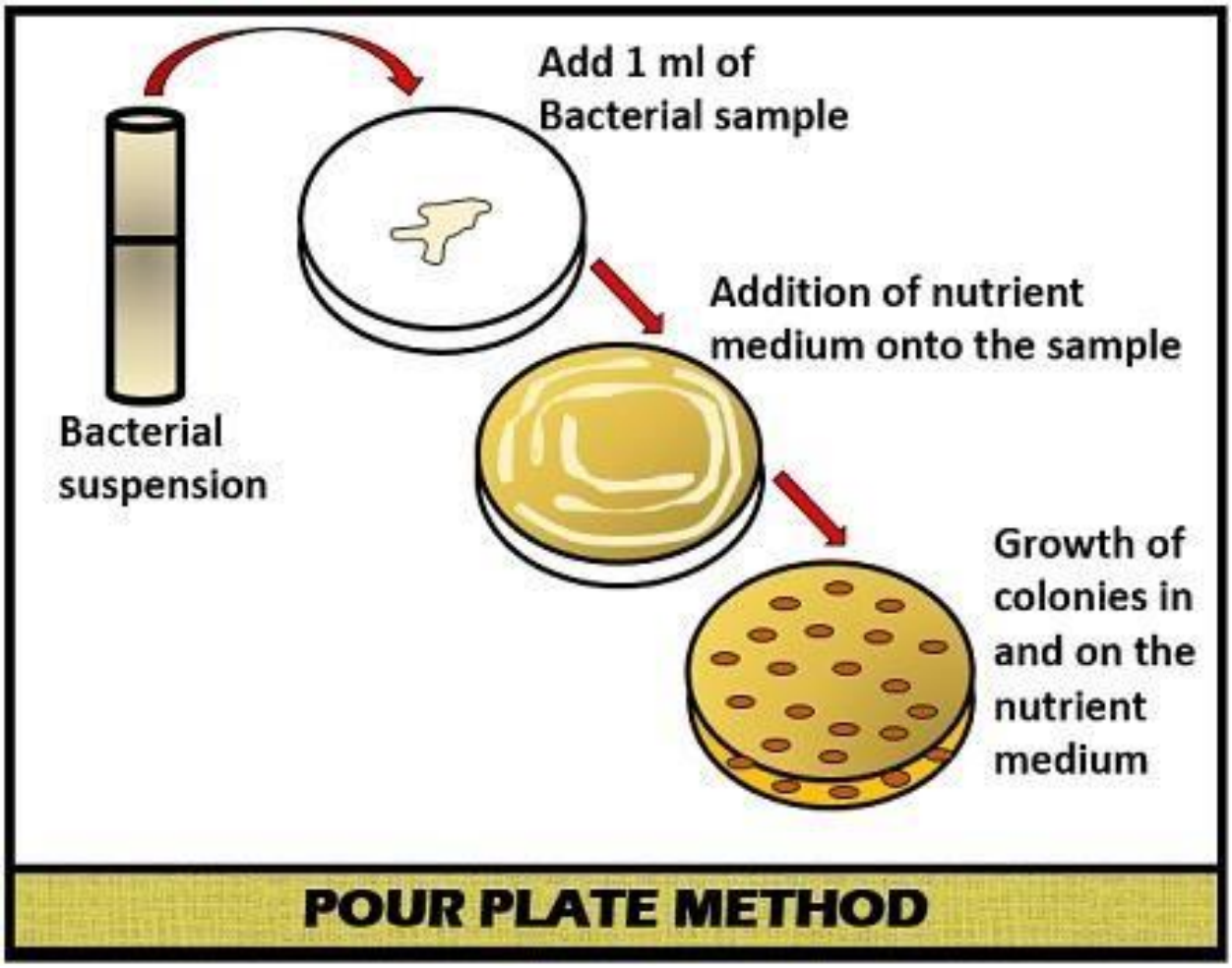
- The plate is then incubated until colonies appear, and the number of colonies is counted.
- The surface of the plate must not be too moist because the added liquid must soak in so the cells remain stationary.
- Volumes greater than about 0.1 ml are avoided in this method because the excess liquid does not soak in and may cause the colonies to coalesce as they form, making them difficult to count.



Pour-plate method

- In the pour-plate method, a known volume (usually 0.1–1.0 ml) of culture is pipetted into a sterile Petri plate.
- Melted agar medium, tempered to just about gelling temperature, is then added and mixed well by gently swirling the plate on the bench top.
- Because the sample is mixed with the molten agar medium, a larger volume can be used than with the spread plate.
- However, with this method the organism to be counted must be able to with stand brief exposure to the temperature of molten agar (45–50 Degree Celsius).

- Here, colonies form throughout the medium and not just on the agar surface as in the spread-plate method.
- The plate must therefore be examined closely to make sure all colonies are counted.
- If the pour-plate method is used to enumerate cells from a natural sample, another problem may arise; any debris in the sample must be distinguishable from actual bacterial colonies or the count will be erroneous.



Growth Factors

- Apart from macro- and micro-nutrients, some microorganisms require additional organic compounds in very small quantities) which are essential for their metabolism.
- These accessory compounds are growth Factor.
- Includes
  - Vitamins
  - Amino acids

- Purines & pyrimidines (for synthesis of nucleic acid)
- Sterols etc.

Growth factors and their functions

Table 5.3 Functions of Some Common Vitamins in Microorganisms

Vitamin	Functions	Examples of Microorganisms Requiring Vitamin[a]
Biotin	Carboxylation (CO_2fixation) One-carbon metabolism	*Leuconostoc mesenteroides* (B) *Saccharomyces cerevisiae* (F) *Ochromonas malhamensis* (A) *Acanthamoeba castellanii* (P)
Cyanocobalamin (B_{12})	Molecular rearrangements One-carbon metabolism—carries methyl groups	*Lactobacillus* spp. (B) *Euglena gracilis* (A) Diatoms and many other algae (A) *Acanthamoeba castellanii* (P)
Folic acid	One-carbon metabolism	*Enterococcus faecalis* (B) *Tetrahymena pyriformis* (P)
Lipoic acid	Transfer of acyl groups	*Lactobacillus casei* (B) *Tetrahymena*spp. (P)
Pantothenic acid	Precursor of coenzyme A—carries acyl groups (pyruvate oxidation, fatty acid metabolism)	*Proteus morganii* (B) *Hanseniaspora*spp. (F) *Parameciums*pp. (P)
Pyridoxine (B_6)	Amino acid metabolism (e.g., transamination)	*Lactobacillus* spp. (B) *Tetrahymena pyriformis* (P)
Niacin (nicotinic acid)	Precursor of NAD and NADP—carry electrons and hydrogen atoms	*Brucella abortus,Haemophilus influenzae* (B) *Blastocladia pringsheimii* (F) *Crithidia fasciculata* (P)
Riboflavin (B_2)	Precursor of FAD and FMN—carry electrons or hydrogen atoms	*Caulobacter vibrioides* (B) *Dictyosteliums*pp. (F) *Tetrahymena pyriformis* (P)
Thiamine (B_1)	Aldehyde group transfer (pyruvate decarboxylation, α-keto acid oxidation)	*Bacillus anthracis* (B) *Phycomyces blakesleeanus* (F) *Ochromonas malhamensis* (A) *Colpidium campylum* (P)

[a]The representative microorganisms are members of the following groups: bacteria (B), fungi (F), algae (A), and protozoa (P).

Factors affecting Microbial Growth

- Microbial growth is greatly affected by chemical and physical nature of their surroundings instead of variations in nutrient levels and particularly the nutrient limitation.
- For successful cultivation of microorganisms it is not only essential to supply proper and balanced nutrients but also it is necessary to maintain proper environmental conditions.
- As bacteria shows divers food habits, it also exhibits diverse response to the environmental conditions.
- Growth and death rates of microorganisms are greatly influenced by number of environmental factors such as water acidity, temperature, oxygen requirement and pH.

Water Acidity

- Water is one of the most essential requirements for life.
- Thus, its availability becomes most important factor for the growth of microorganisms.
- The availability of water depends on two factors - the water content of the surrounding environment and the concentration of solutes (salts, sugars etc.) dissolved in the water.
- In most cases, the cell cytoplasm possesses higher solute concentration in comparison to its environment.
- Thus, water always diffuses from a region of its higher concentration to a region of the lower concentration. This process is called **osmosis**.
- When a microbial cell is placed in hypertonic solution (or, solution of low water activity), it loses water and shrinkage of membrane takes place.
- This phenomenon is called **plasmolysis.**
- Microorganisms show variability in their ability to adapt the habitats of low water activity.
- Microorganisms like S. aureus can survive over a wide range of water activity and are called as **osmotolerant** (as water activity is inversely related to osmotic pressure).
- Most microorganisms grow well only near pure water activity (i.e. around 0.98-1).
- **Halophiles** require high concentration of salts

Temperature

- As temperature influences enzymic reactions it has an important role in promoting or preventing microbial growth.
- Four groups depending on their optimum growth temperature and the temperature range at which they will grow.
- **Thermophiles** have optimum growth *at* 55 °C and a growth range of 30 - 75 °C
- **Mesophiles** have optimum growth *at* 35 °C and a growth range of 10 - 45 °C
- **Psychrotrophs** have optimum growth *at* 20 - 30 °C and a growth range of 0 - 40 °C
- **Psychrophiles** have optimum growth *at* 15 °C and a growth range of -5 – 20 °C

Oxygen

- The atmosphere of earth contains about 20% (v/v) of oxygen. Microorganisms capable of growing in the presence of atmospheric oxygen are called **aerobes** whereas those that grow in the absence of atmospheric oxygen are called as **anaerobes**.
- The micro-organisms that are completely dependent on atmospheric oxygen for growth are called **obligate aerobes** whereas those that do not require oxygen for growth but grow well in its presence are called as **facultative anaerobes.**
- **Aerotolerants** (e.g. Enterococcus faecalis) ignore O_2 and can grow in its presence or absence.
- In contrast, obligate anaerobes (e.g., Bacteroids, Clostridium pastewianum, Furobacterium) do not tolerate the presence of oxygen at all and ultimately die.
- Few microorganisms (e.g., Campylobacter) require oxygen at very low level (2-10%) of concentration and are called as **microaerophiles.** And they are damaged by the normal atmospheric level of oxygen (20%).

pH

- The intracellular pH of any organism must be maintained above the pH limit that is critical for that organism.
- The control of intracellular pH is required in order to prevent the denaturation of intracellular proteins.
- Each organism has a specific requirement and pH tolerance range.
- Most micro-organisms grow best at neutral pH (7.0).
- Yeasts and moulds are typically tolerant of more acidic conditions than bacteria.

IDENTIFICATION AND OBSERVATION OF BACTERIAL CULTURE

Pure Culture Technique

- **Culture :** Act of cultivating microorganisms or the microorganisms that are cultivated
- **Mixed culture :** more than one microorganism
- **Pure culture :** containing a single species of organism.
- A pure culture is usually derived from a mixed culture (one containing many

species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample many times before inoculating the new medium.

Pure cultures are important in microbiology for the following reasons

- θ Once purified, the isolated species can then be cultivated with the knowledge that only the desired microorganism is being grown.
- θ A pure culture can be correctly identified for accurate studying and testing, and diagnosis in a clinical environment.
- θ Testing/experimenting with a pure culture ensures that the same results can be achieved regardless of how many time the test is repeated.
    - θ Pure culture spontaneous mutation rate is low
    - θ Pure culture clone is 99.999% identical

Culture Media

- Bacteria have to be grown or cultured to be identified.
- They have to be is isolated on culture media and obtained as pure culture for study.
- Culture media can be classified in many ways. They are:

♣ Simple media	♣ Selective media
♣ Complex media	♣ Indicator media
♣ Defined media	♣ Assay media
♣ Enriched media	♣ Transport media
♣ Enrichment media	♣ Sugar media
♣ Anaerobic media	♣ Solid medium
♣ Semisolid media	♣ Liquid media

Simple [Basal] media:

Basal media are basically simple media that supports most non-fastidious bacteria. Peptone-water, nutrient broth, and **nutrient agar (NA)** are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

Complex media:

These have added nutrients for special purposes for bringing out certain characteristics or provide special nutrients required for the growth of bacteria.

Sugar media:

Sugar media in microbiology indicates fermentation substance.

Eg: Monosaccharides, Disaccharides, Polysaccharides.

Defined media:

Synthetic or defined media are prepared from pure chemical substances and the exact composition of the medium is known. These are used for study of metabolic requirements.

Enriched medium (Added growth factors):

Addition of extra nutrients in the form of blood, serum, egg yolk, etc, to basal medium makes enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, etc are few of the enriched media. Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. **Chocolate agar** is also known as heated blood agar.

Enrichment media

These media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogens from a mixture of bacteria. While selective media are agar-based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by the addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to making a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH, or a combination of these.

Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation-reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine, or red hot iron filings can render a medium reduced. Before using the medium must be boiled in a water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Selective medium

Principle: Differential growth suppression

Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium is agar-based (solid)

medium so that individual colonies may be isolated.

Examples of selective media include:

Thayer Martin Agar

Mannitol Salt Agar

MacConkey's Agar

Differential / indicator medium: differential appearance:

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony color. Various approaches include incorporation of dyes, metabolic substrates, etc, so that those bacteria that utilize them appear as differently colored colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

Examples of differential media include:

Mannitol salts agar (mannitol fermentation = yellow)

Blood agar (various kinds of hemolysis i.e. α, β and γ hemolysis)

Transport media

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of a specimen, maintain the pathogen to commensal ratio, and inhibit the overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.

Solid medium

Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as **colonies** or in streaks). Solid medium is useful for **isolating bacteria** or for determining the colony characteristics of the isolate.

Semisolid medium

Semisolid medium is prepared with agar at concentrations of 0.5% or less. Semisolid medium has a soft custard-like consistency and is useful for the cultivation of **microaerophilic bacteria** or for the **determination of**

bacterial motility.

Liquid (Broth) medium

These media contain specific amounts of nutrients but don't have a trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of a large number of organisms, fermentation studies, and various other tests. e.g. **sugar fermentation tests, MR-VR broth.**

Microbe Identification

- Identification measures include:
  ♣ **Phenotypic method**
  ♣ **Microscopy method (staining techniques)**
  ♣ **growth on enrichment, selective, differential or characteristic media**
  ♣ **specimen biochemical test (rapid test methods)**
  ♣ **immunological techniques**
  ♣ **molecular (genotypic) methods**.
- After the microbe is identified for clinical samples it is used in **susceptibility tests** to find which method of control is most effective.

Specimen Collection

- Successful identification depends on how the specimen is **collected**, **handled** and **stored**.
- It is important that general **aseptic procedures** be used including **sterile sample containers** and **sampling methods** to prevent contamination of the specimen.

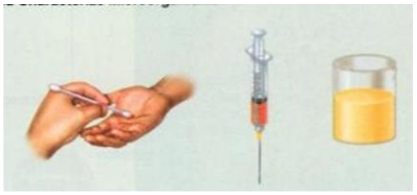
Phenotypic Methods of Identification

- Microbiologists use 5 basic techniques to **grow, examine and characterize microorganisms** in the lab.
- They are called the **5 'I's**: **inoculation, incubation, isolation, inspection and identification**.
- Inoculation: to **culture microorganisms** a tiny sample (inoculum) is introduced into medium (inoculation).
- Isolation involves the **separating one species from another.**

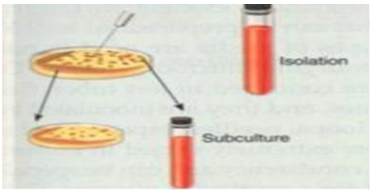
Phenotypic methods of Identification

- **Incubation**: once the media is inoculated it is incubated which means putting the culture in a controlled environment (incubation) to **allow for multiplication**.

- After incubation the organisms are **inspected** and i**dentified** phenotypically,immunologically or genetically.



Specimen collection

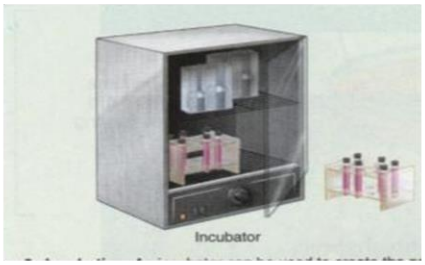


Isolation

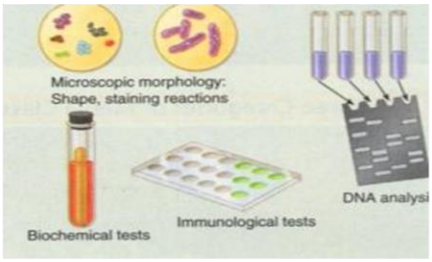
Inoculation

Inspection

5 "I" s



Incubation

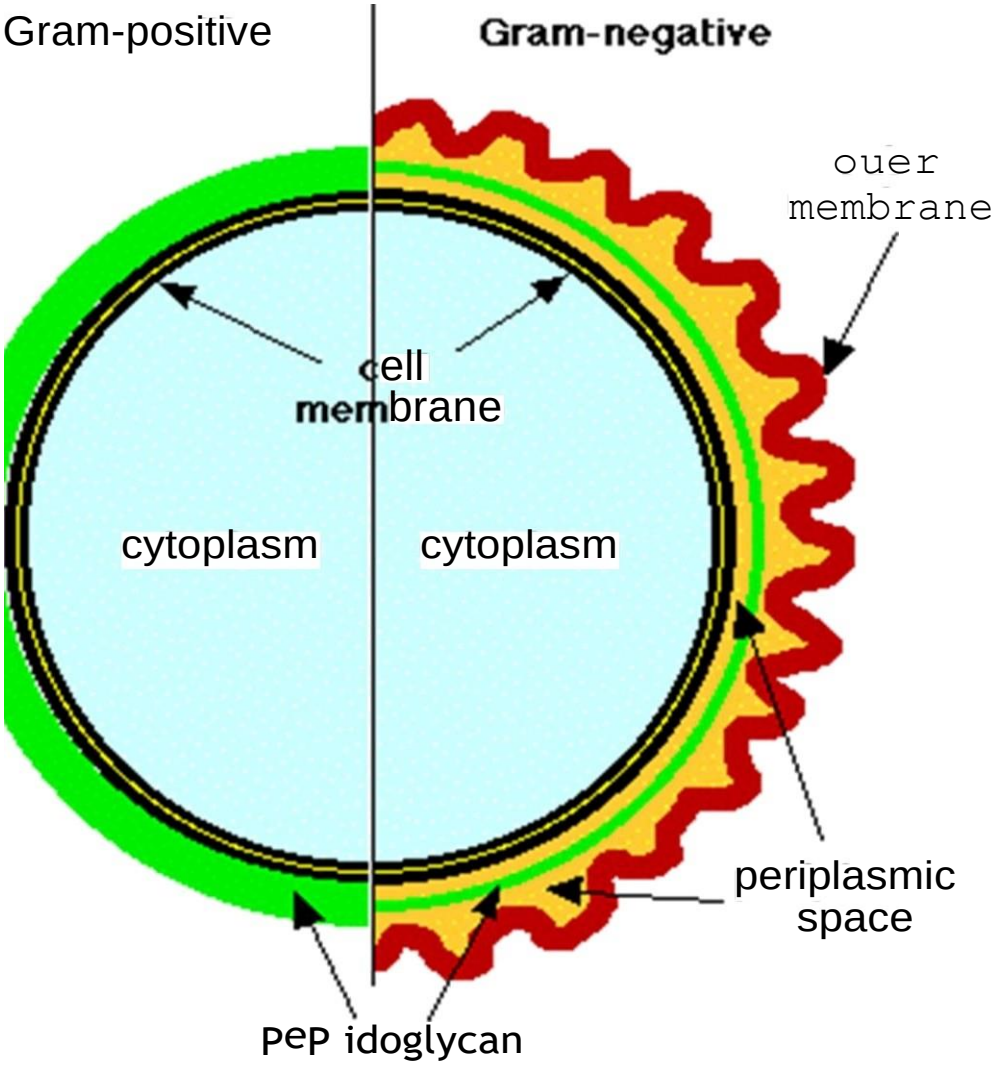


Identification

Staining techniques by Grams Method

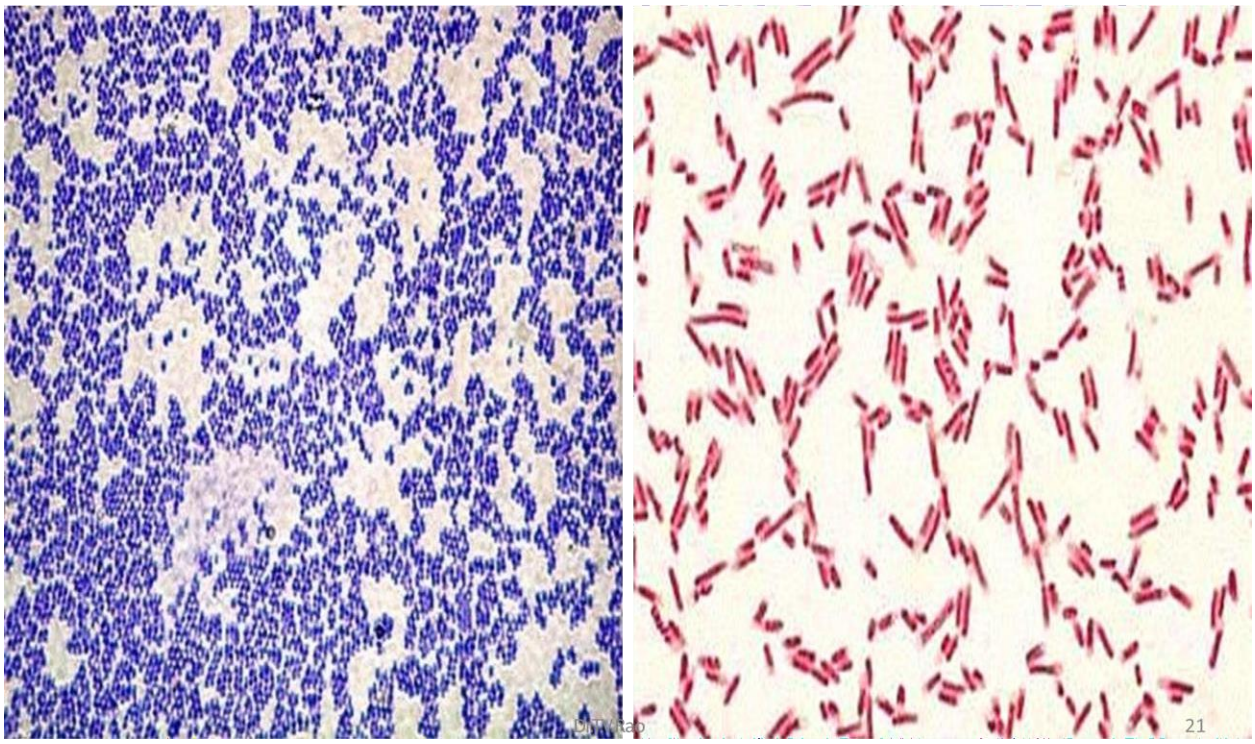
- Make slide by smear, drop, or cytocentrifuge
- Dry, then fix by heat (flame, 10 min at 60ºC) or fix by methanol (95% 1min)
- Gram stain
  - Crystal violet: primary stain
  - Gram's iodine: mordant/fixative
  - Acetone-ethanol: decolorizer
  - Safranin: counterstain

Bacteria differ as per structure


Gram-positive
Gram-negative
ouer membrane
cell membrane
cytoplasm
cytoplasm
periplasmic space
PeP idoglycan

Gram Staining Procedure

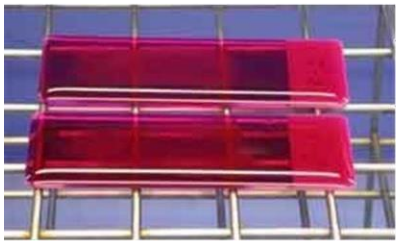
Gm+ve cocci & Gm-ve bacilli



Staining techniques for Mycobacterial spp

- Acid-fast stains
  - for staining of organisms with high degree of fatty (mycolic) acids—waxy
  - render the cells resistant to decolorization: “acid-fast”
  - *Mycobacterium* sp., *Nocardia* sp., *Cryptosporidium* sp. are acid-fast
  - Procedure
    - Ziehl-Neelsen: heat drives in primary stain (carbolfuchsin)
    - Kinyoun: higher conc. of phenol does not require heat
    - Decolorize with acid-alcohol
    - Counterstain with methylene blue or malachite green

1



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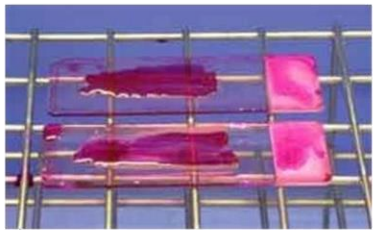

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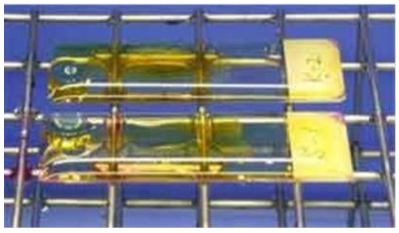

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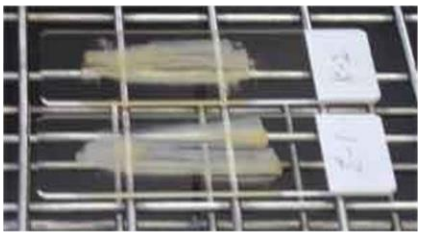

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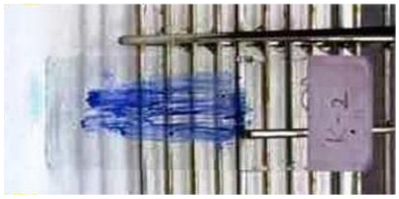


Ziehl-Neelsen stain

- **Media classifications and functions**

  –**Enrichment**

  - used to enhance growth of specific organisms

  –**Selective**

  - contains agents that inhibit the growth of all

  agents except that being sought (dyes, bile salts, alcohols, acids, antibiotics)

  –Differential

  - contains factor(s) that allow certain

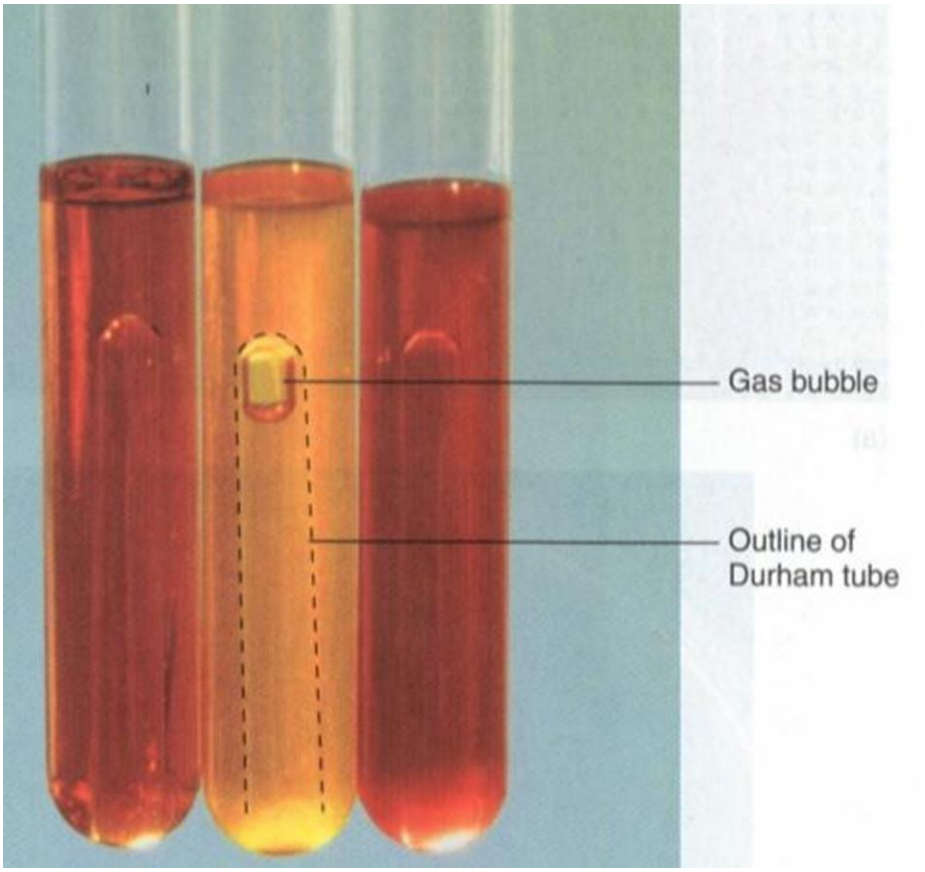
  organisms to exhibit different metabolic characteristics

  Biochemical Tests

  Carbohydrate Fermentation

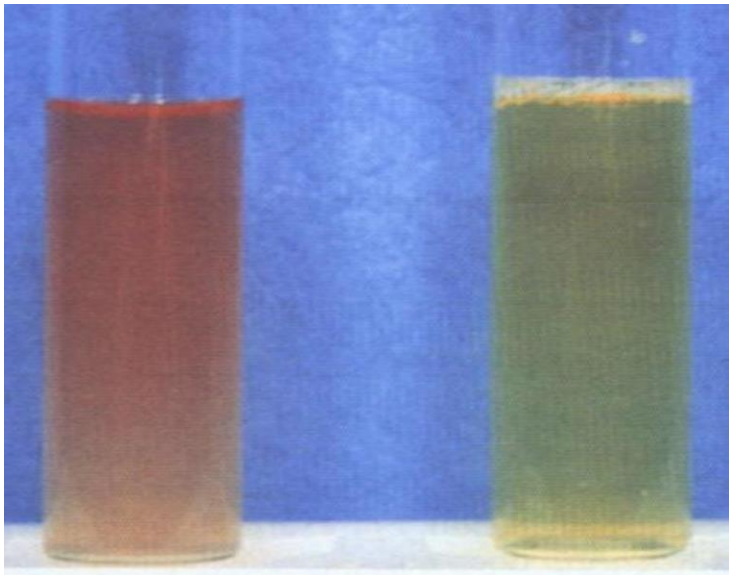
- This medium show f**ermentation** (**acid production**) and **gas formation**.
- The small **Durham tube** for collecting gas bubbles.
- Left- right:
  - Uninoculated negative control
  - Centre, positive for acid (yellow) and gas (open space).

❖ Growth but no gas or acid.



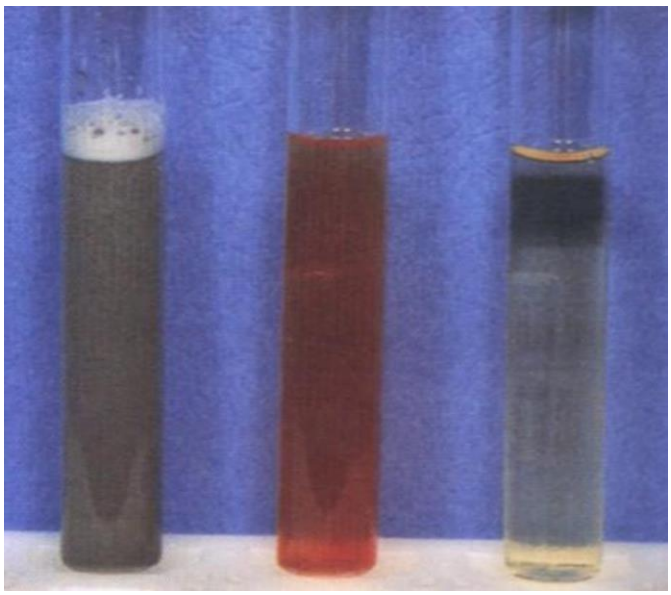
Methyl Red Test

- This is a qualitative test for **acid production**.
- The bacteria is grown in MR-VP broth.
- After addition of several drops of **methyl red solution** a bright red colour is positive and yellow- orange negative.



Nitrate Reduction

- After 24-48 hrs of incubation, **nitrate reagents** are added.
- Left to right:
- Gas formation (positive for nitrate reduction).
- positive for nitrate reduction to nitrite (red colour).
- Negative control



Immunological Methods

- Immunological methods involve the interaction of a microbial **antigen with an antibody** (produced by the host immune system).
- Testing for microbial antigen or the production of antibodies is **often easier** than test for the microbe itself.
- **Lab kits** based on this technique is available for the identification of many microorganisms.

Genotypic Methods

- Genotypic methods involve examining the genetic material of the organisms and has **revolutionized bacterial identification** and classification.
- Genotypic methods include PCR (RT-PCR)use of nucleic acid probes, RFLP and plasmid fingerprinting.
- Increasingly genotypic techniques are becoming the **sole means of identifying** many microorganisms because of its **speed and accuracy**.

UNIT-IV

GENETIC DISORDER:

A genetic disorder is any disorder caused by an abnormality in the genetic makeup of an individual. The genetic abnormality can range from minuscule to major from a discrete mutation in a single base in the DNA of a single gene to a gross chromosomal abnormality involving the addition or subtraction of an entire chromosome or set of chromosomes. Some people inherit genetic disorders from the parents, while acquired changes or mutations in a pre-existing gene or group of genes cause other genetic diseases. Genetic mutations can occur either randomly or due to some environmental exposure.

There are a number of different types of genetic disorders which include:

1. Single gene inheritance

2. Multifactorial inheritance

3. Chromosome abnormalities

4. Mitochondrial inheritance

SINGLE GENE INHERITANCE DISORDERS

Single gene inheritance is also called Mendelian or monogenetic inheritance. Changes or mutations that occur in the DNA sequence of a single gene cause this type of inheritance. There are thousands of known single-gene disorders. These disorders are known as monogenetic disorders (disorders of a single gene).

Single-gene disorders have different patterns of genetic inheritance, including

Autosomal dominant inheritance, in which only one copy of a defective gene (from either parent) is necessary to cause the condition;

Autosomal recessive inheritance, in which two copies of a defective gene (one from each parent) are necessary to cause the condition; and

X-linked inheritance,in which the defective gene is present on the female or X-chromosome. X-linked inheritance may be dominant or recessive.

Some examples of single-gene disorders include cystic fibrosis, alpha- and beta-thalassemias, sickle cell anemia (sickle cell disease),Marfan syndrome, fragile X syndrome, Huntington's disease, and hemochromatosis.

COMMON MULTIFACTORIAL GENETIC INHERITANCE DISORDERS

Multifactorial inheritance is also called complex or polygenic inheritance. Multifactorial inheritance disorders are caused by a combination of environmental factors and mutations in multiple genes. For example, different genes that influence breast cancer susceptibility have been found on chromosomes 6, 11, 13, 14, 15, 17, and 22. Some common chronic diseases

are multifactorial disorders.Examples of multifactorial inheritance include heart disease, high blood pressure, Alzheimer's disease, arthritis, diabetes, cancer, and obesity.

Multifactorial inheritance also is associated with heritable traits such as fingerprint patterns, height, eye color, and skin color.

CHROMOSOMAL ABNORMALITIES

Chromosomes, distinct structures made up of DNA and protein, are located in the nucleus of each cell. Because chromosomes are the carriers of the genetic material, abnormalities in chromosome number or structure can result in disease. Chromosomal abnormalities typically occur due to a problem with cell division.

For example, Down syndrome (sometimes referred to as "Down's syndrome") or trisomy 21 is a common genetic disorder that occurs when a person has three copies of chromosome 21. There are many other chromosomal abnormalities including:

1. Turner syndrome (45,X0),
2. Klinefelter syndrome (47, XXY), and
3. Cri du chat syndrome, or the "cry of the cat" syndrome (46, XX or XY, 5p-).

Diseases may also occur because of chromosomal translocation in which portions of two chromosomes are exchanged.

MITOCHONDRIAL GENETIC INHERITANCE DISORDERS

This type of genetic disorder is caused by mutations in the non-nuclear DNA of mitochondria. Mitochondria are small round or rod-like organelles that are involved in cellular respiration and found in the cytoplasm of plant and animal cells. Each mitochondrion may contain 5 to 10 circular pieces of DNA. Since egg cells, but not sperm cells, keep their mitochondria during fertilization, mitochondrial DNA is always inherited from the female parent.

Examples of mitochondrial disease include

1. Leber's hereditary optic atrophy (LHON), an eye disease;

2. Myoclonic epilepsy with ragged red fibers (MERRF); and

3. Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), a rare form of dementia.

INFECTIONS:

An infection occurs when another organism enters your body and causes disease. The organisms that cause infections are very diverse and can include things like viruses, bacteria, fungi, and parasites.

You can acquire an infection in many different ways, such as directly from a person with an infection, via contaminated food or water, and even through the bite of an insect.

Types of infections

Viral infections

Viruses are very tiny infectious organisms. They're even smaller than bacteria. On the most basic level, a virus is composed of a piece of genetic material that's surrounded by a protein shell. Some viruses may have an additional envelope or other features on their surface.

Viruses are parasitic and require a host cell in which to carry out their life cycle. Once the virus has entered the host cell, it's able to use cellular components to reproduce. New viruses are released from the host cell, a process that'll sometimes cause the host cell to die.

Examples of viral infections include:

Influenza (the flu) ,Common cold,Measles, Rubella, Chickenpox, Norovirus, Polio, Infectious mononucleosis (mono) , Herpes simplex virus (HSV), Human papillomavirus (HPV), Human immunodeficiency virus (HIV), Viral hepatitis, which can include hepatitis A, B, C, D, and E, Viral meningitis, West Nile Virus, Rabies, Ebola.

Possible treatments

- Most of the time, the treatment of viral infections centers on relieving symptoms until your immune system clears the infection.In some cases, antiviral drugs may be available to help treat a viral infection. Some examples of viral infections for which antivirals are available include HIV, herpes, and hepatitis C.
- Some viruses stay with you for life once you've been infected. They can lie dormant within your body and may reactivate. Some examples include herpes simplex virus (HSV) and varicella-zoster virus (VZV).

Bacterial infections

Bacteria are single-celled microorganisms. They're very diverse, coming in a variety of different shapes and sizes. Bacteria can be found in all sorts of environments, including soil, bodies of water, and in or on our bodies. Some can survive extreme temperature. Although there are a great many bacteria in and on our bodies, these bacteria often don't cause disease. In fact, the bacteria in our digestive tract can help us digest our food. However, sometimes bacteria can enter our bodies and cause an infection.

Some examples of bacterial infections include:

- strep throat
- bacterial urinary tract infections (UTIs), often caused by coliform bacteria
- bacterial food poisoning, often caused by E. coli, Salmonella, or Shigella
- bacterial cellulitis, such as due to Staphylococcus aureus (MRSA)
- bacterial vaginosis

- gonorrhea
- chlamydia
- syphilis
- Clostridium difficile(C. diff)
- tuberculosis
- whooping cough
- pneumococcal pneumonia
- bacterial meningitis
- Lyme disease
- cholera
- botulism
- tetanus
- anthrax

Possible treatments

- Bacterial infections are most often treated with antibiotics. Antibiotics are medications that affect bacterial growth. They can either impede bacteria from multiplying or kill them outright.

- There are different classes of antibiotics. The one you're prescribed will depend on what type of bacterium is causing your infection. Additionally, misuse of antibiotics has caused many bacteria to develop resistance to them.

Fungal infections

Fungi are another diverse group of organisms that can include things like yeasts and molds. They can be found throughout the environment, including in the soil, indoors in moist areas like bathrooms, and on or in our bodies. Sometimes fungi are so small that you can't see them with the naked eye. Other times, you're able to see them, such as when you notice mold on your bathroom tile. Not all fungi can make you ill.

But some examples of fungal infections include:

- vaginal yeast infections
- ringworm
- athlete's foot
- thrush
- aspergillosis

- histoplasmosis
- Cryptococcus infection
- fungal meningitis

Possible treatments

- Fungal infections can be treated with antifungal medications. The type of medication that you're prescribed will depend on the type of fungal infection you have.

- For example, a topical antifungal cream may be prescribed for conditions like ringworm or athlete's foot. Oral antifungal medications are also available. More severe fungal infections may require intravenous (IV) antifungal medication.

Parasitic infections

Parasites live on or in a host organism and get food or other nutrients at the host's expense. There are three types of parasites that can cause illness in humans:

Protozoa: small, one-celled organisms

Helminths: larger, worm-like organisms

Ectoparasites: organisms such as fleas, ticks, and lice

Some examples of infections that are caused by parasites include:

Malaria, Toxoplasmosis, Trichomoniasis, Giardiasis, Tapeworm infection, Roundworm infection, Pubic and head lice, Scabies, Leishmaniasis, River blindness.

Possible treatments

As with bacterial and fungal infections, there are specific drugs available to treat a parasitic infection. The type of antiparasitic medication that you'll need to take will depend on the type of parasite that's causing your infection.

Source of infection:

An object is designed as the source of infection, is one in which the agent of infection lives and propagates. Can be man or animal from which the infectious agent is secreted into the outer environment and from there to individuals. In certain circumstances, the outer milieu can be the source of infection where the agent lives as a saprophyte (lives on dead matter)
e.g. mycoses and legionella.

Symptoms of infection

The symptoms of an infection can vary depending on the type of infection that you have. Some symptoms that can indicate you may have an infection include:

- Fever or chills
- Body aches and pains
- Feeling tired or fatigued
- Coughing or sneezing
- Digestive upset, such as nausea, vomiting, or diarrhea
  Some examples of infections that don't always cause symptoms include HPV, gonorrhea, and chlamydia.

Causes of infection transmission

Direct contact

Some, but not all, infections can spread when you come directly into contact with a person who has an infection, whether through touching, kissing, or having sex.

Direct contact with the bodily fluids of a person who has an infection can also spread infections in some instances. This can include things like:

- blood
- nasal secretions
- saliva
- semen
- vaginal secretions

Lastly, some infections can be spread directly from an infected mother to her child either through the placenta or during childbirth.

Indirect contact

Some infectious organisms can be found throughout your environment. You can come into contact with these things and then spread the infection to yourself.

A common example of this is when someone with the flu coughs or sneezes. Influenza virus can then be present in the air or on objects such as door and faucet handles. If you touch a contaminated object and then touch your face, mouth, or nose, you may become infected.

Through contaminated food or water

In some cases, food or water may be contaminated with infectious organisms. You can get these infections by consuming things like:

- foods prepped or prepared in unsanitary conditions
- raw or undercooked foods, such as produce, meats, or seafood
- improperly canned foods
- unpasteurized milks or juices
- foods that have been improperly stored or refrigerated

From an infected animal

Some infections are spread to people from an infected animal. One example is the rabies virus, which you can get if an infected animal bites you.

Another example is toxoplasmosis. You can come down with this parasitic disease from changing an infected cat's litter box.

From a bug bite

There are many different types of biting bugs, including ticks, mosquitoes, and lice. In some cases, you can get an infection if a bug carrying around an infectious microorganism bites you. Some examples include malaria, Lyme disease, and West Nile Virus.

ALL INFECTIONS ARE DIFFERENT

Not all infections are spread in the same way. While one infection may be transmitted via infected blood, another may be transmitted by the bite of an insect. It's always important to consider the specific infection when talking about transmission.

DIAGNOSIS

- blood
- urine
- stool
- nasal or throat
- sputum
- cerebrospinal fluid (CSF)

Your doctor may also use imaging tests, such as an X-ray, CT scan, or MRI scan. In some cases, they may also want to take a biopsy of the affected tissue to examine it.

Preventing infection

There are many actions that you can take to prevent the spread of infections. Be sure to follow the tips below:

- **Practice good hand hygiene.** Wash your hands often, especially before eating or handling food, after using the toilet, and before touching your face or mouth.
- **Get vaccinated.** Many infections can be prevented through vaccines. Examples include, but aren't limited to: measles, whooping cough, and hepatitis B.
- **Avoid sharing personal items.** These include drinking glasses, toothbrushes, and razor blades.
- **Practice safe sex.**
- **Cover cuts or scrapes.** This can lessen the chances that they'll become infected. Don't pick or scratch them.
- **Use insect repellents or sprays.** These products can help you avoid being bitten by mosquitoes or ticks.
- **Be careful with food.** Always prepare food in sanitary conditions, and make sure it's heated to the proper temperature before eating.
- **Avoid wild animals**. Make sure to have any animal bites examined by a doctor.
- **Know before you go.** If you're traveling, be aware of any infections common to the area where you'll be staying. Some of them may even have vaccines available.
- **Cover your mouth when you cough.** If you're sick, be sure to dispose of all used tissues properly. If you don't have a tissue, cough into the crook of your elbow instead of your hand.
- **Stay home if you're sick.** This can prevent you from spreading an infection. Make sure to ask your doctor when you can return to work or school.

IMMUNITY:

Introduction

The immune system is a collection of organs, tissues, cells, and enzymes all united under one goal: protect the body. It detects and responds to a wide variety of pathogens, from viruses to parasitic worms, as well as cancer cells and objects such as wood splinters, distinguishing them from the organism's own healthy tissue. Immune system is made of special cells one of the important cell involved are WBC (leukocytes), which come in two basic types that combine to seek out and destroy disease causing organisms or substances. The two basic types of leukocytes are phagocytes, cells that chew up invading organisms. Lymphocytes, cells that allow the body to remember and recognize previous invaders and help the body destroy them. Dysfunction of the immune system can cause autoimmune diseases, inflammatory diseases and cancer. Immunology is a branch of biology that covers the study of immune systems in all organisms.

The term ‘immunity’ (Latin word ‘ immunitas ’, means freedom from disease) is defined as resistance offered by the host against microorganism(s) or any foreign substance(s).when the immune system does not function efficiently in an individual it leads to infection cause disease the overall ability of the body to fight against the disease causing pathogen is called immunity. It is also known as disease resistance .Lack of immunity is called Susceptibility. IMMUNE RESPONSE is how your body recognizes and defends itself against bacteria, viruses and substances that appear foreign and harmful. Any substance capable of eliciting an adaptive immune response is referred to as an antigen (antibody generator).

Immunity involves both specific and non – specific components. The nonspecific components act as barriers or eliminators of a wide range of pathogens irrespective of their antigenic make-up. Specific components of the immune system adapt themselves to each new disease encountered and can generate pathogen-specific immunity. Immunity types are innate immunity and acquired immunity.

Innate immunity

It is also known as non specific immunity or Natural immunity. Innate immunity is a inborn resistance against infections that the individual possess right from the birth.

Innate immunity is the first line of defence in our immune system. It is non-specific and responds to the common features on all pathogens. It is absolutely essential for survival as it creates barriers to microbial invasion and provides critical biochemical and cellular first responders to infections. Innate immunity refers to non specific productive mechanisms that come into play immediately or within hours of an antigen’s appearance in the body.

Innate immunity has four barriers:

1.Anatomical barriers
2.Physiological barriers
3.Phagocytic barriers
4.Inflammatory barriers

Anatomical barriers:

The Epithelial surfaces form a physical barrier that is impermeable to most infectious agents, acting as the first line of product against invading organisms. The anatomical barriers represented by the skin and mucous membranes which prevent pathogen entry, and the cellular barrier includes phagocytic cells like the macrophage. In skin it prevents the entry of microbes. Mucous membranes are composed of an epithelial layer that secretes mucus, and a connective tissue layer. The mucus is a physical barrier that traps microbes.

Physiological barrier:

The Physiological barriers that contribute to the innate immunity are the body temperature, pH and various soluble secretory products of the mucosa. Normal body inhibit the growth of pathogen. Acidity of gastric secretion (HCL) kills most ingested microbes. Lysosome acts as a antibacterial agent and cleaves the bacterial cell wall.

Phagocytic barrier:

Another important innate protective mechanism is the ingestion of extracellular particulate material by phagocytosis. Phagocytosis is a phenomenon in which there is uptake of material by a cell from its environment. In phagocytosis, a cell’s plasma membrane expands around the particulate material to form large vesicles called phagosomes. Most phagocytosis is

conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages.

Inflammatory barrier:

The Inflammation is an important productive mechanism of host to prevent infection. It is induced in response to tissue damage caused by microorganism, toxins or by mechanical means. The inflammation may be acute; for example, In response to tissue damage or chromic. It is induced in response to tissue damage caused by microorganism toxins or by mechanical means. inflammatory barriers includes histamine, prostaglandin, vasodilation, influx of WBCs.

Acquired Immunity:

The adaptive immune system, also referred as the acquired immune system, is a subsystem of the immune system that is composed of specialized, systemic cells and processes that eliminates pathogens by preventing their growth. The acquired immunity also known as Adaptive immunity or Specific immunity. Immunity that an individual acquires after birth . It has two major group of cells they are lymphocytes and antigen presenting cell. Lymphocytes of the immune system is T-cells mature thymus and B cell mature bone marrow. Antigen presenting cell here the cell that presents captured antigen to immature T-cells. Acquired immunity has two subdivision namely, cell mediated immunity, humoral immunity.

Cell mediated immunity:

The cell-mediated immunity is an immune response that does not involve antibodies. Rather, cell-mediated immunity is the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Cell mediated immune response is controlled by activating T cell. When pathogens are destroyed by the cells without producing antibodies are called cell mediated immunity. It is most effective in removing virus-infected cells, but also participates in defending against fungi, protozoans, cancers, and intracellular bacteria.

Humoral immunity:

The humoral immune response which is controlled by activating b cell ,antibodies .Humoral immunity or HUMOURAL immunity is the aspect of immunity that is mediated by macromolecules found in extracellular fluids such as secreted antibodies, complement proteins, and certain antimicrobial peptides. Humoral immunity is named so because it involves substances found in the body fluids. It contrasts with cell-mediated immunity. Humoral immunity is also referred to as antibody-mediated immunity. Antibodies are the agents of humoral immunity. Antibodies occur in the blood, in gastric and mucus secretions, and in breast milk. Antibodies in these bodily fluids can bind pathogens and mark them for destruction by phagocytes before they are able to infect cells.

MUTATION

- **Mutation**, an alteration in the genetic material (the genome) of a cell of a living organism or of a virus that is more or less permanent and that can be transmitted to the cell’s or the virus’s descendants.

- Mutation in the DNA of a body cell of a multicellular organism (somatic mutation) may be transmitted to descendant cells by DNA replication and hence result in a sector or patch of cells having abnormal function, an example being cancer.
- Mutations in egg or sperm cells (germinal mutations) may result in an individual offspring all of whose cells carry the mutation, which often confers some serious malfunction, as in the case of a human genetic disease such as cystic fibrosis.
- Mutations result either from accidents during the normal chemical transactions of DNA, often during replication, or from exposure to high-energy electromagnetic radiation (e.g., ultraviolet light or X-rays) or particle radiation or to highly reactive chemicals in the environment
- Because mutations are random changes, they are expected to be mostly deleterious, but some may be beneficial in certain environments.
- In general, mutation is the main source of genetic variation, which is the raw material for evolution by natural selection.

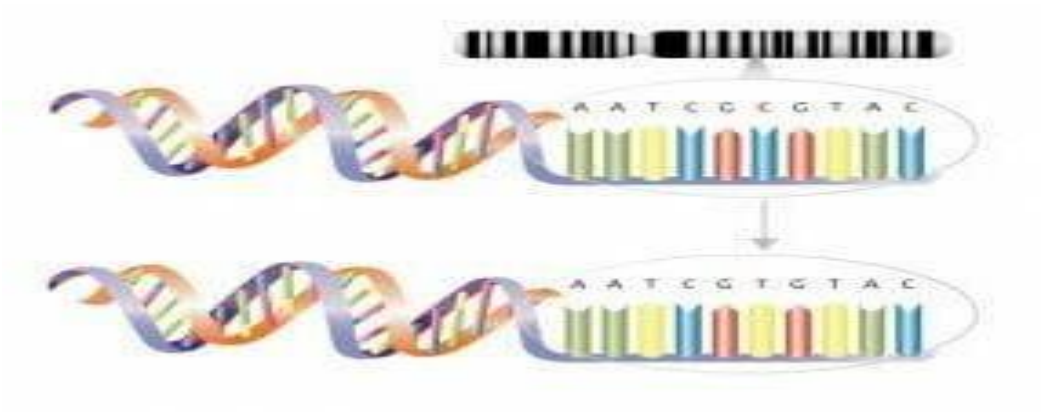
TYPES OF MUTATION :

There are four types of mutation , they are

- Substitution mutation
- Insertion mutation
- Deletion mutation
- Inversion mutation

SUBSTITUTION MUTATION:

- A substitution mutation is a type of replication error during DNA replication which places the wrong nucleotide or sequence of nucleotides in the wrong position.
- A type of substitution mutation, a point mutation, occurs which a single nucleotide is substituted.



Example: sickle cell anemia

- There are two types of substitution mutation, they are
  - Transition
  - Transversion

TRANSITION:

- **Transitions** are interchanges of two-ring purines (A G) or of one-ring pyrimidine (C T): they therefore involve bases of similar shape.
- There are 8 possible types
- A particular base can undergo a single type of transition
- Example: a purine such as adenine may be replaced by the purine guanine.

TRANSVERSION:

- Transversion are interchanges of purine for pyrimidine bases, which therefore involve exchange of one-ring and two-ring structures.
- There are 4 possible types
- A base can undergo two types of transversions
- Example: cytosine, a pyrimidine, is replaced by adenine, a purine

INSERTION:

- In genetics, an **insertion** (also called an **insertion mutation**) is the addition of one or more nucleotide base pairs into a DNA sequence.
- This can often happen in microsatellite regions due to the DNA polymerase slipping.
- An extra nucleotide is inserted into a DNA sequence
- Example: polydactyly

DELETION MUTATION:

- A **deletion mutation** is a mistake in the DNA replication process which removes nucleotides from the genome.
- A **deletion mutation** can remove a single nucleotide, or entire sequences of nucleotides.
- Example: cystic fibrosis, Turner syndrome, and Williams syndrome

INVERSION MUTATION:

- **Inversion** is a type of chromosome rearrangement where a segment of a chromosome gets flipped around.
- These mutations aren't totally awful because there is still the same amount of genetic material.
- Chromosomal mutations all happen during meiosis.
- Example: optiz-kaveggia

SIGNIFICANCE OF MUTATIONS:

- Some mutations may be “neutral”, they won’t have an effect on the protein being built.
- some mutations can be very harmful and cause genetic disorders.
- Some mutations lead to genetic variation in a species.
- Evolution is based on mutation
- Cancer is also the outcome of mutation

CAUSES OF MUTATION:

Genetic Causes:

- ❖ It is due to heridity factors
- ❖ It means mutation from ancestors. It is known as “Germ line mutation”.
- ❖ They are transferred from one generation to another generation by gametes/sex cells.

Environmental causes:

- ❖ Harsh chemicals
- ❖ Uv rays
- ❖ Others external factors damages DNA
- ❖ There are not transferred to next generation because they do not impact sex cells
- ❖ Known as “stomatic mutation.

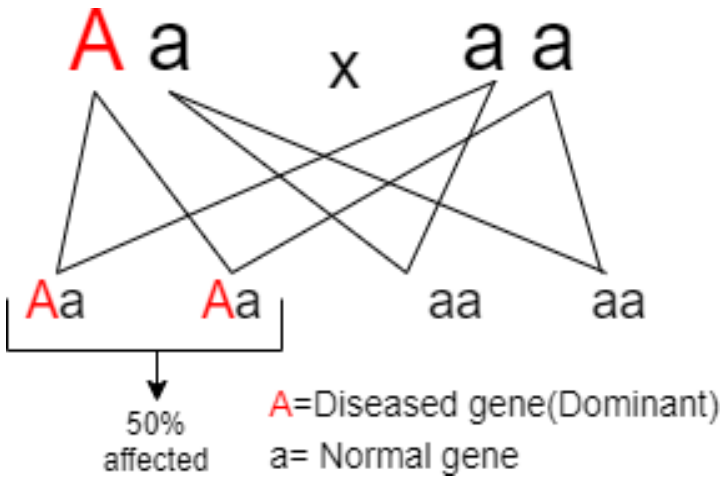
AUTOSOMAL AND X LINED DISORDER:

- **Genetic** condition can A occur when the child inherits one copy of a mutated (changed) gene from one parent.
- Types of Autosomal inheritance:

  1.Autosomal Dominant Inheritance

  2.Autosomal Recessive Inheritance

AUTOSOMAL DOMINANTINHERITANCE:



- Mating is Heterozygous.
- Both sexes are equally affected.
- 50% chance of children to get affected and 50% of children to be normal.
- One of the parent are always affected.
- Unaffected individual does not carry the gene and does not transmit the trait.

Huntington's disease:

- It causes progressive degeneration of neurons in the brain.
- Signs and symptoms usually develop between ages 35 to 44 years.
- It include uncontrolled movements, loss of intellectual abilities, and various emotional and psychiatric problems.
- It is caused by a change (mutation) in the HTT gene. This gene gives instructions for making a protein called huntingtin. The exact function of this protein is unclear, but it appears to be important to nerve cells (neurons) in the brain.

Neurofibromatosis

- It causes tumors to form on nerve tissue(brain, spinal cord and nerves).
- The tumors are usually noncancerous (benign), but sometimes can become cancerous (malignant).
- Symptoms are hearing loss, heart and blood vessel (cardiovascular) problems, loss of vision, and severe pain.
- **NF1 :** The NF1 gene is located on chromosome 17. This gene produces a protein called neurofibromin that helps regulate cell growth. The mutated gene causes a loss of neurofibromin, which allows cells to grow uncontrolled.
- **NF2 :**The NF2 gene is located on chromosome 22, and produces a protein called merlin, which suppresses tumors. The mutated gene causes a loss of merlin, leading to uncontrolled cell growth.

Marfan syndrome

- Marfan syndrome is an inherited disorder that affects connective tissue of the heart and blood vessels, eyes, lungs and skeleton.
- **Marfan syndrome** is **caused** by a mutation in Fibrillin gene on chromosome 15, an essential part of connective tissue that helps make it strong and elastic.
- People with Marfan syndrome are usually tall and thin with disproportionately long arms, legs, fingers and toes.
- Severe condition: If aorta enlarged- the blood vessel that carries blood from your heart to the rest of your body is affected, the condition can become life-threatening.

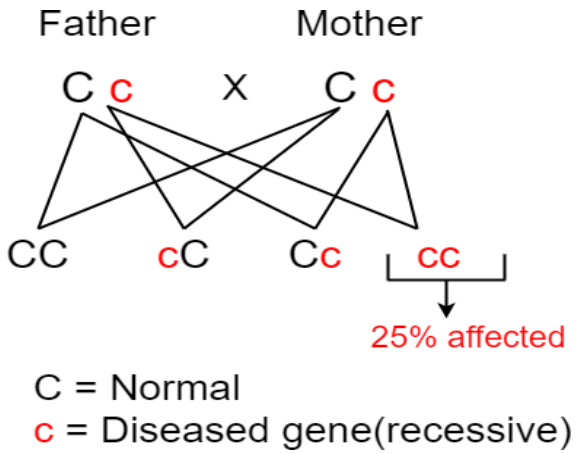
Achondroplasia

- Achondroplasia is a bone growth disorder that causes disproportionate dwarfism.
- During early foetal development, much of your skeleton is made up of cartilage. Normally, most cartilage eventually converts to bone. However, if you have

achondroplasia, a lot of the cartilage doesn't convert to bone. This is caused by mutations in the FGFR3 gene.

- The FGFR3 gene located on chromosome 4 instructs your body to make a protein necessary for bone growth and maintenance. Mutations in the FGFR3 gene affect the protein.
- Main symptoms isa short stature that's significantly below average for age.

Autosomal recessive inheritance:



- Mating is homozygous.
- Both sexes are equally affected.
- 25% chance of children getting affected.
- Both the parents are unaffected but act as carriers.
- When both the copies of genes are abnormal, then the person is affected by disease.
- Consanguineous marriage increases risk.

Cystic fibrosis

- Cystic fibrosis (CF) involves the production of mucus that is much thicker and more sticky than usual.
- It mainly affects the lungs and digestive system.
- The defective CFTR gene(Cystic Fibrosis Transmembrane Regulator) is normally used for producing a protein that controls the flow of salt and water outside of the organs, including the lungs and the pancreas.
- There is no cure, but good nutrition and taking steps to thin mucus and improve mucus expectoration can help.

Sickle cell anemia

- Sickle cell anemia is an inherited red blood cell disorder in which there aren't enough healthy red blood cells to carry oxygen throughout your body.
- Sickle cell anemia is caused by a mutation in the gene that tells your body to make the iron-rich component(hemoglobin).
- The abnormal hemoglobin causes red blood cells to become rigid, sticky and misshapen,which can slow or block blood flow and oxygen to parts of the body.
- Sickle cell anemia can lead to a host of complications like **Stroke, Acute chest syndrome, Pulmonary hypertension, Organ damage, Blindness, Leg ulcers.**

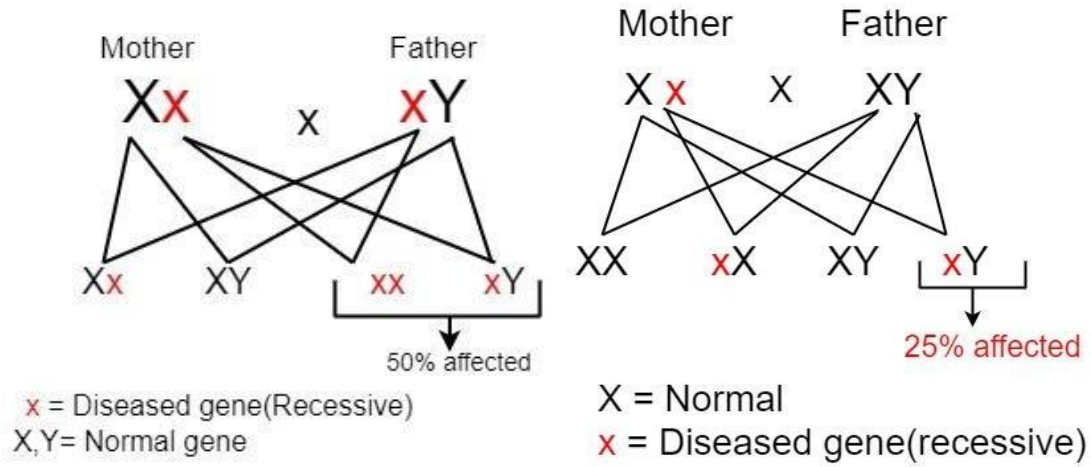
Tay-Sachs disease

- It is caused by the absence of an enzyme that helps break down fatty substances.
- This fatty substances called gangliosides affect the function of the nerve cells. As the disease progresses, the child loses muscle control. Eventually, this leads to blindness, paralysis and death.
- Defects in a gene called HEXA cause Tay-Sachs.
- This HEXA gene located in 15^{th} chromosomes which gives instructions to your body to make an enzyme (a type of protein) known as Hex-A. This enzyme prevents the buildup of a fatty substance in brain and spinal cord.

X-LINKED INHERITANCE:

- It is also known as Sex-Linked inheritance.
- Male - XY and Female – XX.
- This type of disorders transmit only through sex chromosomes.
- Genes on the Y chromosome shows a holandric pattern of inheritance and they are uncommon.
- So under this category all are inherited through the X chromosomes.

X-LINKED RECESSIVE INHERITANCE



- Males are always affected
- Females are carriers.
- No male to male transmission because male transmit only Y chromosome.
- From this,

  Father – Daughter

  Mother – Son

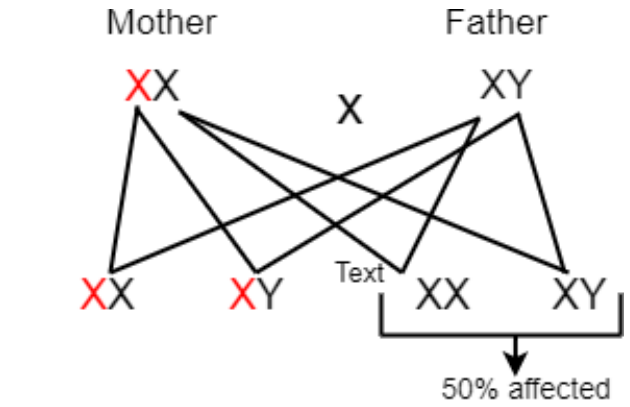
Hemophilia

- Hemophilia is one of a group of inherited bleeding disorders that cause abnormal or exaggerated bleeding and poor blood clotting.
- Hemophilia A is caused by a deficiency of clotting Factor VIII, while hemophilia B(also called Christmas disease) results from a deficiency of clotting Factor IX.

Duchenne muscular dystrophy

- **Duchenne muscular dystrophy** (DMD) that make muscles weaker and less flexibility.
- Duchenne muscular dystrophy is a form of muscular dystrophy. It worsens quickly.
- Early symptoms include delayed ability to sit, stand, walk and speak. Most children with DMD use a wheelchair by their early teens.
- DMD gene is mutated which is mainly used for producing protein called dystropin(used for muscle strength and protects them from injuries).

Autosomal Dominant Inheritance



X = Diseased gene(Dominant)
X,Y= Normal gene

- Practically, the dominant disorders are very rare.
- Mostly affected in females.
- Men have generally milder expression of disease.
- Affected male transmit the trait to all his daughters not to his son.
- The well known example of x-linked dominant disorder is Vitamin-D resistant rickets.

vitamin D-resistant rickets

- **Hypophosphatemic rickets** is a disorder in which the bones become painfully soft and bend easily, due to low levels of phosphate in the blood.
- It cause bowing of the legs, poor bone growth; and short stature. In some affected babies, the space between the skull bones closes too soon (craniosynostosis).
- This caused due to changes in the PHEX gene.
- Treatment involves taking phosphate and calcitriol in order to raise phosphate levels in the blood and promote normal bone formation.

MENDELIAN DISORDERS:

DEFINITION

- Mendelian disorder is a type of genetic disorder primarily resulting due to alterations in one gene or as a result of abnormalities in the genome.
- These genetic disorders are quite rare.
- It may affect one person in every thousand or a million.
- These are one gene disorders.

TYPES OF MENDELIAN DISORDERS:

- According to Mendel's' laws of inheritance, the different types of Mendelian disorders include:

1. Autosomal dominant.
2. Autosomal recessive.
3. Sex-linked dominant.
4. Sex-linked recessive.
5. Mitochondrial.

- **AUTOSOMAL DOMINANT DISORDER:**

Autosomal dominance is a pattern of inheritance characteristic of some genetic diseases.

A single abnormal gene on one of the first 22 non-sex (autosomal) chromosomes from either parent can cause an autosomal disorder.

"**Dominant**" means that a single copy of the disease-associated mutation is enough to cause the disease.

A parent with an autosomal dominant condition has a 50% chance of having a child with the condition.

-More than Half of mendelian phenotypes are Autosomal Dominant

Examples of autosomal dominant diseases include

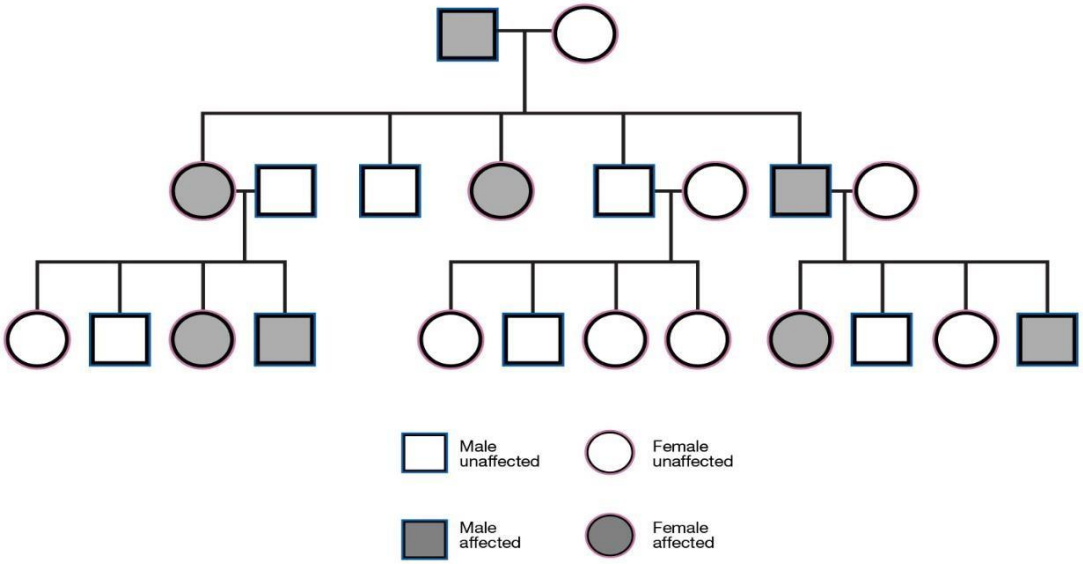
a) Huntington disease-brain disorder ,defect on chromosome no.4.

b) Von Willebrand disease-bleeding disorder , defect on chromosome no.12.

c) Neurofibromatosis-tumour on nerve tissue , NF1 & NF2 gene on chromosome no.21 & 22.

d) Polycystic kidney disease-kidney disorder,PKD1 & PKD2 genes on chromosome 16 & 4

e) Myotonic Dystrophy-muscular dystrophy,deect on chromosome 19.



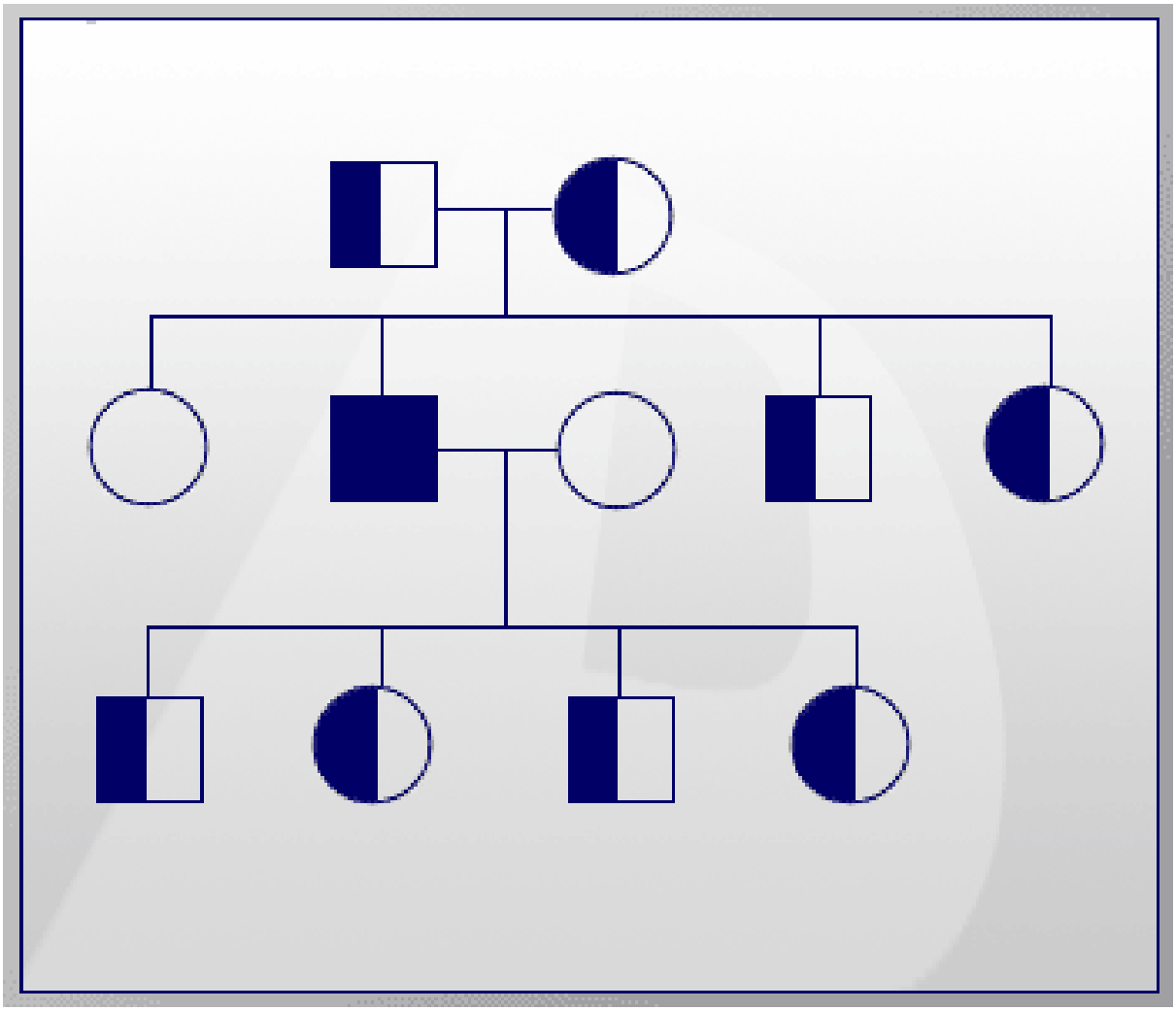
- **AUTOSOMAL RECESSIVE DISORDER:**

- An autosomal recessive disorder means two copies of an abnormal genemust be present in order for the disease or trait to develop.
- These disorders are usually passed on by two carriers.
- To have an autosomal recessive disorder, you inherit two mutated genes, one from each parent.
- There is chance of 25% of children to be affected when both the parents are carriers.
- Examples of autosomal recessive disorders include

a) **cystic fibrosis**-affects lungs and digestive system,defect on chromosome no.7.

b) **sickle cell anemia-,**red blood cell disorder,defect on chromosome no.11.

c) **Tay-Sachs disease**-destruction of nerve cells in brain and spinal cord, defect on chromosome no.15.



*when both the parents are carriers.

25%-Affected 50%-carrier 25%-Unaffected

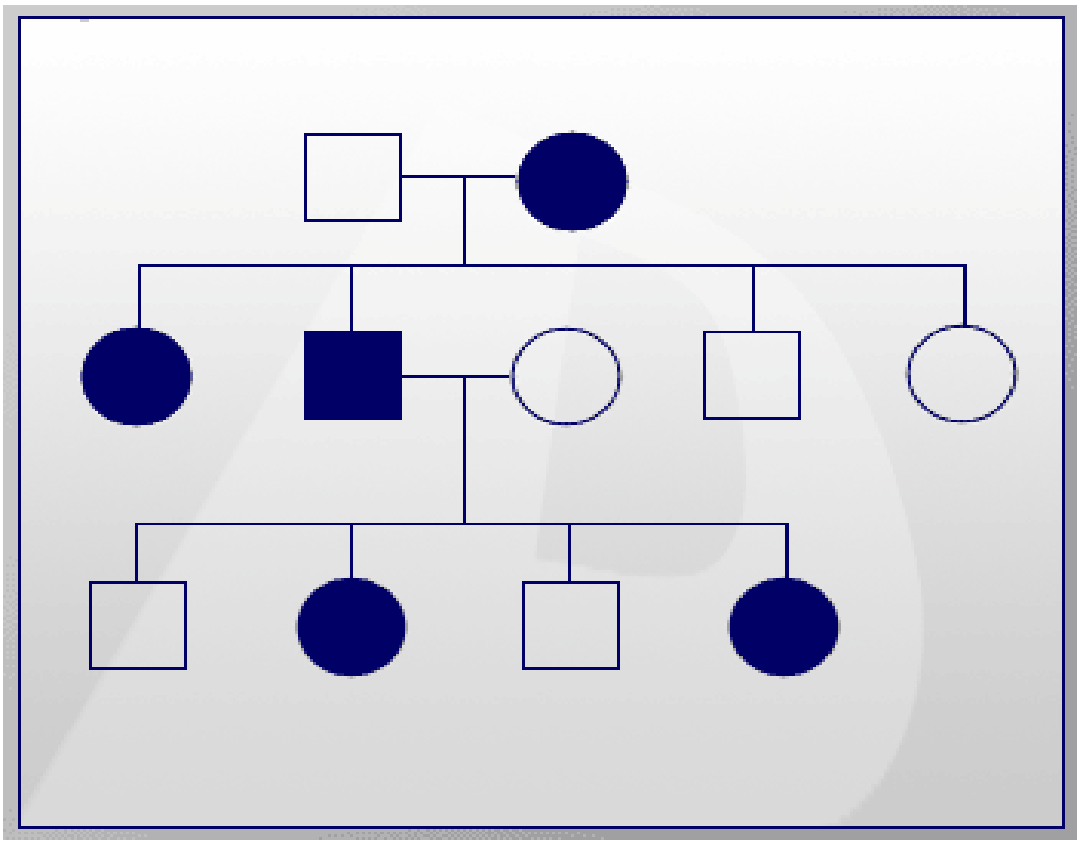
- **SEX-LINKED DOMINANT:**

- It is otherwise called as X-Linked Dominant.
- One abnormal gene on the X chromosome can cause a sex-linked dominant disease.
- Sex-linked dominant is a rare way that a trait or disorder can be passed down through families.
- Some X-linked dominant disorders are so severe that males with the genetic disorder may die before birth.
- 50% of the children will be affected when any one of the parents is affected.
- Males may be severely affected because they only carry one copy of the genes found on the x chromosome.
- Examples of X-linked dominant disorders include

a) Rett syndrome-affects the brain development in girls, defect on x chromosome.

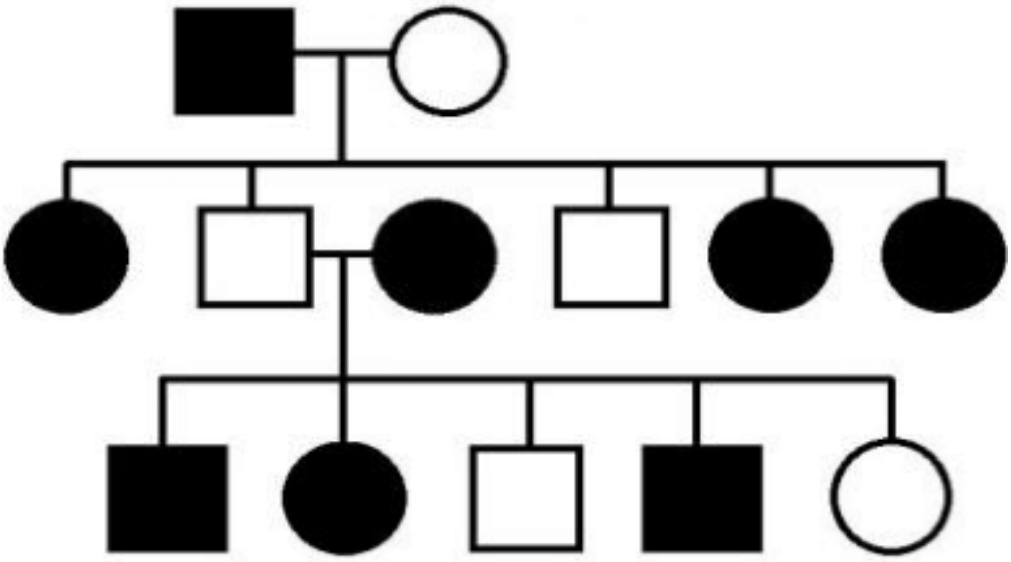
b) X-linked lissencephaly-affects the development of brain and genetalia,often occurs in males.

c) Double-cortex syndrome\Subcortical band heterotopias-neuronal migration disorder,90% females are affected.



*When mother is affected and father is normal

*50%-affected *50%-unaffected *no carriers



*when father is affected and mother is normal.

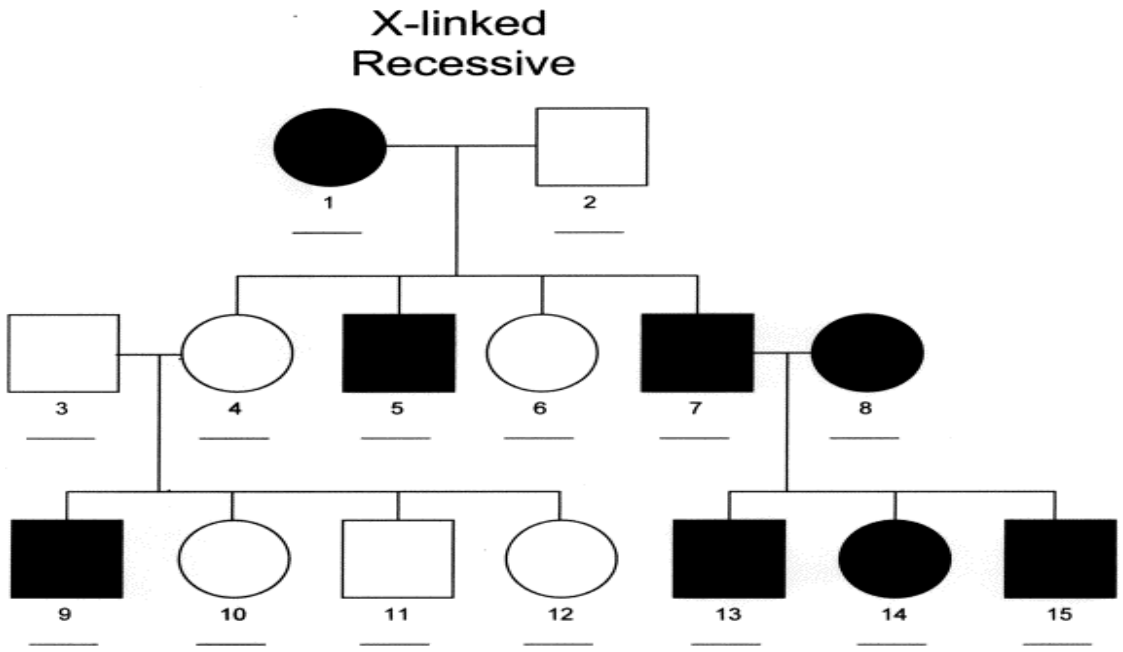
*50%-affected *50%-unaffected *no carrier

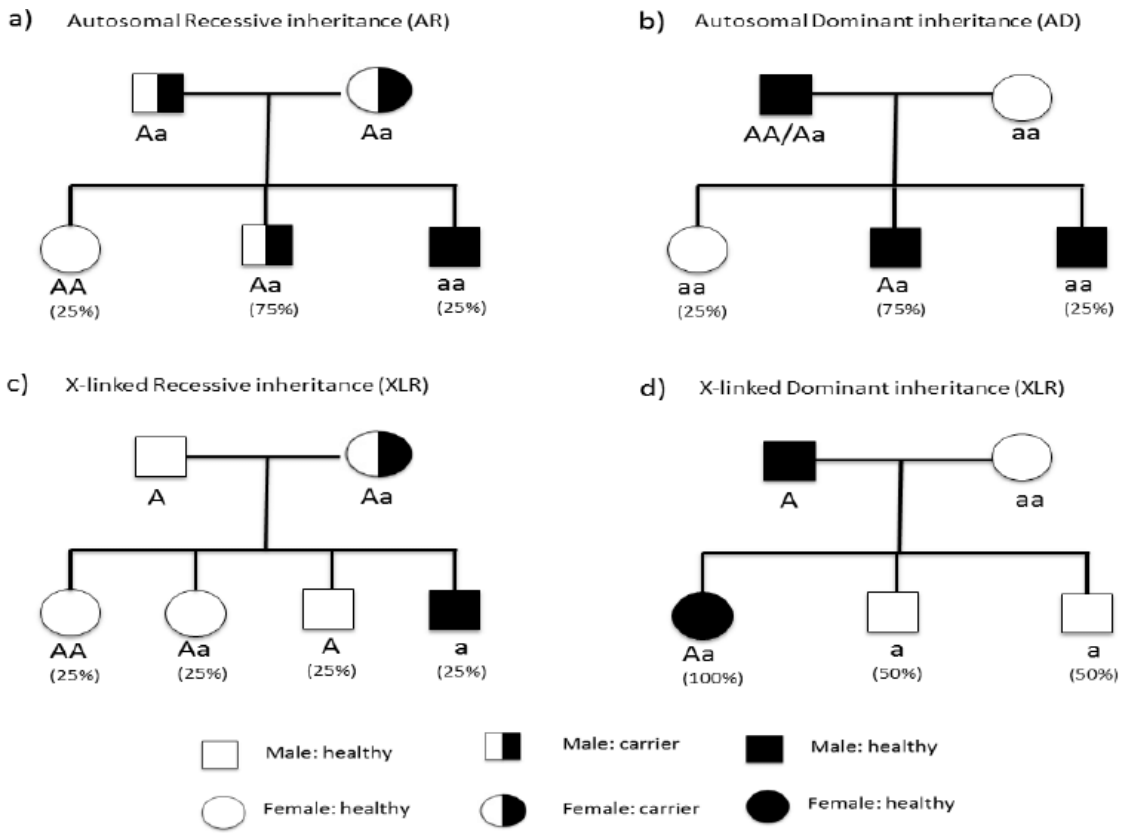
- **SEX-LINKED RECESSIVE:**

- It is otherwise called as X-Linked Recessive.
- X-linked recessive inheritance is a mode of inheritance in which a mutation in a gene on the X chromosome causes the phenotype to be always expressed in males and in females who are homozygous for the gene mutation.
- Females will be carriers because they have two X-chromosomes and one of which is affected.
- 25% of the children are affected and 50% of them are carriers.
- Examples of X-linked recessive conditions include

a) **red-green color blindness**-cannot differentiate between red and green,more common in males.

b) **hemophilia A**-genetic deficiency in clotting factor 8,causes increased bleeding,usually affects male.





- **MITOCHONDRIAL DISORDER:**

- **Mitochondrial** diseases are long-term, genetic, often inherited disorders that occur when **mitochondria** fail to produce enough energy for the body to function properly.
- One in 5,000 individuals has a genetic **mitochondrial disease**.
- A disorder that occurs when structures that produce energy for a cell malfunction.
- A common factor among mitochondrial diseases is that the mitochondria are unable to completely burn food and oxygen to generate energy, which is essential for normal cell function.
- Mitochondrial diseases can be present at birth, but can also occur at any age.
- Mitochondrial diseases can affect almost any part of the body, including the cells of the brain, nerves, muscles, kidneys, heart, liver, eyes, ears or pancreas.
- It is inherited from mother because the egg only contains mitochondria and not the sperm.

CAUSES :

Mitochondrial disorders may be caused by mutations (acquired or inherited), in mitochondrial DNA (mtDNA), or in nuclear genes that code for mitochondrial components. They may also be the result of acquired mitochondrial dysfunction due to adverse effects of drugs, infections, or other environmental causes.

Symptoms:

Symptoms of mitochondrial diseases depend on which cells of the body are affected.

- Poor growth.
- Muscle weakness, muscle pain, low muscle tone, exercise intolerance.
- Vision and/or hearing problems.
- Learning disabilities, delays in development, mental retardation.
- Diabetes.
- Increased risk of infection.

Neurological problems, seizures, migraines, strokes.

DIAGNOSIS:

Diagnosis starts with a series of examinations and tests that may include:

- A review of a patient's family history.
- A complete physical examination.
- A neurological examination.
- A metabolic examination that includes blood and urine tests, and, if needed, a cerebral spinal fluid test

Other tests:

- Magnetic resonance imaging (MRI) or spectroscopy (MRS) for neurological symptoms.
- Retinal exam or electroretinogram(ERG)for vision symptoms.
- Electrocardiogram (EKG) or echocardiogram for symptoms of heart disease.

TREATMENT:

- There's no cure, but physiotherapy and medication can manage symptoms.
- **Vitamins and supplements**, including Coenzyme Q10; B complex vitamins, especially thiamine (B1) and riboflavin (B2); Alpha lipoic acid; L-carnitine (Carnitor); Creatine; and L-Arginine.
- **Exercise**
- **Conserving energy**: Don't try to do too much in a short period of time.

TYPES OF IMMUNE RESPONSE:

The system of animal body , which protects it from various infectious agents and cancer , is called **immune system** .The study of the immune system is known as **immunology**.

The Latin term "**immunis**", meaning "**exempt**" or "**freedom**",gave rise to the English word immunity. It refers to all the mechanisms used by the body for protection from environmental

agents that are foreign to the body. These agents may be microorganisms or their products, certain food items, chemicals, drugs and pollen grains. Immunity is of two types: (a) innate and (b) acquired immunity.

A. **Innate Immunity (Non-specific):** Innate immunity comprises all those natural defense mechanisms with which an organism is protected from infection. As a Strategy, innate immunity consists of various types of barriers that prevent entry of foreign agents into the body. The pathogens that enter into the body, are quickly killed by some components of the immune system. This is the **first line of defense** in most animals. Innate immunity consists of the following four types of barriers.
    a. **Anatomical Barriers**: These barriers block the entry of organisms into the body. The **skin** and the **mucous membrane** lining the respiratory and intestinal as well as the reproductive passages constitute the **barriers.** Mucous material entraps foreign microorganisms. The **ciliary** movements produced by the epithelial lining cells expel out micro-organisms form the body.
    b. **Physiological Barriers**: Factors like **body temperature, pH** and various **body secretions**, prevent the growth of pathogenic micro-organisms. For example, fever response inhibits growth of many pathogens, Acidity of the stomach contents due to **HCL secretion** kills ingested micro-organisms. Lysozyme present in secretions, such as **tears** and **saliva**,digest bacterial cell walls. Certain cells, like **WBC**, when infected with a virus, respond by releasing antiviral proteins, called **interferons**. Interferons, in turn, make the cells in the vicinity resistant to viral infections. As a result, the concerned person exhibit increased resistance to viral infections.
    c. **Phagocytic Barriers**: Phagocytosis is an important mechanism of innate immunity. It is performed by **leucocytes**. In response to pathogenic infections, the total count of leucocytes will increase sharply. Humans contain wandering phagocytes that circulate throughout the body. The most important phagocytes are the **macrophages** and the **neutrophils**. Macrophages are large irregular-shaped cells that engulf microbes, viruses and cellular debris.In response to an infection, monocytes are liberated at the site of infection. These monocytes get converted into macrophages, These cells are provided with **bacteriolytic enzymes** and **free radicals**, which destroy the pathogens.
    d. **Inflammatory Barriers**: Usually an infection or tissue injury results in redness and swelling, along with pain and production of heat that may result in fever. The above phenomenon isknown as **inflammatory response**. This response occurs due to release of chemical alarm signals, notably **histamine**, **serotonin** and **prostaglandins**, by the **damaged mast cells**. At the site of inflammation, theremay be leakage of vascular fluid,which contains serum proteins with antibacterial activity. Further, there is an **influx ofphagocytic cells into the affected area**. These responses inhibit and destroy the invading microorganisms.

Besides the phagocytes, natural killer cells (NK cells) (T Lymphocytes) kill virus-infected cells and some tumor cells of the body by creating **perforin-lined pores** in the plasma membrane of the target cells. These pores allow entry of water into the target cell, which then swells and bursts.

Acquired Immunity (Specific immunity)

Acquired immunity, also known as **adaptive** or **specificimmunity**, is capable or recognizing and selectively eliminating specific microorganisms. Acquired immunity is found only in **vertebrates**. It supplements the protection provided by **innate/natural immunity**. It is generated in response to an **exposure**or **encounter** to the microorganisms in question. Specific defense mechanisms require several days to be activated, following the failure of non-specific defense mechanisms. Adaptive immunity has the following unique features.

- **Specificity**: It is the ability to distinguish differences among various foreign molecules.
- **Diversity**: It can recognize a vast variety of foreign molecules.
- **Discrimination between Self and Non-self**: It is able to recognize and respond to molecules that are foreign (non-self) to the body. At the same time, it can avoid response to those molecules that are present within the body (self-antigens) of the given animal.
- **Memory**: When the immune system encounters a specific foreign agent, e.g., microbe, for the first time, it generates an immune response and eliminates the invader. The immune system retains the memory of this encounter for a prolonged interval. As a result, a second encounter with the same microbe evokes a heightened immune response.

Specific immunity employs two major groups of cells: (a) **lymphocytes**, and (b) **antigen presenting cells**. A healthy individual possesses about a **trillion** of lymphocytes. The lymphocytes are of **two types** viz., **T – lymphocytes** or **T – cells** and **B – lymphocytes** or **B – cells**,Both the types of lymphocytes, as well as the other cells of the immune response, are produced in bone marrow. The process of their production is called **haematopoiesis**. Some immature lymphocytes, destined to become **thymocytes**, migrate via blood to the thymus, where they mature and differentiate as T – cells. The B – cells, on the other hand, mature in the **bone marrow** itself. The B and T cells, together, generate two types of specific immunity, viz., (a) **cell mediated** and (b) **antibody-mediated** or **humoral immunity** respectively.

(a) Cell-mediated Immunity (CMI)

Cell-mediated immunity is the responsibility of a subgroup of T cells, called **cytotoxic T lymphocytes(CTLs)**. An activated cytotoxic lymphocyte is specific to a targetcell, which has been infected, and kill the target cell by a variety of mechanisms. This prevents the completion of life cycle of the pathogen and its growth, since it depends on an intact host cell to do that. Cell-mediated is also involved in killing of cancer cells.

(b) Antibody-mediated Immunity / Humoral Immunity

i. Antibody mediated or humoral immunity involves the synthesis of specific antibody molecules called **immunoglobulins** by the B-lymphocytes. Each antigen has many different Antigenic

determinants, each of which matches a specific antibody and binds to it. The B cells, direct the antibody-mediated immunity. The antibody molecules (**Igs**) may be bound to a cell membrane in the form of receptors or they may remain free. The free antibodies have three main functions viz., 1. **Agglutination**of particular matter, including bacteria and viruses 2. **Opsonisation** or coating over bacteria to facilitate recognition and phagocytosis by the phagocytes and 3.**neutralization** of toxins released by bacteria.

Adaptive immunity may be **active** or **passive**. **Active immunity** is due to the immune response generated in the individual in question by a **pathogen** or **vaccine,** whereas **passive immunity** is conferred by transfer of immune products, like antibodies, etc., from an individual into a non-immune individual.

Hypersensitivity disorders:

- **Hypersensitivity** refers to undesirable reactions produced by the normal immune system, including allergies and autoimmunity .
- These reactions may be damaging, uncomfortable, or occasionally fatal.

CLASSIFICATION

- TYPE I – IMMEDIATE, ATOPIC, ANAPHYLACTIC
- TYPE II – ANTIBODY DEPENDANT
- TYPE III – IMMUNE COMPLEX
- TYPE IV – CELL MEDIATED / DELAYED TYPE OF HYPERSENSITIVITY

TYPE I – IMMEDIATE, ATOPIC, ANAPHYLACTIC

- Type I hypersensitivity is also known as immediate or anaphylactic hypersensitivity.
- The reaction may involve skin (urticariaand eczema), eyes (conjunctivitis), nasopharynx (rhinorrhea, rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis).
- The reaction may cause a range of symptoms from minor inconvenience to death.
- The reaction usually takes 15 - 30 minutes from the time of exposure to the antigen, although sometimes it may have a delayed onset (10 - 12 hours).
- Mediated by IgE antibody to specific antigens
- The primary cellular component in this hypersensitivity is the mast cell or basophil.
- The reaction is amplified and/or modified by platelets, neutrophils and eosinophils.
- Mast cells stimulated and release histamine

CAUSES

- **Allergen:** Allergens are nonparasite antigens that can stimulate a type I hypersensitivity response.

ATOPY

- **Atopy** is the term for the genetic trait to have a predisposition for localized anaphylaxis.
- Atopic individuals have higher levels of IgE and eosinophils.

MECHANISM

- Initial introduction of antigen produces an antibody response. More specifically, the type of antigen and the way in which it is administered induce the synthesis of IgE antibody in particular.
- Immunoglobulin IgE binds very specifically to receptors on the surface of mast cells, which remain circulating.
- Reintroduced antigen interacts with IgE on mast cells causing the cells to degranulate and release large amounts of histamine, lipid mediators and chemotactic factors that cause smooth muscle contraction, vasodilation, increased vascular permeability, broncoconstriction and edema. These reactions occur very suddenly, causing death.

MEDIATORS OF TYPE 1

- Histamine
- Cytokines TNF, IL-1, IL-6.
- Chemoattractants for Neutrophils and Eosinophils.
- Enzymes : tryptase, chymase, cathepsin.
- Changes in connective tissue matrix, tissue breakdown
- Leukotrienes
- Prostaglandins

DIAGNOSIS

- Diagnostic tests for immediate hypersensitivity include skin (prick and intradermal) tests, measurement of total IgE and specific IgE antibodies against the suspected allergens.
- Total IgE and specific IgE antibodies are measured by a modification of enzyme immunoassay (ELISA).
- Increased IgE levels are indicative of an atopic condition, although IgE may be elevated in some non-atopic diseases (e.g., myelomas, helminthic infection, etc.).

TREATMENT

- Drugs.

  › Non-steroidal anti-inflammatories

  › Antihistamines

  › Steroids

  › Theophylline OR epinephrine -prolongs or increases cAMP levels in mast cells which inhibits degranulation.

- Immunotherapy

  › Desensitization (hyposensitization) also known as allergy shots.

  › Repeated injections of allergen to reduce the IgE on Mast cells and produce IgG.

TYPE II – ANTIBODY DEPENDANT

- Type II hypersensitivity is also known as cytotoxic hypersensitivity and may affect a variety of organs and tissues.
- The antigens are normally endogenous, although exogenous chemicals (haptens) which can attach to cell membranes can also lead to type II hypersensitivity.
- Drug-induced hemolytic anemia, granulocytopenia and thrombocytopenia are such examples. Pencillin allergy also belong to this class.
- The reaction time is minutes to hours.
- Type II hypersensitivity is primarily mediated by antibodies of the IgM or IgG classes and complement .
- Phagocytes may also play a role.
- The lesion contains antibody, complement and neutrophils.

DIAGNOSIS

- Diagnostic tests include detection of circulating antibody against the tissues involved and the presence of antibody and complement in the lesion (biopsy) by immunofluorescence.
- The staining pattern is normally smooth and linear, such as that seen in Goodpasture's nephritis (renal and lung basement membrane)
- Treatment involves anti-inflammatory and immunosuppressive agents

TYPE III – IMMUNE COMPLEX

- Antigen antibody immune complexes. IgG mediated
- Large amount of antigen and antibodies form complexes in blood.

- If not eliminated can deposit in capillaries or joints and trigger inflammation.
- The reaction may be general (e.g., serum sickness) or may involve individual organs including skin (e.g., systemic lupus erythematosus, Arthus reaction), kidneys (e.g., lupus nephritis), lungs

  (e.g., aspergillosis), joints (e.g., rheumatoid arthritis) or other organs.
- This reaction may be the pathogenic mechanism of diseases caused by many microorganisms.
- The reaction may take 3 - 10 hours after exposure to the antigen .
- It is mediated by soluble immune complexes.
- They are mostly of the IgG class, although IgM may also be involved.
- The antigen may be exogenous (chronic bacterial, viral or parasitic infections), or endogenous (non-organ specific autoimmunity: e.g., systemic lupus erythematosus, SLE).
- The antigen is soluble and not attached to the organ involved
- PMNs and macrophages bind to immune complexes via FcR and phagocytize the complexes.
- If unable to phagocytize the immune complexes can cause inflammation via C' activation ---> C3a C4a, C5a and "frustrated phagocytes".

DIAGNOSIS AND TREATMENT

- Diagnosis involves examination of tissue biopsies for deposits of immunoglobulin and complement by immunofluorescence microscopy.
- The presence of immune complexes in serum and depletion in the level of complement are also diagnostic.
- Treatment includes anti-inflammatory agents.

TYPE IV – CELL MEDIATED / DELAYED TYPE OF HYPERSENSITIVITY

- Reaction involves **sensitized T cells** and release of its lymphokines as mediators and amplifiers
- Mediated by cells rather than antibodies
- Clinical states: **Contact dermatitis, Transplant rejection, Granuloma**
- Th1 cells release cytokines to activate macrophages causing inflammation and tissue damage.
- Continued macrophage activation can cause chronic inflammation resulting in tissue lesions, scarring, and granuloma formation.

- Response starts after 48 -72 hrs

DIAGNOSIS AND TREATMENT

- Diagnostic tests in vivo include delayed cutaneous reaction (e.g. Montoux test and patch test (for contact dermatitis).
- In vitro tests for delayed hypersensitivity include mitogenic response, lympho-cytotoxicity and IL-2 production.
- Corticosteroids and other immunosuppressive agents are used in treatment.

IMMUNO DEFICIENCY SYNDROME

- ✓ **IMMUNO DEFICIENY SYNDROME** impairs the immune system's ability to defend the body against foreign or abnormal cells that invade or attack it such as bacteria, viruses, fungi and cancer cells
- ✓ These disorders make our body easier to catch serious disease causing pathogens. As a result unusual life threatening diseases or **lymphomas** or other **cancers** may develop.

Immuno deficiency disorders are mainly consists of two stages. One is genetically acquired by birth and the other is obtained by exposure to toxic sources like radiation, exchange of bodily fluids, etc,. Our immune system mainly includes these following organs in which the pathogens mainly invade to destroy the production of antibodies. They are:

- Spleen
- Tonsils
- Bone marrow
- Lymph nodes.

These organs make and release lymphocytes. In white blood cells it is classified as B and T cells [also known as **B lymphocytes and T lymphocytes**].These cells fight invaders called **Antigens**. B cells release antibodies specific to the disease our body detects. T cells destroy foreign or abnormal cells. Example of antigens that our B and T cells might need to fight off includes:

- Bacteria
- Viruses
- Cancer cells
- Parasites.

TYPES OF IMMUNO DEFICIENCY DISORDERS:

An immune deficiency disease occurs when the immune system is not working properly.If you are born with a deficiency or if there is any genetic cause,it is called **PRIMARY IMMUNO DEFICIENCY SYNDROME**. There are more than 100s of primary immuno deficiency disorders.Examples of primary immuno deficiency disorders includes:

- ❖ X-linked agamma globulinemia **[XLA]**
- ❖ Common variable immune deficiency**[CVID]**

- ❖ Severe combined immuno deficiency **[SCID]**which is also known as **alymphocytes** or **a boy in a bubble disease**.

Secondary immuno deficiency disorders happens when an outside source like a toxic chemical or infection attacks our body. The folowing can cause a secondary immuno deficiency disorders:

- Severe burns
- Chemotherapy
- Radiation
- Diabetes
- Malnutrition.

Examples of secondary immuno deficiency disorders includes:

- **AIDS**
- Cancers of the imune system like **leukemia**
- Immune complex diseases like **viral hepatitis**
- **Multiple myeloma**[Cancer of the plasma cells which produce antibodies].

RISKS FOR IMMUNO DEFICIENCY DISORDERS:

- ➢ People who have a family history of primary immune deficiency disorders have a higher than normal risk for developing primary disorders.Anything that weakens your immune system can lead to a secondary immune deficiency disorder. For example: exposure to bodily fluids infected with HIV, or removing the spleen can be the causes.
- ➢ Spleen removal may be necessary because of conditions like cirrhosis of the liver, sickle cell anemia, or trauma of the spleen.
- ➢ Ageing also weakens our immune system. As we age, some of the organs that produce WBC's shrink and produce fewer of them.
- ➢ Proteins are important for our immunity, not enough protein in our diet weaken our immune system.
- ➢ Our body also produces proteins when we sleep that help our body fight against infection. For this reason, lack of sleep reduces our immune defense.
- ➢ Cancers and chemotherapy drugs can also reduce our immunity.

The following diseases and risky conditions that are linked to primary immuno deficiency disorders includes:

- ✓ Ataxia-telangiectasia
- ✓ Chedaik-higashi syndrome
- ✓ Combined immuno deficiency disease
- ✓ Complement deficiencies
- ✓ Digeorge syndrome
- ✓ Hypogammaglobulinemia
- ✓ Job's syndrome
- ✓ Leukocyte adhesion defects

- ✓ Pan hypogammaglobulinemia
- ✓ Bruton's diseases
- ✓ Congenital agamma globulinemia
- ✓ Selective deficiency of IgA
- ✓ Wiskott-aldrich syndrome.

SYMPTOMS FOR IMMUNO DEFICIENCY DISORDERS:

Each disorder have unique symptoms that can be frequent or chronic; some of these symptoms can include:

- Pink eye
- Sineus infections
- Cold
- Diarrhea
- Pneumonia
- Yeast infections.

If these problems don't respond to treatment or we don't completely get better overtime, our doctor might test for an immuno deficiency disorder.

DIAGNOSIS FOR IMMUNO DEFICIENCY DISORDERS:

If we have immuno deficiency disorder then our doctor will ask about the following tests that are taken to check our body conditions: They include:

- Medical history of our body
- Physical examination
- Determination of our T cell count
- Determination of our immunoglobulin levels.

- Vaccines can test our immune system response in what is called an **antibody test.**Our doctor will give us vaccine then they will test our blood fortis response to the vaccine a few days or few weeks later.
- If we don't have an immuno deficiency disorder, our immune system will produce antibodies to fight the organisms in the vaccine, we might have a disorder if our blood test doesn't show antibodies.

TREATMENT FOR IMMUNO DEFICIENCY DISORDERS:

The treatment for each immuno deficiency disorder will depend on the specific conditions:For example: AIDS causes several different infections. Our doctor will prescribe medications for each infection and we may be given an **antiretroviral drug** to treat the HIV infection if appropriate.

Treatment for these disorders commonly includes antibiotics and immunoglobulin therapy. Other **antiviral drugs**, **amantadine** and **acyclovir**, or a drug called **interferon** are used for the treatment of the viral infections caused by immuno deficiency disorders.

If our bone marrow isn't producing enough lymphocytes, our doctor might order a bone marrow [stem cell] transplant.

PREVENTION FOR IMMUNO DEFICIENCY DISORDERS:

- ❖ Primary immuno deficiency disorders can be controlled and treated but they can't be prevented.
- ❖ Secondary disorders can be prevented in a number of ways.For example: It is possible to prevent ourself from getting AIDS by not having unprotected sex with someone who carries HIV.
- ❖ Sleep is very important for a healthy immune system. Adults need about eight hours of sleep per night. It's also important that we have to stay away from people who are sick if their immune system isn't working properly.
- ❖ If we have a contagious immunodeficiency disorders like AIDS, we can keep others healthy by practicing safe sex and not sharing bodily fluids with people who aren't infected.

VIRAL DISEASES

Virus – An Introduction:

- ➢ Viruses are small particles of genetic material (either DNA or RNA) that are surrounded by a protein coat.
- ➢ Some viruses also have a fatty "envelope" covering.
- ➢ They are capable of reproducing only within the living cells of a host.
- ➢ Viruses infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea.

Viral Diseases:

- Viral diseases are extremely widespread infections caused by viruses, a type of microorganism. There are many types of viruses that cause a wide variety of viral diseases.
- The most common type of viral disease is the common cold, which is caused by a viral infection of the upper respiratory tract (nose and throat).

Other common viral diseases include:

- Chickenpox
- Flu (influenza)
- Human immunodeficiency virus (HIV/AIDS)
- Mumps, measles and rubella
- Viral gastroenteritis (stomach flu)

Spread of Viral Diseases:

Viral diseases are contagious and spread from person to person when a virus enters the body and begins to multiply. Common ways that viruses spread from person to person include:

- Breathing in air-borne droplets contaminated with a virus
- Eating food or drinking water contaminated with a virus

- Having sexual contact with a person who is infected with a sexually transmitted virus
- Indirect transmission from person to person by a virus host, such as a mosquito, tick, or field mouse
- Touching surfaces or body fluids contaminated with a virus.

Symptoms of viral diseases:

Symptoms of viral diseases vary depending on the specific type of virus causing infection, the area of the body that is infected, the age and health history of the patient, and other factors. The symptoms of viral diseases can affect almost any area of the body or body system.

Symptoms of viral diseases can include:

- Flu-like symptoms (fatigue, fever, sore throat, headache, cough, aches and pains)
- diarrhea, nausea and vomiting
- Rash
- Sneezing
- Unexplained weight loss

In infants, signs of a viral disease can also include:

- Bulging of the soft spot on the top of the head
- Excessive crying or fussiness
- Excessive sleepiness

Serious symptoms that might indicate a life-threatening condition:

- Chest pain
- High fever (higher than 101 degrees Fahrenheit)
- Shortness of breath, wheezing, or difficulty breathing
- Stiff neck
- Yellowing of the skin and whites of the eyes (jaundice)

Cause of Viral Diseases:

- Viral infections occur when a virus enters the body and invades the inside of the body's cells in order to reproduce.
- If the body's immune system is unable to fight off the virus, it multiplies and spreads to other cells, repeating the process and leading to a widespread infection.

Various ways to become infected with a virus:

- Being bitten by an animal or insect infected with a virus
- Breathing in air-borne droplets contaminated with a virus
- Eating food or drinking water contaminated with a virus
- Having sexual contact with a person who is infected with a sexually transmitted virus
- Sharing needles for tattooing or drug use with an infected person
- Touching surfaces contaminated with a virus

Some Main and Common Viral Diseases:

1. Common Cold:

- ✓ The cold is a common infection of the upper respiratory tract.
- ✓ The common cold is spread when you inhale virus particles from an infected person's sneeze, cough, speech, or loose particles from when they wipe their nose.
- ✓ You can also pick up the virus by touching a contaminated surface that an infected individual has touched.
- ✓ Common areas include doorknobs, telephones, children's toys, and towels.
- ✓ Rhinoviruses (which cause the most colds) can live for up to three hours on hard surfaces and hands.

Human rhinoviruses:

- This group of viruses — of which there are more than 100 types — is by far the most common identified cause of colds. The viruses grow best at the temperature inside the human nose.
- Human rhinoviruses (HRVs) are highly contagious. However, they rarely lead to serious health consequences.

Complications:

The common cold will usually run its course without complication. In some instances it may spread to your chest or ears. The infection can then lead to other conditions such as:

Ear infection: The main symptoms are earaches or a yellow or green discharge from the nose. This is more common in children.

Asthma: Breathing difficulty and/or wheezing that can be triggered by a simple cold.

Chest infection: Infections can lead to pneumonia and bronchitis. Symptoms include lingering cough, shortness of breath, and coughing up mucus.

Strep throat: Strep is an infection of the throat. Symptoms include a severe sore throat and sometimes a cough.

Treatments:

There is no set cure for the cold, but combining remedies may alleviate symptoms.

- Pain relievers such as aspirin and ibuprofen are good for headaches, joint pain, and fever reduction.
- Cough syrups help with persistent coughs and sore throats. Some examples are Robitussin, Mucinex, and Dimetapp.

2. Dengue:

- A mosquito-borne viral disease occurring in tropical and subtropical areas.
- Those who become infected with the virus a second time are at a significantly greater risk of developing severe disease.
- Dengue virus is transmitted by female mosquitoes mainly of the species Aedesaegypti.
- These mosquitoes are also vectors of chikungunya, yellow fever and Zika viruses.

Symptoms of Dengue Fever:

Symptoms, which usually begin four to six days after infection and last for up to 10 days, may include

- Sudden, high fever
- Severe headaches
- Severe joint and muscle pain
- Fatigue
- Nausea
- Vomiting

Treatment for Dengue Fever:

There is no specific medicine to treat dengue infection. If you think you may have dengue fever, you should use pain relievers with acetaminophen and avoid medicines with aspirin, which could worsen bleeding. You should also rest, drink plenty of fluids, and see your doctor.

Preventing Dengue Fever:

The best way to prevent the disease is to prevent bites by infected mosquitoes, particularly if you are living in or traveling to a tropical area.

To protect yourself:

- Use mosquito repellents, even indoors.
- When outdoors, wear long-sleeved shirts and long pants tucked into socks.
- Make sure window and door screens are secure and free of holes. If sleeping areas are not screened or air conditioned, use mosquito nets.

3. EBOLA:

- Ebola is a rare but deadly virus that causes fever, body aches, and diarrhea, and sometimes bleeding inside and outside the body.
- As the virus spreads through the body, it damages the immune system and organs.
- Ultimately, it causes levels of blood-clotting cells to drop. This leads to severe, uncontrollable bleeding.

Causes of Ebola:

- The Ebola virus belongs to the viral family Filoviridae. Scientists also call it Filovirus.
- These virus types cause hemorrhagic fever or profuse bleeding inside and outside the body.

Risk Factors and Transmission:

Unlike other types of viruses, Ebola can't be transmitted through the air or by touch alone. You must have direct contact with the bodily fluids of someone who has it. The virus may be transmitted through:

- blood
- saliva
- sweat
- urine
- vomit

These bodily fluids can all carry the Ebola virus. Transmission can occur via the eyes, nose, mouth, broken skin, or sexual contact.

Other risk factors include:

- exposure to infected objects, such as needles
- interactions with infected animals
- attending burial ceremonies of someone who has died from Ebola
- traveling to areas where a recent outbreak has occurred

Symptoms of Ebola:

Ebola symptoms typically appear within 8 to 10 days after exposure; however, symptoms can appear as early as two days after exposure or take as long as three weeks to appear.

Extreme fatigue is often the first and most prominent symptom. Other symptoms include:

- diarrhea
- fever
- headache
- muscle pain
- stomach pain
- unexplained bleeding or bruising
- vomiting

Treatment for Ebola:

The Ebola virus does not have a cure or vaccine at this time. Instead, measures are taken to keep the person as comfortable as possible. Supportive care measures may include:

- giving medications to maintain blood pressure
- managing electrolyte balances
- providing extra oxygen, if needed
- providing intravenous and/or oral fluids to prevent dehydration
- treating coexisting infections
- preventing other infections from occurring
- administering blood products if indicated

Prevention:

Individuals can take several precautions to protect against Ebola. These steps include:

- avoiding contact with blood and body fluids
- practicing careful hand hygiene, including washing hands with soap and water or an alcohol-based hand sanitizer
- refraining from engaging in burial rituals that involve handling the body of a person who died from Ebola
- wearing protective clothing around wildlife

4. CORONA VIRUS:

- COVID-19 affects different people in different ways. Most infected people will develop mild to moderate illness and recover without hospitalization.
- The virus that causes COVID-19 is in a family of viruses called Coronaviridae.
- Coronaviruses are a group of viruses that can cause disease in both animals and humans. The severe acute respiratory syndrome (SARS) virus strain known as SARS-CoV is an example of a coronavirus. SARS spread rapidly in 2002–2003.
- The new strain of coronavirus is called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus causes coronavirus disease 19 (COVID-19).

- The recent outbreak began in Wuhan, a city in the Hubei province of China. Reports of the first COVID-19 cases started in December 2019.

- Coronaviruses are common in certain species of animals, such as cattle and camels. Although the transmission of coronaviruses from animals to humans is rare, this new strain likely came from bats, though one study suggests pangolins may be the origin.

HOW IT SPREADS:

- The virus that causes COVID-19 is mainly transmitted through droplets generated when an infected person coughs, sneezes, or exhales. These droplets are too heavy to hang in the air, and quickly fall on floors or surfaces.
- You can be infected by breathing in the virus if you are within close proximity of someone who has COVID-19, or by touching a contaminated surface and then your eyes, nose or mouth.

Symptoms:

Most common symptoms:
- fever
- dry cough
- tiredness

Less common symptoms:
- aches and pains
- sore throat

- diarrhoea
- conjunctivitis
- headache
- loss of taste or smell
- a rash on skin, or discolouration of fingers or toes

Prevention:

Protect yourself and others around you by knowing the facts and taking appropriate precautions. Follow advice provided by your local health authority.

To prevent the spread of COVID-19:

- Clean your hands often. Use soap and water, or an alcohol-based hand rub.
- Maintain a safe distance from anyone who is coughing or sneezing.
- Wear a mask when physical distancing is not possible.
- Don't touch your eyes, nose or mouth.
- Cover your nose and mouth with your bent elbow or a tissue when you cough or sneeze.
- Stay home if you feel unwell.

Masks:

- Masks can help prevent the spread of the virus from the person wearing the mask to others. Masks alone do not protect against COVID-19, and should be combined with physical distancing and hand hygiene. Follow the advice provided by your local health authority.

Treatment:

To date, there are no specific vaccines or medicines for COVID-19. Treatments are under investigation, and will be tested through clinical trials.

Self-care:

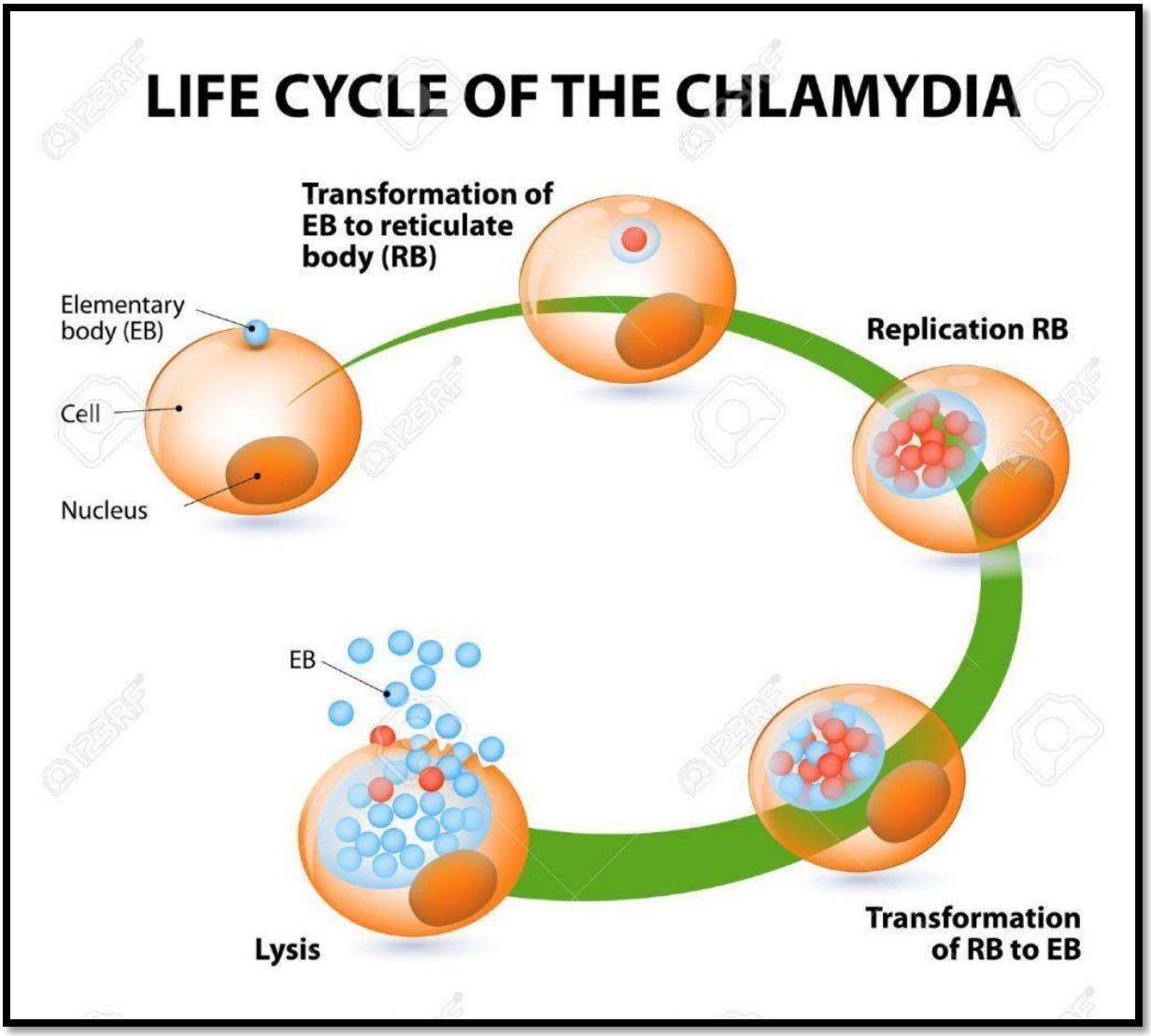
If you feel sick you should rest, drink plenty of fluid, and eat nutritious food. Stay in a separate room from other family members, and use a dedicated bathroom if possible. Clean and disinfect frequently touched surfaces.

Everyone should keep a healthy lifestyle at home. Maintain a healthy diet, sleep, stay active, and make social contact with loved ones through the phone or internet. Children need extra love and attention from adults during difficult times. Keep to regular routines and schedules as much as possible.

It is normal to feel sad, stressed, or confused during a crisis. Talking to people you trust, such as friends and family can help. If you feel overwhelmed, talk to a health worker or counsellor.

CHLAMYDIA:

- Chlamydiae are non motile, obligate, intracellular bacteria.
- They contain DNA, RNA, ribosomes and make their own proteins and nucleic acids.
- Chlamydia is the most common sexually transmitted pathogen worldwide.
- It is caused by the bacteria called Chlamydia Trachomatis which occurs only in humans.
- It can affect both men and women.
- Women can get chlamydia in the cervix, rectum or throat.
- Chlamydia is often known as “Silent Infection”.
- That is because people with chlamydia not experiences symptoms at all.
- Chlamydia infections are asymptomatic (don’t cause noticeable infections).
- People who have chlamydia don’t have outward symptoms at early stages.
- In fact, 90% of women and 70% of men with chlamydia have no symptoms.
- It can cause serious permanent damage to the woman reproductive system which includes pelvic inflammatory disease (PID), ectopic pregnancy, infertility.
- It will be difficult or impossible for her to get pregnant later on.
- It spread by having by having unprotected vaginal, anal or oral sex and by mother to baby by, labour, and nursing.
- If a person is not treated for chlamydia, complications may occur ,woman frequently develop pelvic inflammatory diseae,PID can cause infertility(not being able to get pregnant)
- Chronic pelvic pain, continued spread of disease.
- Repeated infection of the eyes that go without treatment can result in trachoma a common cause of blindness.



❖ The genus chlamydia now contains 12 species,3

of them cause human diseases.

- Chlamydia trachomatis.
- Chlamydia Pneumoniae.
- Chlamydia Psittaci.

C.Trachomatis:

❖ C.trachomatis has 18 immunologically defined Serova's;

- A ,B ,Ba and C cause the eye disease trachoma.
- Trachoma is a chronic conjunctivitis .It was the most important cause of blindness.
- Infection can spread from eye to eye by fingers,
- Shared towels or cloths, coughing and sneezing.

- Symptoms:irritation,redness,lid swelling.
- New born can develop chlamydia eye infection
- Through childbirth.
- D through k can cause sexually transmitted
- Disease (STD).
- L1,L2,L3 cause STD that leads to invasive lymph
- Nodes disease.
- Maternal transmission of C.Trachomatis cause
- Neonatal conjunctivitis&

C.pneumoniae:

- C.pneumoniae can cause pneumonia (especially in children and adults)
- From 6 to 19% of community acquired cases are due to C.pneumoniae.
- C.pneumoniae infection is capable of causing chronic respiratory illness which leads to asthma.
- C.pneumoniae is found in atherosclerotic or normal vascular tissue.
- It is also found in cerebrospinal fluids of patients.

C.Psitacci:

- C.Psitacci causes psittacosis.
- Stains causing human disease are usually acquired from psittacine birds (eg.parrots) causing disseminated disease characterized by the pneumonitis.
- It is a rare cause of pneumonia (psittacosis) that is usually from psittacine birds.
- Chlamydia in joints
- Chlamydia also causes reactive arthritis
- Conjunctivitis.
- It can in both men and women though is more is common in men.

Chlamydia in infants

- Infants born to mother with chlamydia will be born with the disease.
- It can affect infants by causing premature birth,
- Conjunctivitis which may lead to blindness and Pneumonia.

- Conjunctivitis due to chlamydia typically occurs in one week after birth.

Causes:

- A chlamydia infection in the eye can occur through oral or genital contact with the eyes
- New born babies can acquire chlamydia from their mother during birth.
- Unprotected oral sex are the main ways a chlamydia infection can occur.

Diagnosis:

- Chlamydial infection can be diagnosed by ,
- Nucleic acid amplification(NAAT)
- Polymerase chain reaction(PCR)
- Transcription mediated amplification(TMA)
- Strand displacement amplification (SDA).

Transmission:

- Chlamydia is transmitted through vaginal,anal or sex or direct contact with infected person such as conjunctiva.
- Chlamydia is also passed from an infected mother to her baby during vaginal childbirth.

Symptoms in men:

- Many men don’t have symptoms. If symptoms do appear ,it is usually after
- 1 to 3 weeks after transmission.
- Burning sensation during urination.
- Pain in lower abdomen.
- Pain in the testicles.
- Sore throat.
- Cough or fever.

Symptoms in women:

- Vaginal discharge
- Burning sensation during urination.
- Pain in the lower abdomen

- Inflammation of the cervix.
- Bleeding between periods.

Treatment:

- Azithromycin & doxycycline are the antibiotics usually prescribed.
- Tetracyclines or macrolides are also used to treat this condition. These drugs are given intravenously or orally.
- For pregnant woman & children tetracycline should not be used .instead of that Erythromycin is recommended.
- Treatment should continue for 10 to 14 days.

BACTERIAL DISEASE

Introduction:

- Bacterial disease includes any type of illness caused by bacteria.
- It is a type of microorganism is too small and can be seen only with microscope.
- Majority of bacteria do not cause disease and are helpful and even necessary for good health. Such bacteria are known as Good Bacteria or Healthy Bacteria.
- Friendly intestinal bacteria are known as Probiotics. They help breakdown toxins & help to build immunity.
- Bacterial disease occur when pathogenic bacteria get into the body and begin to reproduce &help to build immunity.
- Harmful bacteria may also emit toxins &damage the body.
- One of the bacterial diseases with highest disease burden is tuberculosis caused by Mycobacterium tuberculosis bacteria which kills about 2 million people a year.
- Immunocompromised individuals are more susceptible to pathogenic bacteria.
- Elimination of uncooked wild fish from diets will help to reduce the possibility of mycobacterial infections.

Types of bacteria that causes diseases:

a. Myobacterium Tuberculosis:

- Causes: Tuberculosis
- Symptoms: fatigue, fever, night sweats, cough, weight loss, chest pain.
- Treated with antibiotics.

b. Clostridium Tetani:

- Causes: tetanus.
- Symptoms: muscle spasms, paralysis.
- Treated with antibiotics\vaccine.

c. Streptococcus Pneumoniae:

- Causes: pneumonia
- Symptoms: cough, fever, weight loss, chest pain.
- Treated with antibiotics.

d. Bacillus Anthrasis:

- Causes: Anthrax.
- Symptoms: rash, difficulty breathing.
- Through cuts\wounds.
- Treated with antibiotics.

e. Vibrio Cholera:

- Causes: cholera.
- Symptoms: diarrhea,vomiting,dehydration.
- Through drinking contaminated water.
- Treated with antibiotics.

f. Streptococcus Mutants:

- Causes: tooth decay.
- Bacteria live in mouth.
- Destroys enamel.
- It leads to tooth ache\ loss.

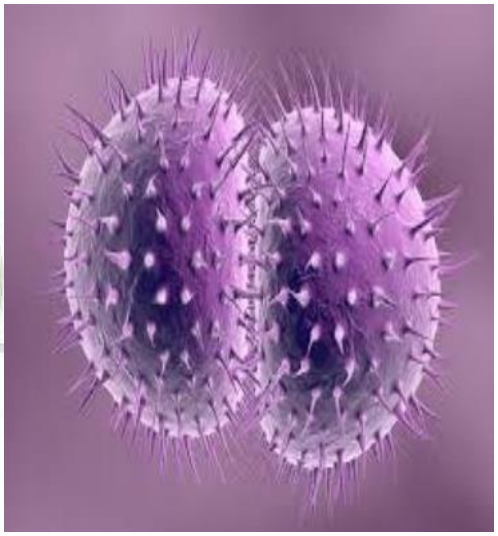
g. Neisseria Gonorrhoeae:

- Causes: gonorrhoea.
- Symptoms: painfull urination, damage to brain and spinal cord.
- Through sexual contact.
- Treated with antibiotics.

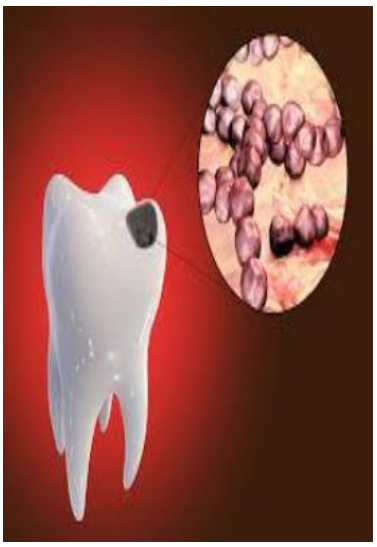
h. E.coli &Salmonella:

- Causes: food poisoning.
    i. Helicobacter pylori:
- Causes gastritis and ulcer.

E.coli &Salmonella

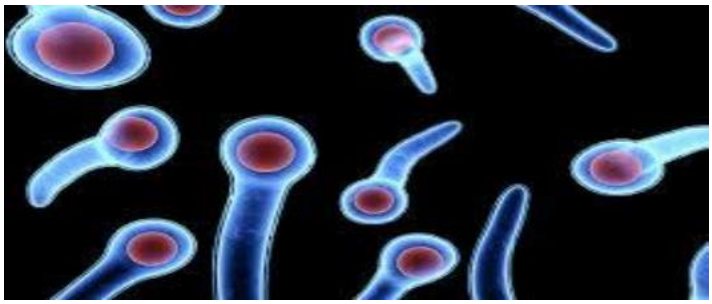


Neisseria Gonorrhoeae

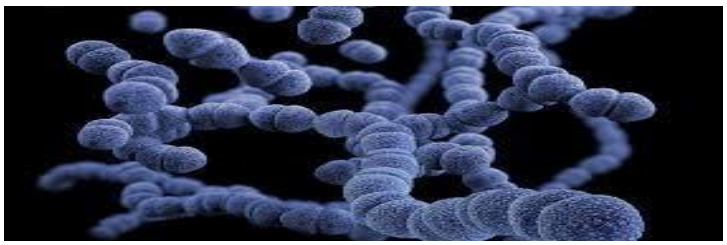


Streptococcus Mutants

Myobacterium Tuberculosis

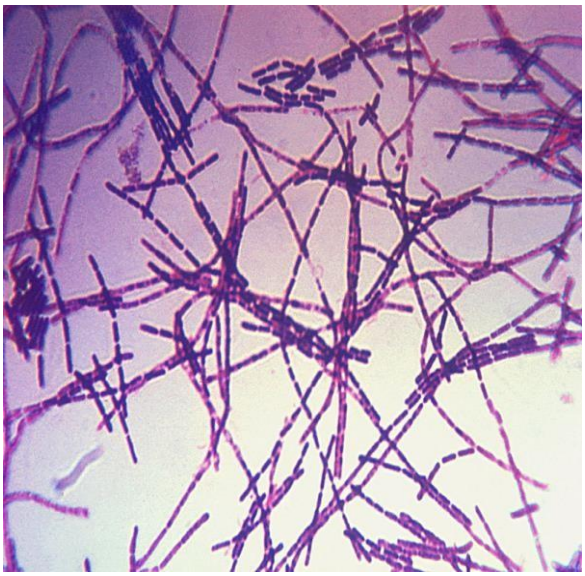


Clostridium tetani



Streptococcus Pneumoniae

Bacillus



Infections caused by bacteria are,

Syphilis:

- It is caused by the bacterium called Treponema Pallidum.
- Transmission of disease occurs through contact with infected person.
- Bacteria enter into the body through minor cuts or abrasion in your skin or mucous membranes.

Leprosy:

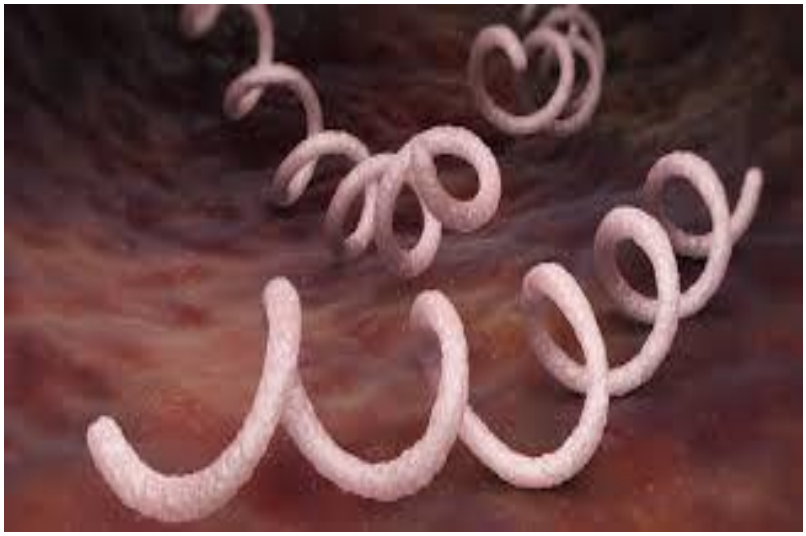
- It is caused by slow growing bacteria called Myobacterium Leprae.
- It is also known as Hansen ’s disease.
- It can affect nerves, skin, eyes, lining of the nasal mucosa.

Diphtheria:

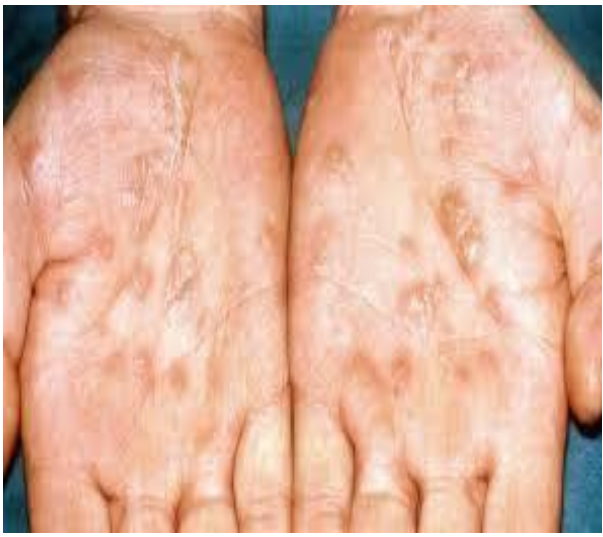
- Diphtheria is a serious infection caused by the strains of bacteria called Corynebacterium Diphtheria that can make toxins &cause people to get very sick.
- It spread from person to person through respiratory droplets,like from coughing or sneezing.

Typhoid :

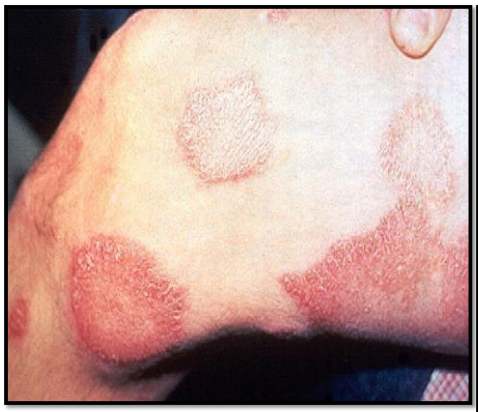
- It is caused by Salmonella Typhi ,which infect humans only.
- Typhoid fever is an infection that spread through contaminated food & water.
- Vaccines are recommended for this fever.



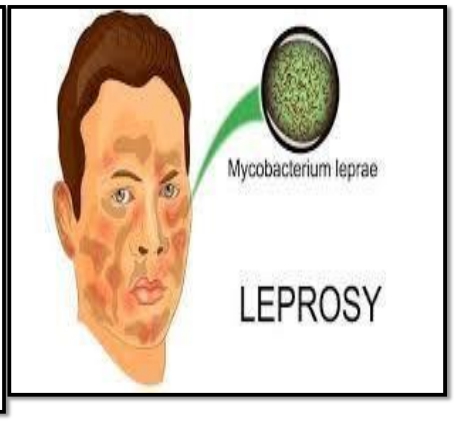
Treponema pallidum



Syphilis caused by Treponema pallidum



Leprosy caused by Myobacterium Leprae



Myobacterium Leprae

Symptoms:

- Diarrhoea
- Nausea &Vomiting
- Irritability
- Fatigue,Fever,Headache,cough,aches,pains.
- Weakness
- Abdominal pain

In Infants,

- Excessive sleeping.
- Excessive crying.
- Difficulty with feeding.

Causes:

- Eating contaminated food.

- Getting bitten by an a infected insect.
- Sharing needles for tattooing.
- Having sexual contact with infected person.
- Inhaling contaminated air borne droplets into the nose and lungs.

Risk factors:

- Eating eggs or meats that are raw or undercooked.
- Malnutrition.
- Not washing your hands frequently, especially after using bathroom.
- Eating expired foods or eating leftovers that have been stored for more than 2 to 3 days.
- Having a chronic disease.
- Significant exposure to a person with bacterial disease.

Complications:

- Coma.
- Kidney failure.
- Septicemia,which is a life threatening blood infection that can lead to a body wide response called sepsis.
- Severe dehydration.
- Electrolyte imbalance.
- Shock.

Treatment:

- Bacterial disease are treated with antibiotics.

Antibiotics work by killing the harmful bacteria or by stopping them from reproducing and spreading.

- General types of antibiotics include ,

❖ Aminoglycosides.
❖ Cephalosporin's.
❖ Macrolides.
❖ Penicillin's.
❖ Quinolones.
❖ Tetracycline's.

Treatment also includes,

- Good nutrition.
- Increased fluids.
- Hospitalization if complications occur.
- Rest.

MYCOPLASMA

INTRODUCTION: Mycoplasma (plural mycoplasmas or mycoplasmata) is a genus of bacteria that lack a cell wall around their cell membranes.This characteristic makes them naturally resistant to antibiotics that target cell wall synthesis (like the beta-lactam antibiotics). They can be parasitic or saprotrophic. Several species are pathogenic in humans, including M. pneumoniae, which is an important cause of "walking" pneumonia and other respiratory disorders, and M. genitalium, which is believed to be involved in pelvic inflammatory diseases. Mycoplasma species are the smallest bacterial cells yet discovered, can survive without oxygen, and come in various shapes. For example, M. genitalium is flask-shaped (about 300 x 600 nm), while M. pneumoniae is more elongated (about 100 x 1000 nm). Hundreds of mycoplasma species infect animals.It is known as mollicutes (mollis-soft ; cutis-skin)

ETYMOLOGY

The term mycoplasma, from the Greek mykes (fungus) and plasma (formed), was first used by Albert Bernhard Frank in 1889 to describe an altered state of plant cell cytoplasm resulting from infiltration by fungus-like microorganisms. Julian Nowak later proposed the genus name Mycoplasma for certain filamentous microorganisms imagined to have both cellular and acellular stages in their lifecycles, which could explain how they were visible with a microscope, but passed through filters impermeable to other bacteria. Later, the name for Mycoplasma was pleuropneumonia-like organisms (PPLO), broadly referring to organisms similar in colonial morphology and filterability to the causative agent (a mycoplasma) of contagious bovine pleuropneumonia.

SPECIES THAT INFECT HUMANS

Species of Mycoplasma, other than those listed below, have been recovered from humans, but are assumed to have been contracted from a non-human host. The following species use humans as the primary host:

- M. buccale
- M. faucium
- M. fermentans
- M. genitalium
- M. hominis
- M. incognitus
- M. lipophilum
- M. orale
- M. penetrans
- M. pirum
- M. pneumoniae

- M. primatum
- M. salivarium
- M. spermatoph

CHARACTERISTICS

Over 100 species have been included in the genus Mycoplasma. Microbes of the class Mollicutes, to which Mycoplasma belongs, are parasites or commensals of humans, animals, and plants. The genus Mycoplasma uses vertebrate and arthropod hosts. Dietary nitrogen availability has been shown to alter codon bias and genome evolution in Mycoplasma and Phytoplasma. Mycoplasmal bacteria are also known as mollicutes. They are the simplest and the smallest free-living prokaryotes. Mycoplasmal bacteria have been found in the pleural cavities of cattle suffering from pleuropneumonia. These organisms are often called MLO (mycoplasma-like organisms) or PPLO (pleuropneumonia-like organisms).

PATHOGENICITY

Produce surface infections - adhere (p1 pili) to the mucosa of respiratory, gastrointestinal & genitourinary tracts with the help of adhesin.Two types of diseases:

- Atypical Pneumonia
- Genital infections

IMPORTANT CHARACTERISTICS OF MYCOPLASMAL BACTERIA

1. Cell wall is absent and plasma membrane forms the outer boundary of the cell.
2. Due to the absence of cell wall these organisms can change their shape and are pleomorphic.
3. Lack of nucleus and other membrane-bound organelles.
4. Genetic material is a single DNA duplex and is naked.
5. Ribosomes are 70S type.

CELL MORPHOLOGY

Due to the lack of a rigid cell wall, Mycoplasmataceae can contort into a broad range of shapes, from round to oblong. They therefore cannot be classified as rods, cocci or spirochetes.

DISEASES CAUED BY MYCOPLASMA

Organism	Disease
M. pneumoniae	Upper respiratory tract disease, tracheobronchitis, atypical pneumonia, (chronic asthma?)
M. hominis	Pyelonephritis, pelvic inflammatory disease, postpartum fever
M. genitalium	Nongonococcal urethritis
U. urealyticum	Nongonococcal urethritis, (pneumonia and chronic lung disease in premature infants?)

LABORATORY DIAGNOSIS

Specimens – throat swabs, respiratory secretions.

Microscopy :

1. Highly pleomorphic, varying from small spherical shapes to longer branching filaments.

2. Gram negative, but better stained with Giemsa,Dienes' stain, crystal-fast violet, orcein or fluorochroming with nucleic acid stain as acredine orange.

LABORATORY FINDINGS OF MYCOPLASMA

- Fried egg colonies (2weeks)…not preferred
- Cold agglutination test…preferred…give quick results.

GENITAL INFECTIONS

- Caused by M. hominis & U. urealyticum
- Transmitted by sexual contact
- Men - Nonspecific urethritis, proctitis, balanoposthitis & Reiter's syndrome
- Women – acute salpingitis, PID, cervicitis, vaginitis

TREATMENT

- Tetracycline, Erythromycin & Clarithromycin – drug of choice
- Resistant to antibiotics which interfere with bacterial cell wall synthesis.
- Newer macrolides & quinolones being used now.

RICKETTSIA

Rickettsia is a genus of nonmotile, Gram-negative, nonspore-forming, highly pleomorphic bacteria that may occur in the forms of cocci (0.1 μm in diameter), bacilli (1–4 μm long), or threads (up to about 10 μm long). The term "rickettsia" has nothing to do with rickets (which is a deficiency disease resulting from lack of vitamin D); the bacterial genus Rickettsia was named after Howard Taylor Ricketts, in honor of his pioneering work on tick-borne spotted fever.

RICKETTSIAL CHARACTERISTICS

- ✓ Obligate intracellular parasite
- ✓ Gram negative pleomorphic bacteria
- ✓ Most are zoonoses spread to humans by arthropods
- ✓ Cannot grow in culture media but cultivable only in living tissue
- ✓ No human to human transmission

PATHOPHYSIOLOGY

Rickettsial organisms are obligate intracellular parasites and invade vascular endothelial cells in target organs, damaging them and producing increased vascular permeability with consequent oedema, hypotension, and hypoalbuminaemia

RICKETTSIAL INFECTIONS (Classified into groups)

- Typhus group
- Spotted fever group
- Scrub typhus

TYPES OF RICKETTSIAL DISEASE

Types of Rickettsial Diseases

Diseases	Rickettsial agent	Insect vectors	Mammalian reservoirs
Typhus group			
a. Epidemic typhus	*R.prowazekki*	Louse	Humans
b. Murine typhus	*R.typhi*	Flea	Rodents
c. Scrub typhus	*O.tsutsugamushi*	Mite	Rodents
Spotted fever group			
a. Indian tick typhus	*R.conorii*	Tick	Rodents, dogs
b. Rocky Mountain spotted fever	*R.rickettsii*	Tick	Rodents, dogs
c. Rickettsial pox	*R.akari*	Mite	Mice
Others			
a. #Q fever	*C.brunetti*	Nil	Cattle, sheep, goats
b. Trench fever	*Rochalimaea Quintana*	Louse	Humans

EPIDEMIC TYPHUS

Rickettsial agent: - *Rickettsia prowazekii*

Insect vector :- Louse

Mammalian Reservoir :- Human, flying squirrels

Transmission :-

Human to human via louse vector, directly in blood, or as the contaminated louse feces is scratched into the bite wound, or inhalation of infected louse feces or dust.

Incubation period: 5-21 days

Symptoms are:

Headache, myalgia, gangrene

CONTROL MEASURES

A. Diagnosis:- based on clinical suspicion with Serology
B. Treatment:- Doxycycline (DOC)
C. Preventive measures:-
Delousing - insecticides
Improvement of personal hygiene and living conditions

ENDEMIC TYPHUS

- Rickettsial agent: -*Rickettsia typhi*
- Insect Vector: Flea
- Mammalian Reservoir: Rodents
- Transmission :-
inhalation of infected louse feces or dust.
inoculation into skin with feces of infected
- Incubation period: 1-2 weeks
- Symptoms:
Gradual onset- fever,
Headache, myalgia, cough
- Rash: > 55% maculopapular rash on trunk

CONTROL MEASURES

A. Diagnosis:- liver enzyme elevated

B. Treatment :- Doxycycline (DOC)

C. Preventive measures :-
Residual insecticides – BHC, Malathion
Rodent control measures in infested areas

SCRUB TYPHUS

- Rickettsial agent : *Orientia tsutugamushi*
- Insect Vector: Mite infective larvae CHIGGERS
- Transmission :- larval forms - chiggers found in areas of scrub vegetations.

  Common in military and Jungle warfare, farmers
- Mammalian Reservoir: -Rodents
- Incubation period: -10-12 days

CONTROL MEASURES

Treatment:- tetracycline (DOC)

Vector control:-

Clearing the vegetation where rats and mice lives

Application of insecticides:- Lindane, Chlordane to the ground and vegetation

Personal prophylaxis :-

Impregnating clothes and blankets with miticial chemicals, i.e. benzyl benzoate.

Mite repellant:- Diethyl toluamide application on exposed skin surfaces.

ROCKY MOUNTAIN SPOTTED FEVER

Rickettsial agent:- R. rickettsii
Insect Vector: - Tick
Mammalian Reservoir: -Rodents, dogs
Incubation period:- 3-7 days
Symptoms:- Abrupt onset fever, chills, headache, myalgia
Rash : first appears in extremities, moves centripetally and involve palm.
Mortality: 70% if left untreated in elderly
Complications:- HSM, jaundice, myocarditis, uremia, ARDS

CONTROL MEASURES

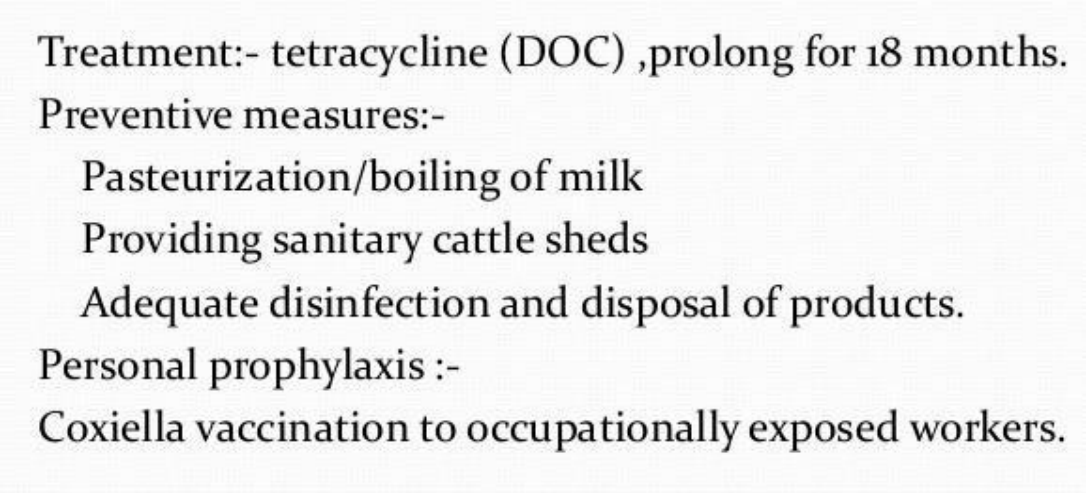
Treatment :- tetracycline (DOC)

Personal prophylaxis :-
Tick infected area should avoided
Disinfection of dogs
Health education about mode of transmission
Clearing the vegetation where rats and mice lives

Q-FEVER

Etiology: *Coxiella burnetti*
Vector : None
Reservoir: Cattle, sheep, goat
MOT: ingestion of dust containing organisms or aerosols excreted in urine, feces, milk etc.
I.P:- 2-3 wks
C/F:- resembles influenza or non bacterial pneumonia
Individuals at risk : food handlers, veterinarians
Infective endocarditis occasionally in chronic Q fever

CONTROL MEASURES



TREATMENT FOR RICKETTSIAL DISEASE

- ✓ Tetracylines
- ✓ Doxycyclines
- ✓ Early treatment critical
- ✓ Prolonged therapy for severe and complicated cases.

FUNGAL DIASEASE

● Fungal infections can affect anyone, and they can appear on several parts of the body. A jock with athlete's foot, a baby with thrush, and a woman with a vaginal yeast infection are just a few examples.

● Fungi are eukaryotic microorganisms characterized by a substance in their cell walls called chitin. Some fungi, like many types of mushrooms, are edible. Other types of fungi, like aspergillus, can be extremely dangerous and lead to life-threatening diseases. A fungal infection is also known as mycosis.

● Fungal infections can be contagious. They can spread from one person to another. In some cases, we can also catch disease-causing fungi from infected animals or contaminated soil or surfaces.

Classification of fungi:

1. Mushroom
2. Yeast
3. Mold

Some common types of fungal infection include:

- athlete's foot

- jock itch

- ringworm

- yeast infection

- onychomycosis, or a fungal infection of the nail

Some types of fungi don't normally cause infections in humans but can cause sickness in people with weakened immune systems. These are called opportunistic infections.

1. **Athlete'sfoot**:

Athlete's foot is also known as tineapedis. It's a type of fungal infection that can affect the skin on feet, as well ashands and nails. The infection is caused by dermatophytes, a group of fungi that can thrive in the warm and humid areas betweentoes.

It's particularly common among athletes and can spread from one person to another. People can also catch it from contaminated surfaces, like a public shower.

Symptoms:

Athlete's foot can cause an itching, stinging, or burning sensation between toes or on other parts of foot. Skin might also crack, peel, or blister.

Diagnosis:

Doctor may recognize athlete's foot by looking at the symptoms on skin. Also,a small area of the skin can be scraped off and tested for the fungus.

Treatment:

There are several topical over-the-counter (OTC) antifungal medications you can be used to treat athlete's foot.

2. **Jock itch:**

Jock itch is also known as tineacruris. It's a fungal infection that can affect the skin on groin area, as well as inner thighs. Like athlete's foot, it's caused by dermatophytes, a group of fungi that thrive in warm and humid areas.This type of infection mostly affects men and boys.

Symptoms:

Common jock itch symptoms include:

- redness
- itchiness
- a burning feeling
- changes in skin color
- flaking or cracking skin

- a rash that gets worse when you exercise

Diagnosis:

Doctor can able to recognize jock itch by looking at the affected skin. To help rule out other conditions, like psoriasis, they may take a scraping of skin cells and have them examined.

Treatment:

Jock itch can usually be treated at home by keeping the area clean and dry and applying OTC antifungal cream, powder, or spray.

3. Ringworm:

Ringworm is a fungal infection that can affect your skin and scalp. Similar to athlete's foot and jock itch, it's caused by dermatophytes. Ringworm is also part of a group of fungi that grow on skin, particularly in damp and humid parts of your body.

Symptoms:

It usually starts as a reddish, itchy, scaly rash. Over time, patches of ringworm can spread and form red rings.

Other signs include:

- patches that get blisters and start to ooze
- bald patches on the scalp
- patches that look like rings with a redder outside edge

Diagnosis:

A simple skin examination can find ringworm. The fungus glows under a black light. A small sample of the affected skin can also be scraped off and sent to a lab for testing.

Treatment:

Like jock itch and athlete's foot, ringworm is often able to be successfully treated with OTC antifungal creams, sprays, gels, or ointments.

4. Yeast infection:

Candida albicans is a type of fungus that can infect skin, mouth, gastrointestinal tract, urinary tract, or genitals.

It’s normal for small amounts of candida albicans to be present on your skin and in your body. But when these fungi multiply too much, they can cause an infection known as a yeast infection.

Symptoms:

If you get a yeast infection in your throat or mouth, it’s called oral thrush. Thrush causes white patches to form in your mouth and throat. People who undergo prolonged antibiotic therapy often develop this type of infection.

In women, vaginal yeast infections are relatively common. They can cause:

- pain
- itchiness
- clumpy discharge
- swelling
- redness

Diagnosis:

To check for oral thrush, doctor use a throat swab to rub the affected areas. Doctor can send the swab to a lab, where technicians will culture it to learn what types of fungi or other microbes are present.

Treatment:

Treatment options will depend on the type of yeast infection.

Thrush can be treated with oral antifungal medications. These can come in the form of lozenges, pills, or mouthwash.

5. Onychomycosis:

Onychomycosis is a common type of fungal infection that affects toenails, fingernails, and nail beds. It’s also known as tineaunguium.

Symptoms:

Toenail fungus usually starts as a small light-colored spot on the nail. As it spreads deeper, it changes the shape and color of nail. Over time, it can cause nail to become thicker and more brittle.

Common signs include:

- scaling under the nail
- white or yellow streaks under the nail
- flakiness or crumbling of the nail
- thick or brittle nail
- lifting off the nail bed

Diagnosis:

Doctor will likely scrape off pieces of the affected nail. They will examine these scrapings under a microscope.

This can help them tell the difference between a fungal infection and other conditions that cause similar symptoms.

Treatment:

It can potentially take weeks to treat fingernail infections and months to treat toenail infections.OTC medications typically aren't effective. Doctor may prescribe a nail lacquer that's brushed on like nail polish or an antibiotic to take by mouth.

Other fungal disease includes:

● Blastomycosis–caused by Blastomyces

● Cryptococcus gattiinfection –caused by Cryptococcus gatti

● Histoplasmosis –caused by Histoplasma

● Pneumocystis pneumonia – caused by Pneumocystis jirovecii

● Sporotrichosis – caused by Sporothrix

PROTOZOAL DISEASE

● Protozoa are unicellular, eukaryotic, heterotrophic organisms. They are either free-living or parasites.They lack a cell wall.

● The protozoa have many stages in their life cycle. Some of the stages of the life cycle are infectious.The cyst stage is dormant and resistant to environmental stress, the trophozoite stage is reproductive and causes disease. Protozoa are heterotrophic and have holozoic nutrition.

Protozoa Classification:

1. **Mastigophora or Flagellates:** They are parasites or free-living.

   - They have flagella for locomotion.
   - Examples: Trypanosoma, Trichomonas, Giardia, Leishmania, etc.

2. **Sarcodina or Amoeboids:** They live in the freshwater, sea or moist soil.

   - The movement is by pseudopodia.
   - Examples: Amoeba, Entamoeba, etc.

3. **Sporozoa or Sporozoans:** They are endoparasitic.

   - They don't have any specialised organ for locomotion.
   - Examples: Plasmodium, Myxidium, Nosema, Globidium, etc.

4. **Ciliophora or Ciliates**: They are aquatic and move actively with the help of thousands of cilia.

   - They have fixed shape due to covering of pellicle.
   - Examples: Paramoecium, Vorticella, Balantidium, etc.

Protozoal Diseases:

1. **Malaria:**

Malaria is a life-threatening disease. It's typically transmitted through the bite of an infected Anopheles mosquito. Infected mosquitoes carry the Plasmodium parasite. When this mosquito bites another person , the parasite is released into bloodstream.The parasites travel to the liver, where they mature. After several days, the mature parasites enter the bloodstream and begin rupture to red blood cells.It releases a toxic substance called hemozoinwhich causes fever. The sporozoite is the infectious stage.

There are four kinds of malaria parasites that can infect humans: Plasmodium vivax, P. ovale, P. malariae, and P. .falciparum. P.falciparumcauses a more severe form of the disease.

Malaria is transmitted by blood, so it can also be transmitted through:

- an organ transplant
- use of shared needle or syringes
- a transfusion
- mother to baby by feeding

Symptoms:

Common symptoms of malaria include,

- shaking chills that can range from moderate to severe
- fever
- sweating
- headache
- nausea
- vomiting
- abdominal pain
- diarrhea
- anaemia

Malaria can cause a number of life-threatening complications. The following may occur:

- swelling of the blood vessels of the brain, or cerebral malaria
- organ failure of the kidney, liver or spleen
- anemia due to the destruction of red blood cells
- Low blood sugar

5. **Amoebiasis or amoebic dysentery:**

E. histolytica is a single-celled protozoan that usually enters the human body when a person ingests cysts through food or water. It can also enter the body through direct contact with fecal matter.

When cysts enter the body, they lodge in the digestive tract. They then release an invasive, active form of the parasite called a trophozite. The parasites reproduce in the digestive tract and migrate to the large intestine.This causes bloody diarrhea, colitis, and tissue destruction.

Symptoms:

- abdominal pain
- bloody stool
- loss of appetite
- nausea
- fever

2. **Balantidiasis:**

Balantidiasis is a food borne illness that is caused by **Balantidium coli**, which is a microscopic protozoan found in feces. People contract balantidiasis by eating food or drinking water that has been contaminated by feces that contain this microorganism. Additionally, people who handle or live near pigs are at an increased risk for contracting this illness because the Balantidium coli microorganisms is often found in pig feces.

Symptoms:

- Intermittent diarrhea
- Constipation
- Vomiting
- Abdominal pain
- Anorexia
- Weight loss
- Headache
- Colitis
- Marked fluid loss

3. **Leishmaniasis:**

Leishmaniasis is a parasitic disease caused by the Leishmania parasite. This parasite typically lives in infected sand flies. People can contract leishmaniasis from a bite of an infected sand fly. The sand flies that carry the parasite typically reside in tropical and subtropical environments.

Leishmaniasis comes in three forms: cutaneous, visceral, and mucocutaneous. Different species of the Leishmania parasite are associated with each form. Cutaneous leishmaniasis causes ulcers on your skin.

With this type of mucocutaneousleishmaniasis, the parasites spread to nose, throat, and mouth. This can lead to partial or complete destruction of the mucous membranes in those areas.

Visceral leishmaniasis is sometimes known as systemic leishmaniasis or kala azar.It usually occurs two to eight months after being bitten by a sand fly. It damages internal organs, such as spleen and liver.

Symptoms:

- Weight loss
- weakness

- Fever that lasts for weeks or months
- enlarged spleen and liver
- decreased production of blood cells
- bleeding
- other infections
- swollen lymph nodes

4. **Trypanosomiasis:**

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. It is caused by infection with protozoan parasites belonging to the genus Trypanosoma. They are transmitted to humans by tsetse fly (Glossina genus) .

Human African trypanosomiasis takes 2 forms, depending on the parasite involved:

- Trypanosomabruceigambiense - A person can be infected for months or even years without major signs or symptoms of the disease.
- Trypanosomabruceirhodesiense - First signs and symptoms are observed a few months or weeks after infection. The disease develops rapidly and invades the central nervous system.

Diagnosis must be made as early as possible to avoid progressing to the neurological stage in order to elude complicated and risky treatment procedures.

Symptoms:

- High fever
- Muscle and joint pain
- Headache
- Weakness
- Fatigue
  If left untreated neurological problems develop, which becomes fatal.

5. **Trichomoniasis:**
   It is caused by a one-celled protozoan organism called Trichomonasvaginalis. It is sexually transmitted disease.As an extracellular pathogen, the parasite mediates adherence to epithelial cells to colonize the human host. In addition, the parasite interfaces with the host immune system and the vaginal microbiota.

Symptoms:

- vaginal spotting or bleeding
- genital burning itching

- genital redness or swelling
- frequent urge to urinate.

Treatment:

Antiprotozoal drugs are used to treat protozoal diseases.They includes,

- Anti-malarial drugs:

Mefloquine, chloroquine, deoxycycline.

- Anti-amoebic drugs:
  Tissue amoebicides, Luminal amoebicides
- Drugs used for Leishmaniasis:
  Amphotericin, Paromomycin, Miltefosine.
- Drugs for Trichomoniasis:
  Metronidazole, Providone- iodine.

HELMINTHIASIS

Helminthiasis, also known as worm infection, is any macroparasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as helminths. There are numerous species of these parasites, which are broadly classified into tapeworms, flukes, and roundworms. They often live in the gastrointestinal tract of their hosts, but they may also burrow into other organs, where they induce physiological damage.

Soil-transmitted helminthiasis and schistosomiasis are the most important helminthiases, and are among the neglected tropical diseases. Helminthiasis has been found to result in poor birth outcome, poor cognitive development, poor school and work performance, poor socioeconomic development, and poverty. Chronic illness, malnutrition, and anemia are further examples of secondary effects.

Soil-transmitted helminthiases are responsible for parasitic infections in as much as a quarter of the human population worldwide. One well-known example of soil-transmitted helminthiases is ascariasis.

Signs and symptoms

The signs and symptoms of helminthiasis depend on a number of factors including: the site of the infestation within the body; the type of worm involved; the number of worms and their volume; the type of damage the infesting worms cause; and, the immunological response of the body. Where the burden of parasites in the body is light, there may be no symptoms. Certain worms may cause particular constellations of symptoms. For instance, taeniasis can lead to seizures due to neurocysticercosis.

Helminths types causing infections

Of all the known helminth species, the most important helminths with respect to understanding their transmission pathways, their control, inactivation and enumeration in

samples of human excreta from dried feces, faecal sludge, wastewater, and sewage sludge are:

- soil-transmitted helminths, including Ascaris lumbricoides (the most common worldwide), Trichuris trichiura, Necator americanus, Strongyloides stercoralis and Ancylostoma duodenale
- Hymenolepis nana
- Taenia saginata
- Enterobius
- Fasciola hepatica
- Schistosoma mansoni
- Toxocara canis
- Toxocara cati

Helminthiases are classified as follows (the disease names end with "-sis" and the causative worms are in brackets):

Roundworm infection (nematodiasis)

- Filariasis (Wuchereria bancrofti, Brugia malayi infection)
- Onchocerciasis (Onchocerca volvulus infection)
- Soil-transmitted helminthiasis – this includes ascariasis (Ascaris lumbricoides infection, trichuriasis (Trichuris infection), and hookworm infection (includes necatoriasis and Ancylostoma duodenale infection)
- Trichostrongyliasis (Trichostrongylus spp. infection)
- Dracunculiasis (guinea worm infection)
- Baylisascaris (raccoon roundworm, may be transmitted to pets livestock and humans)

Tapeworm infection (cestodiasis)

- Echinococcosis (Echinococcus infection)
- Hymenolepiasis (Hymenolepis infection)
- Taeniasis/cysticercosis (Taenia infection)
- Coenurosis (T. multiceps, T. serialis, T. glomerata, and T. brauni infection)

Trematode infection (trematodiasis)

- Amphistomiasis (amphistomes infection)
- Clonorchiasis (Clonorchis sinensis infection)
- Fascioliasis (Fasciola infection)
- Fasciolopsiasis (Fasciolopsis buski infection)
- Opisthorchiasis (Opisthorchis infection)
- Paragonimiasis (Paragonimus infection)
- Schistosomiasis/bilharziasis (Schistosoma infection)

Acanthocephala infection

- Moniliformis infection

Transmission

Helminths are transmitted to the final host in several ways. The most common infection is through ingestion of contaminated vegetables, drinking water, and raw or undercooked meat. Contaminated food may contain eggs of nematodes such as Ascaris, Enterobius, and Trichuris; cestodes such as Taenia, Hymenolepis, and Echinococcus; and trematodes such as Fasciola. Raw or undercooked meats are the major sources of Taenia (pork, beef and venison), Trichinella (pork and bear), Diphyllobothrium (fish), Clonorchis (fish), and Paragonimus (crustaceans). Schistosomes and nematodes such as hookworms (Ancylostoma and Necator) and Strongyloides can penetrate the skin directly. Finally, Wuchereria, Onchocerca, and Dracunculus are transmitted by mosquitoes and flies.

Diagnosis

Specific helminths can be identified through microscopic examination of their eggs (ova) found in faecal samples. The number of eggs is measured in units of eggs per gram. However, it does not quantify mixed infections, and in practice, is inaccurate for quantifying the eggs of schistosomes and soil-transmitted helminths. Sophisticated tests such as serological assays, antigen tests, and molecular diagnosis are also available; however, they are time-consuming, expensive and not always reliable

Prevention

Disrupting the cycle of the worm will prevent infestation and re-infestation. Prevention of infection can largely be achieved by addressing the issues of WASH—water, sanitation and hygiene. The reduction of open defecation is particularly called for, as is stopping the use of human waste as fertilizer. Further preventive measures include adherence to appropriate food hygiene, wearing of shoes, regular deworming of pets, and the proper disposal of their feces. Scientists are also searching for a vaccine against helminths, such as a hookworm vaccine.

Treatment

Broad-spectrum benzimidazoles (such as albendazole and mebendazole) are the first line treatment of intestinal roundworm and tapeworm infections. Macrocyclic lactones (such as ivermectin) are effective against adult and migrating larval stages of nematodes. Praziquantel is the drug of choice for schistosomiasis, taeniasis, and most types of food-borne trematodiases. Oxamniquine is also widely used in mass deworming programmes. Pyrantel is commonly used for veterinary nematodiasis. Artemisinins and derivatives are proving to be candidates as drugs of choice for trematodiasis.

UNIT- V

IDENTIFICATION OF DISEASE PRODUCING ORGANISMS

INTRODUCTION- DISEASE:

- Disease may be defined as abnormality of the structure or function of a part, organ, or system.
- The effects of a disease may be felt by a person or observed by others.
- Diseases may be of known or unknown causes and may show marked variation in severity and effects on an individual.

CATEGORIES OF DISEASE

- Infection
- Degenerative diseases
- Nutritional disorders
- Metabolic disorders
- Immune disorders
- Psychiatric disorders
- Neoplasms

CAUSES OF DISEASE

- Age
- Gender
- Heredity
- Living conditions and habits
- Emotional disturbance

- Physical and chemical damage
- Preexisting illness

STUDY OF DISEASE—pathophysiology

A. Disease terminology

1. Etiology—study of origin or causation

2. Terms related to severity and duration

a. Acute—severe, of short duration

b. Chronic—less severe, of long duration

c. Subacute—intermediate, between acute and chronic

3. Idiopathic—of unknown cause

4. Iatrogenic—results from adverse effects of treatment

5. Epidemiology—study of diseases in populations a. Statistics

(1) Incidence—number of new cases in a population during specific time

(2) Prevalence—number of cases in a population at a given time

(3) Mortality rate—percentage of the population that dies from disease within a given period

b. Categories

(1) Epidemic—widespread in a given region

(2) Endemic—found at lesser level but continuously in a population

(3) Pandemic—prevalent throughout an entire country or the world

TREATMENT AND PREVENTION OF DISEASE

A. Diagnosis—determination of the nature of the illness

1. Symptom—change in body function felt by the patient

2. Sign—change in body function observable by others

3. Syndrome—group of signs and symptoms that characterize a disease

B. Prognosis—prediction of probable outcome of disease

C. Therapy—course of treatment

D. Complementary and alternative medicine (CAM)

E. Prevention of disease—removal of potential causes of disease

INFECTIOUS DISEASE

A. Parasite—organism that lives on or within a host at host's expense

B. Pathogen—disease-causing organism

C. Infection—invasion by pathogens with adverse effects

1. Local—small area

2. Systemic—generalized; usually spread by blood

D. Opportunistic infection—takes hold in a weakened host

E. Communicable infection—can be spread from person to person; is contagious

F. Modes of transmission

1. Direct contact

2. Indirect—touched objects, air, pests a. Vector—animal that transfers organisms from host to host

3. Portals of entry and exit—skin, respiratory, digestive, urinary, and reproductive systems

MICROBIOLOGY—STUDY OF MICROORGANISMS

A. Normal flora—population of microorganisms normally growing on and within the body

B. Bacteria:

a. Oxygen requirements—aerobic, anaerobic, facultative anaerobes

b. Endospores—resistant forms

c. Flagella—used for swimming

d. Pili—short, threadlike; used for attachment

2. Shape and arrangement

a. Cocci—round

(1) Diplococci—pairs (2) Streptococci—chains (3) Staphylococci—clusters

b. Bacilli—straight rods; some produce endospores

c. Curved rods (1) Vibrios—comma shaped (2) Spirilla—corkscrew or wavy (3) Spirochetes—flexible spirals

3. Other bacteria—obligate intracellular parasites a. Rickettsiae b. Chlamydiae

C. Viruses

1. Contain only nucleic acid and protein

2. Obligate intracellular parasites

4. Infectious agents smaller than viruses a. Prions—contain only protein; cause slowgrowing brain diseases

b. Viroids—contain only RNA

D. Fungi—simple, plantlike organisms

1. Yeasts—single cell

2. Molds—filamentous

E. Protozoa—single-cell, animal-like organisms

1. Amebas—dysentery

2. Ciliates

3. Flagellates—African sleeping sickness.

PARASITIC WORMS (HELMINTHS)

A. Roundworms

1. Ascaris

2. Pinworms

3. Hookworms

4. Trichina—transmitted in undercooked meat

5. Filaria—causes filariasis (elephantiasis)

B. Flatworms

1. Tapeworms

2. Flukes

IDENTIFICATION OF PATHOGENS

A. Collection of specimens; accurate labeling and prompt delivery to lab

B. Bacterial isolations and tests

C. Staining techniques (e.g., Gram's, acid-fast)

D. Other methods of identification

1. Growth characteristics

2. Oxygen requirements

3. Fermentation

4. Reactions to test chemicals

5. Serologic (immunologic) tests

MICROBIAL CONTROL

A. Microbes and public health

1. Sewage and garbage disposal

2. Water purification

3. Prevention of food contamination

4. Milk pasteurization

B. Aseptic methods

1. Sterilization—killing of all organisms

2. Disinfection—destruction of all pathogens

3. Antisepsis—pathogens killed or prevented from multiplying (bacteriostasis); safe for living tissue

C. Emergence and spread of microorganisms—factors related to population growth, technology

D. Infection control techniques 1. Body substance precautions (body substance isolation) a. Assume all body fluids potentially infective b. Barriers—gloves, masks, eye protection, gowns c. Handwashing

ANTIGEN-ANTIBODY TECHNIQUE

INTRODUCTION:

- The antigens and the antibodies combine specifically with each other. This interaction between them is called Antigen-Antibody reaction.
- It may be abbreviated as Ag – Ab reaction.

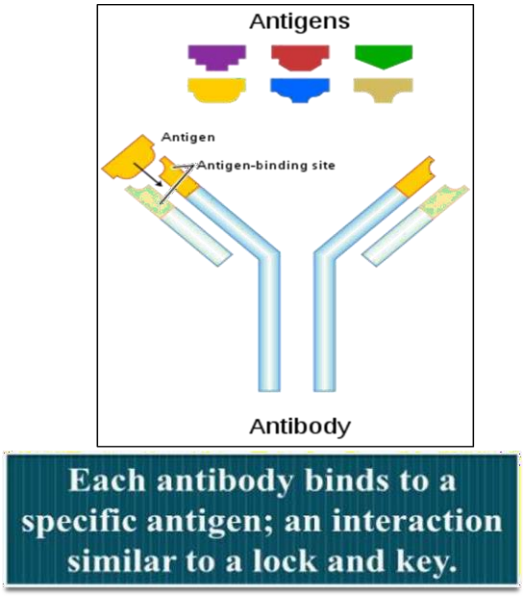
- These form the basis for humoral immunity or antibody mediated immunity.
- These reactions form the basis for detection of infectious disease causing agents and also some non- specific Ag's like enzymes.
- When Ag – Ab reactions occur invitro, they are known as serological reactions.
- The reactions between Ag and Ab occur in three stages.
    - In first stage the reaction involves formation of Ag-Ab complex.
    - The second stage leads to visible events like precipitation, agglutination etc.
    - The third stage includes destruction of Ag or its neutralization

Salient Features of Antigen – Antibody Reaction:

- Specificity of Antigen – Antibody Reaction.
- Immune complex.
- Binding Site of Antigen – Antibody Reaction.
- Binding Force of Antigen – Antibody Reaction.

Specificity of Antigen – Antibody Reaction:

- Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen.



- For example, the antibody produced against kidney antigen will react with only kidney- antigen. A standard lock can be opened by its own key only as one antibody can react with its own antigen.

Immune Complex:

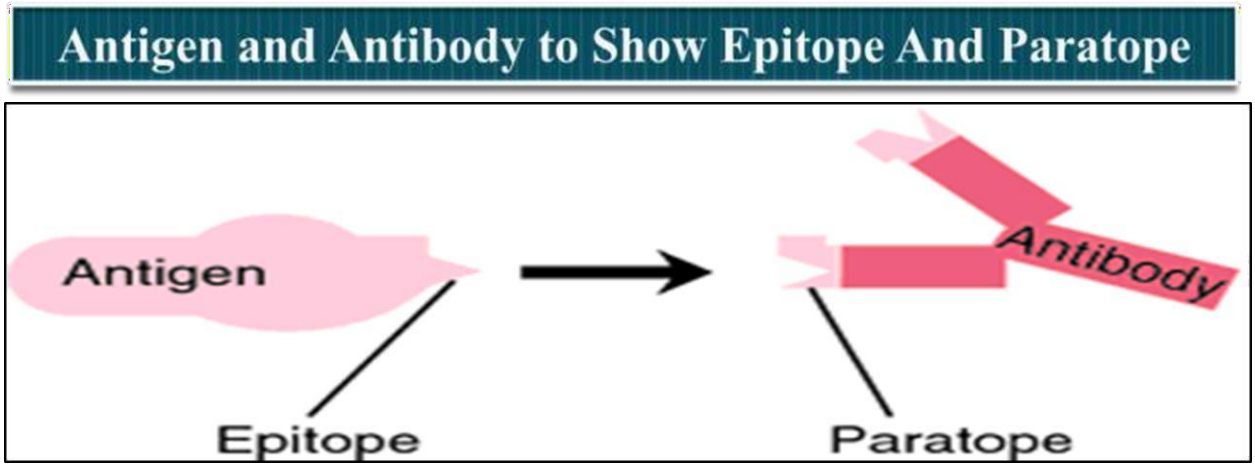
- An immune complex is formed from the integral binding of an antibody to a soluble antigen.
- The bound antigen acting as a specific epitope, bound to an antibody is referred to as a singular immune complex.

$$Ag + Ab \longrightarrow Ag\text{-}Ab \text{ complex}$$

Binding Site of Antigen – Antibody Reaction:

- In antigen - antibody reaction, the antibody attaches with the antigen.
- The part of antigen which combines with antibody is called Epitope.
- An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells.

- The part of an antibody that recognizes the epitope is called a paratope.



Binding Force of Antigen – Antibody Reaction:

- The binding between antigen and antibody in ag – ab reaction is due to three factors namely:
  - Closeness between antigen and antibody.
  - Non – covalent bonds.
  - Affinity of antibody.
- **Closeness between antigen and antibody:** When antigen and antibody are closely fit, the strength of binding is great. When they are apart binding strength low.
- **Non – Covalent Bonds:** The bonds that hold the antigen to the antibody combining site are all non- covalent in nature. These include hydrogen bonds, elec Van der Waals forces and hydrophobic bonds.
- **Affinity of antibody:** Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody.

Properties of Antigen – Antibody Reaction:

The properties of antigen and antibody can be explained with the help of three points. They are:

- Antibody Affinity.
- Antibody Avidity
- Cross reaction.

Affinity of Antibody

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody.

Antibody Avidity

- It is the strength of the bond after the formation of Ag-Ab complexes.
- It is used to denote the overall capacity of antibodies to combine with the multivalent antigen.
- A multivalent Ag has many types of antigenic determinants.
- When injected into the blood, each antigenic determinant stimulates the production of a particular antibody.

Avidity is determined by 3 factors:
1. The binding affinity
2. The valency
3. The structural arrangement

Cross Reaction:

An antiserum raised against an Ag, can also react with a similar Ag of another type. This is called cross reaction and the Ag which produces the cross reaction is called Cross reactive Ag. But the strength of Ab raised against its own Ag is strong. For cross reaction, slow.

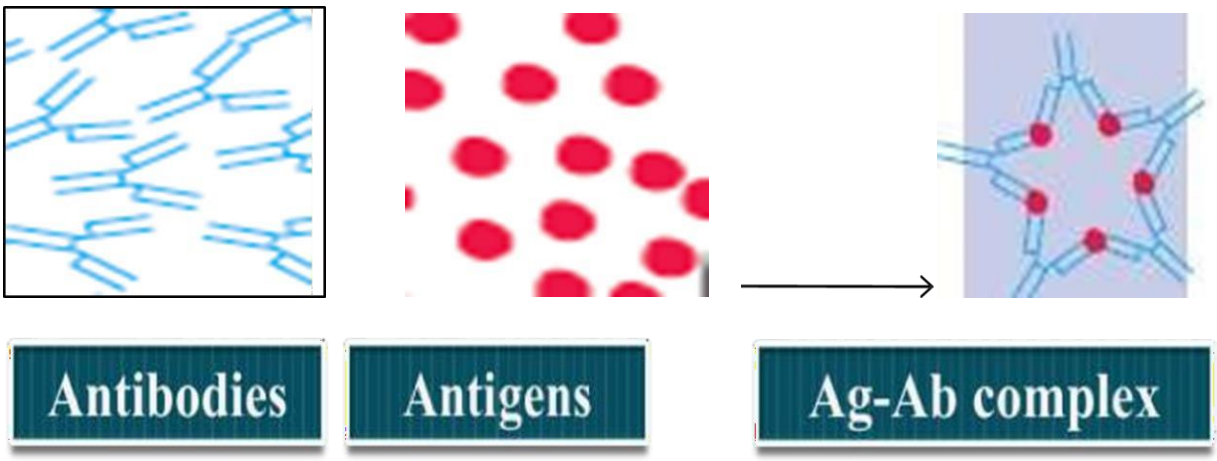
Types of Antigen – Antibody Reaction:

The types of antigen – antibody reactions are:

- Precipitation Reaction.
- Agglutination Reaction.
- Complement Fixation.
- ELISA – Enzyme Linked ImmunoSorbent Assay.
- Immunofluorescence.

Precipitation Reaction

When a soluble Ag combines with its Ab in the presence of an electrolyte (NaCl) at a particular temperature and pH, it forms an insoluble precipitate of Ag-Ab complex. The Ab causing precipitation is called Precipitin and the reaction is called as precipitation reaction.

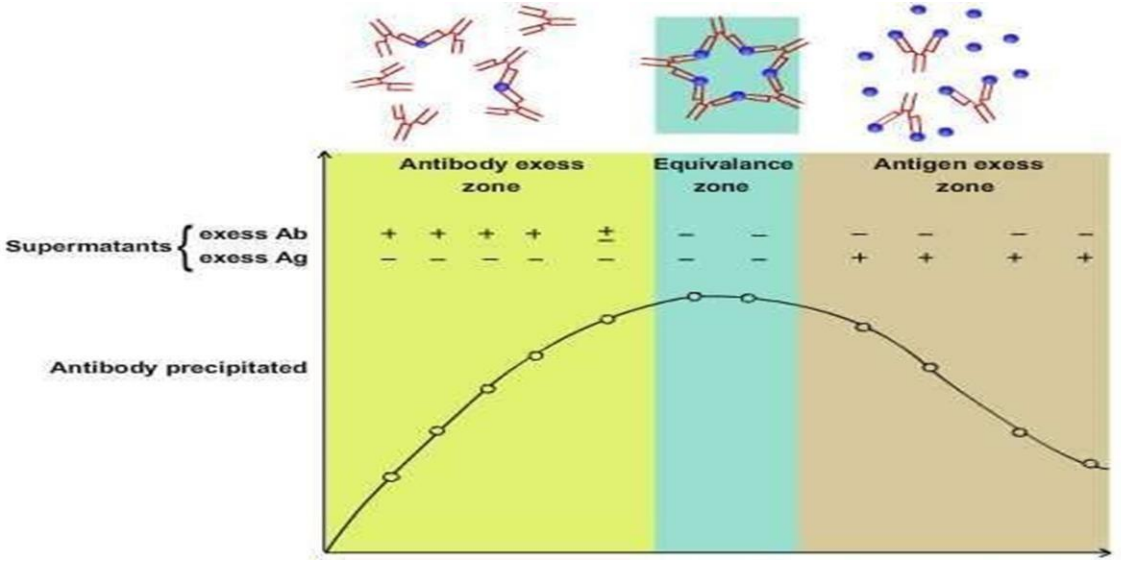


- Function of precipitation reaction: Precipitation occurs in two media:
  - Liquid.
  - Gel.

Precipitation in Liquid:

Antigen – Antibody reaction perform by placing a constant amount of antibody in a series of tubes and adding increased amount of antigen. Antigen – Antibody reacts together resulting in precipitation.

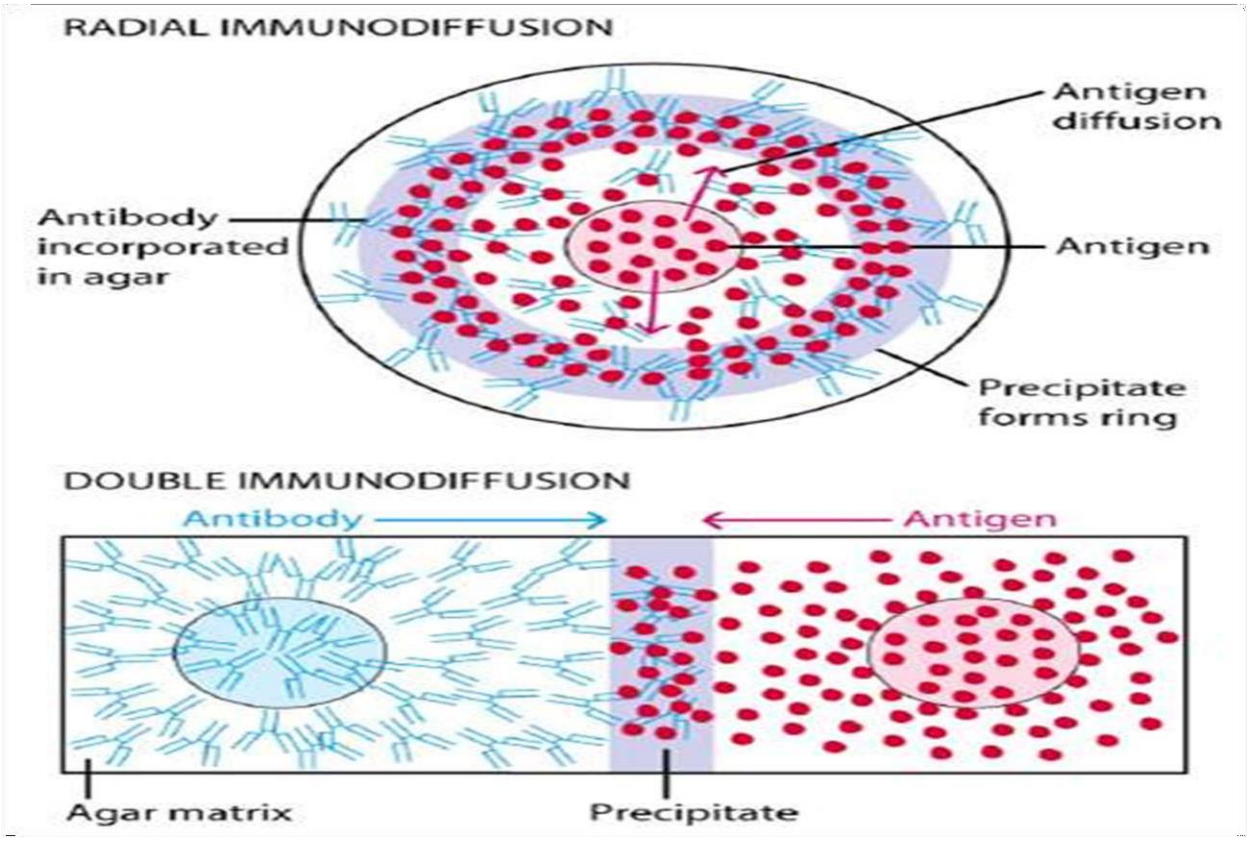
Plotting the amount of precipitate against increasing antigen conc. Yeilds a precipitation curve.



Precipitation in gel:

Radial Immunodiffusion (Mancini):

In these methods agar gel or similar gels are used on plates or petriplates. Both Ag and Ab diffuse freely in the gel system in all directions. At a certain point depending on the rate of diffusion and concentration of the reactants, a zone of equivalence will be formed, which is seen as a visible precipitation.

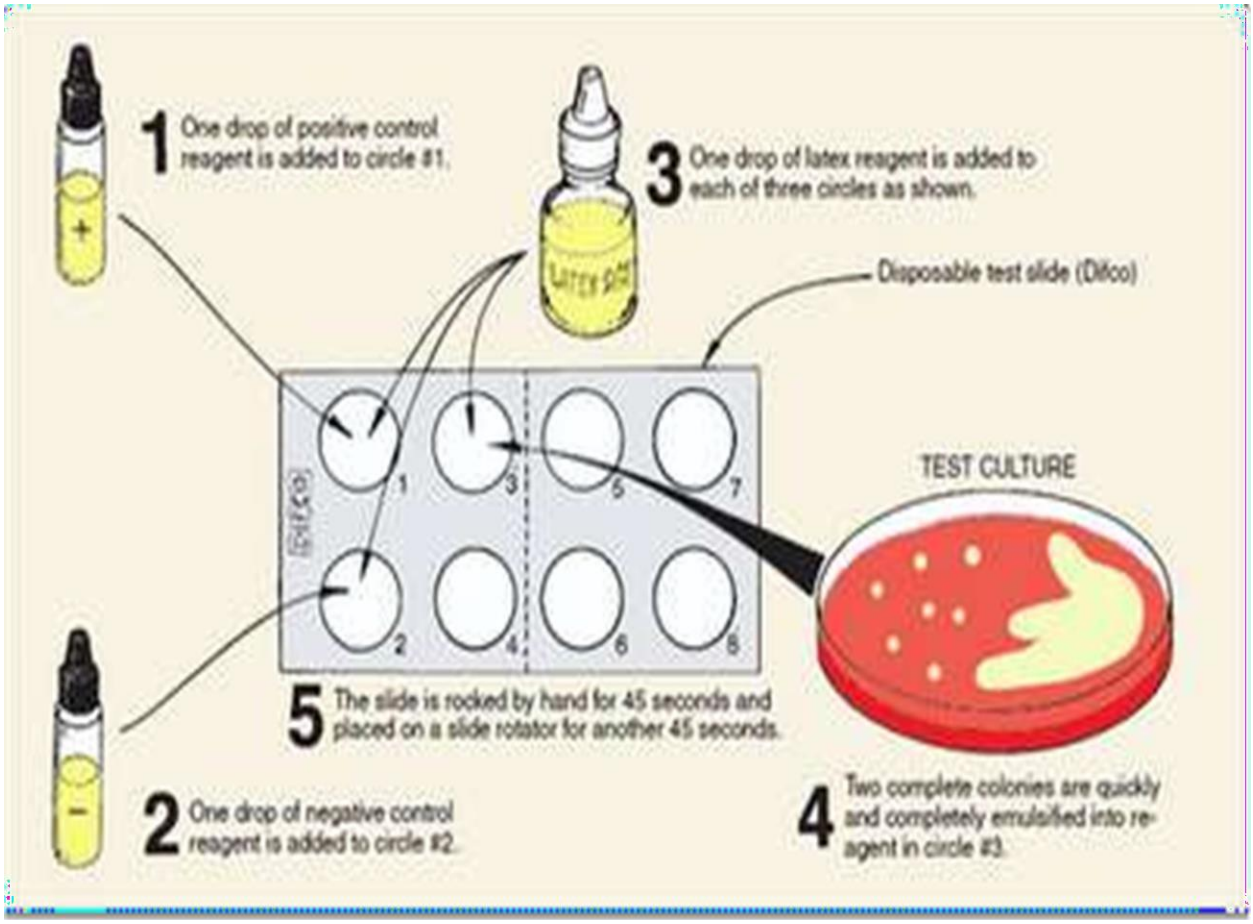


Precipitation reactions in gels

Agglutination Reaction:

- When a particular Ag is mixed with its Ab's in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.
- The Ab of the serum causes the cellular Ag's to form clumps and these are called Agglutinins.
- The particulate antigens that are aggregated are termed Agglutinogens.

➢ Slide agglutination: This is a rapid method to determine the presence of agglutinating antibodies.



Slide Agglutination

- To a uniform suspension of particulate Ag, a drop of saline is added and then a drop of antiserum is added.
- The slide is gently rocked or a fine loop is used to mix the contents. If granulation occurs the test is Positive.
- It takes a minute for the test to complete and is visible to the naked eye. Some times confirmation may be done by observing slide under microscope.
- This test is used for blood grouping (Haemagglutination) and cross matching.

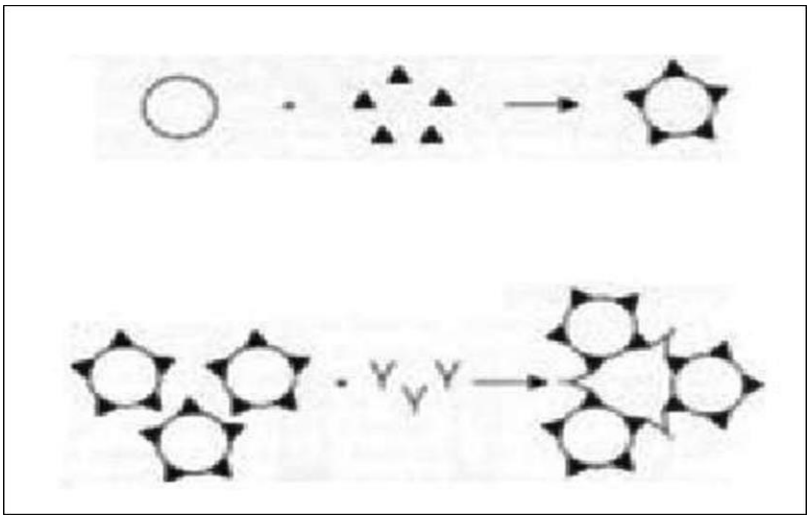
Tube agglutination:

- This is a standard method for quantitative estimation of Ab. The serum containing Ab is diluted serially with saline in several small test tubes, to which a constant volume of Ag suspension is added.
- A control tube is kept which has no antiserum. The tubes are incubated until visible agglutination is observed. The tube showing highest agglutination is referred to as the titre.
- Tube agglutination is employed for the serological diagnosis of typhoid, brucellosis and typhus fever. Widal test is used for the estimation of typhoid fever.
- In this test Ab content of the patient's serum, is measured by adding a constant amount of antigen (Solmonella typhi) to the serially diluted serum.

➢ **Passive agglutination test:**

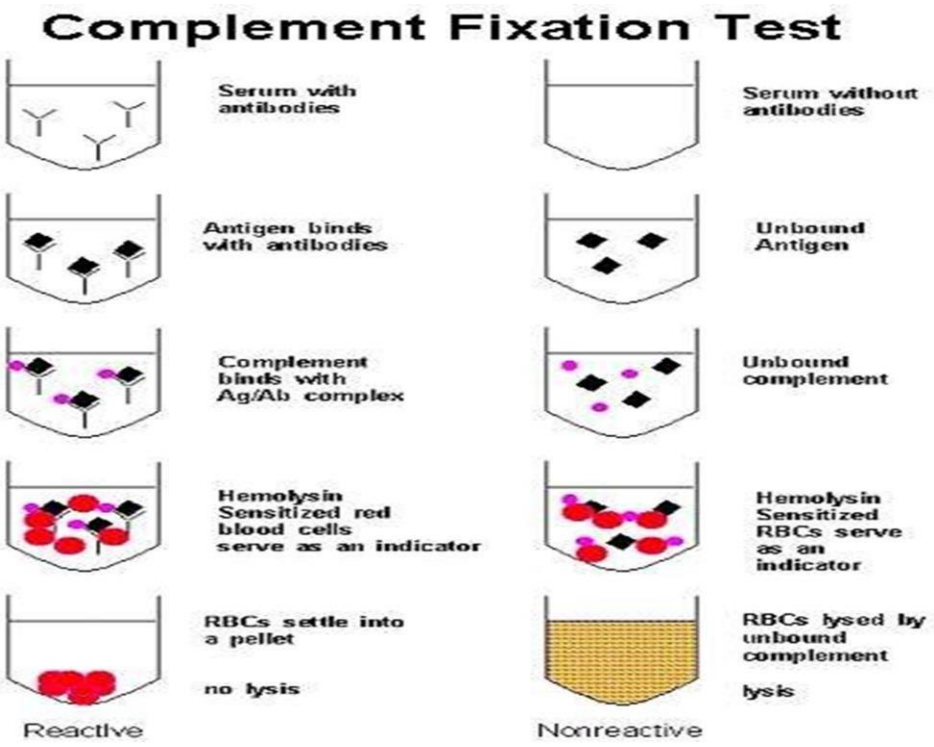
It is similar to haemagglutination test but the physical nature of the reaction is altered.

- The Ag is coated on the surface of a carrier particle and thereby helps to convert a precipitation reaction into an agglutination reaction making the reaction more sensitive. The carrier particles used can be RBC, latex particles or bentonite. Some times RBC coated with polystyrene (tanned RBC) can be used.
- When patients serum is mixed with these, it leads to agglutination. This test is used for the diagnosis of Rheumatoid arthritis.



Complement Fixation:

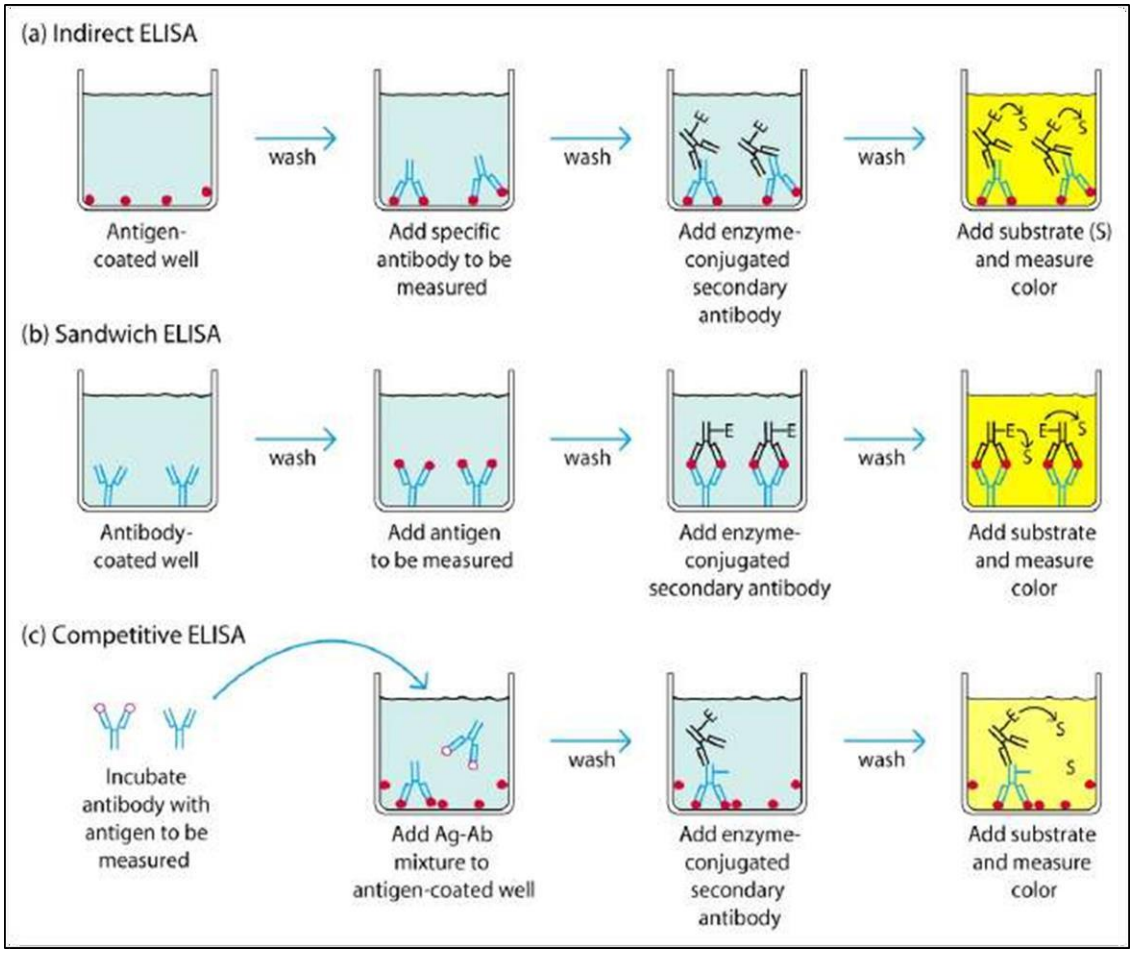
- Lysis of RBC or bacteria requires some non-specific unstable components of fresh serum which are called complement.
- This complement system comprises of 11 proteins and are present in ever individual. They bind to Fc component of Ab involved in Ag-Ab complex. This ability of the Ag-Ab complex to fix complement is used in complement Fixation tests.
- In the first stage, the test Ag and the antiserum (heated to $56^{o}C$ to inactivate complement) are mixed in the presence of known amount of complement. This is incubated at $4^{o}C$ for 18h.
- If Ab specific for the Ag is present in the serum, Ag- Ab complex will be formed that will fix the complement.



ELISA – Enzyme Linked ImmunoSorbent Assay:

ELISA is of 3 types.

- **Indirect ELISA:** This technique is used for the detection of HIV. The envelop proteins are developed by recombinant technology and coated on the surface of the of microtire plates. Suspects serum is added, and unbound proteins are washed off.
- **Sandwich ELISA:** Used to detect the presence of Ag in a sample. The well is coated with Ab specific to the Ag and then suspect serum is added allowed to react. The wells are washed to remove unbound Ag's.



Types of ELISA

Then a labeled Ab against a different epitope of the Ag is added. Unbound Ab's are removed by washing and this is followed by addition of colored substrate and development of color. The intensity of color is directly proportional to the concentration of the Ag in the serum.

- **Competitive ELISA:** Another variation for measuring amounts of antigen is competitive ELISA. In this technique, antibody is first incubated in solution with a sample containing antigen.
- The antigen-antibody mixture is then added to an antigen coated micro titer well.

- The more antigen present in the sample, the less free antibody will be available to bind to the antigen- coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA.

Immunofluorescence:

- Fluorescence is the property of absorbing light rays of one particular wavelength and emitting rays with a different wave length.
- Fluorescent dyes show up brightly under UV light as they convert into visible light.

Coons et al (1942) showed that labeled dyes can be conjugated to Ab's and these labeled antibodies can be used to detect Ag's.

Dyes that are commonly used include:

- Fluorescein
- Phycoerythrin

Immunofluorescence test are of two types.

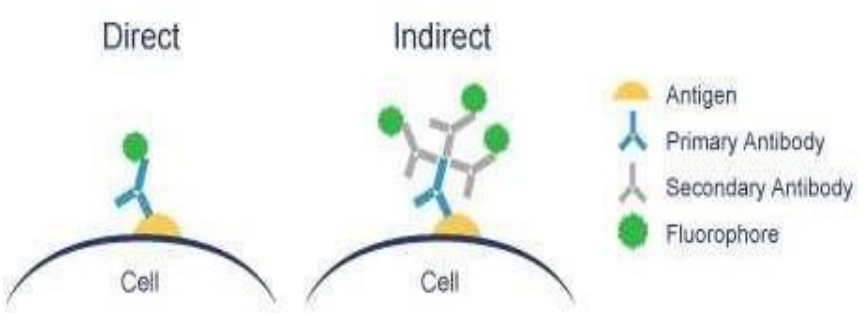
1. Direct immunofluorescence
2. Indirect immunofluorescence

- **Direct immunofluorescence**

Direct immunofluorescence uses a single antibody that is chemically linked to a fluorophore. The antibody recognizes the target molecule and binds to it, and the fluorophore it carries can be detected via microscopy.

- **Indirect immunofluorescence**

Indirect immunofluorescence uses two antibodies; the unlabelled primary antibody specifically binds the target molecule, and the secondary antibody, which carries the fluorophore, recognises the primary antibody and binds to it.



Application of Antigen – Antibody Reaction:

The chief use of antigen-antibody reactions are:

- Determination of blood groups for transfusion.
- Serological ascertainment of exposure to infectious agents.
- Development of immunoassays for the quantification of various substances.
- To detect the presence or absence of protein in serum.
- Determining the characteristics of certain immuno- deficiency disease.

SIMPLE STAINING

Simple staining can define as one of the ordinaries yet popular method which is used to elucidate the bacterial size, shape and arrangement to differentiate the group of bacteria. It stains the bacterial cell uniformly and thus increases the visibility of an organism.
Simple staining sometimes interchangeable with the names like direct, positive or monochrome staining. Now let us understand why simple staining is called by such alternative names.

Refers as Direct staining: Because it is a direct method that directly stains the bacterial cell with a colourless background.

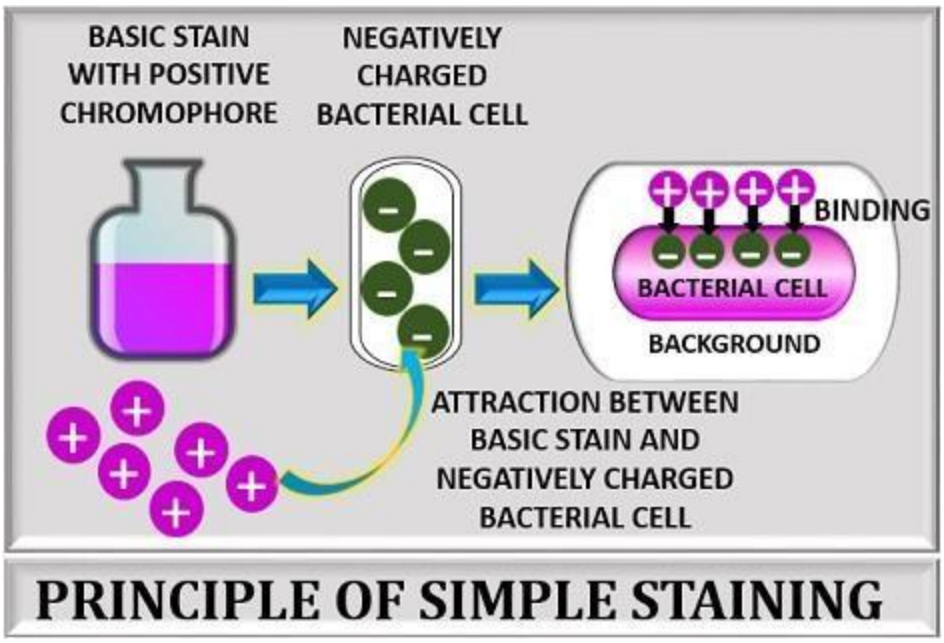
Refers as Positive staining: Because it makes the use of basic dyes which are positively charged and binds with the negatively charged bacterial cell.
Also Refers as Monochrome staining: Because it adds contrast to the specimen by the use of a single stain only.
Simple stains can define as the basic dyes, which are the alcoholic or aqueous solution, diluted up to 1-2%. These can easily release OH^- and accepts H^+ ion, and hence the simple stains are positively charged. As the simple stains are positively charged, they usually refer to as "Positive or Cationic dyes".
It is commonly used to colour most of the bacteria. As the simple stain carry a positive charge, that's why they firmly adhere to a negative bacterial cell by which organism appears coloured with a colourless background.

Examples of simple stain include safranin, methylene blue, crystal violet etc.
The basic stains have different exposure time to penetrate and stain the bacterial cell.

Basic stains	Exposure time to stain the bacteria
Methylene blue	1-2 minutes
Crystal violet	20-60 seconds
Carbol fuschin	15-30 seconds
Safranin	30-60 seconds

Principle

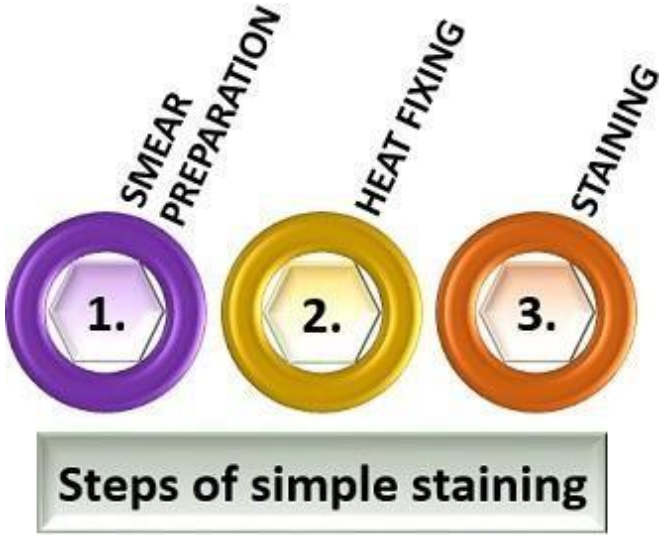
Its principle is based on the principle of producing a marked contrast between the organism and around its surrounding, by the use of basic stain.
A basic dye consists of positive chromophore which strongly attracts to the negative cell components and charged molecules like nucleic acids and proteins.



Procedure of Simple Staining

The method of simple staining involves three steps like:

1. Smear preparation
2. Heat fixing
3. Staining



Smear Preparation

Bacterial smear consists of a thin film of bacterial culture or inoculum. For the preparation of smear, we need to perform the following steps like:

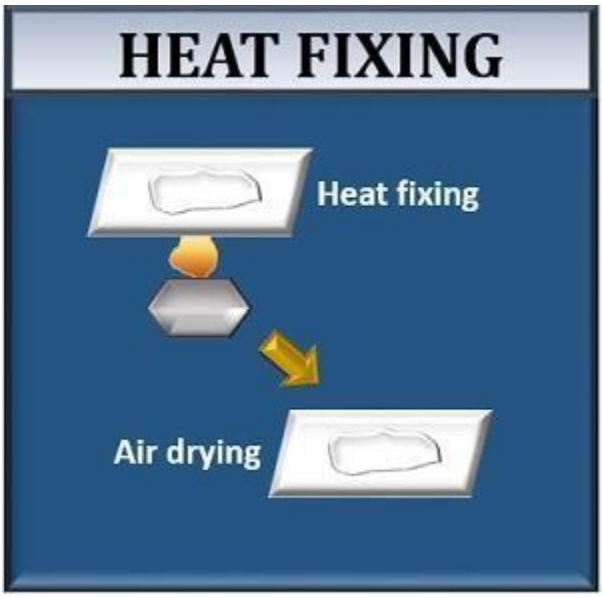
1. Take a clean, grease-free glass slide.
2. Add a drop of distilled water at the centre of the glass slide.
3. Then add inoculum from the bacterial culture with the help of sterilized inoculating loop on the glass slide.
4. After that, mix the inoculum with a drop of distilled water to make a thin film by uniformly rotating the inoculating loop.

Heat Fixing

There are many reasons to perform heat fixing, and it can not be skipped because:

- Heat fixing helps in the fixation of a specimen to the glass slide.
- Heat fixing helps the stain to penetrate into the smear.

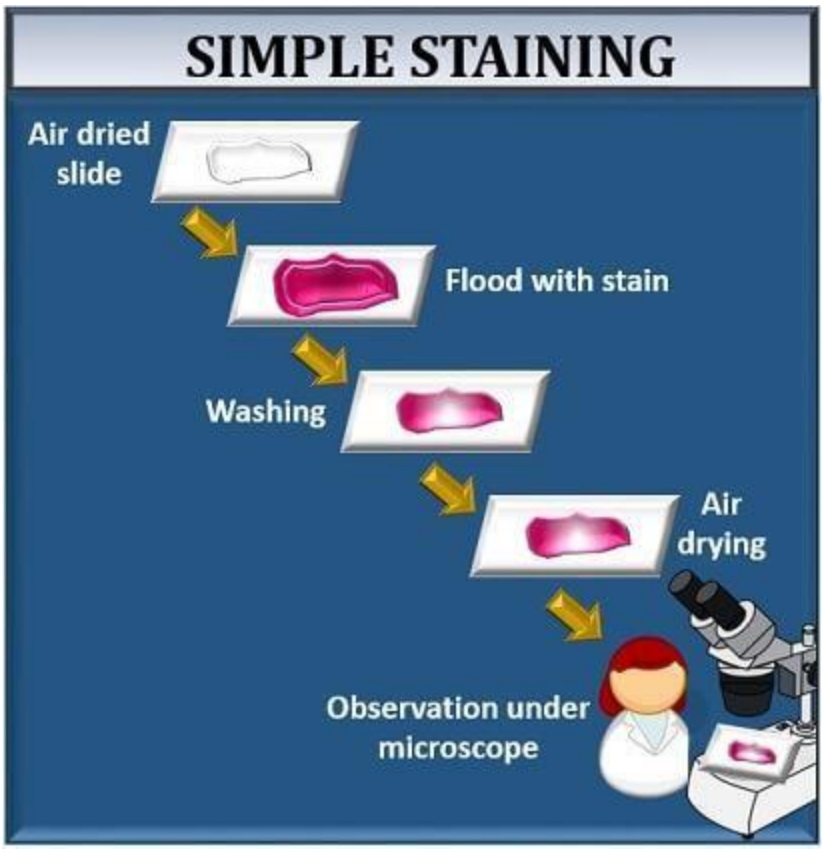
After smear preparation, heat fixes the smear by passing the slides through the flame of Bunsen burner for at least three times. Then, allow the slide to air dry.



Simple Staining of Bacteria

It is the last and the most crucial step which colours the bacterial cells and makes it visible, through which one can identify the morphological characteristics of the bacteria. This stage involves the following steps as follows:

1. Add stain to the heat fixed smear.
2. Allow the stain to stand for at least 1minute so that it can penetrate between the cells.
3. Wash off the glass slide carefully.
4. Blot dry the slide with absorbent paper but do not wipe the slide.
5. Examine the glass slide under the microscope from low to high power objective to get a magnified view of the specimen. One can also add a drop of oil immersion over the stained area of the glass slide and observe it under 100X objective.



Advantages

- Simple staining is a very simple method to perform which stains the organism by a single reagent.
- It is a rapid method which reduces the performance time by taking only 3-5 minutes.
- Simple staining helps to examine or elucidate the bacterial shape, size and arrangement.
- It also helps us to differentiate the bacterial cells from the non-living structures.
- Simple staining can be useful in the preliminary study of the morphological characters of the bacteria.

Disadvantages

- It does not give much information rather than the morphological characteristics of bacteria.
- Through simple staining, we cannot classify a particular type of organism.

GRAM STAINING:

Gram staining method, the most important procedure in Microbiology, was developed by Danish physician Hans Christian Gram in 1884. Gram staining is still the cornerstone of bacterial identification and taxonomic division.

This differential staining procedure separates most bacteria into two groups on the basis of cell wall composition:

1. **Gram-positive bacteria** *(thick layer of peptidoglycan-90% of cell wall*)- stains purple
2. **Gram-negative bacteria** *(thin layer of peptidoglycan-10% of cell wall and high lipid content)* –stains red/pink

Nearly all clinically important bacteria can be detected/visualized using Gram staining method the only exceptions being those organisms;

1. That exists almost exclusively within host cells i.e. Intracellular bacteria (e.g., Chlamydia)
2. Those that lack a cell wall (e.g., Mycoplasma)
3. Those of insufficient dimensions to be resolved by light microscopy (e.g., Spirochetes)

Steps of Gram Staining

Classic Gram staining techniques involve the following steps:

1. Fixation of clinical materials to the surface of the microscope slide either by heating or by using methanol. (# Methanol fixation preserves the morphology of host cells, as well as bacteria, and is especially useful for examining bloody specimen material).
2. Application of the primary stain (crystal violet). Crystal violet stains all cells blue/purple
3. Application of mordant: The iodine solution (mordant) is added to form a crystal violet-iodine (CV-I) complex; all cells continue to appear blue.

4. Decolorization step: The decolorization step distinguishes gram-positive from gram-negative cells.
5. The organic solvent such as acetone or ethanol extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled, gram-positive bacteria. The gram-negative bacteria appear colorless and gram-positive bacteria remain blue.
6. Application of counterstain (safranin): The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue.

Principle of Gram Stain

The differences in cell wall composition of Gram-positive and Gram-negative bacteria account for the Gram staining differences. Gram-positive cell wall contains a thick layer of peptidoglycan with numerous teichoic acid cross-linking which resists the decolorization. In aqueous solutions, crystal violet dissociates into CV+ and Cl – ions that penetrate through the wall and membrane of both Gram-positive and Gram-negative cells. The CV+ interacts with negatively charged components of bacterial cells, staining the cells purple.

When added, iodine (I- or I3-) interacts with CV+ to form large crystal violet-iodine (CV-I) complexes within the cytoplasm and outer layers of the cell.

The decolorizing agent, (ethanol or an ethanol and acetone solution), interacts with the lipids of the membranes of both gram-positive and gram-negative bacteria.

The outer membrane of the Gram-negative cell (lipopolysaccharide layer) is lost from the cell, leaving the peptidoglycan layer exposed. Gram-negative cells have thin layers of peptidoglycan, one to three layers deep with a slightly different structure than the peptidoglycan of gram-positive cells. With ethanol treatment, gram-negative cell walls become leaky and allow the large CV-I complexes to be washed from the cell.

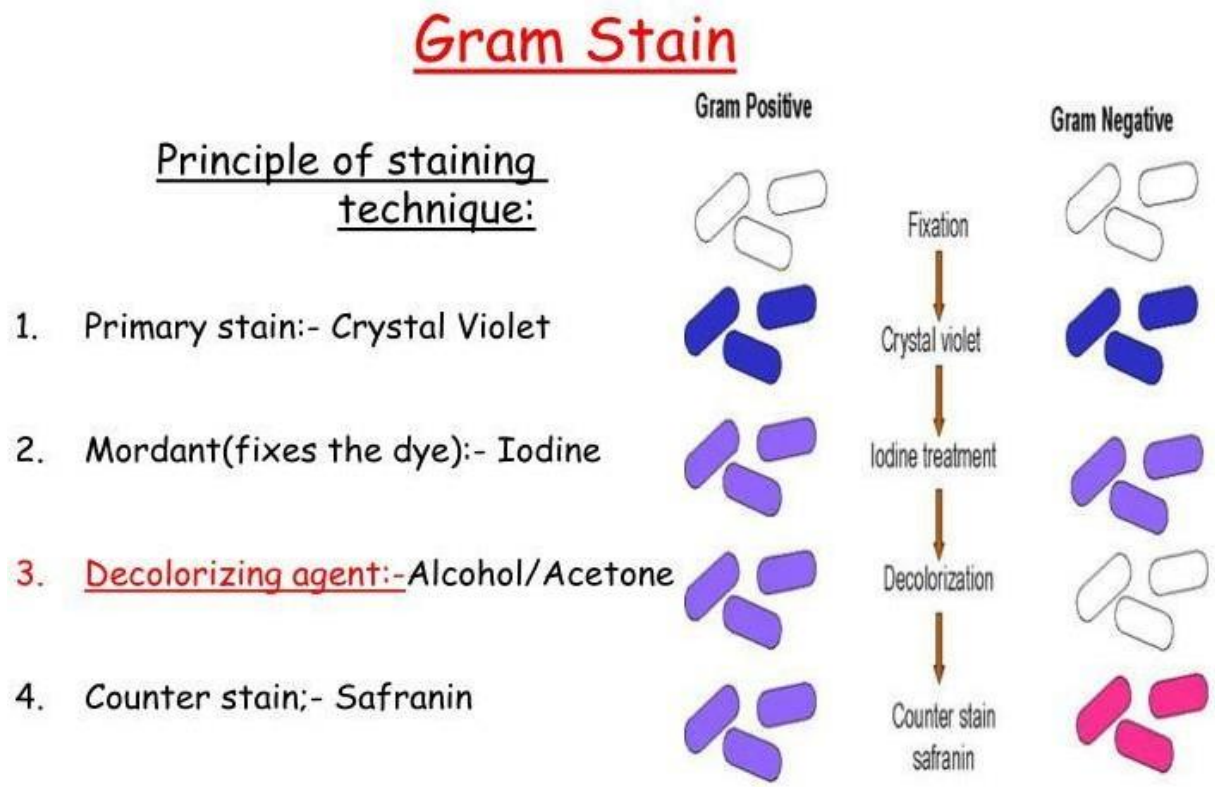
The highly cross-linked and multi-layered peptidoglycan of the gram-positive cell is dehydrated by the addition of ethanol. The multi-layered nature of the peptidoglycan along with the dehydration from the ethanol treatment traps the large CV-I complexes within the cell.

After decolorization, the gram-positive cell remains purple in color, whereas the gram-negative cell loses the purple color and is only revealed when the counterstain, the positively charged dye safranin, is added.

Procedure of Gram Staining

Smear Preparation

Fix material on a slide with methanol or heat. If the slide is heat fixed, allow it to cool to the touch before applying the stain.



Gram Staining Procedure/Protocol:

1. Flood air-dried, heat-fixed smear of cells for 1 minute with crystal violet staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.
2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
3. Flood slide with the mordant: Gram's iodine. Wait 1 minute.
4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
5. Flood slide with decolorizing agent (Acetone-alcohol decolorizer). Wait 10-15 seconds or add drop by drop to slide until decolorizing agent running from the slide runs clear.
6. Flood slide with a counterstain, safranin. Wait 30 seconds to 1 minute.
7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.
8. Observe the results of the staining procedure under oil immersion (100x) using a Bright field microscope.

Results:

- Gram-negative bacteria will stain pink/red and
- Gram-positive bacteria will stain blue/purple.

ACID-FAST BACILLI STAIN

It is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called *Ziehl-Neelsen staining* techniques. Neelsen in 1883 used Ziehl's carbol-fuchsin and heat then decolorized with an acid alcohol, and counter stained with methylene blue. Thus Ziehl-Neelsen staining techniques was developed.

The main aim of this staining is to differentiate bacteria into acid fast group and non-acid fast groups.

Mycobacterium are AFB

- Mycobacterium are Gram-resistant (waxy cell walls), non-motile, pleomorphic rods, related to the Actinomyces. Most Mycobacteria are

found in habitats such as water or soil. However, a few are intracellular pathogens of animals and humans. *Mycobacterium tuberculosis*, along with *M. bovis*,*M. africanum*, and *M. microti* all cause the disease known as tuberculosis (TB) and are members of the tuberculosis species complex. Each member of the TB complex is pathogenic, but *M. tuberculosis* is pathogenic for humans while *M. bovis* is usually pathogenic for animals.

Principle of Acid-Fast Stain

When the smear is stained with carbol fuchsin, it solubilizes the lipoidal material present in the Mycobacterial cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. Then after all cell appears red. Then the smear is decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of lipoidal material in their cell wall which prevents the penetration of decolorizing solution. The non-acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless. Then the smear is stained with counterstain, methylene blue. Only decolorized cells absorb the counter stain and take its color and appears blue while acid-fast cells retain the red color.

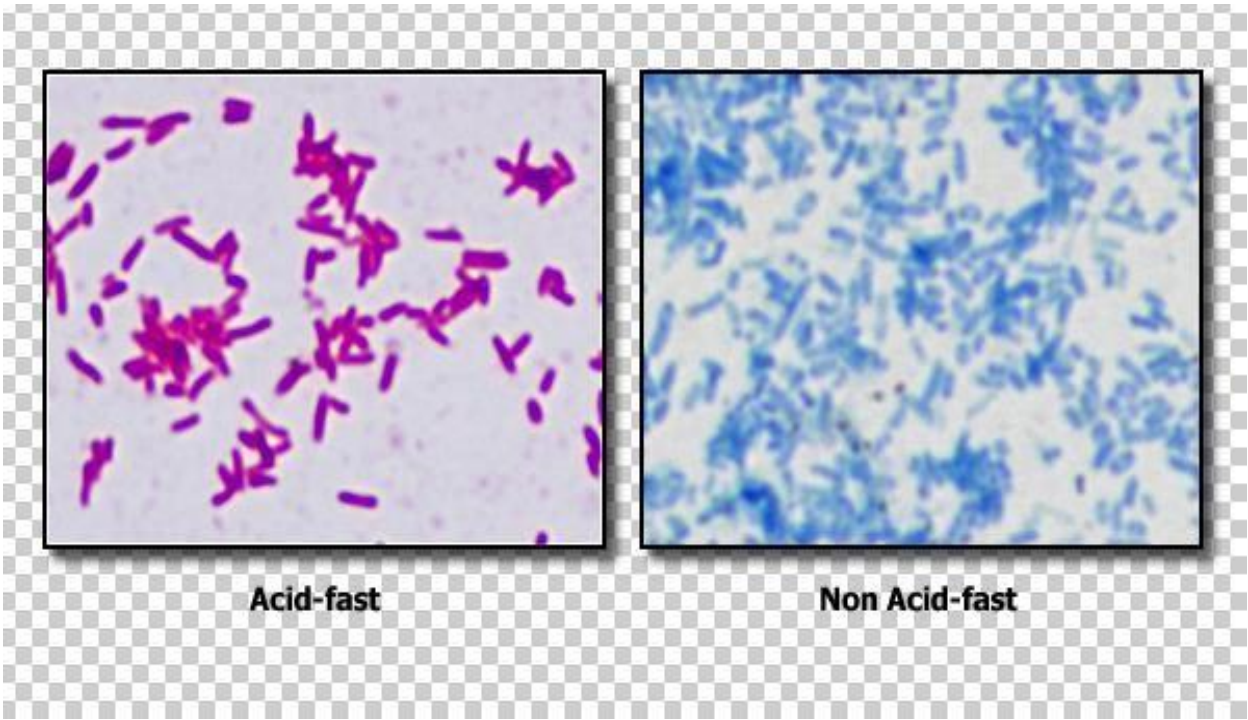
Summary of Acid-Fast Stain

Application	Reagent	Cell colour	
		Acid fast	Non-acid fast
Primary dye	Carbol fuchsin	Red	Red
Decolorizer	Acid alcohol	Red	Colorless
Counter stain	Methylene blue	Red	Blue

Procedure of Acid-Fast Stain

1. Prepare bacterial smear on clean and grease free slide, using sterile technique.
2. Allow smear to air dry and then heat fix. *Alcohol-fixation: This is recommended when the smear has not been prepared from sodium hypochlorite (bleach) treated sputum and will not be stained immediately. M. tuberculosis is killed by bleach and during the staining process. Heat-fixation of untreated sputum will not kill M. tuberculosis whereas alcohol-fixation is bactericidal.*
3. Cover the smear with carbol fuchsin stain.
4. Heat the stain until vapour just begins to rise (i.e. about 60 C). Do not overheat. Allow the heated stain to remain on the slide for 5 minutes. *Heating the stain: Great care must be taken when heating the carbol fuchsin especially if staining is carried out over a tray or other container in which highly fiammable chemicals have collected from previous staining. Only a small fiame should be applied under the slides using an ignited swab previously dampened with a few drops of acid alcohol or 70% v/v ethanol or methanol. Do not use a large ethanol soaked swab because this is a fire risk.*
5. Wash off the stain with clean water. *Note: When the tap water is not clean, wash the smear with filtered water or clean boiled rainwater.*
6. Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolorized, i.e. pale pink. *Caution: Acid alcohol is fiammable, therefore use it with care well away from an open fiame.*
7. Wash well with clean water.
8. Cover the smear with malachite green stain for 1–2 minutes, using the longer time when the smear is thin.
9. Wash off the stain with clean water.
10. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry (do not blot dry).
11. Examine the smear microscopically, using the 100 X oil immersion objective.

How the Acid fast bacteria appear



FLUOROCHROME STAINING:

Auramine-Rhodamine Fluorochrome staining also known as "Truant method of staining", is used to visualize Acid fast bacilli (AFB). Ziehl-Neelsen (hot), Kinyoun (cold) are still widely used methods in developing countries. CDC recommends fluorochrome staining for detecting AFB in primary patient specimens. The acid fastness of Mycobacteria is due to their thick cell wall composed of waxes and lipids that have a high content of mycolic acid.

Fluorescent dyes like Auramine-Rhodamine binds to the mycolic acid present in them and impart bright yellow or orange fluorescence against a greenish background when viewed using a fluorescent microscope. It is also used to stain all Acid fast organisms including the sporozoan parasites.

Principle:

The fluorochrome dye, Auramine-Rhodamine, forms a complex with mycolic acids found in the acid-fast cell wall of organisms which resist decolorization by acid-alcohol.The counterstain, potassium permanganate, renders tissue and

its debris nonfluorescent, thus reducing the possibility of artifacts. The cells visualized under ultraviolet light appear bright yellow or reddish orange.

Reagents:

- Primary Stain: Auramine Rhodamine Solution (Caution: possible carcinogen)
- Decolorizer: 0.5% Acid alcohol (5 ml HCl in 995 ml 70% alcohol). (Caution: Flammable, Corrosive)
- Counter Stain: 0.5% Pottassium Permanganate (0.25 gm in 50 ml). (Caution: Corrosive)

Others:

1. Slide: use only new, unscratched, and clean slides; using old, scratched, or dirty slides can lead to erroneous results.
2. Identifier: Properly label each slide using graphite pencils or use a diamond or tungsten carbide stylus.
3. Slide racks
4. Bunsen burner

Procedure

1.Preparation of Smear:

1. For Sputum : Using a piece of stick, transfer a purulent part of the sputum (containing any yellow caseous material), to a slide and make a thin smear. An area of approximately ½ by 1 inch (or 2-cm square) is recommended.Spread the smear using circular movements. Allow to air dry.

Note: Be sure to prepare smears of suitable thickness. Smears that are too thick may flake during staining and may be difficult to decolorize. Acid-fast organisms that might be present may be obscured. Smears that are too thin may not contain enough sample.

Either condition–too thick or too thin–can lead to erroneous results, particularly false negatives. Here (image 1) the smear in the center is of the proper thickness. Hold a smear about 3 to 4 inches over news-print, if you are just able to read the print, the smear is of proper thickness.

1. For Urine: Make a smear of the deposit from three centrifuged early morning urine sediments.

Allow to air dry and heat fix the specimen. Use of an electric slide warmer is usually the preferred method for heat-fixing smears. An alternate method of heat-fixing is to pass the dried slide, smear facing upward, 2 to 3 times through the blue cone of a burner flame.

2. Staining Method

- Place the fixed smear on a staining rack and flood slide with rhodamine-auramine for 15 minutes. Do not let surface dry. (*Note: Fluorochrome dyes used for acid-fast staining include Auramine O, and Auramine O in combination with another fluorochrome, Rhodamine B).*
- Wash off the stain with distilled water.
- Flood slide with fluorescent decolorizer (i.e acid-alcohol) for 2-3 minutes.
- Rinse thoroughly with distilled water.
- Flood slide with potassium permanganate for 3-4 minutes. Do not allow slide to dry.
- Rinse thoroughly with distilled water and air dry.
- Examine microscopically under the same light source as used for fluorescent microscopy (i.e. a K530 excitation filter and a BG 12 barrier or G-365 excitation filter and an LP 420 barrier filter). Slides can be screened on high power (400X) and verified under oil immersion.

Result and Interpretation:

- **Positive Test –** Acid-fast organisms fluoresce reddish-orange against a dark background.
- **Negative Test –** Non-acid-fast organisms will not fluoresce or may appear a pale yellow, quite distinct from the bright acid-fast organisms.

Auramine-Rhodamine Staining