Microbiology as a Field of Biology

- Microbiology is the study of living organisms of microscopic size, which include bacteria, fungi, algae, protozoa and the infectious agents at the borderline of life that are called viruses
- It includes the study of their distribution in nature, their relationship to each other and to other living organisms, their effects on human beings and on other animals and plants, their abilities to make physical and chemical changes in our environment, and their reactions to physical and chemical agents.
- Microorganisms are closely associated with the health and welfare of human beings, some microorganisms are beneficial and others are detrimental.
- Microorganisms are involved in the making of yogurt, cheese, and wine, in the production of penicillin, interferon and alcohol and in the processing of domestic and industrial wastes.
- Microorganisms can cause disease, spoil food, and deteriorate materials like pipe, glass lenses and wood pilings.
- The word cell was first used more than two centuries ago by an Englishman, Robert Hooks (1635-1703).
- The honeycomb like structure, the concept of the cell as the structural unit of life
- Protoplasm (Greek proto, "first"; plasm formed substance" Introduced to characterize the living material of a cell is a colloidal organic complex consisting largely of protein, lipids, and nucleic acids.
- All biological systems have the following characteristics in common

(1) The ability to reproduce
(2) The ability to ingest or assimilate food substances and metabolize them for energy and growth
(3) The ability to excrete waste products
(4) The ability to react to change in their environment-some time called irritability
(5) Susceptibility to mutation

Microbes and Life Today

- Pathogenic microorganisms are still responsible for a vast spectrum of human illnesses and sufferings. But the development of Microbiology as a science has made outstanding progress.
- Advances in medical Microbiology have made it possible to identify the various pathogens that cause infectious diseases and devise ways to control most of them.

Microbes, Disease and History

- The **bubonic plague** (often called plague or the black death) that swept through Europe during the Middle Ages killed about 25 million people—one-third of the population
- The social and political dislocations were immense, and the resulting terror was particularly intense because the cause of the disaster was unknown. Not until 500 years later, in 1890, did microbiologists identify the causative organism (a bacterium called *Yersinia pestis*)
- Irrespective of where microbiology is placed in the broad field of biology, microorganisms have some characteristics which make them ideal specimens for the study of numerous fundamental life processes.
- This is possible because at the cellular level, many life processes are performed in the same manner whether they be in microbe, mouse, or man
- In microbiology we can study organisms in great detail and observe their life processes while they are actively metabolizing, growing, reproducing, aging, and dying.
- By modifying their environment we can alter metabolic activities, regulate growth, and even change some details of their genetic pattern – all without destroying the organisms.

The Place of Microorganisms in the Living World

- In biology as in any other field, classification means the orderly arrangement of units under study into groups of larger units
- Until the eighteenth century, the classification of living organisms placed all organisms into one of two kingdoms, plant and animal.

Bacteria

- (*sing.*, bacterium) are distinguished by their size
- and prokaryotic cell structure. Instead of the elaborate internal membrane-bound structures seen inside eukaryotic cells, prokaryotes
- Most bacteria are unicellular (single cells) and quite small, even for microorganisms
- A typical bacterial cell has only about one one-thousandth the volume of a typical eukaryotic cell.
- Bacteria are highly diverse. Most species have a characteristic
- They can be spherical, rodshaped, helical, comma-shaped, star-shaped, or even square.
- Some bacteria are motile. Others are not. Some obtain energy by processing organic compounds (foods), as animals.

- Some utilize light energy through photosynthesis
- Bacteria cause a vast spectrum of diseases—from food poisoning and toxic shock syndrome to syphilis and typhoid fever.

Fungi

- (*sing.,* fungus) include organisms we call mushrooms, yeasts, and molds.
- They are eukaryotic, nonphotosynthetic,and either microscopic or macroscopic
- Most fungi are scavengers, are ecologically important because they decompose dead organisms.
- A few fungi are pathogenic to animals and humans.
- Some cause minor infections, such as ringworm and athlete's foot.
- Many fungi are pathogenic to plants. They cause such economically important diseases as corn smut, wheat rust, and potato blight.

Protozoa

- **Protozoa** (*sing.,* protozoon) means ".rst animals." As the name suggests, they are supercially animal-like.
- They resemble bacteria: they are small prokaryotic cells that usually occur singly.
- First discovered they were called **archaebacteria** (ancient bacteria). But they are as distantly related to bacteria as they are to eukaryotes, including humans.

Algae

- **Algae** (*sing.,* alga) are eukaryotic organisms that carry out plantlike photosynthesis.
- Like all eukaryotes, they have a nucleus and membrane-bound organelles, including chloroplasts.
- Some algae are unicellular and microscopic.
- Some consist of many cells and are macroscopic
- Macroscopic alga, or multicellular algae may look superficially like higher plants, but they lack characteristic plant organs, including stems, roots, and leaves

Helminths

- Helminths are worms, and as such they belong to the animal kingdom. Most are also macroscopic.

- The two types of disease-causing helminths are tapeworms and roundworms.
- Flatworms include the beef tapeworm, which can grow to lengths of 30 feet in human intestines are microscopic
- Liver trematodes, which are microscopic.
- Roundworms include hookworms, parasites that were common.

Viruses

- They are not cells. They are merely particles of nucleic acid, either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), packaged in a protein coat and sometimes surrounded by a membrane.
- Viruses are incapable of reproducing themselves.
- They can reproduce only inside a host cell.
- In other words, viruses are obligate intracellular parasites
- They force their hosts to make more viruses.
- Viruses infect animals, plants, and other microorganisms
- Viruses are extremely small, even compared with bacteria.
- The largest viruses are about one-tenth the size of a typical bacterial cell. The smallest are about one thousandth the size of bacteria.
- Viruses cannot be seen through an ordinary microscope, but even the smallest can be seen with an electron microscope.

Branches of microbiology

- Medical Microbiology
- Aquatic Microbiology
- Aero Microbiology
- Food Microbiology
- Agricultural Microbiology
- Industrial Microbiology
- Exomicrobiology
- Geochemical Microbiology
- Analytical Microbiology
- Molecular Biology

Medical Microbiology (Some applied areas)

- Detect causative agents of disease
- Diagnostic procedures for identification of causative agents
- Preventive measures

- Control

Aquatic Microbiology

- Water purification
- Microbiological examination
- Biological degradation of wastes
- Ecology of microbes

Aeromicrobiology

- Contamination and spoilage
- Dissemination of diseases

Food Microbiology

- Food preservation and preparation
- Food borne diseases and their prevention

Environmental Microbiology

- The study of how microorganisms affect the earth and its atmosphere is called environmental microbiology or microbial ecology.

Agricultural Microbiology

- Soil fertility
- Plant and animal diseases

Industrial Microbiology

- Production of medicinal products such as antibiotics and vaccines
- Fermented beverages, industrial chemicals, production of proteins and hormones by genetically engineered microorganisms.

Exomicrobiology

- Exploration for life in outer space
  Geochemical Microbiology
- Coal, mineral and gas formation, propecting for deposits of coal, oil and gas
- Recovery of mineral from low grade ores

Analytical Microbiology

- Microorganism is used to assay quantitatively substances such as vitamins, amino acids and antibiotics
- Determine the potency of all antibiotics at various stages of development
- Measurement of increase in growth or metabolic activity.

History of Microbiology

●Biblical times some customs and practices prevented disease ex. control of lepers in colonies, burial
of human wastes
●400 B.C. Hippocrates -diseases were transmitted from 1 person to another by clothing and objects
●1 B.C. Romans. Belief that invisible animals entered body & caused disease 542, 1347, 17th, 18th
century A.D.
●Periodic epidemics of bubonic plague during the 1347 epidemic the disease was called the Black
Death because of black spots on the skin Killed millions in Europe, one third of the population
within 5 years (25 million) Jews partially protected by sanitary customs
●1600s Microscope invented
Leeuwenhoek discovered microorganisms he called "animalcules“
●1700s Vaccination.
Edward Jenner showed that exposure to pus in cowpox sores gave protection against smallpox
1800s Proof of the germ theory of disease.
Spontaneous generation discredited
●1900s Microbiology developed as a scientific discipline
Development of laboratory methods
Major disease-causing pathogens discovered
Development of chemotherapy and immunology

Spontaneous Generation

●Living organisms arise from nonliving matter Ex. maggots from rotting food, frogs from mud,
microorganisms from meat and vegetable infusions
●Delayed acceptance of germ theory of disease
●Disproved by experiments of Redi, Pasteur and Tyndall
●They showed that organisms only arise only from preexisting living organisms, not from inanimate
matter
●Redi's experiment
●Pasteur's swan-necked flask

The Germ theory of Disease

●Disease is caused by microorganisms --"germs"-- that invade the body

●Developed methods to grow microorganisms as pure cultures
●Used gelatin and agar to solidify growth media
●Allowed study of properties of a single species
●Discovered several pathogenic (disease causing) bacteria
●Ex. Bacillus anthracis -- anthrax
● Mycobacterium tuberculosis -- tuberculosis
● Vibrio cholerae -- cholera

Koch's Postulates

●Proof that a specific microoorganism causes a specific disease
●1. Same microorganism present in all cases of a disease
●2. Must isolate microorganism as a pure culture from diseased animal
●3. Inoculation of healthy animal with pure culture must cause the disease
●4. Same microorganism must be re isolated from the inoculated animal
● Firmly established the germ theory of disease

Other Milestones in Evolution of Microbiology

Effects of the Application of Microbiology on Human Life Expectancy

●Mortality rate was very high due to:
●Pneumonia and influenza, Tuberculosis, Syphilis, Diphtheria Whooping cough, Measles
●Decrease in death from microbial causes mainly due to:
●Development of vaccines to prevent diseases, antibiotics to treat diseases and antiseptics to control
pathogens, Sanitary food handling, Treatment of drinking water and Sewage treatment

Developments in Microbiology

●The late 1800s became known as the golden age of microbiology.
●Advances came rapidly and life was dramatically
improved. But the advances that continued to be made during the twentieth century.
●Four key areas: chemotherapy, immunology, virology, and genetic engineering.

Chemotherapy

●Probably advance in medical microbiology during the 1900s was the development of **chemotherapy**

(treatment of disease with chemicals called drugs).

●Nineteenth-century microbiologists discovered ways to prevent many infections, but not until the twentieth century did they acquire the ability to treat infections once they had started.

●The German physician-chemist Paul Ehrlich is called the father of chemotherapy because he articulated its guiding principle, **selective toxicity.**

●Ehrlich's research on chemotherapy expanded. Sulfa drugs were the first major class of drugs to come into widespread clinical use. These are called **synthetic drugs**

●Antibiotics (chemotherapeutic agents produced by

●microorganisms) were discovered at about the same time as sulfa drugs, but they proved to be more effective 1st medically useful antibiotic

●Penicillin was discovered by the Scottish microbiologist Alexander Fleming (1881–1955) in 1929

●The dramatic effectiveness of Penicillin make it the "wonder drug"

Immunology

●By the days of Pasteur and Koch, immunology was a branch microbiology devoted to developing vaccines for preventing infectious diseases

●Two aspects:

●First, the metabolism and genetic properties of microorganisms are remarkably similar to those of plants and animals, including humans

●Second, microorganisms, especially bacteria, are especially suitable for experimental investigation:

●They are easy to culture and they grow rapidly.

● Under proper conditions certain bacteria can double their numbers every 20 minutes

Virology

●**Virology,** the study of viruses, began in 1892

●Russian microbiologist Dmitri Iwanowski discovered tobacco mosaic virus

●Iwanowski was studying a disease of tobacco plants called tobacco mosaic disease.

●Viruses could not be seen, even under the most powerful microscopes Until the electron microscope was developed in the 1930s, we knew viruses existed, but little more.

●Development of vaccines after their identification

Genetic Engineering and Genomics

●Intensive laboratory studies on microorganisms have led development of a remarkable set of
techniques, collectively called **genetic engineering** or **recombinant DNA** technology
●With this technology researchers can obtain DNA(the cell's genetic material) from one organism,
manipulate it in the laboratory, and introduce DNA into another cell where it will exert its effect.
●Recombinant DNA technology has also lead to the ability to decipher and read an organism's
genetic material DNA

The Future

●Microbiology is an active experimental science, little more than a hundred years old. Its pattern of
accelerating progress likely to continue.
●Medical microbiology and virology will need to solve pressing problems of resistance of pathogens
to antibiotics evolution of new viruses that cause new diseases.
●The increasing development on **bioremediation** (use of microorganisms to degrade toxic
chemicals) to clean up our environment.

Prokaryotes

●First appeared about 3.5 billion years ago and remained that only life on earth for about 2 billion
Years; even today, they still dominate the biosphere
● About 4000 species are recognized, although about 400,000 species are believed to exist
●Metabolically and structurally diverse
●Differences from eukaryotic cells:
●Smaller
●Lack membrane bound organelles
●Have cell walls that are structurally different
●Simpler genomes
●Methods of genetic replications, protein synthesis, decomposition

Impact of prokaryotes on the Earth

●A small percentage cause disease
●Perform decomposition important to the carbon, nitrogen, phosphorus, and sulfur cycles
●Symbiotic relationships - mitochondria and chloroplast may have evolved from such symbiotic relationships
Branches prokaryotic evolution
Two main branches of prokaryotic (domains)

Archaeabacteria (archaea):

●16S and 18S segments of ribosomal RNA identical to human ribosomal RNA - evidence for evolution
●Inhibit extreme environments (e.g. hot springs, salty ponds) similar to early conditions on Earth
●Believed to have a common ancestry with eukaryotes

Eubacteria:

●More modern and believed to have evolved later than archaeabacteria
●More prominent than archaeabacteria and differ in structure, biochemistry, physiological character

Characteristic of Microorganisms

●Morphological characteristics
●Size, Shape, Arrangement, Structure
●Chemical composition
●Cultural characteristics
●Metabolic characteristics
●Antigenic characteristics
●Genetic characteristics
●Pathogencity
●Ecological characteristics

Morphological Characteristics

●Microorganisms are very small and their size is usually expressed in um. One um is equal to .001 mm
●The use of electron microscopy provides magnification of thousands of diameters and makes it possible to see fine details of cell structure.

Size

●0.75 – 1.25 um	Cocci
●0.70 - 3 um	Haemophilus
●2 - 3 um	Escherechia Coli
●3 - 8 um	B. anthracis
●20-30 um	Leptospira, Borrelia

Shape

●Spherical (Spheroids)	Cocci – Coccus
●Cylindricals (Rods)	Bacilli – Bacillus
●Spirillar (Helical)	Spirillia - Spirillum
●Curved	Vibrio
●Pleomorphic	Peoteus, Francisella

Arrangements

●Single	E. coli, Salmonella
●Chains	Streptococcus (Streptococcus equi)
	Streptobacilli (Streptococcus aureus)
●Diplococci	Diplobacilli
●Tetrads	(Tetracocci)

●Sarcinae – Cuboidal arrangements
●Trichomes – Beggiata, Saprospira

Structures

●Structures external to the cell wall

●Cell wall and cytoplasm membranes

●Structures internal to the cell walls

Structures external to CW

●Glycocalyx
●Flagella
●Axial filaments
●Fimbriae
●Pili

●Trichomes

Glycocalyx

●Substances that surround the cell wall
●Viscous, gelatinous layer
●Made up of
- Polysaccharides
- Polypeptides
- Polysaccharides + Polypeptides
- Prepared in side the cell

●If Glycocalyx is organized and firmly attached to cell wall – Capsule
●If unorganized and loosely attached to cell wall – slime layer

Functions of Capsule

●Virulence
●Protection against phagocytosis
●Attachment to various surfaces
●Protection against dehydration
●Source of nutrition
●Inhibit movement of nutrients from the cell

Flagella

●Flagellum – Whip like structure
●Some prokaryotic cells have flagella
●Much thinner than flagella or cilia of EU
●Arrangements
- Monotrichous – Single polar
- Amphitrichous – Single at each end
- Lophotrichous - Two or more at one or both poles
- Peritrichous- All over the entire surface

Structure of Flagellum

●Filament – Long outer most region, constant in diameter – protein, flagellin
●Hook – slightly wider, consist of a different protein
●Basal body – Anchors the flagella to CW and plasma membrane – Composed of a small central rod inserted into a series of rings
●Gram negative bacteria – two pairs of rings

•Gram positive only inner rings

Functions of Flagella

●Organ of motility
●Movement towards favourable environments
●Movement away from the adverse environment

Axial Filaments

●Spirochaetes move by means of axial filaments
●Bundles of fibrils that arise at the end of the cell beneath the outer sheath and spiral around the cell
●Axial filaments which are anchored at one end of the spirochaetes have structure similar to flagella

Fimbriae

●Many gram negative bacteria contain hair like appendages
●Shorter straighter and thinner than flagella
●At poles of the bacterial cells are evenly distributed over the entire surface – 100s per cell
●Attachments

Pili (Pilus)

●Longer than fimbriae
●1 to 2 per cell
●Join bacteria prior to the transfer of DNA from one cell to another – sex pili

Trichomes

●Tube like structures around different bacterial cells
●Secreted by the bacteria
●Protection

MEASUREMENT OF BACTERIAL GROWTH

- Cell Count
- Cellular Mass
- Cellular Activity

1. STANDARD PLATE COUNT

- When bacteria are placed on a solid medium, ideally each colony is founded by only a single bacterial cell (obviously, given clumping and various cell arrangements, this ideal is not always met)
- Thus, addition of a known quantity of bacterial cells to a solid medium should produce the same number of colonies
- This result can be employed backward so that the number of colonies grown on solid medium can be used to estimate the number of individual bacteria that were added to the solid medium
- This number, in turn, may be employed to estimate the concentration of bacteria.

A.Pourplate

The pour-plate method is employed for bacterial-cell enumeration and isolation

- In the pour-plate method of addition of cells to solid medium contained within a petri dish, cells are added to melted (but not too hot) solid medium
- The melted solid medium is then poured into a petri dish and allowed to harden
- Colonies appear both within, beneath, and on top of the agar

B.Spreadplate

The spread plate method is employed for bacterial-cell enumeration and isolation

- In the spread-plate method of addition of cells to solid medium, a small volume of culture is dropped onto the surface of agar that has already hardened in a petri dish
- The volume is then spread around the agar surface
- Colonies will grow solely on the surface of the agar
- This technique is advantageous particularly when cells are sensitive to exposure to relatively high temperatures plus the method does not require a prior melting of the solid medium.

2. MEMBRANE FILTER COUNT

●Measures living bacteria
●Use of membrane filters with known uniform pore size

3.DIRECTMICROSCOPICCOUNT

Measures total bacterial count
●Use of Petroff Hausers counting chambers
●A more direct means of enumerating bacteria is done by viewing them through a microscope
●This method's limitations are that only relatively high concentrations of bacteria may be enumerated and the method cannot distinguish living from dead bacteria.
●Most probable number method (MPN method)
●Organisms that cannot grow on solid media can still be enumerated using the most probable number method
●Once again, cultures are diluted, here into a suitable broth medium
●"The more tubes that show growth, especially at greater dilutions, the more organisms were present in the sample."
●Particularly, at some greater dilution there will be on average no organisms added per tube of broth, while at lesser dilutions there will be organisms in every tube; the middle dilution at which the transition is made from all tubes inoculated with organism to few or none represents approximately the inverse of the concentration of organisms in the original culture

4. Turbidity

●The degree of turbidity (cloudiness) exhibited by a broth culture gives an indication of the number of organisms present
●Degree of turbidity varies with organisms, conditions, and phase of growth so use of turbidity as a form of enumeration requires previous standardization
●As with direct microscopic counts, use of turbidity is limited to relatively high cell concentrations

5. Biochemical Determinations

● Measures living bacteria
●Measurement of specific chemical changes in the media
●The rate of use of substrates or liberation of metabolic products also can be employed as methods of enumeration, though just as with turbidity, prior standardization is necessary
● For example measurement of acid or alkali or other end points in the media

6. Dry Weight

●measures total bacterial counts
●A time-consuming method of culture-mass determination involves drying cells (removing water) and then weighing them

NATURAL MICROBIAL POPULATION (MIXED CULTURES)

●Cough
●Sneeze
●Faeces
●Skin Scrapings
●Soil Samples
●Air Samples
●Water Samples
●Milk Samples

Selective Methods for Pure Culture

●Chemical Methods
●Physical Methods
●Biological Methods

1. Chemical Methods

●Use of Special Carbon or nitrogen Source
●Use of Dilute Media
●Use of Inhibitory or Toxic Chemicals – Selective Media

2. Physical Method

●Heat Treatment
●Incubation Temperature
●pH of the Medium
●Cell Size
●Cell Motility

3. Biological Methods

●Use of Laboratory Animals as Selective Media
●Mice for Streptococcus Pneumoniae

MAINTENANCE AND PRESERVATION OF PURE CULTURES

- Stock Culture Collection
- Laboratory Cultures
- Research Work
- Assay Tools for Vitamins and Amino Acids
- Production of Vaccines and Anti Sera
- Antitumour Agents, Enzymes, Organic Chemicals etc.

Considerations

- Amount of Labour Involved
- Storage Space
- To Maintain Cultures alive
- To Maintain Cultures Uncontaminated
- To Prevent any Change in the Characteristics

1. Periodic Transfer to the Fresh Media

- Culture Media – Slow Growth (vary with species)
- Storage Temperature (vary with species)
- Time Interval (vary with species)
- Disadvantages – Variants, Mutants

2. Overlaying Cultures with Mineral Oil

- Sterile Mineral Oil
- Duration Varies with Species (1 Month – 2 Years)
- Less Laborious
- Changes in the Characteristics can still occur

3. Lyophilization (Freeze Drying)

- Dense Cell suspension in vials
- High Vaccum Line
- Dehydration of Bacteria with Minimum Damage to Delicate cell Structures
- Vials an Sealed under Vaccum
- Stored in Refrigerator
- Stored for More than 30 years
- Minimum Space Required
- Transportation easy

4. Storage at Low Temperature

●Dense Cultures Containing a Cryoprotective Agent such as Glycerol
●Sealed in small Ampoules or Vials, Frozen at –150 °C
●Stored in Liquid Nitrogen –196 °C
●10 – 30 Years or more without undergoing changes in characteristics
●Expensive

BACTERIAL GENETICS

●Genetics is the study of the inheritance (heredity) and the variability of the characteristics of an organism
●Exact transmission of genetic information from parents to their progeny
●Variability of the inherited characteristics can be accounted for by a change either in the genetic makeup of a cell or in environmental conditions
Study of Microbial Genetics
●Genetic principals are universal
●The study of microbial genetics has contributed much to what we know about the genetics of all organisms
●There are distinct advantages in the use of bacteria for genetic experiments
Bacterial cultures contain millions of individual cells. Rare genetic events can be discovered
Rapid growth rates of microbes, the relative ease of growing bacteria and their viruses in a constant, controlled environment
Great diversity of metabolic types among microorganisms
●In addition to the inheritance of characteristics, changes are associated with two fundamental properties of the cell or organism, namely genotype and the phenotype

Genotype refers to the genetic constitution of the cell

Phenotype is the expression of the genotype in observable properties

●The genotype of a culture of cells remains relatively constant during growth
●However, it can change by mutation
●This change results in an alteration in the properties or phenotype of the cells
●Genotype represents the heritable total potential characteristics
●Phenotype represents the characteristics expressed
●Bacteria like the cells of higher organisms, carry genetic information – their genotype
●The extent to which this information is expressed depends on the environment
●Depending on the presence or absence of oxygen during growth

●The presence or absence of oxygen determines which enzymes function and which not

GENOTYPIC CHANGES

●The genotype of a cell is determined by the genetic information contained in its characteristics (or chromosomes).
●The chromosome is divided into genes
●A gene is a functional unit of inheritance it specifies the formation of a particular polypeptide as well as various types of RNA
●Each gene consists hundreds of nucleotide pairs
●Any gene is capable of changing or mutating to a different form
●A mutation is a change in the nucleotide sequence of a gene
●A cell or an organism which shows the effects of a mutation is called a mutant
●In nature, mutations are rare events which occur at random and arise spontaneously with no regard to environmental conditions
●Generally the mutants in a cell population are masked by the greater numbers of unmutated cells

Types of Mutation

●Changes in the purine-pyrimidine base sequence of a gene can occur, resulting in mutation
●Two common types are point mutations and frame shift mutations
●Point mutations occur as a result of the substitution of one nucleotide for another in the specific nucleotide sequence of a gene
●Transition type of point mutation is the replacement of a purine by a pyrimidine or vice versa
●This base-pair subsitution may result in one of three kinds of mutations affecting the translation process
●The altered gene triplet produces a codon in the mRNA which specifies an amino acid different from the one present in the normal protein This mutation is called a mis-sense mutation
●The altered gene triplet produces a chain terminating codon in mRNA, resulting in premature termination of protein formation during translation. This is called a non-sense mutation.
●These mutations result from an addition or loss of one or more nucleotides in a gene and are termed insertion or deletion mutations
●This results in a shift of the reading frame

How Mutation Occur

●Mutations most commonly occur during DNA replication

●Some mutations occur as the result of damages by ultraviolet(UV)light or x-rays
●Any agent that increases the mutation rate is called a mutagen
●Mutation obtained by use of a mutagen are said to be induced rather than spontaneous
●UV light causes mutation under both natural and laboratory conditions

Radiation

●The major effect of UV light is to cause the formation of dimers by cross-linking between adjacent pyrimidine
●These cross-linked residues disrupt the normal process of replication by preventing the various polymerases
●When x-rays interact with DNA, the result is usually a break in the phospho-diester backbone of the nucleic acid

How Mutations are Repaired

●Cells contain specific enzymes which can repair damaged DNA
●Many kinds of bacterial cells and yeasts have been shown to possess an efficient photoreactivating mechanism for repairing damage caused by UV radiation
●Photoreactitation occurs when cells exposed to lethal doses of UV light are immediately exposed to visible light
●A special enzyme designated PRE, induced by visible light, splits or unlinks the dimers formed because of exposure to UV light and restores the DNA to its original state
●Some bacteria have enzymes, called endonucleases and exomucleases, that excise or cut out a damaged segment of DNA
●The other enzyme, polymerases and ligases, repair the resulting break by filling in the gap and joining the fragments topgether
●The process by which E. cell repairs large amounts of DNA damage is called inducible or SOS repair
●Includes diverse responses such as the ability to repair pyrimidine dimers, to induce various prophages,
●to shut off respiration, and to delay pyrimidine dimers,
●to induce various prophages, to shut off respiration, and to delay septum formation during cell division
●All the responses are coordinately regulated
●The process is a very efficient one
●However, it tends to insert mismatched bases and thus is error-prone and introduces additional mutation

Mutation Rate

●The rate of mutation is the probability that a gene will mutate at any particular cell division. Thus the mutation rate is generally defined as the average number of mutations per cell per division
●It is expressed as a negative exponent per cell division
●For example, is there is one chance in a million that a gene will mutate when the cell divides, the mutation rate for any single gene equals 10-5 per cell division
●Generally, the mutation rate for any single gene ranges between 10 –3 and 10 –9 per cell division

Phenotypes of Bacterial Mutants

●An increased tolerance to inhibitory agents,particularly antibiotics(antibiotics, or drug resistant mutants)
●An altered fermentation ability or increased or decreased capacity to produce product
●Nutritionally deficient, that is, that require a more complex medium for growth than the original culture from which they were derived(auxotrophic mutants)
●Changes in colonial form or ability to produce pigments
●A change in the surface structure and composition of the microbial cell (ant. mutants)
●Resistant to the action of bacteriophages
●Change in morphological features, for example, the loss of ability to produce spores, capsules, or flagella
●Have lost a particular function but retain the intracellular enzymatic activities to catalyze the reactions of the function, for example, loss of a permease (cryptic mutants)
●A wild-type phenotype under one set of conditions and a mutant phenotype under another (conditionally expressed mutants)

Practical Implications with the Microbial Mutants

●Some microorganisms are known to develop resistance to certain antibiotics because of mutation
●Originally effective for the control of a bacterial infection become less effective or ineffective as antibiotic-resistant mutants appear
●Possible to isolate biochemical mutants capable of producing large yields of product
●This is important in industry
●Yield of penicillin in commercial production dramatically increase through selection of mutant strains of Penicillium

●The maintenance of pure culture of typical microorganism species requires that occurrence of mutation be prevented; otherwise, the culture will no longer be typical
●Microbial mutants have been extensively used in the investigation of various biochemical processes, particularly biosynthetic reactions.
●Mutants with blocks or impairment at different enzymatic steps have been used to unravel metabolic sequences

Designation of Bacterial Mutants

●Each genotype is given a lowercase, italicized, three-letter code
●A mutation which affects proline synthesis is designated pro
●Mutation in a number of different genes may exhibit identical phenotypes
●Number may be added sequentially to designate particular mutations that is as each mutation is isolated, it is assigned a number that identifies it in bacterial pedigress, for example pro A52 is the 52d isolate of the *Escherichia* coli

BACTERIAL RECMBINATION

●Formation of a new genotype by reassortment of genes following an exchange of genetic material between two different chromosome
●Have similar genes at corresponding sites
●These are called homologous is chromosomes and are from different individual
●Progeny from recombination have combinations of genes different from those that are present in the parents
●In bacteria, genetic recombination results from three types of gene transfer
●Transfer of genes between cells that are in physical contact with one another
●Transfer of genes from one cell to another by a bacteriophage
●Transfer of ce-l free or @naked@ DNA from one cell to another
●Recombination the cells do not fuse, and usually only a portion of the chromosome from the donor cell (male|) is transferred to the recipient cell (female)
●The recipient cell thus becomes a merozygote a zygote that is partial diploid
●Once merozygote transformation has occurred recombination can take place
●Inside the recipient cell the donor DNA fragment is positioned alongside the recipient DNA
●Homologous genes are adjacent
●Enzymes act on the recipient DNA, causing nicks and excision of a fragment
●The donor DNA is integrated into the recipient chromosome in place of the excised DNA
●The recipient cell then becomes the recombinant cell because its chromosome contains DNA of both the donor and the recipient cell

●The excised DNA pieces from the recipient chromosome are probably broken down by specific enzymes
●The genetics of plants and animals depends upon the regular cycle of sexual reproduction in these organisms
●An opportunity for different mutants of a species to mate with each other and produce new individuals with new combinations of mutations
●Mating or conjugation in E. coll is radically different from sexual mating in higher organisms
●It is not a reproductive process that occurs regularly at each generation
●It does not involve meiosis since bacterial cells are haploid, nor does it involves the fusion of gametes
●It involves the transfer of some DNA from one cell to another followed by separation of the mating pair of cells
●In conjugation it is possible for large segments of the chromosome and in special cases the entire chromosome, to be transferred

Sex Factors

●There is sexual differentiation in E.coll
●Male cells contain a small circular piece of DNA which is in the cytoplasm and not part of the chromosome, called the sex factor or F factor (fertility factor)
● These cells are referred to as F and are donors in mating
●Female cells lack this factor and are labled F
●They are recipient cells
●The male replicates its sex factor, and one copy of it is almost always transferred to the female recipient
●The F cell is converted to an F cell and is itself capable of serving as a donor
●The transfer of the F factor in an formation of in an F x F cross occurs with a frequency that approaches 100 percent
●Transfer of the F factor is independent of the transfer of chromosomal genes
●Since the transfer of the F factor is independent, it follows that the F factor DNA replicates independently of the F donor cell's normal chromosome
●One or more sex pill are produced by each F cell
●Sex pili seems to act to bind an F cell to an F cell and then to retract into the F cell, pulling the F cell into close contact
Extrachromosomal Genetic Elements
●Plasmids are circular pieces of DNA that are extra genes
●Either replicating autonomously or integrating into the bacterial DNA chromosome and are called episomes
●F cell of E. coli was called an episome because it can alternately exist in the F

Transduction

●Most bacteriphages, the virulent phages undergo a rapid lytic growth cycle in their host cells.
●They inject their nucleic acid, usually DNA, into the bacterium, where it replicates rapidly and also directs the synthesis of new phage proteins. Within 10 to 20 min, depending on the phage, the new DNA combines with the new proteins to make whole phage particles, which are released by destruction of the cell wall and lysis of the cell
●However some bacteria viruses, the temperate phages, which do not lyse the cell, carry DNA that can behave as a kind of episomes in bacteria; like other episomes, such as the F, these viral genomes can become integrated into the bacterial genome
●They are then known as prophages
●Bacteria that carry prophages (lysogenic bacteria) can be induced with ultraviolet light and other agents to make the prophages start to replicate rapidly and go through a lytic growth cycle, resulting in lysis of the cell with release of new phage particles
●Phage particles may become filled with cell chromosom DNA or amixture of chromosomal and phage DNA (rather than comparatively with phage DNA, as a normally the case).
●Such aberrant phages can attach to other bacteria and introduce bacterial DNA from one cell to another

Generalized Transduction

●If all fragments of bacterial DNA from any region of the bacterial chromosome have a chance to enter a transducting phage, the process generalized transduction. In this process, as the phage begins the lytic cycle, viral enzymes hydrolyze the bacterial chromosome into many small pieces of DNA. Any part of the bacterial chromosome may be incorporated into the phage head during phage assembly and is usually not associated with any viral DNA
●Generalized transduction, like bacterial conbjugation and transformation also provides a means for mapping bacterial genes, since the fragments transferred by a bacteriophage are often large enough to contain hundreds of genes.
●Certain temperate phage strains can transfer only a few restricted genes of the bacterial chromosome. More specifically, the phages transduce only those bacterial genes adjacent to the prophage in the bacterial chromosome.
●Rusticated transduction occurs when a bacteriophage genome, after becoming integrated as prophage in the DNA of the host bacterium, again becomes free upon induction and takes with it into the phage head a small adjacent piece of the bacterial chromosome. When such a phage infects a cell, it carries with it the group of nbacterial genes that has become part of it

●Cell free or DNA containing a limited amount of gene form is transferred from one bacterial cell to another
●Donor – Not all lysis or chem. Extraction – Recip cell – Recomb- Transformed contain but grown in the presence of dead cells, culture filtrates or cell extracts of clearly related strain
●After DNA enter into a cell – one straned is immediately degraded by deoxyribonucleases, the other undergoes base pairing with the homolegous portion of the recipient ch. – integrated
●Only closely related strains of bact
●Late logarithmic phase of growth
●Transformable bacteria are said to be competent to take up and incorporate donor DNA
●The uptake process has been found to be an energy requiring mechanism because it can be inhibited by agents that interfere with energy metabolism
●Transformation between bacterial strain of low virulence
●Can give rise to transformed cells of high virulence
●Extremely useful in genetic studies of bacteria in the laboratory

BACTERIAL CELL WALL

●The tough, rigid cell walls of bacteria protect them from mechanical damage and osmotic lysis
●Differences in the structure and chemical composition of the cell walls of bacterial species account for variation in their pathogenicity
●Influence other characteristics including staining properties

●Peptidoglycan, a polymer unique to prokaryotic cells, imparts rigidity to the cell wall
●This polymer is composed of chains of althernating subunits of N-acetylglucosamine and N-acetylmuramic acid cross-linked by short tetrapeptide side chains and peptide cross-bridges
●Bacteria can be divided into two major groups, Gram-positive and Gram-negative, on the basis of colour when stained by the Gram method
●This colour reaction is determined by the composition of the cell wall
●Gram-positive bacteria which stain blue have a relatively thick uniform cell wall which is composed mainly of peptidoglycan and teichoic acids
●Gram-negative bacteria, which stain red, have cell walls with a more complex structure, consisting of an outer membrane and a periplasmic space containing a comparatively small amount of peptidoglycan
●The outer membrane is a protein-containing asymmeterical lipid bilayer.
●The structure of the inner surface of the membrane resembles that of the cytoplasmic membrane, whereas that of the outer surface is composed of lipopolysaccharide (LPS) molecules
●Low molecular weight substances such as sugars and amino acids enter through specialized protein channels, known as porins, in the outer membrane
●The outer membrane LPS, the endotoxin of Gram-negative bacteria, is released only after cell lysis
●The major components of LPS molecules are core polysaccharides bound to lipid A and long external polysaccharide side chains.
●The polysaccharide side chains of the LPS molecules stimulate antibody production and correspond to the somatic (O) antigens used for serotyping of Gram-negative cells
●Lipid A is the molecular component in which endotoxic activity resides
●The outer membrane excludes hydrophobic molecules and renders Gram-negative bacteria resistant to some detergents which are lethal to most Gram-positive bacteria.

CYTOPLASMIC MEMBRANE

●Flexible structures composed of phospholipids and proteins
●Bacterial cytoplasmic membrane, with the exception of those present in mycoplasmas, do not contain sterols
●The inner and outer faces of cytoplasmic membranes are hydrophilic which the interior is hydrophobic
●Only a limited range of small molecules such as water, oxygen, carbon dioxide and some lipid-soluble compounds can enter bacterial cells by passive diffusion.
●Two major functions of the cytoplasmic membrane
●The active transport of nutrients into the cell

●Elimination of waste metabolites, require the expenditure of energy
●The cytoplasmic membrane is also the site of electron transport for bacterial respiration, of phosphorylation systems and of enzymes and carrier molecules that function in the biosynthesis of DNA, cell wall polymers and membrane lipids.

CYTOPLASM

●An aqueous fluid containing the nuclear material, ribosomes, nutrients and the enzymes and other molecules
●Synthesis, cell maintenance and metabolism
●Storage granules may be present under certain environmental conditions, usually those unfavourable for bacterial growth
●These granules, which may be composed of starch, glycogen, polyphosphate or other compounds, can often be identified using particular dyes.

RIBOSOMES

●All protein synthesis takes place on ribosomes
●Composed of ribonucleoproteins and are up to 25 nm in size
●They consist of two subunits, a larger 505 subunit and a smaller 30S subunit
●The Svedberg (S) unit is a measure of sedimentation rate, which is dependent on both the size and shape of a particle
●Ribosomal RNA is complexed with many different proteins and accounts for about 80% of the RNA of the cell.
●Smaller amounts of transfer RNA (tRNA) and messenger RNA (mRNA) account for the remaining cellular RNA
●Ribosomes may be present either in the cytoplasm or associated with the inner surface of the cytoplasmic membrane
●During active bacterial growth and rapid protein synthesis, individual ribosomes are joined by mRNA into long chains known as polysomes.

NUCLEAR MATERIAL

●Composed of a single haploid circular chromosome containing double-stranded DNA
●Small amounts of protein and RNA are also associated with nuclear material
●The genes in the bacterial chromosome code for all the vital functions of the cell
●Bacterial genomes vary in size depending on the species
●Because of its length, the bacterial chromosome is extensively folded to form a dense body which can be seen by electron microscopy

●The nuclear material can also be demonstrated by light microscopy when stained by the Feulgen method which is specific for DNA..
●During replication, the DNA helix unwinds and both daughter cells, produced by binary fission, receive a copy of the original genome
●Plasmids, small circular pieces of DNA which are separate from the genome
●Capable of autonomous replication
●Several different plasmids may be present in individual bacterial cells
●Copies of plasmids can be transferred from cell to cell during binary fission or through conjugation
●Plasmid DNA may code for characteristics such as antibiotic resistance and exotoxin production
●Not essential for bacterial survival.

Spores and Sporulation

●Certain species of bacteria produce spores
●Within the cell (endospores) or external to the cell (exospores)
●Spores are metabolically dormant forms
●Under appropriate conditions can undergo germination and out growth to form a vegetative cell

Endospores

●Thick walled, highly refractile bodies
●Produced by Bacillus, Clostridium and few other genera
●Shape and location vary depending on the species

Characteristics of Endospores

●Endospores are extremely resistant to dessication, staining, disinfectants, radiation and heat
●Resistance is due to
- Low water contents (20 %)
- Large amounts of dipicolinic acid (DPA),unique compound undetectable in the vegetative cells (10 – 15 % of spores dry wt)
- Large amounts of calcium, located in the core
- Calcium DPA complex – role in heat resistance
- Synthesis of DPA and uptake of calcium takes place during advanced stages of sporulation

Exospores

●Cells of methane oxidising genus, Methylosinum form xospores
●Spores external to the vegetativ cell
●By budding at one and
●Dessication and heat resistant
●Do not contain DPA

Growth and Cell Division

Definition

●Microbial growth is defined not in terms of cell size but as the increase in the number of cells which occurs by cell division.

Cell Division

●Cell division in bacteria, unlike cell division in eukaryotes, usually occurs by binary fission or some
times by budding
●In binary fission, a cell duplicates its components and divides into two cells
●The daughter cells become independent where a septum (partition)grows between them and they
separate
●DNA synthesis also is continuous and replicates the single bacterial chromosome shortly before the
cell divides
●The chromosome is attached to the cell membrane, which grows and separates the replicated
chromosomes
●Replication of the chromosome is completed before cell division
●In some species, incomplete separation of the cells produces linear chains (linked bacilli), tetrad
(Cuboidal groups of four cocci), sarcinae (singular sarcina; groups of eight cocci in a cubical packet),
or grapelike clusters (staphylococci)
●Streptococci from chains when grown on artificial media but exist as single or paired cells when
isolated from a rapidly growing lesion in an infected human host.
●Cell division in yeast and a few bacteria occurs through budding

Phases of Growth

●Consider a population of organisms introduced into a fresh, nutrient-rich medium (plural media)

Lag Phase

●In the lag phase, the organisms do not increase significantly in number, but they are metabolically
active
●Growing in size, synthesizing enzymes
●Incorporating various molecules from the medium
●During this phase the individual organisms increase in size, and they produce large quantities of
energy in the form of ATP
●The length of the lag phase is determined in part by characteristics of the bacterial species

Log Phase

●Population growth occurs at an exponential or logarithmic (log) rate
●During the log phase, the organisms divide at their most rapid rate
●A regular, genetically determined interval called the generation time
●The population of organisms doubles in each generation time
●The number of cells in a culture would increase in a stair-step pattern, exactly doubling every 20
minutes – a hypothetical situation called synchronous growth
●Non synchronous growth appears as a smooth line, not as steps, on a graph

Stationary phase

● Stationary phase is a steady-state equilibrium where the rate of cell growth (division) is exactly
balanced by the rate of cell death
● Cell death (or, at least, lack of cell growth) occurs because of a loss of limiting nutrients (due to
their incorporation into cells during log-phase growth) or a build-up of toxins (due to their release
during log-phase growth, e.g., fermentative products)
● Note that the simplest conditions that will result in a stationary phase is when both the rate of cell
increase and the rate of cell death together equal zero (i.e., cells neither die nor are born)

Decline phase (death phase)

●Stationary phase, in a standard bacterial growth curve, is followed by a die-off of cells

- Cell death in bacteria cultures basically means that the cells are unable to resume division
  following their transfer to new environments
- Typically this die-off occurs exponentially, i.e., such that cell number graphed against time, using
  a semi-log scale for cell number, results in a straight line
- This death occurs because vegetative cells can survive exposure to harsh conditions (few nutrients
  or too-many toxins) for only so long

Factors affecting bacterial growth

- The kinds of organisms found in a given environment and the rates at which they grow can be
  influenced by a variety of factors, both physical and biochemical
- Physical factors include pH, temperature, oxygen concentration, moisture, hydrostatic pressure,
  osmotic pressure, and radiation
- Nutritional (biochemical) factors include availability of carbon, nitrogen, sulfur, phosphorus, trace
  elements, and, in some cases, vitamins

Physical Factors

1. pH

- pH scale measures hydrogen ion (H+) concentration and that low pHs correspond with high
  concentrations of hydrogen ions (1-14)
- Optimum pH
- Optimum pH is that pH at which a given organism grows best
- The range over which most organisms can grow tends to vary over no more than a single pH unit in
  either direction (e.g., from pH 6 to pH 8 for an organism whose pH optimum is pH 7)

Acidophiles

- Optimum pH is relatively to highly acidic

Neutrophiles

● Optimum pH ranges about pH 7, plus or minus approximately 1.5 pH units (5.5-8.5)

Alkaphiles

● Optimum pH is relatively towards basic

2. Optimum temperature

● Optimum temperature is the temperature at which an organism grows best
● Psychrophilic, mesophilic, and thermophilic bacteria
● Typically the range in temperature over which a bacterium can grow is about 30ºC.

Psychrophiles

● Cold-adapted organisms are called psychrophiles
● The cut-off temperature for a psychrophile is a 20ºC or colder temperature optimum
● Psychrophiles may additionally be termed obligate or facultative with obligate psychrophiles unable
to grow above 20ºC, but facultative psychrophiles are able to grow

Mesophiles

● Organisms whose optimum growth temperatures is found between 20ºC to 40ºC are termed
mesophiles
● Human pathogens, which must be able to grow at the approximately 37ºC body temperature, are
mesophiles

Thermodurics

● Mesophilic organisms that can endure brief exposures to relatively high temperatures are termed
thermoduric
● These are one category of the organisms that survive following inadequate heating of foods and
may thereby contribute to the spoilage of foods that have been heated (e.g., Pasteurization) to kill
microorganisms

Thermophiles

High-temperature-adapted organisms are called thermophiles

3. Oxygen requirement

● Organisms differ in their requirements of molecular oxygen (i.e., O2) as well as other atmospheric
gasses (e.g., carbon dioxide)
● Categories of oxygen requirements include:
● Obligate aerobes
● Obligate anaerobes
● Microaerophiles
● Facultative anaerobes
● Aerotolerant anaerobes

Obligate aerobes

● Organisms that are unable to generate ATP via fermentation are termed obligate aerobes
● This term is somewhat misleading because some of these organisms can still grow in the absence of
molecular oxygen by employing alternative final electron acceptors to their electron transport
systems
● The bottom line, then, for an obligate aerobe is a dependence on an electron transport system for
their generation of ATP as well as a tolerance for atmospheric oxygen (which otherwise can serve
as a poison).

Obligate anaerobes

● Organisms that are unable to detoxify atmospheric oxygen are termed obligate anaerobes because
they cannot grow (nor, often, even survive) in the presence of oxygen
● Obviously, obligate anaerobes must possess means for ATP generation that do not require
molecular oxygen, e.g., fermentation pathways

Microaerophiles

● These are organisms that grow best when small amounts of oxygen are present
● That is, less (typically much less) than atmospheric concentrations, but more than those
concentrations tolerable by obligate anaerobes

Facultative anaerobe

● Facultative anaerobes can generate ATP by either aerobic or anaerobic means and have the means
to detoxify oxygen
● These organisms tend to exist in environments in which oxygen concentrations are uncertain, and
serve as the oxygen scavengers in environments
● For example, the lumen of the large intestine is mostly anaerobic because:
● The body does not actively oxygenate the lumen of the large intestine
● Oxygen scavengers such as Escherichia coli remove what oxygen manages to leak into this
environment
● Facultative anaerobes tend to grow better/faster when O2 is present.

Aerotolerant anaerobe

● These are organisms that are unable to utilize molecular oxygen in their ATP generation but
otherwise possess the means to detoxify oxygen
● This allows, if nothing else, the safe passage of these organisms through aerobic environments
● Lactobacillus spp. for example generate their ATPs by fermentation regardless of the oxygen
concentrations of the environment

Strict anaerobe

● A strict anaerobe is unable to replicate, and may even die given the presence of oxygen
● This inability or fragility results from a lack of enzymes necessary to detoxify molecular oxygen

Capnophiles

● These are organisms whose optimum growth requires relatively high concentrations of carbon

dioxide

4. Osmotic Pressure

● The concentration of dissolved substances in the environment can impact on the growth and
survival of cells
● Environments containing large concentrations of dissolved substances draw water out of cells,
causing a shrinkage of the cytoplasm volume, a phenomenon termed plasmolysis
● Plasmolysis interferes with growth and this is why highly osmotic environments prevent bacterial
growth (e.g., brine, the high sugar concentrations in jellies and jams, salting of meats)
● Halophiles
● Require high concentrations of dissolved salts to grow are termed halophiles
● Depending on organism, the salt concentrations required range from those of sea water up to those
Of brine

Nutritional Factors

All forms of life from microorganisms to human share certain nutritional requirements to grow, repair
themselves, and to replicate:

(i) Source of energy

➢Chemotrophs
➢Phototrophs

(ii) Source of electrons for metabolism

➢Lithotrophs (inorganic compounds)
➢Chemolithorophs
➢Photolithotrophs

(iii) Source of Carbon – Synthesis of cellular components

➢Autotrophs (Carbon dioxide)
➢Heterotrophs (Organic compounds).

(iv) Source of Nitrogen – Cellular components and enzyme systems

➢Atmosphoric nitrogen
➢Inorganic nitrogen compounds
➢Organic nitrogen compounds
▪Nitrogen may be obtained from inorganic sources (e.g., nitrate ions, NO3-, or ammonium ion, NH4+) or from organic sources (i.e., amino acids or nucleotides)
▪In contrast, organisms such as ourselves obtain nitrogen solely from organic sources
▪Many microorganisms (e.g., E. coli) can satisfy all of their nitrogen needs from inorganic sources
▪Fastidious microorganisms may require one or more amino acid in their growth medium because they are unable to synthesize that amino acid.

(v) Oxygen – Provided in various forms

➢Water
➢Molecular oxygen
➢Component items of various nutrients

(vi) Sulfur – Needed for synthesis of amino acids Cysteine, Cystine and methionine
➢Organic sulfur compound
➢Inorganic sulfur compound
➢Elemental sulfur
➢Like nitrogen, sulfur may be obtained from organic sources (sulfur-containing amino acids) or from inorganic sources.

(vii) Phosphorus – Essential component of nucleotides, nucleic acids, phospholipids, teichoic acids

➢Unlike nitrogen and sulfur, phosphorous is typically obtained by microorganisms in its inorganic form, i.e., the phosphate ion (PO43-).

(viii) Metal ions –

Living organisms rquires, K, Ca, Mg,Fe, for growth

(ix) Trace elements –

Zn, Cu, Mn, Mo, Ni, B and Co

- Components of some of the culture media to support bacterial growth
- Trace elements are required in relatively small quantities
- Typically these elements are employed as enzyme cofactors, e.g., metals to which enzymes must complex with in order to function properly.

(x) Vitamins and Vitamin Like Compound

- Some synthesis their entire requirements of vitamins in the culture medium but others can do so.
- Some microorganisms require various vitamins as well as additional organic factors (e.g., specific amino acids)
- Vitamins are essentially organic equivalents of trace elements, i.e., organic molecules required in relatively small amounts which various enzymes must complex with in order to function properly
- While many microorganism can synthesize vitamins, others cannot and an inability to synthesize vitamins contributes to an organism's fastidiousness since vitamins consequently must be supplied in the organism's growth medium.

(xi) Exoenzymes

- Microorganisms employ enzymes as well as transport proteins to bring substances into their cytoplasm
- If an organism possesses sufficient kinds of enzymes, they can break down or build up just about anything, though no organism possesses all possible enzymes so consequently no organism is capable of breaking down nor building up everything
- In addition to enzymes found within their cells, many bacteria (as well as fungi and ourselves) produce enzymes that are secreted from cells into the surrounding medium
- These enzymes typically are employed to break down nutrients found outside of cells (extracellularly) so that the breakdown products may be taken up into the cell and used.
- Many of these enzymes are harmful and represent exotoxins produced by disease-causing microorganisms, especially Gram-positive bacteria
- To either be broken down in catabolic reactions
- Used as building blocks to produce more complex substances via anabolic pathways

(xii) Water –

All nutrients must be in aqueous solution before entry in the cells

➢High specific heat – provides resistence tosudden temperature change
➢Chemical reactant – require for many hydrolytic reactions.

culture medium

●To successfully grow microorganisms, one must employ a culture medium that contains all of the nutrients required by an organism (as well as conditions that meet an organism's physical requirements)
●Media may be differentiated in various ways

Broth versus solid media

●Broth media is liquid while solid media typically has agar added as a solidifying agent
●Semi-solid media also exists that contains insufficient quantities of agar to fully solidify the media
Solid medium
●Solid media contains agar (1.5 – 2%), which is a compound that goes into water solution at temperatures approaching boiling, and then, once in solution, solidifies the medium at room (<40ºC) temperature
●Subsequent exposure to high temperature (i.e., boiling) will melt the medium
●Exposure to relatively low temperatures (i.e., >40ºC), however, will not melt the medium, thus allowing incubation of solid medium at various temperatures (compare to gelatin which liquefies at 37ºC)
●Once boiled, agar-containing medium will stay liquid at 45ºC
●This allows solid medium to be poured into various vessels at temperatures that will not kill most cells (nor melt vessels), followed by a solidification of the medium
●Semi solid media contain agar 0.5 % or less, soft custard like consistency
●Cultivation of microaerophilic bacteria and for determination of bacterial motility

Synthetic medium

A synthetic medium is prepared in the laboratory from reasonably well-defined ingredients

●By contrast, a non synthetic medium could be something like soil or sewage or ocean mud, i.e., something obtained directly from the environment
●A defined synthetic medium is produced only from well-defined, relatively pure ingredients
●A defined synthetic medium for growing Proteus vulgaris and Escheria coli

General Purpose Media
●For the growth of most of the common bacteria
●Nutrient broth – Liquid media
●Nutrient agar – Solid media

Complex media (chemically undefined media)

●A second approach to producing a synthetic medium is to employ ingredients that are not well-defined nor pure
●Such ingredients additionally may vary from batch to batch
●For example, complex media may contain extracts from animals (e.g., beef, hearts, milk, etc.), plants (e.g., soy beans), or microorganisms (e.g., yeast)
●Complex media may additionally include very complex ingredients such as blood

Selective media

Enhance the growth and predominance of a particular type of bacteria

●Act to inhibit the growth of other kinds of microorganisms
●MacConkey's agar for Enterobacteriacae
●Staph. 110 for staphylococci
●Use of antibiotics – Penicillin for Mycoplasma

Differential media

●Differential media contains ingredients that allow different microorganisms to look reproducibly different
●Consequently, one may determine whether a given organism is present in a culture on the basis of colony morphology alone
●Confusingly, many media employed in microbiological laboratories are both differential and selective
●That is, many media both inhibit the growth of certain microorganisms while making those organisms that it allows to grow produce easily differentiated colony morphologies.
●Blood agar – To differentiate hemolytic and non hemolytic bacteria

Assay Media

●Media of prescribed composition
●Determination of production potential of bacteria I.e., vitamins, amino acids, antibiotics etc.

Media for Enumeration of Bacteria

●Specific types of media for determining the bacterial counts i.e., in milk water etc.
●Specific composition

Media for Characterization of Bacteria

●To determine the type of growth produced by bacteria
●To determine their ability to produce certain chemical changes

Maintenance Media

●Maintenance of viability and physiological characteristics
●Media supporting slow growth

Preparation of Media

●Naturally occurring substances like skim milk, meat yeast extract etc.
●Dissolved in appropriate volume of distilled water
●Adjustment of pH
●Solid media – agar is added and the medium is boiled
●Dispensed into tubes or flasks

Bacterial Colonies

Colony-forming unit (CFU)
Whether a single cell or a clump of cells or whatever, what grows into an isolated colony is termed a colony-forming unit
●When doing standard plate counts, what is being estimated are numbers of colony-forming units
●The number of colonies that may be counted per petri dish (and therefore the number CFUs that may be enumerated) is limited by statistics (at the low end) and space on the dish (at the upper end) to between 30 and 300 colonies/CFUs per plate

●Sterilization

–Autoclaving for thermo stable ingredients
–Filteration for thermo labile ingredients

Serial dilution

●For a given agar surface, there are only so many colonies that may be present before it becomes impossible to accurately count the number of colonies present
●This number serves as a limit on the concentration of bacteria in cultures that may be enumerated using the standard plate-count technique

●How to get around this limitation? This may be achieved by diluting cultures before enumerating them
●By keeping track numerically of the degree of diluting employed, the concentration of bacteria in the pre-diluted culture may be estimated
●Due to limitations in the size of the volumes that may be conveniently handled (i.e., both very large volumes and very small volumes are difficult to handle), relatively large dilutions are typically handled serially
●For example, a 10-fold dilution followed by a second 10-fold dilution (i.e., 1 ml from a parent culture diluted to 9 ml diluent followed by mixing followed by 1 ml from the diluted culture diluted to an additional 9 ml diluent followed by mixing) gives a total of a 100-fold dilution

Measurement of Bacterial Growth

●Cell Count
●Cellular Mass
●Cellular Activity

1. STANDARD PLATE COUNT

●When bacteria are placed on a solid medium, ideally each colony is founded by only a single bacterial cell (obviously, given clumping and various cell arrangements, this ideal is not always met)
●Thus, addition of a known quantity of bacterial cells to a solid medium should produce the same number of colonies
●This result can be employed backward so that the number of colonies grown on solid medium can be used to estimate the number of individual bacteria that were added to the solid medium
●This number, in turn, may be employed to estimate the concentration of bacteria present in a culture

A. Pour plate

The pour-plate method is employed for bacterial-cell enumeration and isolation

●In the pour-plate method of addition of cells to solid medium contained within a petri dish, cells are added to melted (but not too hot) solid medium
●The melted solid medium is then poured into a petri dish and allowed to harden
●Colonies appear both within, beneath, and on top of the agar

B. Spread plate

The spread plate method is employed for bacterial-cell enumeration and isolation

●In the spread-plate method of addition of cells to solid medium, a small volume of culture is dropped onto the surface of agar that has already hardened in a petri dish
●The volume is then spread around the agar surface
●Colonies will grow solely on the surface of the agar
●This technique is advantageous particularly when cells are sensitive to exposure to relatively high temperatures plus the method does not require a prior melting of the solid medium.

2. MEMBRANE FILTER COUNT

● Measures living bacteria
●Use of membrane filters with known uniform pore size

3. DIRECT MICROSCOPIC COUNTS

Measures total bacterial count

●Use of Petroff Hausers counting chambers
●A more direct means of enumerating bacteria is done by viewing them through a microscope
●This method's limitations are that only relatively high concentrations of bacteria may be enumerated and the method cannot distinguish living from dead bacteria.
●Most probable number method (MPN method)
●Organisms that cannot grow on solid media can still be enumerated using the most probable number method
●Once again, cultures are diluted, here into a suitable broth medium
●"The more tubes that show growth, especially at greater dilutions, the more organisms were present in the sample."
●Particularly, at some greater dilution there will be on average no organisms added per tube of broth, while at lesser dilutions there will be organisms in every tube; the middle dilution at which the transition is made from all tubes inoculated with organism to few or none represents approximately the inverse of the concentration of organisms in the original culture

4.Turbidity
●The degree of turbidity (cloudiness) exhibited by a broth culture gives an indication of the number of organisms present

●Degree of turbidity varies with organisms, conditions, and phase of growth so use of turbidity as a form of enumeration requires previous standardization
●As with direct microscopic counts, use of turbidity is limited to relatively high cell concentrations

5. Biochemical Determinations
● Measures living bacteria
●Measurement of specific chemical changes in the media
●The rate of use of substrates or liberation of metabolic products also can be employed as methods of enumeration, though just as with turbidity, prior standardization is necessary
● For example measurement of acid or alkali or other end points in the media

6. Dry Weight
●measures total bacterial counts
●A time-consuming method of culture-mass determination involves drying cells (removing water) and then weighing them

Natural Microbial Population (Mixed Cultures)
●Cough
●Sneeze
●Faeces
●Skin Scrapings
●Soil Samples
●Air Samples
●Water Samples
●Milk Samples

Selective Methods for Pure Culture
●Chemical Methods
●Physical Methods
●Biological Methods

Chemical Methods
●Use of Special Carbon or nitrogen Source
●Use of Dilute Media
●Use of Inhibitory or Toxic Chemicals – Selective Media

Physical Method
●Heat Treatment
●Incubation Temperature
●pH of the Medium
●Cell Size
●Cell Motility

Biological Methods
- Use of Laboratory Animals as Selective Media
- Mice for Streptococcus Pneumoniae

Maintenance and Preservation of Pure Cultures
- Stock Culture Collection
- Laboratory Cultures
- Research Work
- Assay Tools for Vitamins and Amino Acids
- Production of Vaccines and Anti Sera
- Antitumour Agents, Enzymes, Organic Chemicals etc.

Considerations
- Amount of Labour Involved
- Storage Space
- To Maintain Cultures alive
- To Maintain Cultures Uncontaminated
- To Prevent any Change in the Characteristics

1. Periodic Transfer to the Fresh Media
- Culture Media – Slow Growth (vary with species)
- Storage Temperature (vary with species)
- Time Interval (vary with species)
- Disadvantages – Variants, Mutants

2. Overlaying Cultures with Mineral Oil
- Sterile Mineral Oil
- Duration Varies with Species (1 Month – 2 Years)
- Less Laborious
- Changes in the Characteristics can still occur

3. Lyophilization (Freeze Drying)
- Dense Cell suspension in vials
- High Vaccum Line
- Dehydration of Bacteria with Minimum Damage to Delicate cell Structures
- Vials an Sealed under Vaccum
- Stored in Refrigerator
- Stored for More than 30 years
- Minimum Space Required
- Transportation easy.

4. Storage at Low Temperature
- Dense Cultures Containing a Cryoprotective Agent such as Glycerol
- Sealed in small Ampoules or Vials

●Frozen at –150 oC
●Stored in Liquid Nitrogen –196 oC
●10 – 30 Years or more without undergoing changes in characteristics
●Expensive

Control of Microorganisms

1. Control by Physical Agents
2. Control by Chemical Agents
3. Control by Antibiotics and Chemotherapeutic Agents

Mode of Action

●The manner in which antimicorbial agents inhibit or kill can be attributed to the following kinds of actions:

1. Damage to the cell wall or inhibition of cell-wall synthesis
2. Alteration of the permeability of the cytoplasmic membrane
3. Alteration of the physical or chemical state of proteins and nucleic acids
4. Inhibition of enzyme action
5. Inhibition of protein or nucleic acid synthesis

Physical Agents

A. High Temperature

●Microorganisms can grow over a wide range of temperature
●Very low temperature characteristic of psychrophiles
●Every type has an optimum, minimum and maximum growth temperature
●Very high growth temperature characteristic of thermophiles
●Temperature above maximum – generally kills all microbes
●Temperature below the minimum – produces stasis, may be considered preservative
●High temperature combined with moisture is one of the most effective methods of killing microorganisms
●Moist heat kills microorganisms by coagulating their proteins
●Much more rapid and effective than dry heat
●Dry heat destroys microorganisms by oxidizing their chemical constituents
●Spores of Cl.botulinum are killed in 4 to 20 min by moist heat at 120°C, four hours required during dry heat
●Spores of B. anthracis are destroyed in 2 to 15 min by moist heat at 100°C, 1 to 2 hours at 150°C is required during dry heat
●Vegetative cells are much more sensitive to heat than are spores
●Cells of most bacteria are killed in 1 to 10 min at 60 to 70°C (moist heat).

1. Moist Heat

The application of moist heat for inhibiting or destroying microorganisms is discussed by the method used to obtain the desired result.

Steam Under Pressure

●Heat in the form of saturated steam under pressure is the most practical dependable agent for sterilization
●Steam under pressure provides temperatures above those obtainable by boiling
●The laboratory apparatus designed to use steam under regulated pressure is called an autoclave
●A double-jacked steam chamber equipped with devices which permit the chamber to be filled with saturated steam and maintained at a designed temperature and pressure for any period of time.
●The autoclave is an essential unit of equipment in every microbiology laboratory

2. Fractional Sterilization (tyndallization)

●Some microbiological media, solutions of chemicals and biological materials cannot be heated above 100 °C without being damaged
●It is possible to sterilize them by fractional sterilization (tyndallization).

3. Boiling Water

Contaminated material or objects exposed to boiling water cannot be sterilized with certainty

- Vegetative cells will be destroyed within minutes by exposure to boiling water
- Boiling water cannot be (and is not) used in the laboratory as a method of sterilization

4. Pasteurization

milk, cream and certain alcoholic beverages (bear and wine) are subjected to a controlled heat treatment (called pasteurization) Which kills microorganisms of certain types but does not destroy all organisms

5. Dry Heat: Hot Air Sterilization

●Dry-heat, or hot-air, sterilization is recommended where it is either undesirable or unlikely that steam under pressure will make direct and complete contact with the materials to be sterilized
●This is true of certain items of laboratory glassware, such as Petri dishes and pipettes, as well as oils, powders, and similar substances
●The apparatus employed for stove oven
●Exposure to a temperature of 160°C is sufficient for sterilization.

6. Incineration

●Destruction of microorganisms by burning is practical routine in the laboratory when the transfer needle is introduced into the flame of the Bunsen burner
●The transfer needle is sterilized, care should be exercise to prevent spattering
●Incineration is used for the destruction of carcasses, infected laboratory animals, and other infected materials to be disposed of.

B. Low Temperature

●Temperature below the optimum for growth depress the rate of metabolism, and it if the temperture is sufficiently low, growth and metabolism cease.

●Low temperature are useful preservation of cultures, since microorganisms have a unique capacity for surviving extreme cold.
●Agar-slant cultures of some bacteria, yeasts, and molds are customarily stored for long periods of time at refrigeration temperatures of about 4 to 7 oC.
●Many bacteria and viruses can be maintained in a deep-freeze unit at temperature from –20 to –70 oC
●Liquid nitrogen at a temperature of –196 oC, is used for preserving cultures of many viruses and microorganisms

C. Desiccation

●Desiccation of the microbial cell causes a cessation of metabolic activity, followed by a decline in the total viable population
●Desiccation varies on the following factor
1. The kind of microorganism
2. The material in or on which the organisms are dried
3. The completeness of the drying process
4. The physical conditions to which the dried organisms are exposed e.g., light, temperature, and humidity.

D. Osmotic Pressure

●When two solutions with differing concentrations of solute are separated by a semi permeable membrane, there will occur a passage of water,through the membrane, in the direction of the higher concentration
●Thus if cells are exposed to solutions with higher solute concentration, water will be drawn out of the cell, the process is called plasmolysis
●The reverse process, that is, passage of water from a low solute concentration into the cell, is termed plasmoptysis.

E. Radiation

●Energy transmitted through space in a variety of forms is generally called radiation
●The most significant type of radiation is probably electromagnetic radiation of which light and x-rays are examples
●Application of ionizing radiation to sterilize biological material called cold sterilization

1. Ultraviolet Light

●The ultra violet portion of the spectrum includes all radiations from 150 to 3900 A
●Wavelengths around 2650 A have the highest bactericidal efficiency
●Many lamps are available which emit a high concentration of ultraviolet light in the most effective region, 2600 to 2700 A
●Germicidal lamps, which emit ultraviolet radiations, are widely used to reduce microbial populations

Mode of Action

●Ultraviolet light is absorbed by many cellular materials but most significantly by the nucleic acids, where it does the most damage

●The absorption and subsequent reaction are predominantly in the pyrimidines of the nucleic acid

2. X-Rays (Roentgen Rays)

●X-rays are lethal to microorganisms and higher forms of life

●Unlike ultraviolet radiation, they have considerable energy and penetration ability

●Impractical for purposes of controlling microbial populations

●They are very expensive to produce in quantity

●They are difficult to utilize efficiently, since radiations are given off in all directions from their point of origin

3. Gamma Rays

●Gamma radiation are high-energy radiations emitted from certain radioactive isotopes

●These isotopes are potential sources of gamma radiations

●Gamma rays are similar to x-rays but are of shorter wavelength and higher energy

●They are capable of greater penetration into matter, and they are lethal to all life, including microorganisms

4.Cathode Rays(Beam Radiation)

●When a high voltage potential is established between a cathode and an anode in an evacuated tube, the cathode emits beams of electrons, called cathode rays

●The election accelerator, a type of equipment which produces the high-voltage electron beam

●Today for the sterilization of surgical supplies, drugs and other materials

F. Surface Tension and Interfacial Tension

●The interface, or boundary , between a liquid and a gas is characterized by unbalanced forces of attraction between the molecules in the surface of the liquid and in the interior

●Changes in surface tension may alter the permeability

●Characteristics of cytoplasmic membrane,causing leakage of cellular substances

G. Filtration

Bacteriological Filters

●The mean pore diameter in these bacteriological filters ranges from approximately one to several micrometers

●Most filters are available in several grades based on the average size of the pores

●In recent years a new type of filter termed the membrane or molecular filter has been developed whose pores are of a uniform diameter

●Membrane or molecular filters are composed of biologically inert cellulose esters

●They are prepared as circular membranes of about 150 um thickness and contain millions of microscopic pores of very uniform diameter

●The development of high-efficiency particulate air (HEPA) filters has made it possible to deliver clean air to an enclosure
●This type of air filtration together with a system of laminar airflow is now used extensively to produce dust-and bacteria-free air

Characteristics of an Ideal Antimicrobial Chemical Agent

●Antimicrobial activity
●Solubility
●Stability
●Non toxicity to humans and other animals
●Homogenecity
●Non combination with extraneous organic material
●Toxicity to microorganisms at room or body temperatures
●Capacity to penetrate
●Noncorroding and nonstaining
●Deodorizing ability
●Detergent capacities
●Availability

General Terms

●Sterilization
●Disinfectant
●Antiseptic
●Sanitizer
●Germicide(Microbicide)
●Bactericide
●Bacteriostasis
●Antimicrobial Agent

Major Groups

Phenol and Phenolic Compounds

Phenol has the additional distinction of being the standard against which other disinfectants of a similar chemical structure are compared to determine their antimicrobial activity. The procedure used is called the phenol-coefficient technique

Alochols

●Methyl alcohol is less bactericidal than ethyl alcohol
●Propyl and isopropyl alcohols in concentrations ranging from 40 to 80 % are bactericidal for vegetative cells

Halogens

Iodine is one of the oldest and most effective germicidal agents

The element is traditionally used as a germicidal agent in a form referred to as tincture iodine

Cholrine and Chlorine Compounds

●Chlorine, either in the form of gas or in certain chemical combinations, represents one of the most widely used disinfectants.
●The chloramines represent another category of chlorine compounds used as disinfectants, sanitizing agents, or antiseptics

Heavy Metals and their Compounds

●The most effective are mercury, silver and copper

Dyes

●These are triphenylmethane and acridine dyes
●Triphenylmethane Dyes, Malachine green, Brilliant green and Crystal Violet
●Gram positive organisms are more susceptible than gram negative organisms
●Inhibition of gram positive organisms

Acridine Dyes

Acriflavine and Tryptoflavine

Synthetic Detergents

●Surface-tension depressants, or wetting agents, employed primarily for cleaning surfaces are called detergents
●Soap is example
●Soap is a poor detergent in hard water
●Many new more efficient cleaning agents have been developed called surfactants or synthetic detergents

1. Anionic, 2. Cationic 3. Nonionic

Quarterly Ammonium Compounds

●Most compounds of the germicidal cationic-detergent class are quarterly ammonium salts
●The bactericidal power of the quaternaries is exceptionally high against Gram-positive bacteria
●Also active against gram negative bacteria
●Low toxicity and high solubility in water
●Stability in solution and noncorrosive
●Extensively used on floors, walls and other surfaces in hospitals and public places
●Dairy egg and fishing industries
●Denaturation of proteins, interference with glycolysis and membrane damage

Aldehydes

●Aldehydes, several of the low-molecular-weight compounds are antimicrobial
●Two of the most effective are formaldehyde and glutaraldehyde
●Both are highly microbicidal, and both have the ability to kill spores (sporicidal)

Gaseous Agents

●The main agents currently used for gaseous sterilization are ethylene oxide, B-propiolactone, and formaldehyde

Characteristics of Antibiotics

2.They should prevent the ready development of resistant forms of the parasites

3.They should not produce undesirable side effects in the host, such as sensitivity or allergic reactions, nerve damage, or irritation of the kidneys and gastrointestinal tract
1.They should have the ability to destroy or inhibit many different species of pathogenic microorganisms. This is what is meant by a broad-spectrum antibiotic
4. They should not eliminate the normal microbial flora of the host, because doing so may upset the 'balance of nature' and permit normally nonpathogenic microbes, or particularly pathogenic forms normally restrained by the usual flora, to establish a new infection

Mode of Action
- Inhibition of cell-wall synthesis
- Damage of the cytoplasmic membrane
- Inhibition of nucleic acid and protein synthesis
- Inhibition of specific enzyme systems

Inhibition of Cell Wall Synthesis
- Inhibition of the biosynthesis of the peptidoglycan cell-wall structure are the penicillins, cephalosporins, cycloserine, vancomycin and bacitracin
- Some antibiotics exert their antimicrobial effect by inhibiting biosynthesis of the peptidoglycan polymer, resulting in the inhibition of cell-wall formation

Penicillins
1.Ampicillin
2.Cephalosporins
3. Cycloserine
4.Bacitracin
5.Vancomycin

Damage to Cytoplasmic Membrane
- Several polypeptide antibiotics produced by Bacillus spp. have the ability to damage cell-membrane structure
- They adversely affect the normal permeability characteristics of the cell membrane
- Polymyxins, gramicidins, and tyrocidines
- The polymyxins are particularly effective against Gram-negative organisms while the tyrocidines and gramicidins are more effective against Gram-positive organisms

Inhibition of Nucleic Acid and Protein Synthesis
- Synthesis of these substances involves a number of intricate biochemical reactions

1. Streptomycin
2. Tetracyclines
3.Chloramphenicol
4. Erythromycin

Inhibition of Specific Enzyme Systems

●Many bacteria require p-aminobenzoic acid (PABA) as a precursor to their synthesis of the essential coenzyme tetrahydrofolic acid (THFA)

●PABA is a structural part of the THFA acid molecule

●The selective action of sulfonamides is explained by the fact that the PABA molecule and a sulfonamide molecule are so very similar that the sulfonamide may enter the reaction in place of the PABA and block the synthesis of a essential cellular constituent

Antifungal Antibiotics

●Nystatin is an antifungal agent useful in the therapy of nonsystemic fungal infections. It is produced during fermentation by a strain of Streptomyces noursei

●The antimicrobial activity of nystatin is restricted to yeasts and other fungi, e.g., Condida, Aspergillus, Penicillyum, and Batrytis

●Griseofulvin is obtained from Penicillium griseofulvin

●It is used in the treatment of many superficial fungus infections of the skin and body surfaces and also effective in the treatment of some systemic (deep-seated) mycoses

Antiviral Chemotherapeutic Agents

●Chemotherapeutic agent, in order to attack the virus, must enter the host cells

●Also the agent must not be toxic to the host cell while exerting an inhibiting action on the virus

●More promising of the Chemotherapeutic agents for treating viral diseases interferon

●Interferons are small glycoprotein substances

●The antiviral action of interferon is attributed to interference of protein synthesis

●Recent advances in recombinant DNA techniques have increased the availability of interferon

●Acycloguanosine is a nucleoside analog which is active against the herpes virus in animals

●Inhibition of nucleotide utilization

●Amantadine is a low-molecular-weight compound which is very effective against influenza A virus

●The mode of action of amantadine is that of interfering with the un-coating of virus particles and the subsequent release of their nucleic acids

Bacterial Genetics

●Genetics is the study of the inheritance (heredity) and the variability of the characteristics of an organism

●Exact transmission of genetic information from parents to their progeny
●Variability of the inherited characteristics can be accounted for by a change either in the genetic makeup of a cell or in environmental conditions

Study of Microbial Genetics
●Genetic principals are universal
●The study of microbial genetics has contributed much to what we know about the genetics of all organisms
●There are distinct advantages in the use of bacteria for genetic experiments
Bacterial cultures contain millions of individual cells
Rare genetic events can be discovered
Rapid growth rates of microbes, the relative ease of growing bacteria and viruses in a constant, controlled environment
Great diversity of metabolic types among microorganisms
●In addition to the inheritance of characteristics, changes are associated with two fundamental properties of the cell or organism, namely genotype and the phenotype
Genotype refers to the genetic constitution of the cell
Phenotype is the expression of the genotype in observable properties
●The genotype of a culture of cells remains relatively constant during growth
●However, it can change by mutation
●This change results in an alteration in the observable properties or phenotype of the cells
●Genotype represents the heritable total potential characteristics
●Phenotype represents the characteristics expressed
●Bacteria like the cells of higher organisms, carry genetic information – their genotype
●The extent to which this information is expressed depends on the environment
●Depending on the presence or absence of oxygen during growth
●The presence or absence of oxygen determines which enzymes function and which do not

Genotypic Changes
●The genotype of a cell is determined by the genetic information contained in its characteristics (or chromosomes).
●The chromosome is divided into genes
●A gene is a functional unit of inheritance; it specifies the formation of a particular polypeptide as well as various types of RNA
●Each gene consists hundreds of nucleotide pairs
●Any gene is capable of changing or mutating to a different form
●A mutation is a change in the nucleotide sequence of a gene
●A cell or an organism which shows the effects of a mutation is called a mutant

●In nature, mutations are rare events which occur at random and arise spontaneously with no regard to environmental conditions
●Generally the mutants in a cell population are masked by the greater numbers of unmutated cells

Types of Mutation

●Changes in the purine-pyrimidine base sequence of a gene can occur, resulting in mutation
●Two common types are point mutations and frame shift mutations
●Point mutations occur as a result of the substitution of one nucleotide for another in the specific nucleotide sequence of a gene
●Transition type of point mutation is the replacement of a purine by a pyrimidine or vice versa
●This base-pair substitution may result in one of three kinds of mutations affecting the translation process
●The altered gene triplet produces a codon in the mRNA which specifies an amino acid different from the one present in the normal protein This mutation is called a mis-sense mutation
●The altered gene triplet produces a chain terminating codon in mRNA, resulting in premature termination of protein formation during translation. This is called a non-sense mutation.
●These mutations result from an addition or loss of one or more nucleotides in a gene and are termed insertion or deletion mutations
●This results in a shift of the reading frame

How Mutation Occur

●Mutations most commonly occur during DNA replication
●Some mutations occur as the result of damages inflicted by ultraviolet(UV)light or x-rays
●Any agent that increases the mutation rate is called a mutagen
●Mutation obtained by use of a mutagen are said to be induced rather than spontaneous
●UV light causes mutation under both natural and laboratory conditions

Radiation

●The major effect of UV light is to cause the formation of dimers by cross-linking between adjacent pyrimidine
●These cross-linked residues disrupt the normal process of replication by preventing the various polymerases
●When x-rays interact with DNA, the result is usually a break in the phospho-diester backbone of the nucleic acid

How Mutations are Repaired

●Cells contain specific enzymes which can repair damaged DNA
●Many kinds of bacterial cells and yeasts have been shown to possess an efficient photo- reactivating mechanism for repairing damage caused by UV radiation

●Photo-reactivation occurs when cells exposed to lethal doses of UV light are immediately exposed to visible light
●A special enzyme designated PRE, induced by visible light, splits or unlinks the dimers formed because of exposure to UV light and restores the DNA to its original state
●Some bacteria have enzymes, called endonucleases and exomucleases, that excise or cut out a damaged segment of DNA
●The other enzyme, polymerases and ligases, repair the resulting break by filling in the gap and joining the fragments together
●The process by which E. cell repairs large amounts of DNA damage is called inducible or SOS repair
●Includes diverse responses such as the ability to repair pyrimidine dimers, to induce various prophages,
●to shut off respiration, and to delay pyrimidine dimers,
●to induce various prophages, to shut off respiration, and to delay septum formation during cell division
●All the responses are coordinately regulated
●The process is a very efficient one
●However, it tends to insert mismatched bases and thus is error-prone and introduces additional mutation

Mutation Rate

●The rate of mutation is the probability that a gene will mutate at any particular cell division. Thus the mutation rate is generally defined as the average number of mutations per cell per division
●It is expressed as a negative exponent per cell division
●For example, is there is one chance in a million that a gene will mutate when the cell divides, the mutation rate for any single gene equals 10-5 per cell division
●Generally, the mutation rate for any single gene ranges between 10 –3 and 10 –9 per cell division

Phenotypes of Bacterial Mutants

●An increased tolerance to inhibitory agents, particularly antibiotics(antibiotics, or drug resistant mutants)
●An altered fermentation ability or increased or decreased capacity to produce some end product
●Nutritionally deficient, that is, that require a more complex medium for growth than the original culture from which they were derived(auxotrophic mutants)
●Changes in colonial form or ability to produce pigments
●A change in the surface structure and composition of the microbial cell (antigenic mutants)
●Resistant to the action of bacteriophages
●Change in morphological features, for example, the loss of ability to produce spores, capsules, or flagella

●Have lost a particular function but retain the intracellular enzymatic activities to catalyze the reactions of the function, for example, loss of a permease (cryptic mutants)
●A wild-type phenotype under one set of conditions and a mutant phenotype under another (conditionally expressed mutants)

Practical Implications with the Microbial Mutants

●Some microorganisms are known to develop resistance to certain antibiotics because of mutation
●Originally effective for the control of a bacterial infection become less effective or ineffective as antibiotic-resistant mutants appear
●Possible to isolate biochemical mutants capable of producing large yields of an end product.
●This is important in industry
●Yield of penicillin in commercial production dramatically increase through selection of mutant strains of Penicillium
●The maintenance of pure culture of typical microorganism species requires that occurrence of mutation be prevented; otherwise, the culture will no longer be typical
●Microbial mutants have been extensively used in the investigation of various biochemical processes, particularly biosynthetic reactions.
●Mutants with blocks or impairment at different enzymatic steps have been used to unravel metabolic sequences

Designation of Bacterial Mutants

●Each genotype is given a lowercase, italicized, three-letter code
●A mutation which affects proline synthesis is designated pro
●Mutation in a number of different genes may exhibit identical phenotypes
●Number may be added sequentially to designate particular mutations that is as each mutation is isolated, it is assigned a number that identifies it in bacterial pedigrees, for example pro A52 is the 52d isolate of the *Escherichia* coli

Bacterial Recombination

●Formation of a new genotype by re-assortment of genes following an exchange of genetic material between two different chromosome
●Have similar genes at corresponding sites
●These are called homologous chromosomes and are from different individual
●Progeny from recombination have combinations of genes different from those that are present in the parents
●In bacteria, genetic recombination results from three types of gene transfer
●Transfer of genes between cells that are in physical contact with one another
●Transfer of genes from one cell to another by a bacteriophages
●Transfer of ce-l free or @naked@ DNA from one cell to another
●Recombination the cells do not fuse, and usually only a portion of the chromosome from the donor cell (male) is transferred to the recipient cell (female)

●The recipient cell thus becomes a merozygote a zygote that is partial diploid
●Once merozygote transformation has occurred recombination can take place
●Inside the recipient cell the donor DNA fragment is positioned alongside the recipient DNA
●Homologous genes are adjacent
●Enzymes act on the recipient DNA, causing nicks and excision of a fragment
●The donor DNA is integrated into the recipient chromosome in place of the excised DNA
●The recipient cell then becomes the recombinant cell because its chromosome contains DNA of both the donor and the recipient cell
●The excised DNA pieces from the recipient chromosome are probably broken down by specific enzymes
●The genetics of plants and animals depends upon the regular cycle of sexual reproduction in these organisms
●An opportunity for different mutants of a species to mate with each other and produce new individuals with new combinations of mutations
●Mating or conjugation in E. coll is radically different from sexual mating in higher organisms.
●It is not a reproductive process that occurs regularly at each generation
●It does not involve meiosis since bacterial cells are haploid, nor does it involves the fusion of gametes
●It involves the transfer of some DNA from one cell to another followed by separation of the mating pair of cells
●In conjugation it is possible for large segments of the chromosome and in special cases the entire chromosome, to be transferreds

Sex Factors

●There is sexual differentiation in E.coll
●Male cells contain a small circular piece of DNA which is in the cytoplasm and not part of the chromosome, called the sex factor or F factor (fertility factor)
● These cells are referred to as F and are donors in mating
●Female cells lack this factor and are labled F
●They are recipient cells
●The male replicates its sex factor, and one copy of it is almost always transferred to the female recipient
●The F cell is converted to an F cell and is itself capable of serving as a donor
●The transfer of the F factor in an formation of in an F x F cross occurs with a frequency that approaches 100 percent
●Transfer of the F factor is independent of the transfer of chromosomal genes
●Since the transfer of the F factor is independent, it follows that the F factor DNA replicates independently of the F donor cell's normal chromosome
●One or more sex pill are produced by each F cell
●Sex pili seems to act to bind an F cell to an F cell and then to retract into the F cell, pulling the F cell into close contact

Extrachromosomal Genetic Elements

●Plasmids are circular pieces of DNA that are extra genes

●Either replicating autonomously or integrating into the bacterial DNA chromosome and are called episomes

●F cell of E. coli was called an episome because it can alternately exist in the F Transduction

●Most bacteriphages, the virulent phages undergo a rapid lytic growth cycle in their host cells.

●They inject their nucleic acid, usually DNA, into the bacterium, where it replicates rapidly and also directs the synthesis of new phage proteins. Within 10 to 20 min, depending on the phage, the new DNA combines with the new proteins to make whole phage particles, which are released by destruction of the cell wall and lysis of the cell

●However some bacteria viruses, the temperate phages, which do not lyse the cell, carry DNA that can behave as a kind of episomes in bacteria; like other episomes, such as the F; these viral genomes can become integrated into the bacterial genome;

●They are then known as prophages

●Bacteria that carry prophages (lysogenic bacteria) can be induced with ultraviolet light and other agents to make the prophages start to replicate rapidly and go through a lytic growth cycle, resulting in lysis of the cell with release of new phage particles

●Phage particles may become filled with cell chromosom DNA or amixture of chromosomal and phage DNA (rather than comparatively with phage DNA, as a normally the case).

●Such aberrant phages can attach to other bacteria and introduce bacterial DNA from one cell to another

Generalized Transduction

●If all fragments of bacterial DNA from any region of the bacterial chromosome have a chance to enter a transducting phage, the process generalized transduction. In this process, as the phage begins the lytic cycle, viral enzymes hydrolyze the bacterial chromosome into many small pieces of DNA. Any part of the bacterial chromosome may be incorporated into the phage head during phage assembly and is usually not associated with any viral DNA

●Generalized transduction, like bacterial conbjugation and transformation also provides a means for mapping bacterial genes, since the fragments transferred by a bacteriophage are often large enough to contain hundreds of genes.

●Certain temperate phage strains can transfer only a few restricted genes of the bacterial chromosome. More specifically, the phages transduce only those bacterial genes adjacent to the prophage in the bacterial chromosome.

●Rusticated transduction occurs when a bacteriophage genome, after becoming integrated as prophage in the DNA of the host bacterium, again becomes free

upon induction and takes with it into the phage head a small adjacent piece of the bacterial chromosome. When such a phage infects a cell, it carries with it the group of nbacterial genes that has become part of it

●Cell free or DNA containing a limited amount of gene form is transferred from one bacterial cell to another

●Donor – Not all lysis or chem. Extraction – Recip cell – Recomb- Transformed contain but grown in the presence of dead cells, culture filtrates or cell extracts of clearly related strain

●After DNA enter into a cell – one straned is immediately degraded by deoxyribonucleases, the other undergoes base pairing with the homolegous portion of the recipient ch. – integrated

●Only closely related strains of bact

●Late logarithmic phase of growth

●Transformable bacteria are said to be competent to take up and incorporate donor DNA

●The uptake process has been found to be an energy requiring mechanism because it can be inhibited by agents that interfere with energy metabolism

●Transformation between bacterial strain of low virulence

●Can give rise to transformed cells of high virulence

●Extremely useful in genetic studies of bacteria in the laboratory

Fungi (Fungus)

- A group of eucaryotic – great practical and scientific interest to Microbiologists
- Many fungi are of microscopic dimensions
- Multi—cellular fungi familiar to us
- Blue and green growth on rotting oranges & lemons
- Whitish gray growths on bread and jam
- Mushrooms in the fields
- Diversity of morphological appearance

Fungi

- Molds filamentous and multi-cellular
- Yeasts – Usually unicellular
- Eucaryotic spore-bearing – lack chlorophyll
- Reproduce both sexually and asexually

Importance of Fungi

- Heterotrophic organisms – require organic compounds for nutrition
- Feed on dead organic matter – Saprophytes
- Saprophytes decompose complex plant and animal wastes
- Breaking these into simpler chemical compounds
- Returned to the soil – Increasing its fertility - Beneficial
- Preparation of beer, wines, production of antibiotics – penicillin

- Ripening of cheese
- Undesirable – decompose timber, textiles, foods and other materials
- Saprophytic fungi – industrial fermentations
- As parasites (living in or on another organism) – cause diseases in plants, humans and animals
- Less common than bacterial and viral diseases in human and animals
- Much important in causing diseases of plants
- Tools for the physiologists, biophysicists, geneticists and bio-chemists – highly suitable for the study of some biological processes

Important characteristics of fungi

- The thallus (thalli) – Body of a fungus – consists of a single cell as in the yeasts
- More typically the thallus consists of filaments 5-10 µ across, which are commonly branched
- Yeast cells or mold filaments – surrounded by a true cell wall (exception slime molds)
- Some fungi are dimorphic – 2 forms
- Some pathogenic fungi – unicellular and yeast-like form in their host, when growing saprophytically in soil or on a laboratory medium-filamentous mold form
- Identification – demonstration of dimorphism

Morphology of Fungi

- Yeast cells are larger than most of the bacteria
- Size variable: 5-30 µ or more in length
- Commonly egg shaped, some elongated, some spherical
- Each species has a characteristic shape
- Considerable variation in size and shape of individual cells – age and environment
- Yeast have no flagella or other organelles of locomotion
- Thallus of molds– consists of 2 parts: Mycelium (mycelia) and the spores (resistant, resting or dormant cells)
- Mycelium – complex of several filaments called hyphae (hypha)
- New hyphae arise from a spore
- Spore germination puts on a germ tube or tubes
- Germ tubes elongate and branch to form hyphae
- Each hyphae is about 5-10 µ wide (bacteria – 1µm in diameter)
- Hyphae are composed of an outer tube-like wall surrounding a cavity (lumen) filled with protoplasm
- Between the protoplasm and the wall is the plasmalemma
- Plasmalemma – a double-layered membrane which surrounds the protoplasm

- Hyphal wall – microfibrils composed of chitin; true cellulose occurs only in the walls of lower fungi
- Wall matrix – proteins, lipids and other substances
- Soluble nutrients are absorbed through the walls
- Insoluble nutrients – first digested externally by secreted enzymes and then absorbed

Reproduction

Asexual Sexual

Asexual Reproduction

(Somatic or Vegetative reproduction)

- Does not involve the union of nuclei, sex cells or sex organs
- May be accomplished lby
- Fixxion of somatic cells-2 similar daughter
- Budding of somatic cells or spores-each bud, a small outgrowth developing into a new individual
- Fragmentation or disjointing of the hyphal cell- each fragment becoming a new organism
- Spore formation Asexual spores – dissemination of species, many types of asexual spores
- Sporangiospores – Single celled spores formed within sacs called sporangia (Sing- Sporangium) at the end of special hyphae (Sporangiophores)
    - Aplanospores – Non motile
    - Zoospores – Motile-due to flagella
- Conidiospores or conidia (Sing. Conidium)
- Microconidia – Small single celled conidia
- Macroconidia – Large multicelled conidia
- Conidia are formed at the tip or side of a hypha
- Oidia (Sing. Oidium) or arthrospores – Single celled spores formed by disjointing of hyphal cells
- Chlamydospores – thick walled single celled spores – highly resistant, formed from cells of the vegetative hypha
- Blastospores – Spores formed by budding

Sexual Reproduction

- Fusion of compatible nuclei of 2 parent cells
- Joining of 2 cells and fusion of their protoplasts
- Fusion of haploid nuclei to form a diploid nucleus
- Followed by meiosis-again reducing the number of chromosomes to haploid
- Sex organs, if present called gametangia (gametangium)
- May form differentiated sex cells (gametes)

- Or may contain one or more gamete nuclei
- If male and female gametangia are morphologically different, the male gametangium is called antheridium (antheridia) and the female gametangium is called oogonium (oogonia)

Various methods of sexual reproduction

- Gametic copulation: Fusion of naked gametes, one or both of which are motile
- Gamete-gametangial copulation: Two gametangia come into contact but do not fuse, the male nucleus migrates through a pore or fertilization tube into the female gametangium
- Gametangial copulation: Two gametangia or their protoplasts fuse and give rise to a zygote that develops into a resting spore
- Somatic copulation: Fusion of somatic or vegetative cells
- Spermatization: Union of a special male structure called spermatium (spermatia) with a female receptive structure. The spermatium empties its contents into female receptive structure
- Sexual spores produced by the fusion of 2 nuclei, occur less frequently, later and in smaller number than the asexual spores
- types of sexual spores
- Sexual spores produced by the fusion of 2 muclei, occur less frequently, later and in smaller number than the asexual spores. Several types of sexual spores:
- Ascospores: Single-celled spores produced in a sac called ascus
- Basidiospores: Single-celled spores borne on a club-shaped structure called Basidium
- Zygospores: Large, thick walled spores formed when the tips of 2 sexually compatible hyphae of certain fungi fuse together
- Oospores: Formed within a special female structure, the oogonium. Fertilization of the eggs by male gametes gives rise to oospores
- Asexual and sexual spores surrounded by highly organized protective structure called fruiting bodies. A single fungus may produce asexual and sexual spores by several methods at different times and under different conditions, however, the spores are quite constant in their structure and the method by which these are produced—identification and classification of fungi

PHYSIOLOGY

- Resistant: Able to withstand certain extreme environmental conditions than other microorganisms i.e. sugar concentrations (4-5%, bacteria 0.5-1%) – Jams & molds
- Can tolerate more acidic conditions

- Some yeasts are facultative-Aerobic and anaerobic
- Molds and many yeasts are usually aerobic
- Fungi—wide range of temperature
- Most saprophytic species—optimum 22-30°C
- Pathogenic species—optimum 30-37°C
- Some fungi will grow at near zero°C—spoilage of meat and vegetables in cold storage
- Variety of materials for nutrition
- Cannot use inorganic carbon compounds such as CO2 as their sole carbon source
- Carbon from an organic source such as glucose
- Some species can use inorganic compounds of nitrogen such as ammonium salts
- All fungi can use organic nitrogen
- Cell wall—chitin, cellulose (Bacteria-peptidoglycan)
- Antibiotic sensitivity—griseofulvin

Cultivation of Fungi

- Same general methods used for bacteria
- Grow aerobically on usual bacteriological media
- Temperature 20-30 °C
- Most of them grow more slowly
- Media for bacteria as well as fungi—overgrown by bacterial contaminants in a mixed inoculum
- Better to use selective media
- Acidic pH 5-6
- High cocentration of sugars
- Sabourand—maltose and peptone as principal ingredients

Assays and applications of immune response

- Status of immunity
- Diagnosis – Serodiagnosis
- Measurements of humoural antibodies
- Cannot seen, behaviour in the presence of specific antigen or pathogen.
- Many tests depend on the formation of visible reaction by cross linking of antigen and antibody in the form of large complex

Precipitation tests

- Reaction between a soluble antigen and the solution of its homologous antibody
- Reaction is manifested by formation of visible precipitate at the interference
- Inhibited by excess of either antigen or antibody

- Equivalence zone → concentration of antigen and antibody where complete precipitation occures.
- Factors
- Electrolytes
- pH
- Temperature
- Time
- Diagnosis of infectious diseases
- Serological screening of various pathogens
- Identification of blood on cloths
- Post-martum diagnosis of anthrax (Ascoli Test)
- Detection of adultration of food
- Antigen → prepared by making an extract from bacterial cells, tissues or other suitable materials
- Immunodiffusion test
    - Ring test
    - Single diffusion
    - Double diffusion
    - Immunodiffusion in petri plate
- Single radial immuno diffusion in ager
- Previous tests were qualitative
- Antigen can also be quantitated
- Ring of precipetate forms, diameter proportional to andibodies
- Antigen concertation simple and sensitive method
- Immunoelectrophorosis
- An electrochemical process – suspended particles with a net electric charge migrate (in sol/ager gel) under the influence of an electric current. Positively charged substances travel to the cathode, negatively charged ones go to the anode →called electrophoretic mobility, applied to the study of antigen-antibody reactions —immuno electrophoresis
- Rocket immuno-electrophoresis
- Combination of immluno-electrophoresis and single redial immuno-diffusion
- Electrophoretic migration of antigen from wells into an ager gel which contains specific intiserum
- Results in→Rocket shaped precipitate, the height of each Rocket is proportional to the concentration of antigen in the well
- B. Agglutination tests
- Relatively easy, simple and a method of choice — cellular antigen (particulate antigen). Measurement of immune response and diagnostic value

- Macroscopic – small test tubes
- Microscopic – slide
- Measurement of agglutination is the simplest way to estimate the quantity of that antibody in the serum. Small amounts of antibody can be detected
- Agglutination reaction has been extended to include a wide variety of antigens by attaching soluble antigens to the surface of inert particles such as betonies, latex, glass beads, RBC. The role of these particles is passive. Once coated they react as if they possess the antigen specificity of the coating antigen RBC convenient carriers of antigen. Specific antibodies are addled to antigen coated RBC – antibody bridges are formed between neighboring RBCs – large aggregates of RBC produced.
- →Passive or indirect haemagglutination
- Tube agglutination: Most commonly used method
    - Presence of specific agglutinins in serum
    - Approximate concentrations – serum may be diluted and antigen mixed with it, control tube without serum → incubation – cont. no agglitination. If so?
- Macroscopic Slides agglutination Rapid – Agglutinating antibodies. A drop of danse suspension of organism (antigen) on slide + serum → agglutination in 2-4 minutes
- Microscopic slide agglutination
- Serial dilutions of serum one loop of each dilution on a cover glass + loop of antigen
- Cover glass is placed over a concave slide – incubate for 1 hour – exam. Under microscope for clumping
- Whole Blood Agglutination
- Similar to rapid serum test, whole blood. Pullorum disease → 2 minutes
- Agglutination adsorption tests:
- Hemagglutination tests (HA): Certain viruses ability to agglunate RBC from certain species of animals
- Hemagglutination inhibition (HI):
- Complement Fixation Tests
- Based on the presence of complement fixing antibodies in serum
- Antibodies produced in response to antigen
- In the presence of antibodies, complement causes lyses of the specific bacterial cells
- Specific bacterial antibodies are present in the serum or not
- If the antigen and antibodies in serum are specific (capable of union) the complement is said to be fixed (used)
- The second system is an indicator system (Rabbit antibodies against sheep RBC) are added along with sheep RBCs

- If complement is available, lyses of RBCs
- If complement is fixed – no hemolysis
- Hemolysis — Negative test
- Radioimmunoassays (RIAs)
- The sensitivity with which antigen may be detected has been increased by the use of radioactive-labeled antibody or pure antigen
- Rapid and precise
- Direct method employs labeled antibodies which can be precipitated with the antigen
- In indirect method by labeling the antigen, the amount of radioactivity in an antigen antibody precipitate measures the antigen binding capacity of the immune sera
- Enzymes Linked Immune Sorbet Assay
- To couple enzymes to antibody to antibody or antigen for use in immuno assay
- As sensitive as immuno assays
- ELISA less expensive and safer
- Fluorescent antibody technique
- Rapid proceduse for the identification of unknown infectious agent
- Based on behaviour of certain dyes which fluoresce (glow) when exposed to certain wavelengths of light
- Cell mediated immunity
- Tuberculin, brucellin and some skin tests — detection of delayed type IV hypersensitivity
- After 18 hour and may persist for days
- The activated T lymphocytes proliferate and release soluble mediators called lymphokines which recruit and activate other host cells
- One of the hymphokines is called migration inhibition factor (MIF), important in inflammatory response
- It prevents the migration of hymphocytes away from the focus of immune response
- Interferon another lymphokines

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Spores and Sporulation

- Certain species of bacteria produce spores
- Within the cell (endospores) or external to the cell (exospores)
- Spores are metabolically dormant forms

- Under appropriate conditions can undergo germination and out growth to form a vegetative cell

Endospores

- Thick walled, highly refractile bodies
- Produced by Bacillus, Clostridium and few other genera
- Shape and location vary depending on the species

Characteristics of Endospores

- Endospores are extremely resistant to dessication, staining, disinfectants, radiation and heat
- Resistance is due to
  - Low water contents (20 %)
  - Large amounts of dipicolinic acid (DPA),unique compound undetectable in the vegetative cells (10 – 15 % of spores dry wt)
  - Large amounts of calcium, located in the core
  - Calcium DPA complex – role in heat resistance
  - Synthesis of DPA and uptake of calcium takes place during advanced stages of sporulation

Exospores

- Cells of methane oxidising genus, Methylosinum form xospores
- Spores external to the vegetativ cell
- By budding at one and
- Dessication and heat resistant
- Do not contain DPA

Host-microbial interaction

•Infectious disease interaction between pathogen microbial and host. All infectious at some surface of the host. Mucous membranes of respiratory tract, intestinal, urogenital, tract.

•In most infections pathogen organ penetrate the body surface

Internal organs:

Localized

Generalized

1.Toxins

Endotoxins

Exotoxins

2. Antiphagocytic factors

3. Plasmids

4. Hyaluronidase

5. Streptokinase

Blood plasminogen → is a protease that dissolves the fibrin of blood clot.

6. Deoxyribonuclease (D Nase) its ability to destroy DNA.

7. Coagulase
   Fibrinogen → Fibrin
   The fibrin coats the cell wall and protect them from phagocytosis.
8. Protein A → To bind antibodies
9. H_2O_2 and NH_3
10. Iron chelators → to compete for available iron

Natural Resistance:
a)Species resistance
   Mammals →X Fish or reptiles
   Frog →Cold blooded →Resist to anthrax
   Most mammals →Resistant to Myc. Avium
b)Racial Resistance
c) Individual Resist

External defense mechanisms
Skin, Coughing, sneezing, Mucous membranes, weeping transferring, perspiration, peristalsis

Immunity
•Body possess a complex system for freedom from potentially harmful organisms
•Immune system is a complex network of nonspecific and highly specific mechanisms for regulating interactions
•Nonspecific means- interaction constitute natural immunity
•Highly specific means- interaction that can recognize fine molecular fingerprints
Natural or Innate Immunity
(Non- Specific Immune Response)
•Innate immunity can be seen to comprise four types of defensive barriers:
1. Anatomic
2. physiologic
3. phagocytic,
4. inflammatory
1. **Anatomic Barriers**
➢Mechanical barrier retards entry of microbes
➢Skin is the first line of defense against infection
➢The epidermis which contain tightly packed epithelial cells filled with keratin.
➢The dermis composed of connective tissue, blood vessels, hair follicles and sweat gland**.**
➢ Mucous membranes

Normal flora compete with microbes for attachment sties and nutrients. Mucus entraps foreign microorganisms. Cilia propel microorganisms out of body.

2. **Physiologic barriers**

Temperature

Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens.

Low pH

Acidity of stomach content kills most ingested microorganism.

3. Chemical mediators

➢Lysozyme cleaves bacterial cell wall, Enzyme found in many body fluids and secretions. Anti-microbial action – lysis of G+ bacterial cell wall by hydrolysing peptidoglycen.

➢Interferon induces antiviral state in uninfected cells.

➢Complement lyses microorganisms or facilitates phagocytosis

COMPLEMENT SYSTEM

•The complement system is a major triggered enzyme plasma system.

•It coats microbes with molecules that make them more susceptible to engulfment by phagocytes.

•Vascular permeability mediators increase the permeability of the capillaries to allow more plasma and complement fluid to flow to the site of infection.

•They also encourage polys to adhere to the walls of capillaries (**margination**) from which they can squeeze through in a matter of minutes to arrive at a damaged area.

•Once phagocytes do their job, they die and their "corpses," pockets of damaged tissue, and fluid form pus

Complement system

➢Lactoferrin – Red iron containing protein in milk and most of the secretions that wash mucosal surfaces

➢Transferrin(Serum)

4. Phagocytic/endocytic barriers

➢Various cells internalize (endocytose) and break down foreign macro-molecules

➢ Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill, and digest whole microorganisms.

Phagocytic cell

Phagein-to eat Kytos-hollow vessel

A. Leukocytes

a) Polymorphonuclear granulocytes
i) Neutrophils
ii) Eosinophil
iii)Basophil
b). Agranulocyte
i) Lymphocytes-derived from lymphoid organs, bone marrow smaller than monocytes, large nucleusT & B cell
ii) Monocytes- Bone marrow-larger than granulocytes

B) Plasma cells

- Location-lymphoid organ (lymph nodes, spleen, thymus)
- Derived from B lymphocytes
- Produce Abs

C) Macrophages

- Transformed from monocytes
- Have numerous cytoplasmic granules
- Phagocytic

–Wandering- Alveolar-Lungs
Peritoneal-Abdomin
–Fixed - Histiocytes-Connective Tissues
- Kupffer cells

Phagocytosis

•Polymorphonuclear granulocytes (mainly neutrophils) → Front line of internal defense, Billions in blood, life few days, replaced by new one from the bone marrow.

•Enzymes and antimicrobial substances for killing and degradation of bacteria-contained in membrane-bound organelles- lysosomes.

•Macrophages – Preursor monocytes.

•Unlike the polymorphs, macrophages are long lived – can persist in tissues for weeks or months.

•Widely distributed throughout the body under certain conditions macrophages can synthesize DNA and multiply.

•Differentiated in the tissues – histiocytes liver – Kupffer cells, Lungs – Alveolar macrophages.

•Macrophages also have lysosomes with bactericidal substances.
G+ rapidly destroyed

G- Persist more, relatively resistant

Mycobacterium tuberculosis Brucella abortus, multiply in the phagocytes.

7. Inflammatory barriers

➢Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity

➢Influx of phagocytic cells into the affected area

Inflammation

•Nonspecific factors combine to combat an invasion of pathogens

•Activate and fix complement

•Chemotactic complement derived factors attract lenkocytes to the site

•Anaphylatoxin causes degranulation of tissue basophils (mast cells)

•These release histamine and serotonin

•Constriction of smooth muscles e.g. in bronchioles and blood vessels

•Increased capillary permiability

•Promotes the passage of plasma and leukocytes into the affected tissue

•Plasma contains microbicidal substances – inhibit replication and growth of pathogens

•Local swelling-accumulation of large number of phagocytic cells

•Erythema, Heat- Increased blood flow, enzymatic activity, release of bacterial Endotoxins, Most important in resisting infection by pyogenic bacteria

Specific Immune Response (Adaptive Immunity)

Adaptive or specific immunity is capable of recognizing and selectively eliminating specific foreign microorganisms or molecules (foreign antigens).

Unlike nonspecific (innate) immune responses, adaptive immune responses are reactions towards specific antigenic challenges and display four characteristic attributes:

1. Antigenic specificity

Distinguish differences among soluble and insoluble antigens; distinguish antibodies that differ only in a single amino acid

2. Diversity

Is capable of generating tremendous diversity in its recognition molecules

3. Immunologic memory

Once immune system has recognized and responded to an antigen it exhibits immunologic memory- second contact with the same Ag induces higher immune reactivity

4. Self/nonself recognition

Immune system responds only to foreign antigens, indicating that it is capable of distinguishing self

from nonself, is limited to nonself Ag.

Cells of the Immune System

An effective immune response involves two major groups: Lymphocytes and antigen presenting cells

1. Lymphocytes

• Lymphocytes derived from lymphoid organs, manufactured within the bone marrow

•Characteristics of specificity, diversity, memory and self/nonself recognition are mediated by the

lymphocytes

•Able to recognize Ag by means of membrane receptors specific for the foreign material. Two major

populations of lymphocytes

a) B Lymphocytes

• When they leave bone marrow, each expresses a unique antigen-binding receptor on their membrane

• Mature in Bursa of Fabricius in poultry, lymphoid tissues around intestines in mammals

• Differentiate into antibody (Ab) producing and memory B cells

• Memory B cell have a longer life span than native cells because it has already been encountered with antigen

• The binding of antigen to the antibody causes the cell to divide rapidly, its progeny differentiates into memory cells and effector cells called Plasma cells

• It has been estimated that a plasma cell can secrete more than 2000 molecules of antibody per second

• Response for humoral immune response

b) T Lymphocytes

• T Lymphocytes also arise in the bone marrow while B Lymphocytes mature in the bone marrow

• T cell migrate to thymus to mature

• T cell expresses a unique antigen binding molecule during maturation called the T-cell receptor

• T-cell receptor can recognize antigen that is bound to cell membrane protein called Major Histocompatibility Complex (MHC)

• MHC is responsible for antigen presentation

• The T-cell proliferates and differentiates into memory T-cell and various effector T-cells

• There are two well defined subpopulations of T-cell (T-helper and T-cytotoxic)

• MHC produces cytokines after activation which play important role in activating B-cells

2. Antigen presenting cell

• It is first internalized antigen either by phagocytosis or by endocytosis

• The specialized cells include :

Macrophages, B-Lymphocytes and dendritic cells (These cells participate in the cutaneous

immune response and migrate from skin to lymph nodes) Langerhans cells

• Activation of both the humoral and cell-mediated branches of the immune systems requires cytokines produced by TH cells

• It is essential that activation of TH cells be carefully regulated because an inappropriate TH cell response to self- components can have fatal autoimmune consequences

• To insure careful regulation, the TH cell can be activated following antigen recognition

• When the antigen is displayed together with MHC molecules on the surface of specialized cells called *antigen-presenting cells* (***APCs)***

• Antigen-presenting cells, which include macrophages, B lymphocytes, and dendritic cells, are distinguished by their expression of a particular type of MHC molecule

• These specialized cells internalize antigen, either by phagocytosis or by endocytosis, and then re-express a part of that antigen, together with the MHC molecule, on their membrane.

Humoral Immune Response

• Antibody-mediated immunity is an indirect response to a microbial pathogen

• Microbial antigens stimulate B cells to transform into plasma cells

• Plasma cells produce antibodies

• The antibodies secreted react with the inciting antigens

• The actual mediators of the response are not the B cell but the Abs

• When an antigen makes contact with a B cell, it triggers a chain of events that culminates in the production of antibodies to react with the antigen

• The first step in this process is the specific attachment of an antigen to a B cell

• This is the act of antigen recognition, and it is highly specific because of special antigen receptors that B cells have on their surfaces

• There may be 100,000 such receptors on a B cell

• Reaction with an antigen triggers a complex process of cellular differentiation that changes the relatively small B lymphocyte into a larger, highly complex cell called the plasma cell

• It is the plasma cell that actually synthesizes and secretes the specific immunoglobulins. The first phase of differentiation is conversion of small B cells (approximately 10 μm diameter) to rapidly dividing cells known as lymphoblasts

•At this stage, some lymphoblasts continue differentiating into plasma cells and others revert to small lymphocytes. The ultimate stage of B-cell differentiation is the plasma cell, which is the major antibody producer

• Plasma cells lack the cell surface immunoglobulin receptors that are characteristic of the B cells from which they are derived, but they have an extremely complex cytoplamic organization

COMPLEMENT SYSTEM

• The complement system is a major triggered enzyme plasma system

• It coats microbes with molecules that make them more susceptible to engulfment by phagocytes

• Vascular permeability mediators increase the permeability of the capillaries to allow more plasma and complement fluid to flow to the site of infection

•They also encourage polymorphs to adhere to the walls of capillaries (**margination**) from which they can squeeze through in a matter of minutes to arrive at a damaged area.

•Once phagocytes do their job, they die and their "corpses," pockets of damaged tissue, and fluid form pus complement system

Immunoglobulins (Ig)

Glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies.

•The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field

GENERAL FUNCTIONS OF IMMUNOGLOBULINS

•A. Antigen binding

Immunoglobulins bind specifically to one or a few closely related antigens.

➢Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host.

➢The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind.

•B. Effector Functions

➢Frequently the binding of an antibody to an antigen has no direct biological effect.

➢The antibodies have the variety of these effector functions.

Such effector functions include:

1. Fixation of complement - This results in lysis of cells and release of biologically active molecules
2. Binding to various cell types –

- Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins.
- This binding can activate the cells to perform some functions.
- Some immunoglobulins also bind to receptors on placental trophoblasts, which result in transfer of the immunoglobulin across the placenta.
- As a result, the transferred maternal antibodies provide immunity to the fetus and newborn

BASIC STRUCTURE OF IMMUNOGLOBULINS

A. Heavy and Light Chains

➢All immunoglobulins have a four chain structure as their basic unit.

➢They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD)

B. Disulfide bonds

1. Inter-chain disulfide bonds

➢The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions

➢The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

2. Intra-chain disulfide binds

Within each of the polypeptide chains there are also intra-chain disulfide bonds.

C. Variable (V) and Constant (C) Regions

After the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences. These are the:

•1. Light Chain - VL (110 amino acids) and CL (110 amino acids)

•2. Heavy Chain - VH (110 amino acids) and CH (330-440 amino acids)

D. Hinge Region

This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

E. Domains

Three dimensional images of the immunoglobulin molecule

•It is folded into globular regions each of which contains an intra-chain disulfide bond . These regions are called domains

•1. Light Chain Domains

•2. Heavy Chain Domains

F. Oligosaccharides

➢Carbohydrates are attached to the CH2 domain in most immunoglobulins.

➢However, in some cases carbohydrates may also be attached at other locations.

IMMUNOGLOBULIN FRAGMENTS:

•STRUCTURE/FUNCTION RELATIONSHIPS

A. Fab

Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain disulfide bond.

➢This results in the formation of two identical fragments that contain the light chain and the domains of the heavy chain.

➢Antigen binding - These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody.

➢Each Fab fragment is monovalent whereas the original molecule was divalent.

➢An antibody is able to bind a particular antigenic determinant because it has a particular combination of VH and VL.

➢Different combinations of a VH and VL result in antibodies that can bind a different antigenic determinants

B. Fc

➢Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a CH2 and CH3 domain.

➢This fragment was called Fc because it was easily crystallized.

➢Effector functions - The effector functions of immunoglobulins are mediated by this part of the molecule

➢Different functions are mediated by the different domains in this fragment.
➢Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen.

STRUCTURE AND SOME PROPERTIES OF IG CLASSES

A. IgG

➢1. Structure
All IgG's are monomers (7S immunoglobulin). The subclasses differ in the number of disulfide bonds and length of the hinge region.
➢2. Properties
Most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.
➢a) IgG is the major Ig in serum - 75% of serum Ig is IgG
➢b) IgG is the major Ig in extravascular spaces
➢c) Placental transfer - IgG is the only class of Ig that crosses the placenta. Transfer is mediated by receptor on placental cells for the Fc region of IgG. Not all subclasses cross equally; IgG2 does not cross well.
➢d) Fixes complement - Not all subclasses fix equally well; IgG4 does not fix complement
➢e) Binding to cells - Macrophages, monocytes, PMN's and some lymphocytes have Fc receptors for the Fc region of IgG.
•A consequence of binding to the Fc receptors on PMN's, monocytes and macrophages is that the cell can now internalize the antigen better.
•The antibody has prepared the antigen for eating by the phagocytic cells.
•The term **opsonin** is used to describe substances that enhance phagocytosis

B. IgM

1. Structure
Exists as a pentamer (19S immunoglobulin) but it can also exist as a monomer.
➢In the pentameric form all heavy chains are identical and all light chains are identical.
➢IgM has covalently bound via a S-S bond called the J chain. This chain functions in polymerization of the molecule into a pentamer.
2. Properties
➢a) IgM is the third most common serum Ig.
➢b) IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.
➢c) As a consequence of its pentameric structure, IgM is a good complement fixing Ig.
➢d) As a consequence of its structure, IgM is also a good agglutinating Ig .

➢IgM antibodies are very good in clumping microorganisms for eventual elimination from the body

C. IgA

1. Structure

Serum IgA is a monomer but IgA found in secretions is a dimer

➢When IgA is found in secretions is also has another protein associated with it called the secretory piece or T piece.

➢Unlike the remainder of the IgA which is made in the plasma cell, the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions.

➢The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions.

2. Properties

➢a) IgA is the 2nd most common serum Ig.

➢b) IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.

➢c) Normally IgA does not fix complement, unless aggregated.

➢d) IgA can binding to some cells - PMN's and some lymphocytes.

D. IgD

1. Structure

IgD exists only as a monomer.

2. Properties

➢a) IgD is found in low levels in serum; its role in serum uncertain.

➢b) IgD is primarily found on B cell surfaces where it functions as a receptor for antigen.

➢IgD on the surface of B cells has extra amino acids at C-terminal end for anchoring to the membrane. It also associates with the Ig-alpha and Ig-beta chains.

➢c) IgD does not bind complement.

E. IgE

1. Structure

➢IgE exists as a monomer and has an extra domain in the constant region.

2. Properties

➢a) IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells.

➢b) Involved in allergic reactions - As a consequence of its binding to basophils an mast cells, IgE is involved in allergic reactions.

➢Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.

➢d) IgE does not fix complement

Clinical Implications of Immunoglobulin Classes

IgG

1. Increases in:

a) Chronic granulomatous infections
b) Infections of all types
c) Hyperimmunization
d) Liver disease
e) Malnutrition (severe)
f) Dysproteinemia
g) Disease associated with hypersensitivity granulomas, dermatologic disorders, and IgG myeloma
h) Rheumatoid arthritis

2. Decreases in:

a) Agammaglobulinemia
b) Lymphoid aplasia
c) Selective IgG, IgA deficiency
d) IgA myeloma
e) Bence Jones proteinemia
f) Chronic lymphoblastic leukemia

IgM

1. Increases (in adults) in:

a) Trypanosomiasis
b) Actinomycosis
c) Carrión's disease (bartonellosis)
d) Malaria
e) Infectious mononucleosis
f) Lupus erythematosus
g) Rheumatoid arthritis
h) Dysgammaglobulinemia (certain cases)

2. Decreases in:

a) Agammaglobulinemia
b) Lymphoproliferative disorders (certain cases)
c) Lymphoid aplasia
d) IgG and IgA myeloma
e) Dysgammaglobulinemia
f) Chronic lymphoblastic leukemia

IgA

1. Increases in:

a) Cirrhosis of the liver (most cases)
b) Certain stages of collagen and other autoimmune disorders such as rheumatoid arthritis and lupus erythematosus
c) Chronic infections not based on immunologic deficiencies
d) IgA myeloma

2. Decreases in:
a) Hereditary ataxia telangiectasia
b) Immunologic deficiency states (*e.g.*, dysgammaglobulinemia, congenital and acquired agammaglobulinemia, and hypogammaglobulinemia)
c) Malabsorption syndromes
d) Lymphoid aplasia
e) IgG myeloma
f) Acute lymphoblastic leukemia
g) Chronic lymphoblastic leukemia

IgD

1. Increases in:
a) Chronic infections
b) IgD myelomas

IgE

1. Increases in:
a) Hay fever
b) Asthma
c) Anaphylactic shock
d) IgE-myeloma

2. Decreases in:
a) Congenital agammaglobulinemia
b) Hypogammaglobulinemia due to faulty metabolism or synthesis of immunoglobulins

Cells of the Immune System

A variety of white blood cells or leukocytes, participate in the development of an immune response of the cells, only the lymophocytes posses the attributes of diversity, specificity, memory and self/non self recognition

All the other cells play accessory roles, serving to activate lymphocytes, to increase the effectiveness of antigen clearance by phagocytosis, or to secrete various immune effector molecule.

Lymphoid cells

- Lymphocytes are the white blood cells responsible for the immune response

- Their characteristics account for the immune system's attributes of diversity, specificity, memory and self/non self recognition
- Constitute 20-40% of the body's white blood cells, circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs
- The lymphocytes can be broadly subdivided on the basis of functions and cell-membrane components into the three populations B cells, T cells and Null cells
- B and T lymphocytes that have not interacted with antigen-referred to as *virgin, naïve, or unprimed*-are resting cells
- These cells are only about 6 μm in diameter; their cytoplasm forms a barely discernible rim around the nucleus
- These resting lymphocytes have a short life span (from a few days to a few weeks) and undergo programmed cell death
- Interaction of unprimed B or T lymphocytes with antigen, in the presence of certain cytokines, rescues the cells from programmed cell death.

An effective immune response involves two major groups: Lymphocytes and antigen presenting cells.

C. Null Cells

- A small group of peripheral-blood lymphocytes, called null cells, fail to express the membrane molecules that distinguish T and B lymphocytes
- These cells also fail to display antigen-binding receptors of either the T- or B-cell lineage and therefore lack the attributes of immunologic specificity and memory
- One functional population of null cells called *natural killer (NK)* cells are large, granulated lymphocytes; these cells constitute 5-10% of the peripheral-blood lymphocytes

- Null cells display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor
- NK cell were subsequently shown to play an important role in host defense against tumor cells.

Organs of the Immune system

- A number of morphologically and functionally diverse organs have various functions in the development of an immune response
- These organs can be divided on the basis of function into the primary (or central) and secondary (or peripheral) lymphoid organs
- Immature lymphocytes generated during hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs
- Only after a lymphocytes has matured within a primary lymphoid organ is the cell immunocompetent
- In mammals, the primary lymphoid organs are the bone marrow, where B-cell maturation occurs and the thymus, where T-cell maturation occurs.

Primary Lymphoid Organs

a. <u>Thymus</u>

- T-cell progernitors formed during hematopoiesis enter the thymus gland as immature thymocytes and mature there to become antigen-committed, immunocompetent T cells
- The thymus is a flat, bilobed organ situated above the heart
- Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called *trabeculae*
- Each lobule is organized into two compartments: the outer compartment, or cortex, is densely packed with thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymocytes

- Both the cortex and medulla of the thymus are crisscrossed by a three-dimensional network of stromal cells composed of epithelial cells, interdigitating dendritic cells, and marcophages, which make up the framework of the organs and contribute to thymocyte maturation.

Bone Marrow

- In birds a lymphoid organ called the bursa of Fabricius is the primary site of B-cell maturation
- There is no bursa in mammals and no single counterpart to it as a primary lymphoid organ
- Instead, regions of the bone marrow and possibly of other lymphoid tissues serve as the "bursal equivalent" where B-cell maturation occurs
- Because B-cell development in mammals does not take place in a single anatomic structure, it is difficult to study B-cell development in mammals, and much remains unknown about this process.

Secondary Lymphoid Organs

- As blood circulates under pressure, the fluid component of the blood (*plasma*) seeps through the thin wall of the capillaries into the surrounding tissue
- Much of this fluid, called *interstitial fluid,* returns to the blood through the capillary membranes
- The remainder of the interstitial fluid, now called *lymph*, flows from the connective tissue spaces into a network of tiny open lymphatic capillaries and then into a series of progresively larger collecting vessels called *lymphatic vessels.*
- The largest lymphatic vessel, the *thoracic duct*, empties into the left subclavian vein near the heart
- In this way the lymphatic system functions to capture fluid lost from the blood and return it to the blood

- When foreign antigen gains entrance into the tissues, it is picked up by the lymphatic system and carried to various organized lymphoid tissues, which trap the foreign antigen
- As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes.
- Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen
- Various types of organized lymphoid tissues are located along the vessels of the lymphatic system
- Some lymphoid tissue in the lung and lamina propria of the intestinal wall consists of diffuse collections of lymphocytes and marcophages
- Other lymphoid tissue is organized into structures called *lymphoid follicles,* which consist of aggregates of various cells surrounded by a network of draining lymphatic capillaries.

Lymph Nodes

- Lymph nodes are encapsulated bean-shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells
- Clustered at junctions of the lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces
- The overall architecture of a lymph node provides an ideal microenvironment for lymphocytes to effectively encounter and respond to trapped antigens.
- Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, *paracortex*, and medulla each of which provides a distinct microenvironment

- The innermost layer of a lymph node, the *medulla,* is more sparsely populated with lymphocytes, but many of these are plasma cells actively secreting antibody molecules.

Spleen

- The spleen is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity
- Unlike lymph nodes, which are specialized to trap localized antigen from regional tissue spaces, the spleen is adapted to filtering blood and trapping blood-borne antigens, and thus can respond to systemic infections
- The spleen is surrounded by a capsule that sends a number of projections (trabeculae) into the interior to form a compartmentalized structure
- The compartments are of two types, the *red pulp and white pulp*, which are separated by a diffuse *marginal zone.*
- The splenic red pulp consists of a network of sinusoids populated with macrophages and numerous erythrocytes; it is the site where old and defective red blood cells are destroyed and removed
- Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin
- The splenic white pulp surrounds the arteries, forming a *periarteriolar lymphoid sheath (PALS)* populated mainly by T lymphocytes
- The marginal zone is rich in B cells organized into primary lymphoid follicles.
- Unlike the lymph nodes, the spleen is not supplied by afferent lymphatics draining the tissue spaces
- Instead, blood-borne antigens are carried into the spleen through the splenic artery, which empties into the marginal zone
- As antigen enters the marginal zone, it is trapped by dendritic cells, which carry the antigen to the periarteriolar lymphoid sheath.

Mucosal-Associated Lymphoid Tissue

- The mucous membranes lining the digestive, respiratory and urogenital system are the major sites of entry for most pathogens
- The defense of these vulnerable membrane surfaces is provided by organized lymphoid tissues known collectively as *mucosal-associated lymphoid tissue (MALT)*
- Structurally these tissues range from loose clusters of lymphoid cells with little organization in the lamina propria of intestinal villi to organized structures such as the tonsils, appendix and Peyer's patches.
- The functional importance of MALT in the body's defense is attested to by its large population of antibody-producing plasma cells, whose number far exceeds that of plasma cells in the spleen, lymph nodes and bone marrow combined
- The tonsils are found in three locations: lingual at the base of the tongue; palatine at the side of the back of the mouth; and nasopharngeal (adenoids) in the roof of the nasopharynx
- All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and fibers interspersed with lymphocytes, macrophages, granulocytes and mast cells.
- The B cells are organized into follicles and germinal centers; the latter are surrounded by regions showing T-cell activity
- The tonsils play a role in defense against antigens entering through the nasal and oral epithelial routes
- Peyer's patches consist of 30 – 40 lymphoid nodules on the outer wall of the intestines
- These structures also contain follicles from which germinal centers develop upon antigenic stimulation
- The follicles, which are very close to the intestinal mucosal epithelium, are thought to be the sites where antigens penetrate the intestinal

epithelium, thus facilitating accumulation of antigen within organized lymphoid structures.

- The epithelial cells of mucous membranes play an important role in promoting the immune response by delivering small samples of foreign antigen from the lumina of the respiratory, digestive and urogenital tracts to the underlying mucosal-associated lymphoid tissue
- This antigen transport is carried out by specialized cells, called *M cells*.
- The outermost layer, the cortex, contains lymphocytes (mostly B cells) and macrophages arranged in primary follicles
- Following antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center
- Intense B-cell activation and differentiation into plasma and memory B cells occurs in the germinal centers of lymph nodes
- Beneath the cortex is the *paracortex*, which is populated with T lymphocytes and also contains dendritic cells thought to have migrated from tissues to the node.

ANTIGEN OR IMMUNOGEN

➢Immunogenicity is the ability to induce humoral or cell mediated immune response.

➢Antigenicity is ability to combine specifically with the end products of humoral or cell mediated

immune response.

➢All molecules having immunogenicity also have antigenicity but the reverse is not possible.

FACTOR INFLUENCING IMMUNOGENICITY

➢The immune system must be able to recognize viruses, bacteria, bacterial products, fungi and

parasites as immunogens.

➢Proteins and polysaccharides are the most potent immunogens of humoral immunity.

➢Lipids and nucleic acids generally do not serve as immunogens of humoral immunity.

➢Only proteins and some lipids serve as immunogens for CM immunity.

➢These proteins must be processed into small peptides and then presented with MHC molecules.

➢Including all the above factors there are certain biological factors also which influence

immunogenicity.

CONTRIBUTION OF IMMUNOGEN TO IMMUNOGENICITY

✓Foreignness

✓Molecular size

✓Chemical composition

✓Antigen processing and presentation

FOREIGNNESS

➢The degree of immunogenicity depends upon the degree of foreigness.

➢A molecule must be recognized as non self by biological system.

➢Ability to recognize as self/non self arises during lymphocytic development.

➢The greater the difference between two species the greater will be the immunogenicity.

MOLECULAR SIZE

➢The best immunogen tend to have a size approaching 100000(lac) daltons.

➢Substances having molecular size less than 5000-10000 daltons are poor immunogens.

CHEMICAL COMPOSITION

➢Synthetic homopolymers tend to lack immunogenicity regarless of their size.

➢Copolymers having two or more different amino acids are immunogenic.

➢The addition of aromatic amino acid tends to enhance immunogenicity of these synthetic polymers.

PROCESSING & PRESENTATION

➢For the development of HI and CMI, interaction of T cells and antigen processed with MHC in

necessary.

➢For TH cells antigen must be presented with class II MHC molecule on an APC.

➢For TC cells antigens must be presented with class I MHC molecule on an altered self cell.

CONTRIBUTION OF BIOLOGICAL SYSTEM TO IMMUNOGENICITY

✓Genotype of recipient.

✓Dose and route of immunogen

✓Adjuvants

GENOTYPE

•The genotype of an immunized animal influences the type of immune response the animal menifests.

•The response of an animal to antigen influenced by the genes that encode B cells and T cells receptors.

•These are also influenced by genes that encode various proteins of the immune regulatory

mechanisms.

DOSAGE AND ROUTE

•An insufficient dose will not stimulate a immune response either because it fails to activate enough

lymphocytes or because it induces a non responsive state.

•An excessively high dose also can fail to induce a immune response because it causes lymphocytes to
enter in a non responsive state.

•Mostly a single dose will not induce a strong response so repeat administration over a period of week is required to stimulate immune response.

•Such repeated administration increases the clonal proliferation of specific B cells or T cells.

•Such repeated doses are called as BOOSTER dose.

•The route of administration influences the immune organs and cells involve in the response.

•Intravenous, Intradermal, Subcutaneous, Intramuscular and Intraperitoneal

ADJUVANTS

•Adjuvants are the substances that when mixed with antigen and injected with them, enhance the immunogenicity of that antigen.

•Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or small amounts of an antigen are available.

Functions

✓Enhance immunogenicity.

✓Prolong antigen persistence.

✓Enhance co stimulatory signals.

✓Induce granuloma formation.

✓Stimulate lymphocytic proliferation non specifically.

EPITOPES

•Epitopes are descrete sites on an antigen which are recognized by lymphocytes rather than the whole
antigen.

•These are immunologically active regions of antigen that bind to antigen specific memberane

receptors on lymphocytes or secreted antibodies.

B CELL EPITOPES

•Their size ranges widely, some are small (peptides) and some are large (proteins).

•A small peptide epitope interacts with a deep, narrow groove in the antibody molecule.

•A large proteins epitope interacts with larger, flatter surface on antibody molecule.

T CELLS EPITOPES

•These are basically composed of amino acid sequence.

•T cell epitopes rendered the immune system by antigen processing, which fragments the proteins into small peptides that combines with class I MHC or class II MHC molecules.

•The resulting peptide MHC complexes are then displayed on the surface of altered self cells or antigen presenting cells.

HAPTENS

•Haptens are small organic molecules that are antigenic but not immunogenic.

•The conjugate formed by coupling a hapten to a large carrier protein is immunogenic and elicits production of antihapten antibodies when injected into an animal.

•Example is drug allergy (penicillin allergy)

(MHC)Major Histocompatibility Complex

• A tightly linked cluster of genes, whose products play a role in intercellular recognition and in discrimination between self and nonself

- MHC is a region of multiple loci that play major role in determining whether transplanted tissue is accepted as self(Histocompatible) or rejected as foreign(Histoincompatible)

MHC plays a central role

In the development of both

1. Humoral and Cell-mediated immune responses

T cells reorganization

- Most antigen only when it is combined with an MHC molecule
- MHC molecules play a critical role in antigen recognition by T cells
- MHC molecules act as antigen presenting structures,
- MHC partially determines the response of an individual to an Antigen of infectious organism
- MHC has therefore been implicated :

In the susceptibility to 1. disease 2.In the development of autoimmunity

General organization and inheritance of genes

- The concept that the rejection of foreign tissue is the result of an immune response to cell-surface molecules (now called histocompatibility antigens) originated from the work of Peter Gorer in the mid-1930s
- Snell called these genes ***"histocompatibility genes"***
- Snell was awarded the ***Nobel Prize*** in 1980 for this work, attesting to the significance of these observations

Location and Functions of MHC Regions

- Location and Functions of MHC Regions within a long continuous stretch of DNA on chromosome 6 in humans and chromosome 17 in mice
- MHC is referred to as

HLA complex in human and H-2 complex in mice

- Although the arrangement of genes is somewhat different, in both cases the MHC genes are organized into regions encoding three classes of molecules

Class I MHC genes

• Encode glycoprotiens expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of peptide antigens to Tc cells

Class II MHC genes

• Encode glycoprotiens expressed primarily on antigen-presenting cells (macrophages, dendritic cells, and B cells), where they present processed antigenic peptides to Th cells

Class III MHC genes

Generally encode various secreted proteins that have immune functions, including complement system and molecules involved in information

MHC haplotypes

The loci constituting the MHC are highly polymorphic; that is, many alternative forms of gene, or alleles, exist at each other locu

• Most individuals inherit the alleles encoded by these closely linked loci as two sets, one from each parent. Each set of alleles is referred to as a haplotype

MHC MOLECULES AND GENES

• Class I and class II MHC molecules are membrane-bound glycoproteins and are closely related in both structure and function

• Class III MHC molecules are group of unrelated proteins and don't share structural similarity and common function with class I and II molecules

Structure of class I molecules

Class I MHC molecules contains

1. large ∝ chain
2. smaller **β2-microglobulin** molecule

α-CHAIN OF CLASS I MOLECULE

• Has three external domains α-1.α-1 2.a-2 3.a-3

• Each containing approximately 90 amino acid

• A transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged (hydrophilic) amino acids; and a cytoplasmic anchor segment of 30 amino acids

• β2 –microglubullin is similar to the ∝3 external domain;

• The ∝1 and ∝2 domains interact to form a platform of eight antiparallel β strand spanned by two long ∝-helical regions

• The ∝3 domains and β2-microglubbulin are organized into two β sheets each formed by antiparrallel β strands of amino acids

• This structure known as a immunoglobulin fold, is characteristic of immunoglubin domains.

• In the absence of β2-microglubulin, the class I MHC ∝ chain is not expressed on the cell membrane **Structure of class II MOLECULES**

• It contain 2 different polypeptide chains

33 kDa α chain

28kDa β chain

Associated by noncovalent interaction

Each chain in a class II molecule contains two external domains

1. ∝1 domains
2. ∝2 domains

Regulation of MHC expression

• Class II MHC genes are expressed only in a limited number of cell type, and the level of

expression of both class I and class II genes differs among cell types

• The interferons and tumor necrosis factor have each been shown to increase expression of class I

MHC molecules on cells

• The expression of MHC molecules is also regulated by various cytokines

Other cytokines influence MHC expression only in certain cell types; for example, IL-4 increases

expression of class II molecules by resting B cells

MHC and Immune Responsiveness

• Early studies by B. Benacerraf in which guinea pigs were immunized with simple synthetic

antigens first showed that the ability of an animal to mount an immune response, as measured by

the production of serum antibodies, is determined by MHC haploty

•Dependence of immune responsiveness on the class II MHC reflects the central role of class II

MHC molecules in presenting antigen to Th cells

MHC and Disease Susceptibility

• An extensive body of information links certain HLA alleles to susceptibility to certain diseases. A number of different diseases have been associated with particular MHC alleles, among them important are some :

1. Autoimmune diseases
2. Disorders of the complement system
3. Some neurological disorders
4. Several different allergies

• By comparing the frequency of that allele in the patients with the allele frequency in the general population, the ***relative risk*** may be calculated

• A relative risk value of *1* means that the HLA allele is expressed with the same frequency in the patient and general population; therefore, the allele confers no increase risk for the disease

• By contrast, a relative risk value substantially ***above 1*** indicates an association between the HLA allele and the disease, implying that persons are more likely to acquire the disease

Relative risk

•Relative risk is a measure of the increased susceptibility of an individual with the allele over those lacking the allel

Immune System Disorders

Hypersensitivity (Allergy): An abnormal response to antigens.
Four Types of Hypersensitivity Reactions:
Type I (Anaphylactic) Reactions
■Type II (Cytotoxic) Reactions
■Type III (Immune Complex) Reactions
■Type IV (Cell-Mediated) Reactions

Type I (Anaphylactic) Reactions

◆Occur within minutes of exposure to antigen
◆Antigens combine with IgE antibodies
◆IgE binds to mast cells and basophils, causing them to undergo *degranulation* and release several mediators:
☞Histamine: Dilates and increases permeability of blood vessels (swelling and redness), increases mucus secretion (runny nose), smooth muscle contraction (bronchi).
☞Prostaglandins: Contraction of smooth muscle of respiratory system and increased mucus secretion.
☞Leukotrienes: Bronchial spasms.
◆Anaphylactic shock: Massive drop in blood pressure. Can be fatal in minutes.
Mast Cells and the Allergic Response
Mast Cells and the Allergic Response

Type II (Cytotoxic) Reactions

◆Involve activation of complement by IgG or IgM binding to an antigenic cell.
◆Antigenic cell is lysed.
◆Transfusion reactions:
☞ABO Blood group system: Type O is universal donor. Incompatible donor cells are lysed as they enter bloodstream.
☞Rh Blood Group System: 85% of population is Rh positive. Those who are Rh negative can be sensitized to destroy Rh positive blood cells.
•Hemolytic disease of newborn: Fetal cells are destroyed by maternal anti-Rh antibodies that cross the placenta.

Type III (Immune Complex) Reactions

◆Involve reactions against *soluble* antigens circulating in serum.
◆Usually involve IgA antibodies.
◆Antibody-Antigen immune complexes are deposited in organs, activate complement, and cause inflammatory damage.
☞Glomerulonephritis: Inflammatory kidney damage.
◆Occurs with slightly high antigen-antibody ratio is present.
Immune Complex Mediated Hypersensitivity

Type IV (Cell-Mediated) Reactions

◆Involve reactions by T_D memory cells.
☞First contact sensitizes person.
☞Subsequent contacts elicit a reaction.
◆Reactions are *delayed* by one or more days (delayed type hypersensitivity).
☞Delay is due to migration of macrophages and T cells to site of foreign antigens.
◆Reactions are frequently displayed on the skin: itching, redness, swelling, pain.
•Tuberculosis skin test
•Poison ivy
•Metals
•Latex in gloves and condoms (3% of health care workers)
◆Anaphylactic shock may occur.

Assays and applications of immune response

●Status of immunity

●Diagnosis – Serodiagnosis

Measurements of humoural antibodies

●Cannot be seen, behaviour in the presence of specific antigen or pathogen.

●Many tests, depend on the formation of visible reaction by cross linking of antigens and antibodies in the form of large complex.

A. **Precipitation tests**

●Reaction between soluble antigens and the solution of its homologous antibodies

●Reaction is manifested by formation of visible precipitate at the interference

●Inhibited by excess of either antigen or antibody

●Equivalence zone → concentration of antigen and antibody where complete precipitation occurs.

●Ab → Precipitins.

Factors

–Electrolytes

–pH

–Temperature

–Time: Complex forms within minutes but slowly develops into visible precipitate → 1 – 2 days

●Diagnosis of infectious diseases

●Serological screening of various pathogens

●Identification of blood on cloths, etc.

●Post-martum diagnosis of anthrax (Ascoli Test)

●Detection of adultration of food

●Antigen → prepared by making an extract from bacterial cells, tissues or other suitable materials

Immunodiffusion test

1. Ring test
2. Single diffusion
3. Double diffusion
4. Immunodiffusion in Petri plate

Single radial immuno-diffusion in agar

●Previous tests were qualitative

●Antigen can also be quantitated

●Ring of precipitate forms, diameter proportional to antibodies or antigen concentration

●Simple and sensitive method

Immuno-electrophorosis

●An electrochemical process – suspended particles with a net electric charge migrate (in sol/agar gel) under the influence of an electric current

●Positively charged substances travel to the cathode

●Negatively charged ones go to the anode

●Called electrophoretic mobility

●Applied to the study of antigen-antibody reactions —immuno electrophoresis

Rocket immuno-electrophoresis

●Combination of immuno-electrophoresis and single redial immuno-diffusion

●Electrophoretic migration of antigen from wells into an ager gel which contains specific antiserum

●Results in→Rocket shaped precipitate, the height of each Rocket is proportional to the concentration of antigen in the well

B. Agglutination tests

●Relatively easy, simple and a method of choice

●Cellular antigen (particulate antigen)

●Measurement of immune response and diagnostic value

a) Macroscopic – small test tubes

b) Microscopic – slides

● Measurement of agglutination is the simplest way to estimate the quantity of specific antibodies in the serum

● Small amounts of antibody can be detected

● Agglutination reaction has been extended to include a wide variety of antigens by attaching soluble antigens to the surface of inert particles such as latex, glass beads, RBC

● The role of these particles is passive

● Once coated they react as if they possess the antigen specificity of the coating antigen

● RBC convenient carriers of antigen

● Specific antibodies are added to antigen coated RBC

● Antibody bridges are formed between neighboring RBCs

● Large aggregates of RBC produced

Whole Blood Agglutination

●Similar To Rapid Serum Test, Whole Blood. Pullorum Disease → 2 Minutes

●Agglutination Adsorption Tests:

●Hemagglutination Tests (HA): Certain Viruses have ability to agglutinate RBC From Certain Species

of Animals

●Hemagglutination Inhibition (HI):

Passive or indirect haemagglutination

Tube agglutination

● Most commonly used method

● Presence of specific agglutinins in serum

● Approximate concentrations

● Serum may be diluted and antigen mixed with it

● Control tube without serum → incubation

Macroscopic slides agglutination

● Rapid – agglutinating antibodies

● A drop of dense suspension of organisms (antigen) on slide + serum → agglutination in 2-4 minutes

Microscopic slide agglutination

●Serial dilutions of serum-one loop of each dilution on a cover glass + loop of antigen

●Cover glass is placed over a concave slide – incubate for 1 hour – exam. under microscope for clumping

Complement Fixation Tests

●Based on the presence of complement fixing antibodies in serum

●Antibodies produced in response to antigen

●Specific bacterial antibodies are present in the serum or not

●In the presence of antibodies, complement causes lysis of the specific bacterial cells

●If the antigen and antibodies in serum are specific (capable of union) the complement is said to be fixed (used)

●The second system is an indicator system (Rabbit antibodies against sheep RBC) are added along with sheep RBCs

●If complement is available, lysis of RBCs

●If complement is fixed – no hemolysis

●Hemolysis — Negative test

Radioimmunoassays (RIAs)

●The sensitivity with which antigen may be detected has been increased by the use of radioactive-labeled antibodies or pure antigens

●Rapid and precise

●Direct method employs labeled antibodies which can be precipitated with the antigen

●In indirect method by labeling the antigen, the amount of radioactivity in an antigen antibody precipitate measures the antigen binding capacity of the immune sera

Enzymes Linked Immune Sorbet Assay (ELISA)

●To couple enzymes to antibody to antibody or antigen for use in immuno assay

●As sensitive as immuno assays

●ELISA less expensive and safer

Fluorescent antibody technique

●Rapid proceduse for the identification of unknown infectious agents

●Based on behaviour of certain dyes which fluorescen (glow) when exposed to certain wavelengths of

light

Cell mediated immunity

●Tuberculin, brucellin and some skin tests — detection of delayed type IV hypersensitivity

●After 18 hour and may persist for days or even months

●The activated T lymphocytes proliferate and release soluble mediators called lymphokines which recruit and activate other host cells

●One of the hymphokines is called migration inhibition factor (MIF), important in inflammatory response

●It prevents the migration of hymphocytes away from the focus of immune response

●Interferon another lymphokines

VACCINE AND VACCINATION

Definition of Vaccine

A preparation of live, weakened or killed pathogen (bacterium or virus, or of a portion of the pathogen's structure) that upon administration stimulates antibody production or cellular immunity against the pathogen but is incapable of causing severe infection

Insurance Against Disease

"Vaccines are used to prevent or reduce problems that can occur when a flock or herd is exposed to field disease organisms"

Price to Be Paid for protection Against a Potential Threat

- Price of the vaccine
- Time spent for administering the vaccines
- Losses due to vaccine reactions *(live vaccines)*
- Losses due to localized tissue damage *(killed vaccines)*

Insurance Against Disease

• If the risk of a particular disease is low in the area, it makes little sense to vaccinate against that disease

• Costs may outweigh the benefits

Types of Vaccines

• Must infect an idividual

Live Vaccines

• Multiply in its body to produce immunity

• Preferably with minimal reactions

Advantages of Live Vaccines

• Ease of administration

• Low price

• Rapid onset of immunity

• Broader scope of protection because chickens/animals are exposed to all stages of the replicating virus

Disadvantages *(Live vaccines)*

• Problems with uniform vaccine application

• Excessive vaccine reactions

• Unwanted spread of the vaccine virus

• Extreme handling requirements needed to maintain viability of the vaccine organism

• Stimulation for short time

• Relatively low level of immunity

Killed Vaccines

• Prepared from bacteria or viruses that have been inactivated and processed

• Prepared from a portion of the pathogen

• Cannot spread from bird-to-bird

• Requires individual injection

• Adjuvants enhance the immune response

• Stimulates the immune system for a longer period of time

Advantages of killed Vaccines

- Administration of a uniform dose *(birds/animals are individually injected)*
- Safety *(inactivated organism)*
- Development of uniform levels of immunity *(each bird/ animal receives the same dose)*
- No chance for spread of vaccine organism
- Increased product stability
- Choice of a wider variety of virus strains
- High level of immunity

Disadvantages *(killed vaccines)*

- Increased costs *(labor and product)*
- Slower onset of immunity
- Narrower spectrum of protection
- Localized tissue damage at site of injection

Vaccination Failure

"A vaccination failure occurs when, following vaccine administration, the chickens/animals do not develop adequate antibody titer levels and/or are susceptible to a field disease outbreak"

- When a vaccination fails
- Natural inclination is to blame the vaccine

Causes of Vaccine Failure

***1. <u>High level of maternal antibodies</u>*:**

- Interferes with the multiplication of live vaccines
- Reducing the amount of immunity produced

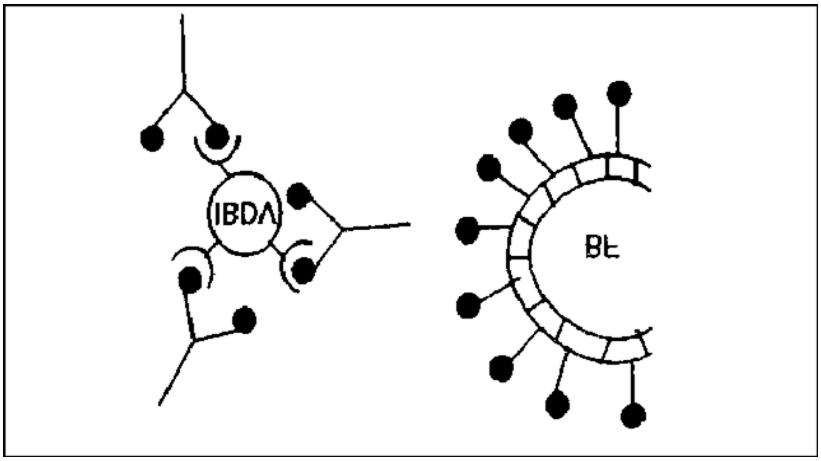
Causes of Vaccine Failure

2. <u>Stress</u>

- Environmental extremes; *(temperature, relative humidity)*
- Inadequate nutrition
- Parasitism

• Other diseases

Gumboro Live Vaccine to High mAb Flock



3 . Improper handling or administration

• Check and record lot numbers

• Check expiry dates on the vials

• Store and handle vaccines as recommended by the manufacturer (cold chain)

• Vaccinate quickly; "*Infectious bronchitis vaccine loses 50 percent of its potency in warm conditions in under 1 hour* "

4. Improper strains or serotypes of vaccine

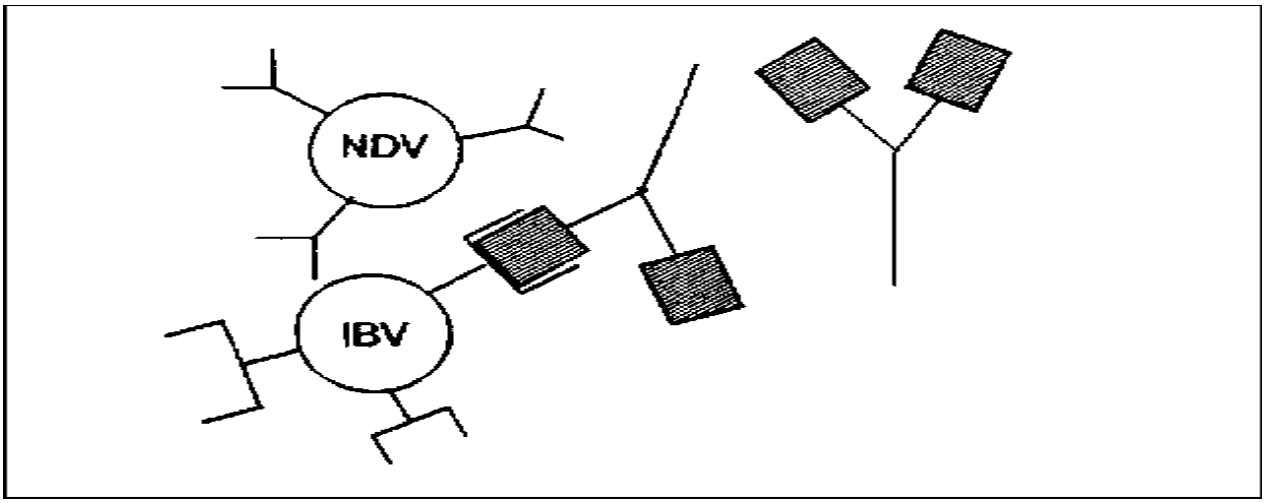
•Do not stimulate protective immunity

•Infectious bronchitis

•Infectious bursal disease

High Immune Response for IB

Low Immune Response for ND

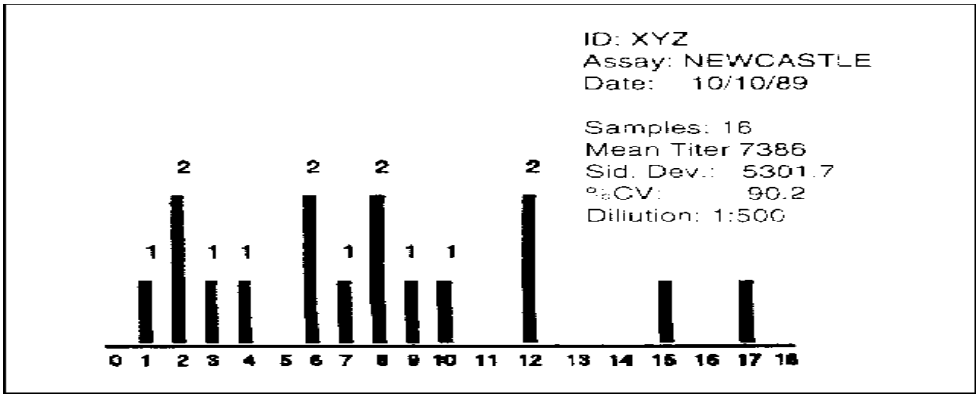


- Protective levels of antibodies
- Antibody titers must be determined for each vaccine/disease
- Good antibody levels of one vaccine does not assure protection for other diseases

5.Poor distribution of vaccine

- Live vaccine; *(water or spray route)*
- Chickens /animals being *"missed"*
- Transmission of the vaccine from bird to bird
- Excessive rolling-type reactions of long duration
- Delayed immunity Killed vaccine
- *Misses"* with killed vaccines will result in chickens with no protection

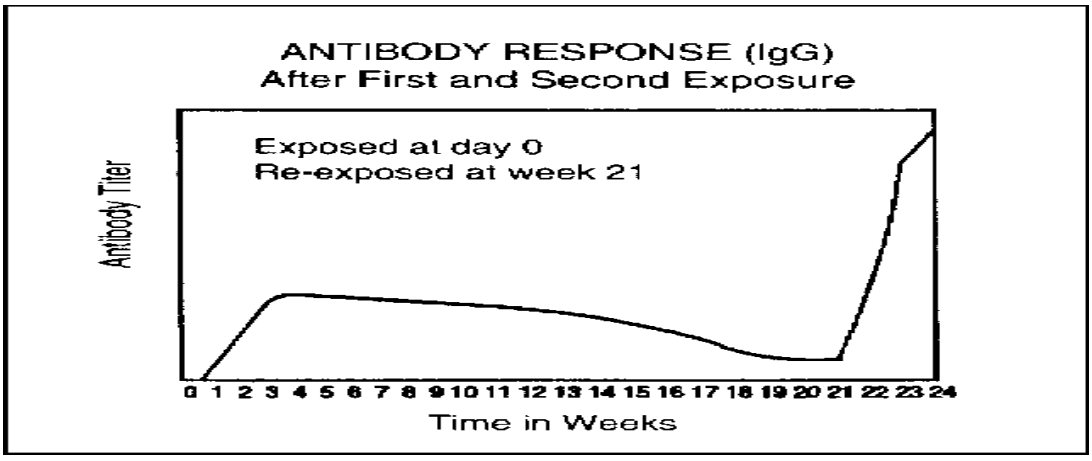
Poor Vaccine Distribution



6. Health status of the flock/ herd

- Incubating the disease at the time of vaccination

- Time is needed for antibody production to begin and reach protective levels
- Immunoglobulins G, M, and A are first detected approximately 4 to 5 days following exposure
- Additional days are required for titers to reach protective levels



7. *Immunosuppression*

- Infections
- Infectious bursal disease
- Mareks' disease
- CRD
- Coccidiosis
- Feed with high levels of mycotoxins

8.Vaccine quality

- Low vaccine titer Contaminated etc
- Assurance of cold chain
- Proper shipment from manufacturer to end user

Conclusions

- Objective should be disease prevention
- Good quality chicks/ animals
- Good quality feed and water
- Strict management practices
- Effective biosecurity procedures

Vaccination Schedule

- Basic Consideration
- Type of birds/ animals
- Prevalence of disease
- Magnitude of disease
- Chick quality
- Nature of feed
- Type of housing
- Concentration of poultry in the area
- Biosecurity measures

Viral Diseases of Poultry

- Newcastle disease
- Infectious bronchitis
- Infectious laryngeotracheitis
- Infectious bursal disease
- Fowl pox
- Avian influenza
- Hydropericardium syndrome
- Marek's disease
- Egg drop syndrome
- Reo virus disease
- Swollen head syndrome
- Inclusion body hepatitis

Bacterial Diseases of Poultry

- Infectious coryza
- Avian pasteurellosis (Cholera)
- Mycoplasma gallisepticum
- Mycoplasma synoviae

• Salmonellosis

AUTOIMMUNE DISEASES

●When your body is attacked – perhaps by a virus or germs or a nail you stepped on – your immune system defends you

●It sees and kills the germs that might hurt you

●But when the system doesn't work right, this process can cause harm

●Immune cells can mistake your body's own cells as invaders and attack them

●This "friendly fire" can affect almost any part of the body

●It can sometimes affect many parts of the body at once

●This is called autoimmunity (meaning self-immunity)

Causes of Autoimmunity

●No one knows why the immune system treats some body parts like germs

●We do know that you can't catch autoimmune diseases from another person

●Most scientists think that our genes and things in the environment are involved

●If you have a certain gene or combination of genes, you may be at higher risk for autoimmune diseases

●But you won't get the disease until something around you turns on your immune system

●This may include the sun, infections, drugs, or in some women, pregnancy

Problems caused by Autoimmunity

●Autoimmunity can affect almost any organ or body system

●The exact problem one has with autoimmunity (or its diseases) depends on which tissues are targeted

●It skin is the target, you may have skin rashes, blisters, or color changes

●If it's the thyroid gland, you may be tired, gain weight, be more sensitive to cold and have muscle aches

●If it's the joints, you may have joint pain, stiffness, and loss of function

●You may know which organ or system is affected from the start

●But you may not know the site of the attack

●In many people, the first symptoms are fatigue, muscle aches and low fever

Where does Autoimmunity Strikes?

●Because autoimmune diseases can affect almost any organ or system of the body, one way to group them is by the body system (s) they attack. The following is a list (not inclusive) of body systems and the autoimmune diseases that can affect them

Blood and blood vessels

●Autoimmune hemolytic anemia

●Pernicious anemia

●Systemic lupus erythematosus

Digestive tract (including the mouth)

●**Autoimmune hepatitis**

●Primary biliary cirrhosis

●Ulcerative colitis

Eyes

●Sjogren's syndrome

●Type 1 diabetes mellitus

●Uveitis

Glands

●Thyroiditis

●Type 1diabetes mellitus

Heart

●Myocarditis

●Rheumatic fever

●Scieroderma

●Systemic lupus erythematosus

Joints

●Ankylosing spondyitis

●Rheumatoid arthritis

●Systemic lupus erythematosus

Kidneys

●Glomerulonephritis

Lungs

●Rheumatoid arthritis

Muscles

●Polymyositis

Autoimmune Diseases Diagnosed

●Autoimmune diseases often don't show a clear pattern of symptoms at first

●So diagnosing them can be hard

●But with time, a diagnosis can usually be made by using:

Medical History

●The doctor will ask about your symptoms and how long you have had them

●Your should tell your doctor if you have a family member with autoimmune disease.

●You may not have the same disease as your family member

●But having a family history of any autoimmune disease makes you more likely to have one

Physical exam

● During the exam, the doctor will check for any signs

●Inflamed joints, swollen lymph nodes, or discolored skin might give clues

●**Medical tests--** No one test will show that you have an autoimmune disease

●But doctors may find clues in a blood sample

●For example, people with lupus or rheumatoid arthritis often have certain auto antibodies in their blood

●Auto antibodies are blood proteins formed against the body's own parts

●Not all people with these diseases have auto antibodies

●And some people without autoimmune disease do have them

●So blood tests alone may not always help

●But if a person has disease symptoms and auto antibodies, the doctor can be more sure of a diagnosis

●The key is patience

●Your doctor may be able to diagnose your condition quickly based on your history, exam, and test results

●But the process often takes time

●It may take several visits to find out exactly what's wrong and the best way to treat it

Autoimmune Diseases Treated

●Autoimmune takes many forms

●There are also many treatment for it

●Treatment depends on the type of disease, how severe it is, and its symptoms

●Generally, treatments have one of three goals

Relieving symptoms

● If your symptoms both you, your doctor may suggest treatments that give some

●Relieving symptoms may be as simple as taking a drug for pain relief

●It may also be as involved as having surgery

Preserving organ function

● When autoimmune disease threaten organs, treatment may be needed to prevent damage

● Such treatments may include drugs to control an inflamed kidney in people with lupus

● Insulin injections can regulate blood sugar in people with diabetes

● These treatments don't stop the disease

● But they can save organ function

● They can also help live with disease complications

Targeting disease mechanisms

● Some drugs may also be used to target how the disease works

● In other words, they can suppress the immune system

● These drugs include cyclophosphamide(cytoxan*)and cyclosporine (Neoral and Sandimmune)

● The same immune-suppressing drug may be used for many diseases

● Your doctor may not prescribe a treatment

● If your symptoms are mild, the risks of treatment may be worse than the symptoms

● You may choose to put off treatment for now

● But you should watch for signs the disease is progressing

● You need to catch changes before they lead to serious damage

Immunomodulation, Immunoseppression & Immunostimulation

Stimulation of the immune system

● There are various situations where it is desirable to enhance immune system. These includes:

a) Enhancement of resistance to infection and
b) Treatment of immunosuppressive conditions

● Immuno-stimulants very according to the way in which these are used. These includes

1. Bacteria and Bacterial Products:

● Many bacteria used for immuno-stimulation

● These bacteria are readily phagocytosed by macrophages and so stimulate cytokine synthesis

● So their immuno-stimulating effects are due to the release of a mixture of cytokines

● The most potent of these cytokine synthesis enhances is BCG, the live attenuated vaccine strain of

Mycobacterium bovis

(A) BCG produces a generalized increase of both

1. B-cell and T-cell mediated responses
2. Enhances phagocytosis
3. Enhances graft rejection and
4. Enhances resistance to infection

(B) Anaerobic coryneforms

1. Promotes antibody formation when administered as a killed suspension
2. Promotes macrophages activities

Similarly staphylococci cell walls, or its some components and products froms

(d) Bordetella Pertussis

(e) Brucella Abortus

(f) Bacillus subtilis

(g) Klebsiella pneumoniae have immuno-stimulating activity

2. Complex carbohydrates

● Certain complex carbohydrates derived from yeasts

● Namely zymosan, glucans, aminated polyglucose and lentinans, can increase phagocytic activities

by activating macrophages

● They may function as adjuvants and potentiate resistance to infections

● Acemannan, a complex carbohydrate desired from the plant is a pontent cytokine synthesis enhancer

with antitumor and antiviral activities

● It has been used to treat feline leuhemia and fibrosarma in cats and dogs

● It also have alribity to enhance wound dealing

3. Immune-enhancing drugs

● A broad-spectrum anthelmintic, levamisole stimulates the immune-system like thymic hormone

(thymopoitin)

● Thymopoitin i.e. it stimulates T-cell differentiation and response to antigens

● Thus levamisole enhancs bovine lymphocyte blastogenesis

● It also enhances interferon production and increases FcR activity in bovine macrophages

● The effect of levamisole are greatest in animals with depressed

● T-cell function. But little effect on normal animals

● So it maybe used in treatment of chronic infections and neoplastic diseases

4.Vitamins

● Vitamin E affects immune response and disease resistance

● Supplementation of Vit.E with diets in cows prior to calving prevents neutrophils decline function and macrophage function that normally occurs in the immediate postparturient period.

● Supplementation with Vit. A and E in chickens can reduce mortality from a challenge from E-coli in chicken and streptococcus pneumoniae in mice.

● Vit. E promotes B-cell proliferation, especially in Ist immune response

● The mode of action is unclear but it may provide stimulus

5.Cytokines

●Major cytokines, IL-1, IL-2, colony-stimulating factors have been tested as immune stimulating gents

●Administration of purified cytokines has minimal effects because naturally in animals different cytokines are produced to stimulate the immune system

6. Interferons

● These are antiviral agents. The administration of interferons stimulate some cellular functions such as Neutrophils activity and so promote disease resistance

1. Recombinant human interferon (RHUIFN-α) is effective for rhinopneumonitis (Bovine herpesvines-1) and rotavirus induced diarrhea in calves

2. Recombinant bovine interferon (RBOIFN-α) is used to treat BHV-1 and Pasteurella haemolytica it is effective when given orally

3. Recombinant bovine interferon (RBOIFN-α) is effective against Salmonella typhimurium

4. They increase the antibody response and cell (neutrophils) mediated or killing of infection these are more effective on immunosuppressed or stressed animals

7. Interleukine-2

●Recombinant forms of IL-2 when administered to animals at the same time as animals were vaccinated, since increased level of protection eg.

(i) RHUIL-2 with BH-V-1 vaccine

(ii)RHUIL-2 with A-pleuropneumonia bacteria

● Intramammary infusions of RBOIL-2 indunce local macrophage and neutrophils infiltration and increase mastitis cure rates

●It may also be used for treatment of carcinomas

●Unfortunately IL-2 is highly toxic. It causes side effects like diarrhea and fever

8. Other cytokines

● IL-1 and G-M-C & F. RBOIL-1β is effective adjuvant in BHV-1 immunization

● RBO G-M-C&F increase neutrophils functions and enhancing their ability to phgocytose in infections (Staphylococcus aurus)

Immune System Disorders

●Hypersensitivity (Allergy): An abnormal response to antigens.

●Four Types of Hypersensitivity Reactions:

- Type I (Anaphylactic) Reactions
- Type II (Cytotoxic) Reactions
- Type III (Immune Complex) Reactions
- Type IV (Cell-Mediated) Reactions

Type I (Anaphylactic) Reactions

- Occur within minutes of exposure to antigen
- Antigens combine with IgE antibodies
- IgE binds to mast cells and basophils, causing them to undergo *degranulation* and release several mediators:
- Histamine: Dilates and increases permeability of blood vessels (swelling and redness), increases mucus secretion (runny nose), smooth muscle contraction (bronchi).
- Prostaglandins: Contraction of smooth muscle of respiratory system and increased mucus secretion.
- Leukotrienes: Bronchial spasms.
- Anaphylactic shock: Massive drop in blood pressure. Can be fatal in minutes.
- Mast Cells and the Allergic Response

Type II (Cytotoxic) Reactions

- Involve activation of complement by IgG or IgM binding to an antigenic cell
- Antigenic cell is lysed
- Transfusion reactions:
- ABO Blood group system: Type O is universal donor. Incompatible donor cells are lysed as they enter bloodstream
- Rh Blood Group System: 85% of population is Rh positive. Those who are Rh negative can be sensitized to destroy Rh positive blood cells
- Hemolytic disease of newborn: Fetal cells are destroyed by maternal anti-Rh antibodies that cross the placenta

Type III (Immune Complex) Reactions

- Involve reactions against <u>*soluble*</u> antigens circulating in serum

●Usually involve IgA antibodies

●Antibody-Antigen immune complexes are deposited in organs, activate complement, and cause inflammatory damage

●Glomerulo nephritis: Inflammatory kidney damage.

●Occurs with slightly high antigen-antibody ratio is present

●Immune Complex Mediated Hypersensitivity

Type IV (Cell-Mediated) Reactions

●Involve reactions by TD memory cells

●First contact sensitizes person

●Subsequent contacts elicit a reaction

●Reactions are *delayed* by one or more days (delayed type hypersensitivity)

●Delay is due to migration of macrophages and T cells to site of foreign antigens

●Reactions are frequently displayed on the skin: itching, redness, swelling, pain

●Tuberculosis skin test

●Poison ivy

●Metals

●Latex in gloves and condoms (3% of health care workers)

●Anaphylactic shock may occur

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