Biyani's Think Tank

Concept based notes

Immunology, Microbiology and Biotechnology

(B.Sc. Part-II)

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Author

Syllabus

Section-A

Immunology

1. Immunology: Definition, types of immunity: innate and acquired: humoral and cell mediated.
2. Antigen: Antigenicity of molecules, haptens.
3. Antigen-antibody reactions: Precipitation reaction, agglutination reaction, neutralizing reaction, complement and lytic reactions and phagocytosis.
4. Cells of Immunit : Macrophages, Lymphocytes (B- and T- types) T- Helper cells. T-Killer cells, plasma cells and memory cells.
5. Mechanism of humoral or antibody mediated immunity.

Section-B

Microbiology

1. Brief introduction to the history of Microbiology : work of Anatomy Van Leeuwenhoek : theory of spontaneous generation : Germ theory of fermentation and disease : Work of Louis Pasteur, John Tynadal, Rober-Koch and Jenner.
2. The Prokaryota (Bacteria) :
   Structural organization :
   i. Size shapes and patterns of arrangement
   ii. Structural organization :

   Slime layer (capsule) : cell envelopses : cytoplasmic membrane (inner membrane). cell wall (outer membrane) of Gramm negative and Gram-positive bacteria : mesosomes : cytoplasmic organization : Cell projection : flagella dn pili.

3. Genetic material of bacteria : (i) Chromosome (ii) Replication of bacterial DNA
4. Reproduction in Bacteria: Axexual reproduction binary fission, budding endospore formation, exospore and cyst formation: Sexual reproduction conjugation.
5. Microbial nutrition culture of bacteria
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   b. Nitrogen and minerals
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6. Bacteria of medical importance :
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      a. Cocci : Staphylococci , Streptococci
      b. Bacilli : Dioptheria : Tetanus.

i. Gram-Negative :
   a. Cocci : Gonnorrhea, Meningitis
   b. Bacilli : Dirrhea

ii. Mycobacteria : Tuberculosis, Leprosy

7. AIDS and hepatitis (with emphasis on B type). The causatie agents. Transmission, Pathogencity, Laboratory, diagnosis, treatment and prevention.

Section-C

Biotechnology

1. Concept of gene : Muton, recon and cistron : gene expression, lac operon and gene mutation.
2. Eugenics and genetic counselling.
3. History, scope and significance of biotechnolgy, Major areas of biotechnology.
4. Vectors for gene transfer (polasmids and phages). Basic concepts of cell and tissue culture.
5. Basic concepts of animals cell and tissue culture and hybridoma technology.
6. Monoclonal antibodies and their applications.
7. Protosplast fusion in prokaryotes and eukaryotes.
8. Recombinant DNA technology and its application.
9. Bacteria and genetic engineering (outline idea only) : benefits of genetic engineering : potential hazards and regulations of genetic engineering.
10. Transgenic animals, their uses and biotechnology
11. Brief account of cloning, Genomic research its advantages and disadvantages.
12. Biotechnology in Medicine (outline idea only) : P.C.R. (Genema0chine) Antibiotics. Vaccines, Enzymes, Vitamines., Steriodis. Artificial Blood.
13. Environmental Biotechnology (outline idea only) : Metal and petroleum recovery pest control waste-water treatment.
14. Food drink and diary Biotechnology (outline idea only) : Fermented food production : diary products, alcoholic beverages and vinegar microbial spoilage and food preservation.

Immunology

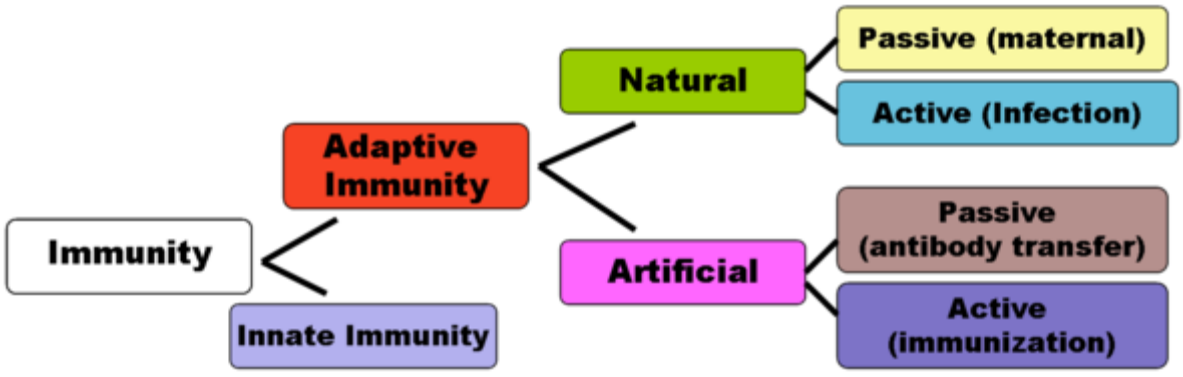
Q. 1. Define immunology?

Ans. The branch of biomedicine concerned with the structure and function of the immune system, innate and acquired immunity, the bodily distinction of self from nonself, and laboratory techniques involving the interaction of antigens with specific antibodies.

Q.2. Explain the types of immunity?

Ans. Immunity is a biological term that describes a state of having sufficient biological defences to avoid infection, disease, or other unwanted biological invasion. Immunity involves both specific and non-specific components. The non-specific components act either as barriers or as eliminators of wide range of pathogens irrespective of antigenic specificity. Other components of the immune system adapt themselves to each new disease encountered and are able to generate pathogen-specific immunity.

Innate immunity, or nonspecific, immunity is the natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches. Microbes first encounter the epithelial layers, physical barriers that line our skin and mucous membranes. Subsequent general defenses include secreted chemical signals (cytokines), antimicrobial substances, fever, and phagocytic activity associated with the inflammatory response. The phagocytes express cell surface receptors that can bind and respond to common molecular patterns expressed on the surface of invading microbes. Through these approaches, innate immunity can prevent the colonization, entry, and spread of microbes.



Adaptive immunity is often sub-divided into two major types depending on how the immunity was introduced. **Naturally acquired immunity** occurs through contact with a disease causing agent, when the contact was not deliberate, whereas **artificially acquired**

immunity develops only through deliberate actions such as vaccination. Both naturally and artificially acquired immunity can be further subdivided depending on whether immunity is induced in the host or passively transferred from a immune host. **Passive immunity** is acquired through transfer of antibodies or activated T-cells from an immune host, and is short lived -- usually lasting only a few months -- whereas **active immunity** is induced in the host itself by antigen, and lasts much longer, sometimes life-long. The diagram below summarizes these divisions of immunity.

Q. 3 Describe the mechanism of natural immunity.

Ans

Innate immunity provides broad defenses against infection and the barriers include

Physical, Chemical and Physiological and Biological barriers

- An invading microbe must penetrate the external barrier formed by the skin and mucous membranes, which cover the surface and line the openings of an animal's body.
- If it succeeds, the pathogen encounters the second line of nonspecific defense, innate cellular and chemical mechanisms that defend against the attacking foreign cell.

Physical Barriers

The skin and mucous membrane provide first-line barriers to infection.

- **Intact skin** is a barrier that cannot normally be penetrated by bacteria or viruses, although even minute abrasions may allow their passage.

  The epithelial cells contan some fixed Lymphocytes which fight against the microbes lying on the skin.

- **The mucous membranes** that line the digestive, respiratory, and genitourinary tracts bar the entry of potentially harmful microbes.
  - Cells of these mucous membranes produce mucus, a viscous fluid that traps microbes and other particles.
  - In the trachea, ciliated epithelial cells sweep out mucus with its trapped microbes, preventing them from entering the lungs.
- Beyond their role as a physical barrier, the skin and mucous membranes counter pathogens with chemical defenses.
  - In humans, for example, secretions from sebaceous and sweat glands give the skin a pH ranging from 3 to 5, which is acidic enough to prevent colonization by many microbes.
  - Microbial colonization is also inhibited by the washing action of saliva, tears, and mucous secretions that continually bathe the exposed epithelium.
    - All these secretions contain antimicrobial proteins.
    - One of these, the enzyme ***lysozyme***, digests the cell walls of many bacteria, destroying them.
- Microbes present in food or water, or those in swallowed mucus, must contend with the highly acidic environment of the stomach.
  - The acid destroys many microbes before they can enter the intestinal tract.

- One exception, the virus hepatitis A, can survive gastric acidity and gain access to the body via the digestive tract.

Biological Barrieres

Phagocytic cells and antimicrobial proteins function early in infection.

- Some microbes have adaptations that allow them to evade destruction by ***phagocytes***.
  - The outer capsule of some bacterial cells hides their surface polysaccharides and prevents phagocytes from attaching to them.
  - Other bacteria are engulfed by phagocytes but resist digestion, growing and reproducing within the cells.
- Natural killer (NK) cells do not attack microorganisms directly but destroy virus-infected body cells.
  - They also attack abnormal body cells that could become cancerous.
  - NK cells attach to a target cell and release chemicals that bring about apoptosis, or programmed cell death.
- Four types of white blood cells are phagocytic.
- The phagocytic cells called **neutrophils** constitute about 60–70% of all white blood cells (leukocytes).
  - Cells damaged by invading microbes release chemical signals that attract neutrophils from the blood.
  - The neutrophils enter the infected tissue, engulfing and destroying microbes there.
  - Neutrophils tend to self-destruct as they destroy foreign invaders, and their average life span is only a few days.
- ***Monocytes,*** about 5% of leukocytes, provide an even more effective phagocytic defense.
  - After a few hours in the blood, they migrate into tissues and develop into macrophages, which are large, long-lived phagocytes.
  - Some macrophages migrate throughout the body, while others reside permanently in certain tissues, including the lungs, liver, kidneys, connective tissues, brain, and especially in lymph nodes and the spleen.
- The ***fixed macrophages*** in the spleen, lymph nodes, and other lymphatic tissues are particularly well located to contact infectious agents.
  - Microbes that enter the blood become trapped in the spleen, while microbes in interstitial fluid flow into lymph and are trapped in lymph nodes.
  - In either location, microbes soon encounter resident macrophages.
- ***Eosinophils***, about 1.5% of all leukocytes, contribute to defense against large parasitic invaders, such as the blood fluke, Schistosoma mansoni.
  - Eosinophils position themselves against the external wall of a parasite and discharge destructive enzymes from cytoplasmic granules.
- ***Dendritic cells*** can ingest microbes like macrophages. However, their primary role is to stimulate the development of acquired immunity.

Chemical Barriers

- A variety of proteins function in innate defense either by attacking microbes directly or by impeding their reproduction.
  - In addition to **lysozyme**, other antimicrobial agents include about 30 serum proteins, known collectively as the complement system.
    - Substances on the surface of many microbes can trigger a cascade of steps that activate the **complement system**, leading to lysis of microbes.
- Another set of proteins that provide innate defenses are the **interferons**, which defend against viral infection.
  - These proteins are secreted by virus-infected body cells and induce uninfected neighboring cells to produce substances that inhibit viral reproduction.
  - Interferon limits cell-to-cell spread of viruses, helping to control viral infection.
  - Because they are nonspecific, interferons produced in response to one virus may confer short-term resistance to unrelated viruses.
  - One type of interferon activates phagocytes.
  - Interferons can be produced by recombinant DNA technology and are being tested for the treatment of viral infections and cancer.
- Damage to tissue by a physical injury or the entry of microbes leads to the release of chemical signals that trigger a localized inflammatory response.
- One of the chemical signals of the inflammatory response is **histamine**, which is stored in mast cells in connective tissues.
  - When injured, mast cells release their histamine.
  - Histamine triggers both dilation and increased permeability of nearby capillaries.
  - Leukocytes and damaged tissue cells also discharge prostaglandins and other substances that promote blood flow to the site of injury.
  - Increased local blood supply leads to the characteristic swelling, redness, and heat of inflammation.
  - Blood-engorged leak fluid into neighboring tissue, causing swelling.
- Enhanced blood flow and vessel permeability have several effects.
  - First, they aid in delivering clotting elements to the injured area.
    - Clotting marks the beginning of the repair process and helps block the spread of microbes elsewhere.
  - Second, increased blood flow and vessel permeability increase the migration of phagocytic cells from the blood into the injured tissues.
    - Phagocyte migration usually begins within an hour after injury.
- ***Chemokines*** secreted by many cells, including blood vessel endothelial cells and monocytes, attract phagocytes to the area.

Physiological barrier

1. Phagocytosis

- Microbes that penetrate the first line of defense face the second line of defense, which depends mainly on phagocytosis, the ingestion of invading organisms by certain types of white cells.
- Phagocyte function is intimately associated with an effective inflammatory response and also with certain antimicrobial proteins.

- Phagocytes attach to their prey via surface receptors found on microbes but not normal body cells.
- After attaching to the microbe, a phagocyte engulfs it, forming a vacuole that fuses with a primary lysosome. This is called as phagolysosome. The microbes are digested with lysosomal enzymes and the undigested materials are carried to outside through residual bodies.
  - Microbes are destroyed within lysosomes in two ways.
    - Lysosomes contain nitric oxide and other toxic forms of oxygen, which act as potent antimicrobial agents.
    - Lysozymes and other enzymes degrade microbial components.

Inflammatory Responses

The body may also mount a systemic response to severe tissue damage or infection.

- Injured cells secrete chemicals that stimulate the release of additional neutrophils from the bone marrow.
- In a severe infection, the number of white blood cells may increase significantly within hours of the initial inflammation.
- Another systemic response to infection is fever, which may occur when substances released by activated macrophages set the body's thermostat at a higher temperature.
  - Moderate fever may facilitate phagocytosis and hasten tissue repair.

- Certain bacterial infections can induce an overwhelming systemic inflammatory response leading to a condition known as septic shock.
  - Characterized by high fever and low blood pressure,
  - Clearly, while local inflammation is an essential step toward healing, widespread inflammation can be devastating.

Summary:

The nonspecific defense systems, *the first line of defense*, the *skin* and *mucous membranes*, prevents most microbes from entering the body.

The *second line of defense* uses *phagocytes, natural killer cells, inflammation, and antimicrobial proteins* to defend against microbes that have managed to enter the body.These two lines of defense are nonspecific in that they do not distinguish among pathogens.

Invertebrates also have highly effective innate defenses.

- Insect hemolymph contains circulating cells called hemocytes.
  - Some hemocytes can phagocytose microbes, while others can form a cellular capsule around large parasites.
  - Other hemocytes secrete antimicrobial peptides that bind to and destroy pathogens.
- Current evidence suggests that invertebrates lack cells analogous to lymphocytes, the white blood cells responsible for acquired, specific immunity in vertebrates.
- Certain invertebrate defenses do exhibit some features characteristic of acquired immunity.
  - Sponge cells can distinguish self from nonself cells.
  - Phagocytic cells of earthworms show immunological memory, responding more quickly to a particular foreign tissue the second time it are encountered.

Q. 4 What is the main difference between humoral and cell mediated immunity?

Ans. Humoral immunity is acquired by B-lymphocytes and T-helper lymphocytes as antigen is engulfed by antigen processing cell (APC) e.g., B-cells then a part of antigen (epitope) is presented to T-helper cells in association with MHC-II (Major Histocompatibility Complex) then T-helper cell activates B-cell to produce effector cell (plasma cell) and memory cell then plasma cell secrete antibodies which neglify harmful or toxic effects of the above antigen.

Cell mediated immunity can be shown by every cell of the body while in the above case only APC can show humoral response whole foreign body/ antibody is engulfed by the cell then epitope is represented to cytotoxic T-lymphocytes(CTLs) then CTLs secrete certain chemicals which kill the cell along with foreign body. When B cells and T cells are activated by a pathogen, memory B-cells and T- cells develop. Throughout the lifetime of an animal these memory cells will "remember" each specific pathogen encountered, and are able to mount a strong response if the pathogen is detected again.

Q.5 Explain Antigens.

Ans. An Antigen is defined as "*any foreign substance that elicits an immune response (e.g., the production of specific antibody molecules) when introduced into the tissues of a susceptible animal and is capable of combining with the specific antibodies formed"*.

Chemical Property of Antigen

- Generally proteins or polysaccharides with m.w. greater than 10,000daltons.
- May be Nucleoprotein, lipoprotein, glycoprotein from any biological sources or synthetic polysaccharides or polypeptides.

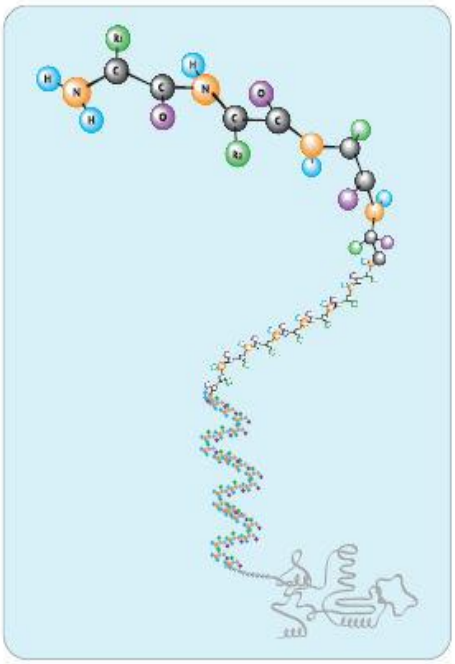
The basic principle of any immunochemical technique is that a specific antibody will combine with its specific antigen to give an exclusive antibody-antigen complex.

Immune responses may also be generated against smaller substances, called ***haptens***, if these are chemically coupled to a larger carrier protein, such as bovine serum albumin, keyhole limpet hemocyanin (KLH) or other synthetic matrices. A variety of molecules such as drugs, simple sugars, amino acids, small peptides, phospholipids, or triglycerides may function as haptens. Thus, given enough time, just about any foreign substance will be identified by the immune system and evoke specific antibody production.

However, this specific immune response is highly variable and depends much in part on the size, structure and composition of antigens. Antigens that elicit strong immune responses are said to be strongly immunogenic.

The small site on an antigen to which a complementary antibody may specifically bind is called an *epitope*. This is usually one to six monosaccharides or 5–8 amino acid residues on the surface of the antigen. Because antigen molecules exist in space, the epitope recognized by an antibody may be dependent upon the presence of a specific three-dimensional antigenic conformation (e.g., a unique site formed by the interaction of two native protein loops or subunits), or the epitope may correspond to a simple primary sequence region. Such epitopes are described as conformational and linear, respectively.

The range of possible binding sites is enormous, with each potential binding site having its own structural properties derived from covalent bonds, ionic bonds and hydrophilic and hydrophobic interactions.



For efficient interaction to occur between the antigen and the antibody, the epitope must be readily available for binding. If the target molecule is denatured, e.g., through fixation, reduction, pH changes or during preparation for gel electrophoresis, the epitope may be altered and this may affect its ability to interact with an antibody. For example, some antibodies are ineffective in Western blot but very good in immunohistochemistry because in the latter procedure, a complex antigenic site might be maintained in the tissue, whereas in the former procedure the process of sample preparation alters the protein conformation sufficiently to destroy the antigenic site and hence eliminate antibody binding. Thus, the epitope may be present in the antigen's native, cellular environment, or only exposed when denatured. In their natural form they may be cytoplasmic (soluble), membrane associated, or secreted. The number, location and size of the epitopes depends on how much of the antigen is presented during the antibody making process.

If a gene product of interest is present in extremely low concentrations, one may choose to use known nucleotide sequence information to derive a corresponding peptide for generating sequence-specific antibodies. In some instances, peptide antigens have advantages over whole protein antigens in that the antibodies generated may be targeted to unique sequence regions. This is especially useful when investigating proteins that belong to families of high sequence homology.

Characteristics of a Good Antigen Include:

- Areas of structural stability and chemical complexity within the molecule.
- Significant stretches lacking extensive repeating units.
- A minimal molecular weight of 8,000–10,000 Daltons, although haptens with molecular weights as low as 200 Da have been used in the presence of a carrier protein
- The ability to be processed by the immune system.
- Immunogenic regions which are accessible to the antibody-forming mechanism.
- Structural elements that are sufficiently different from the host.
- For peptide antigens, regions containing at least 0% of immunogenic amino acids: K, R, E, D, Q, and N.

- For peptide antigens, significant hydrophilic or charged residues.

Q.6 Mention the properties of antigens?

Ans. Two main properties of antigens are:

- ***Specificity***: Ability to react specifically with antibodies or cells
- ***Immunogenicity***: Ability to induce a humoral or cell mediated immune response is immunogenicity, which is caused by the immunogens.
- ***Antigenicity***: It is different to the immunogenicity. It is the ability to combine specifically with the final products of immune responses (like antibodies or T- Cell receptors)

All those cause immunogenicity can have the capacity of antigenicity, but it is not necessary that all the antigens are immunogens.

Q.7 Short notes on the following terms?

Ans

a. Immunogen: A substance that induces a specific immune response and the phenomenon is known as immunogenicity. Immunogens are usually proteins or polysaccharides. This includes parts (coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, and other microorganisms. Lipids and nucleic acids are antigenic only when combined with proteins and polysaccharides. Non-microbial exogenous (non-self) antigens can include pollen, egg white, and proteins from transplanted tissues and organs or on the surface of transfused blood cells.

b. FACTORS INFLUENCING IMMUNOGENICITY

1. Foreignness
The immune system normally discriminates between self and non-self such that only foreign molecules are immunogenic.

2. Size
There is not absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.

3. Chemical Composition
In general, the more complex the substance is chemically the more immunogenic it will be. The antigenic determinants are created by the primary sequence of residues in the polymer and/or by the secondary, tertiary or quaternary structure of the molecule.

4. Physical form
In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.

5. Degradability
Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens, see below) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

c. **Adjuvants :** Substances that can enhance the immune response to an immunogen are called adjuvants. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

d. Epitope or Antigenic Determinant
That portion of an antigen that combines with the products of a specific immune response, like antibody. The immune cells do not interact with the entire antigen or immunogen.

Q.8 Describe different types of antigen?

TYPES OF ANTIGENS

A. T-independent Antigens
T-independent antigens are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help In general, polysaccharides are T-independent antigens. The responses to these antigens differ from the responses to other antigens.

Properties of T-independent antigens

1. Polymeric structure
These antigens are characterized by the same antigenic determinant repeated many times as illustrated in Figure 1.

2. Polyclonal activation of B cells
Many of these antigens can activate B cell clones specific for other antigens (polyclonal activation). T-independent antigens can be subdivided into Type 1 and Type 2 based on their ability to polyclonally activate B cells. Type 1 T-independent antigens are polyclonal activators while Type 2 are not.

3. Resistance to degradation
T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.

B. T-dependent Antigens
T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are

characterized by a few copies of many different antigenic determinants as i

II> Based on the capacity of antigenicity, antigens can be of two types:

1. **Incomplete antigen:** Able to bind with antibodies but unable to produce immune response,

   e.g. Haptens

2. **Complete Antigen:** High m.w. antigens having both immunogenicity and antigenicity, eg.

   Hapten- Carrier Complex

III> Antigens can be species specific if antibody recognizes all tissues of donor species. Some antibodies are able to recognize the organ specific antigens, and are helpful in forensic medicine and for product identification in food industry. They may be of following types:

a. ***Alloantigens/ isoantigens***: A type of tissue- specific antigen which is present in one individual of a species but not in another is called as alloantigen.

   e.g. Human sera contains isoantibodies against the RBC isoantigens of other persons (A, B, AB)

b. Natural or Microbial vs. Artificial Antigens

Natural antigens are mainly of microbial origin, present almost everywhere in air, water, soil etc.

Ex. Pathogenic bacteria, virus, ricketsias , fungi etc.

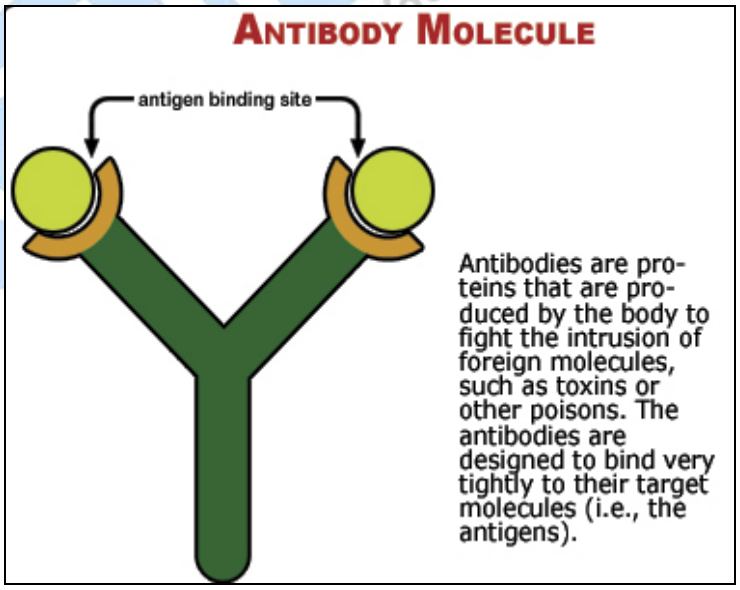
Artificial antigens are vaccines, which are suspensions of killed, living and attenuated cultures of microorganisms.

Ex. Typhoid vaccines, Salk & Sabin Vaccine, Polio Vaccine etc.

Q.9 Explain the structure of antibody and its function?

Ans. An **antibody**, also known as an **immunoglobulin**, is a large Y-shaped protein used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The antibody recognizes a unique part of the foreign target, termed an antigen.[[1][2]] Each tip of the "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can *tag* a microbe or an infected cell for attack by other parts of the immune system, or can neutralize its target directly (for example, by blocking a part of a microbe that is essential for its invasion and survival). The production of antibodies is the main function of the humoral immune system.

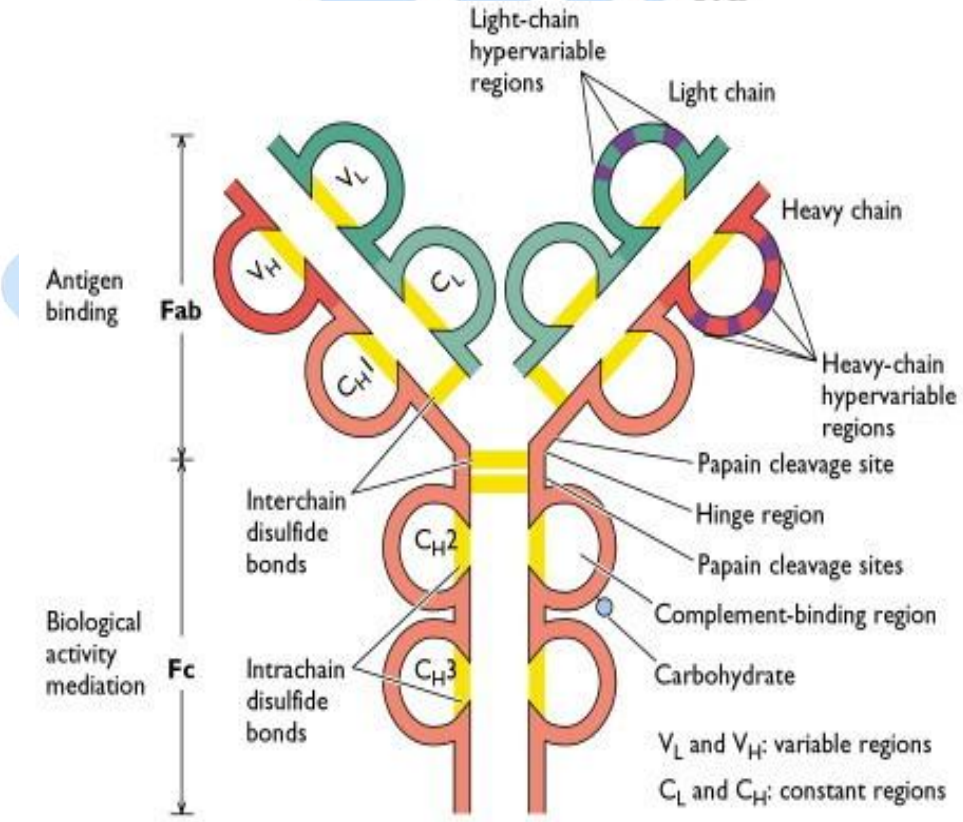
Antibodies are produced by a type of white blood cell called a plasma cell. Antibodies can occur in two physical forms, a soluble form that is secreted from the cell, and a membrane-bound form that is attached to the surface of a B cell and is referred to as the B cell receptor (BCR). The BCR is only found on the surface of B cells and facilitates the activation of these cells and their subsequent differentiation into either antibody factories called plasma cells, or memory B cells that will survive in the body and remember that same antigen so the B cells can respond faster upon future exposure.In most cases, interaction of the B cell with a T helper cell is necessary to produce full activation of the B cell and, therefore, antibody generation following antigen binding. Soluble antibodies are released into the blood and tissue fluids, as well as many secretions to continue to survey for invading microorganisms.



Antibodies are glycoproteins belonging to the immunoglobulin superfamily; the terms *antibody* and *immunoglobulin* are often used interchangeably Antibodies are typically

made of basic structural units—each with two large heavy chains and two small light chains. There are several different types of antibody heavy chains, and several different kinds of antibodies, which are grouped into different *isotypes* based on which heavy chain they possess. Five different antibody isotypes are known in mammals, which perform different roles, and help direct the appropriate immune response for each different type of foreign object they encounter

Though the general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with slightly different tip structures, or antigen binding sites, to exist. This region is known as the *hypervariable region*. Each of these variants can bind to a different target, known as an antigen.This enormous diversity of antibodies allows the immune system to recognize an equally wide variety of antigens. The large and diverse population of antibodies is generated by random combinations of a set of gene segments that encode different antigen binding sites (or *paratopes*), followed by random mutations in this area of the antibody gene, which create further diversity.Antibody genes also re-organize in a process called class switching that changes the base of the heavy chain to another, creating a different isotype of the antibody that retains the antigen specific variable region. This allows a single antibody to be used by several different parts of the immune system.



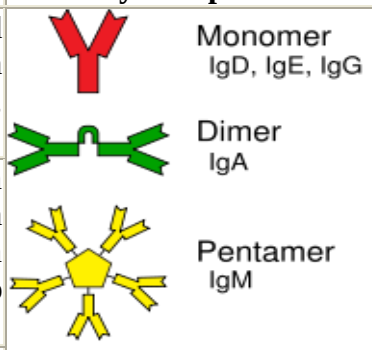
Functions

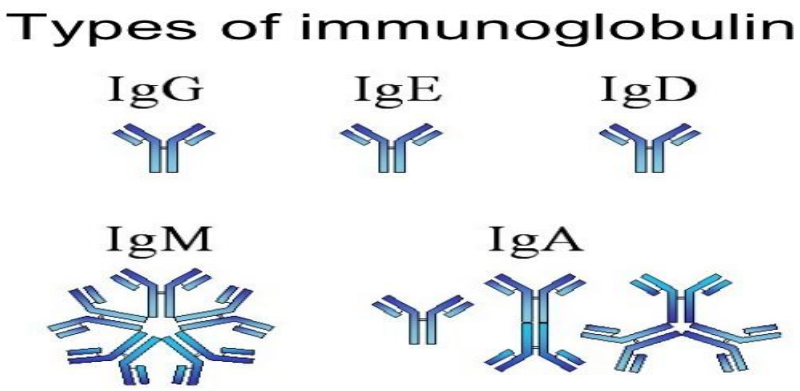
Activated B cells differentiate into either antibody-producing cells called plasma cells that secrete soluble antibody or memory cells that survive in the body for years afterward in order to allow the immune system to remember an antigen and respond faster upon future exposures.

At the prenatal and neonatal stages of life, the presence of antibodies is provided by passive immunization from the mother. Early endogenous antibody production varies for different kinds of antibodies, and usually appear within the first years of life. Since antibodies exist freely in the bloodstream, they are said to be part of the humoral immune system. Circulating antibodies are produced by clonal B cells that specifically respond to only one antigen (an example is a virus capsid protein fragment). Antibodies contribute to immunity in three ways: they prevent pathogens from entering or damaging cells by binding to them; they stimulate removal of pathogens by macrophages and other cells by coating the pathogen; and they trigger destruction of pathogens by stimulating other immune responses such as the complement pathway.

Q. 10. What are different types of antibodies?

Ans. The different types can be summarised as follows

Name	Types	Description	Antibody Complexes
IgA	2	IgA is a secretory antibody and expressed in dimer IgA. It is found in mucosal areas, such as the gut, respiratory tract and urogenital tract.	
IgD	1	IgD functions as mainly as an antigen receptor on B cells that have not been exposed to antigens. It has been shown to activate basophils and mast cells to produce antimicrobial factors.	
IgE	1	IgE binds to allergens and triggers histamine release from mast cells and basophiles, and is involved in allergy. Also protects against parasitic worms.	
IgG		IgG provides the majority of antibody-based immunity against invading pathogens.	
IgM	1	IgM expressed on the surface of B cells and in a secreted form with very high avidity.	



IgD – exists as a monomer and is attached to the external surface of the B cell where it functions as an antigen receptor and is important in B cell activation

b. IgM – exists in a monomer or pentamer form.

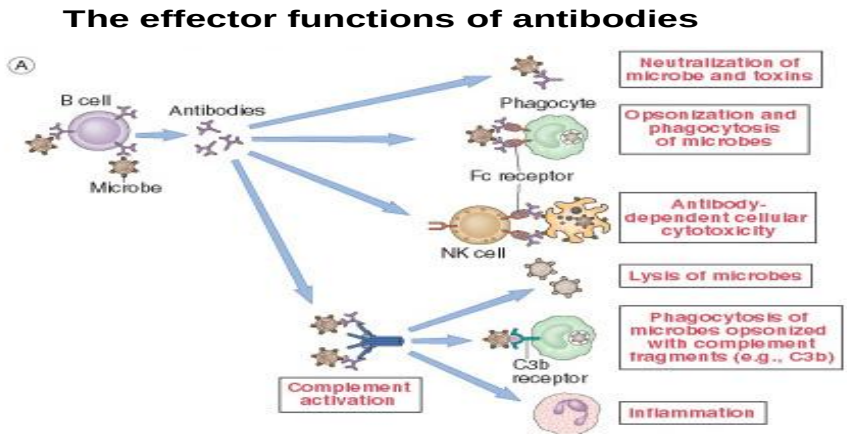
i. monomer attached to the B cell surface and is an antigen receptor

ii. pentamer circulates in blood plasma and is released by plasma cells during the primary response. It presence in the blood indicates current infection and it acts as a potent agglutinating agent and readily fixes and activates complement

c. IgG –exists as a monomer and is the most abundant and diverse antibody in plasma and accounts for 75-85% of circulating antibodies. Can protect against bacteria, viruses, and toxins and can fix complement. It is the main antibody of both primary and secondary responses.

d. IgA – is a dimer in plasma and is also called secretory IgA because it is present in body secretions such as saliva, sweat, intestinal juice, and milk and helps prevent pathogens from gaining access to the body.

e. IgE – a monomer that is secreted by epithelial plasma cells and almost never found in the blood. They bind to mast cells and basophils when activated by antigen and it causes those cells to release histamine and other chemicals that mediate inflammation and allergic reactions. Blood IgE levels rise dramatically during allergic reactions or chronic gastrointestinal tract parasites.



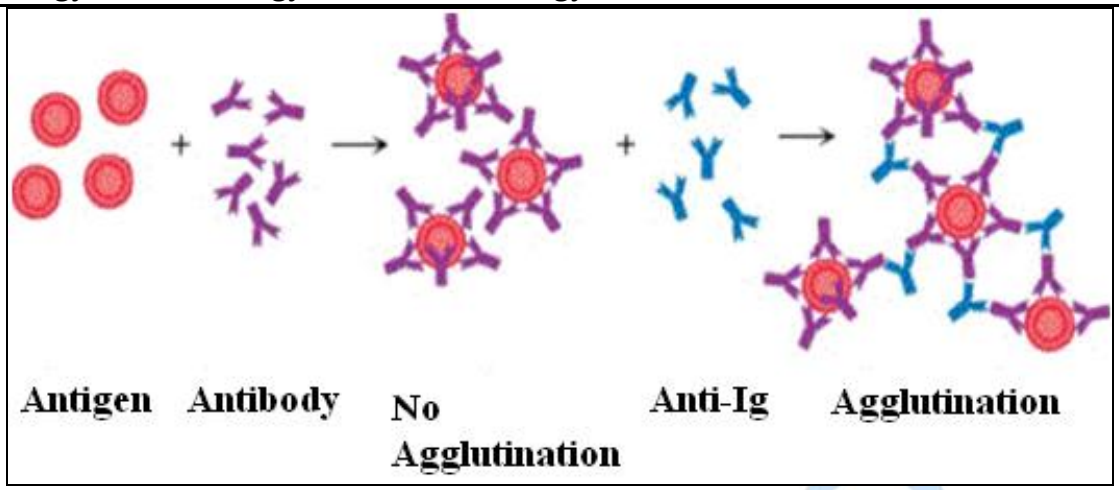
Q.11 What is Antigen and antibody reactions?

Ans. Anibody molecules are **multivalent** and antigens are also often **multivalent**. This multivalency tends to increase the strength of the interaction, and this really represents the true state of affairs. This overall binding energy that results in the binding of a multivalent antibody with a multivalent antigen is called the **functional affinity** or the **avidity.The antigen-antibody reaction can be of mainly following types-**

i. **Agglutination Reactions**
ii. **Precipitation Reactions**
iii. **Immunoassays**
iv. **Immunofluorescence**

i. Agglutination Reactions

Agglutination reactions involve the formation of complexes between antigen and antibodies.such type of reactions are used to identify human blood group types. The following diagram illustrates the agglutination reaction.



ii. **Precipitation Reactions**

This describes the reaction between **soluble** antibody and **soluble** antigen in which an insoluble product results. Please note the discussion describing the effects of antibody excess, antigen excess, and the zone of optimal proportions (equivalence zone) on the production of a precipitate. (Remember that precipitation is a secondary phenomenon. Ag-Ab reactions may occur and form **soluble immune complexes** even without the production of a visible precipitate!)

Precipitation reactions can be done in a variety of ways:

In test tubes

In agarose gels:

- As double diffusion
- As single diffusion
- After electrophoresis

iii. **Immunoassays**: Solid phase immunoassays;

ELISA – Enzyme linked immunosorbant assay

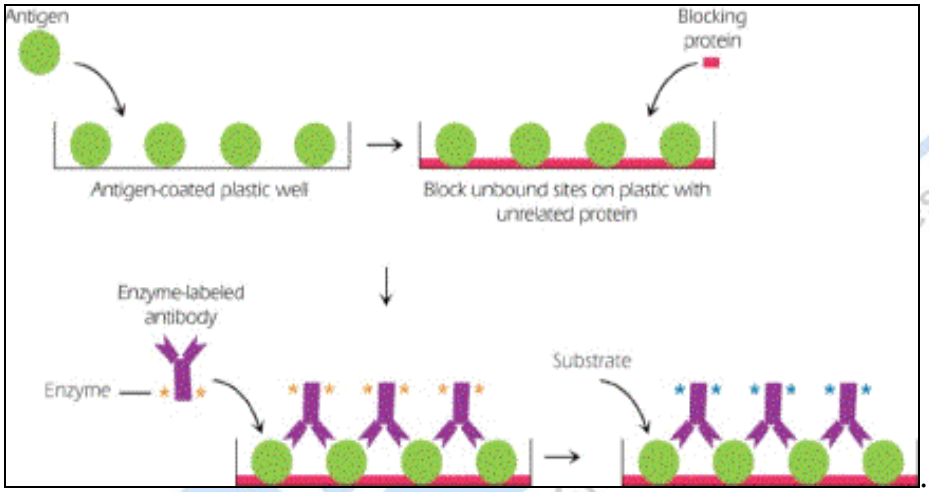
In ELISA a reagent antibodies or reagent binding proteins is required that have been "tagged" with an enzyme label. This means that the enzyme has been covalently coupled to the protein reagent.

a. Typical enzymes include: Horseradish peroxidase or Alkaline phosphatase

b. Reagent proteins include antibodies such as goat-anti-human-IgG. Or, bacterial proteins that bind to antibodies such as Staphylococcal protein A or Streptococcal

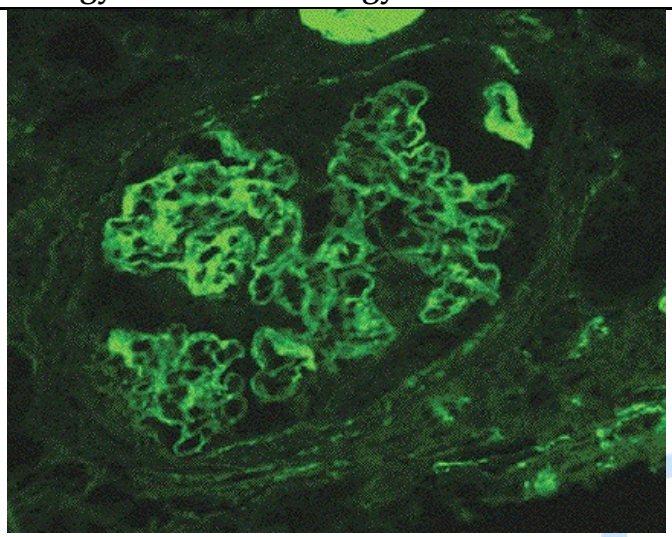
protein G. Biotin and Avidin can also be used ---- avidin has several high-affinity binding sites for biotin thus it can be used to bridge molecules that have been "tagged" with biotin.

2. Some kind of "solid phase" to which proteins can stick is required. This would usually be some kind of plastic microtiter plate.



iv. **Immunofluorescence**

In these assays, antibodies or other reagent proteins are "tagged" or labeled with fluorescent dyes. These fluorescent reagents can then be used to stain samples mounted onto microscope slides and the slides can be examined using a fluorescent microscope.



Typical fluorescent labels include FITC, TRITC, PE and many others.Cells in suspension can also be stained fluorescently and then analyzed by **fluorescence-activated cell sorting** or **FACS analyis**.

Q.12 What are B cells?

Ans. An immune system is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens and tumor cells. It detects a wide variety of agents, from viruses to parasitic worms, and needs to distinguish them from the organism's own healthy cells and tissues in order to function properly. Detection is complicated as pathogens can evolve rapidly, and adapt to avoid the immune system and allow the pathogens to successfully infect their hosts.

B Cells: B cells are lymphocytes that play a large role in the humoral immune response (as opposed to the cell-mediated immune response, which is governed by T cells). The principal functions of B cells are to make antibodies against antigens, perform the role of antigen-presenting cells (APCs) and eventually develop into memory B cells after activation by antigen interaction. B cells are an essential component of the adaptive immune system.

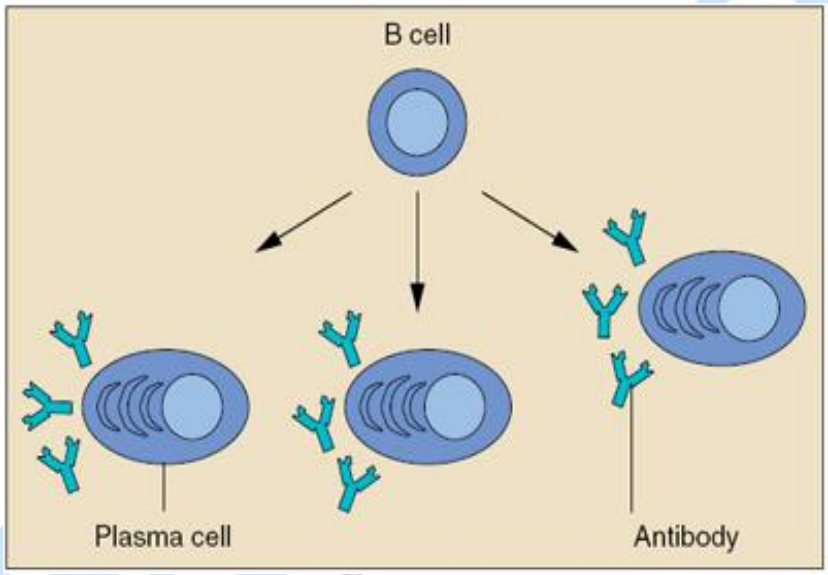
The abbreviation "B", in B cell, comes from the bursa of Fabricius in birds, where they mature. In mammals, immature B cells are formed in the bone marrow, which is used as a acronym for the cells' name

Development of B cells

Immature B cells are produced in the bone marrow of most mammals. Rabbits are an exception; their B cells develop in the appendix-sacculus rotundus. After reaching the IgM+ immature stage in the bone marrow, these immature B cells migrate to the spleen,

where they are called transitional B cells, and some of these cells differentiate into mature B lymphocytes.

B cell development occurs through several stages, each stage representing a change in the genome content at the antibody loci. An antibody is composed of two identical light (L) and two identical heavy (H) chains, and the genes specifying them are found in the 'V' (Variable) region and the 'C' (Constant) region. In the heavy-chain 'V' region there are three segments; V, D and J, which recombine randomly, in a process called VDJ recombination, to produce a unique variable domain in the immunoglobulin of each individual B cell. Similar rearrangements occur for light-chain 'V' region except there are only two segments involved; V and J. The list below describes the process of immunoglobulin formation at the different stages of B cell development.



Functions

The human body makes millions of different types of B cells each day that circulate in the blood and lymphatic system performing the role of immune surveillance. They do not produce antibodies until they become fully activated. Each B cell has a unique receptor protein (referred to as the B cell receptor (BCR)) on its surface that will bind to one particular antigen. The BCR is a membrane-bound immunoglobulin, and it is this molecule that allows the distinction of B cells from other types of lymphocyte, as well as being the main protein involved in B cell activation. Once a B cell encounters its cognate antigen and receives an additional signal from a T helper cell, it can further differentiate into one of the two types of B cells listed below (plasma B cells and memory B cells). The B cell may either become one of these cell types directly or it may undergo an intermediate differentiation step, the germinal center reaction, where the B cell will hypermutate the variable region of its immunoglobulin gene ("somatic hypermutation") and possibly undergo class switching. Other functions for B cells include antigen presentation, cytokine production and lymphoid tissue organization.

B cell types

A suspected plasma cell. Plasma cells are normally detected in tissue rather than circulation.

- **Plasma B cells** (also known as *plasma cells*, *plasmocytes*, and *effector B cells*) are large B cells that have been exposed to antigen and produce and secrete large amounts of antibodies, which assist in the destruction of microbes by binding to them and making them easier targets for phagocytes and activation of the complement system. They are sometimes referred to as *antibody factories.* An electron micrograph of these cells reveals large amounts of rough endoplasmic reticulum, responsible for synthesizing the antibody, in the cell's cytoplasm. These are short lived cells and undergo apoptosis when the inciting agent that induced immune response is eliminated. This occurs because of cessation of continuous exposure to various colony stimulating factors required for survival.

- **Memory B cells** are formed from activated B cells that are specific to the antigen encountered during the primary immune response. These cells are able to live for a long time, and can respond quickly following a second exposure to the same antigen.

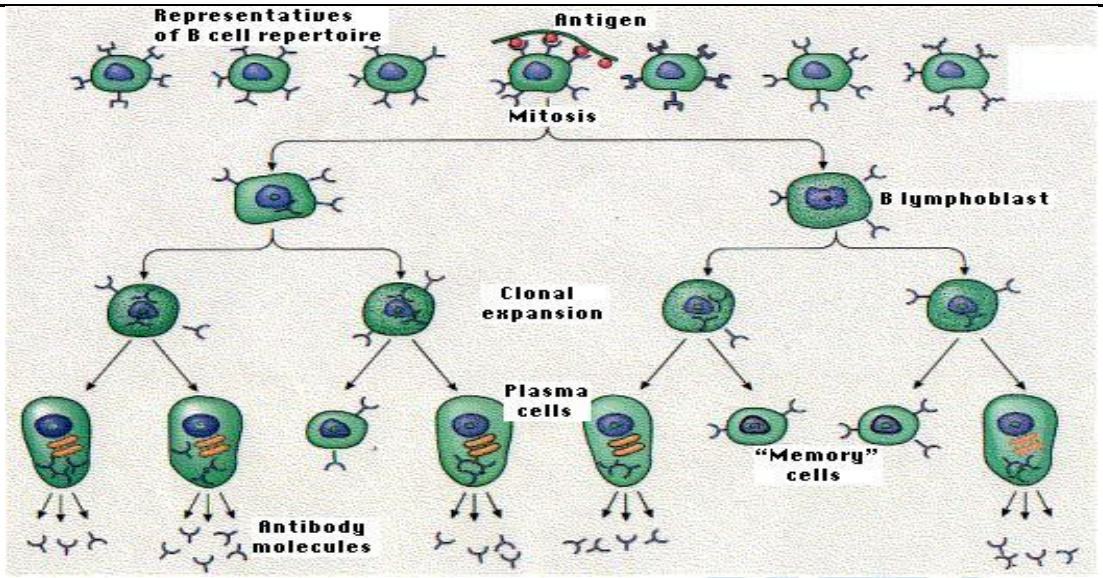
- **B-1 cells** express IgM in greater quantities than IgG and their receptors show polyspecificity, meaning that they have low affinities for many different antigens. Polyspecific immunoglobulins often have a preference for other immunoglobulins, self antigens and common bacterial polysaccharides. B-1 cells are present in low numbers in the lymph nodes and spleen and are instead found predominantly in the peritoneal and pleural cavities

Q. 13 What is clonal selection theory?

Ans. The ability of the immune system to respond to an antigen exists **before** it ever encounters that antigen. The immune system relies on the prior formation of an incredibly diverse population of:

- **B cells** (B lymphocytes) each with its surface covered with thousands of identical copies of a **receptor for antigen** (the B-cell receptor for antigen = **BCR**)
- **T cells** (T lymphocytes) each with its surface covered with thousands of identical copies of a T-cell receptor for antigen (**TCR**)

The specificity of both BCRs and TCR is that the epitope to which a given receptor can bind, is created by a remarkable genetic mechanism. Each receptor is created even though the epitope it recognizes may never have been present in the body. If an antigen with that epitope should enter the body, those few lymphocytes able to bind to it will do so. If they also receive a second **co-stimulatory signal**, they may leave G_0 of the cell cycle and begin repeated rounds of mitosis (the cells are now called lymphoblasts). In this way, **clones** of antigen-specific lymphocytes (B and T) develop providing the basis of the immune response.



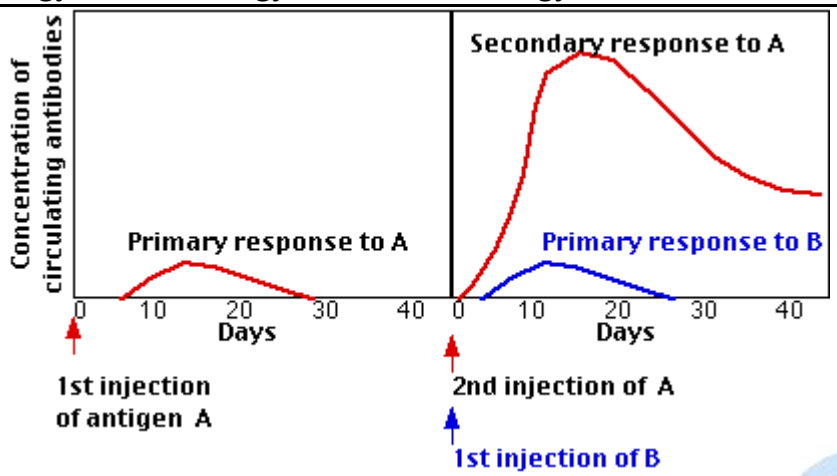
This drawing illustrates the activation of the one B cell in a pool of B cells whose BCR is specific for an epitope (small dark spheres) on the antigen. This phenomenon is called **clonal selection** because it is antigen that selects particular lymphocytes for clonal expansion. Clonal selection leads to the eventual production of:

- a pool of antibody-secreting plasma cells. Plasma cells are B-cells that have tooled up for massive synthesis and secretion of an antibody. The antibody is the secreted version of the BCR. (For clarity, each BCR is shown with a single binding site for the epitope; actually, the BCRs are IgM and each has 10 identical binding sites.
- A pool of **"memory" cells**. These are B lymphocytes with receptors of the same specificity as those on the original activated B cell.

Q. 14. Describe immunological memory and differentiate between primary and secondary immune response.

Ans. **The** immune system has its immunological memory. Whenever a person faces the infection of any antigen first time, the response generated first time is called as the primary immune response. During this response by the clonal selection following the phase of recognition, the specific antibodies are produced and secreted into the body stream for neutralizing the effect of the antigens.

After recovering from an infection, the concentration of antibodies against the infectious agent gradually declines over the ensuing weeks, months, or even years. A time may come when antibodies against that agent can no longer be detected. Nevertheless, the individual often is still protected against a second case of the disease; that is, the person is still **immune**. In fact, a second exposure to the agent usually calls forth a more rapid and larger response to the antigen. This is called the **secondary response**.



The graphs demonstrate the main features of the secondary antibody response. A second injection of **antigen A** produces a secondary response. But the response is specific — not simply the result of a general enhancement of the immune system — because the simultaneous injection of a new antigen (**B**) produces only a primary response to it.

The secondary response reflects a larger number of antigen-specific cells — called **memory cells** — than existed before the primary response. During the initial expansion of clones, some of the progeny cells neither went on dividing nor developed into plasma cells. Instead, they reverted to small lymphocytes bearing the **same BCR** on their surface that their ancestors had. This lays the foundation for a more rapid and massive response the next time the antigen enters the body.

To provide long-lasting immunity, memory cells must have long life spans if we assume that they never divide again unless they encounter the appropriate antigen. Some of the lymphocytes that circulate from lymph nodes to blood and back again do appear to be long-lived. In fact, there is evidence in humans that some small lymphocytes survive at least 20 years; perhaps for life.

Q.15 What are T cells?

Ans. T cells or T lymphocytes belong to a group of white blood cells known as lymphocytes, and play a central role in cell-mediated immunity. They can be distinguished from other lymphocyte types, such as B cells and natural killer cells (NK cells) by the presence of a special receptor on their cell surface called *T cell receptors* (TCR). The abbreviation *T*, in *T cell*, stands for thymus, since this is the principal organ responsible for the T cell's maturation. Several different subsets of T cells have been discovered, each with a distinct function.

Types of T-cells

- **Helper**

T helper cell (T_H cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages, among other functions. These cells are also known as CD^+ T cells because they express the CD protein on their surface. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of Antigen Presenting Cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response. These cells can differentiate into one of several subtypes, including T_H1, T_H2, T_H, T_H17, or T_{FH}, which secrete different cytokines to facilitate a different type of immune response. The mechanism by which T cells are directed into a particular subtype is poorly understood, though signalling patterns from the APC are thought to play an important role.

- **Cytotoxic**

Cytotoxic T cells (T_C cells, or CTLs) destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as $CD8^+$ T cells since they express the CD8 glycoprotein at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body. Through IL-10, adenosine and other molecules secreted by regulatory T cells, the $CD8^+$ cells can be inactivated to an anergic state, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis.

- **Memory**

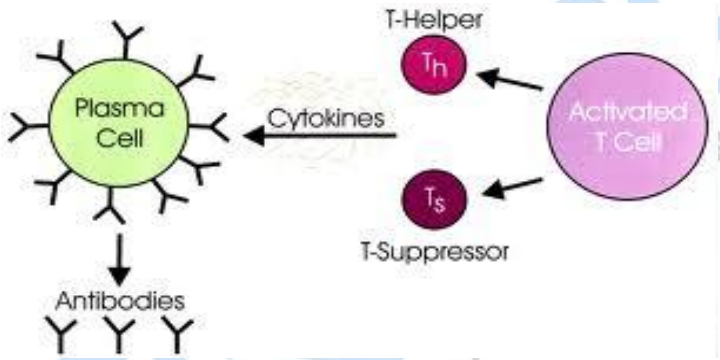
Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections. Memory T cells comprise two subtypes: central memory T cells (T_{CM} cells) and effector memory T cells (T_{EM} cells). Memory cells may be either CD^+ or $CD8^+$. Memory T cells typically express the cell surface protein CD5RO

- **Regulatory**

Regulatory T cells (T_{reg} cells), formerly known as **suppressor T cells**, are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress auto-reactive T cells that escaped the process of negative selection in the thymus. Two major classes of CD^+ regulatory T cells have been described, including the naturally occurring T_{reg} cells and the adaptive T_{reg} cells. Naturally occurring T_{reg} cells (also known as $CD^+CD25^+FoxP^+$ T_{reg} cells) arise in the thymus and have been linked to interactions between developing T cells with

both myeloid (CD11c+) and plasmacytoid (CD12+) dendritic cells that have been activated with TSLP. Whereas the adaptive T_{reg} cells (also known as Tr1 cells or Th cells) may originate during a normal immune response. Naturally occurring T_{reg} cells can be distinguished from other T cells by the presence of an intracellular molecule called FoxP. Mutations of the *FOXP* gene can prevent regulatory T cell development, causing the fatal autoimmune disease IPEX.

Natural killer T cells (NKT cells) are a special kind of lymphocyte that bridges the adaptive immune system with the innate immune system. Unlike conventional T cells that recognize peptide antigen presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1d. Once activated, these cells can perform functions ascribed to both T_h and T_c cells (i.e., cytokine production and release of cytolytic/cell killing molecules). They are also able to recognize and eliminate some tumor cells and cells infected with herpes viruses.



Development in the thymus

All T cells originate from haematopoietic stem cells in the bone marrow. Haematopoietic progenitors derived from haematopoietic stem cells populate the thymus and expand by cell division to generate a large population of immature thymocytes. The earliest thymocytes express neither CD nor CD8, and are therefore classed as *double-negative* (CD^-CD8^-) cells. As they progress through their development they become *double-positive* thymocytes (CD^+CD8^+), and finally mature to *single-positive* (CD^+CD8^- or CD^-CD8^+) thymocytes that are then released from the thymus to peripheral tissues.

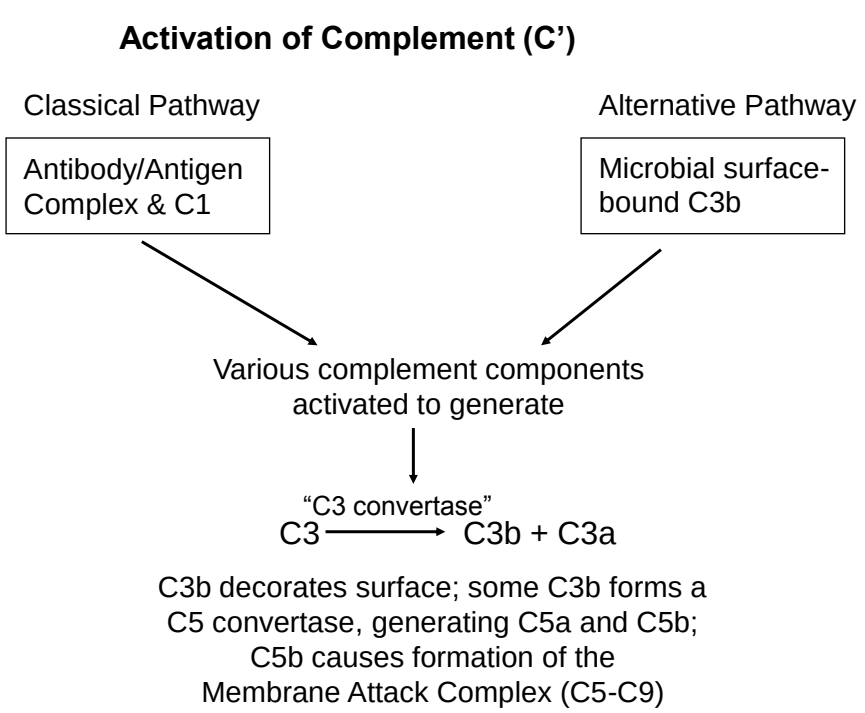
About 98% of thymocytes die during the development processes in the thymus by failing either **positive selection** or **negative selection**, whereas the other 2% survive and leave the thymus to become mature immunocompetent T cells.

The thymus contributes more naive T cells at younger ages. As the thymus shrinks by about % a year throughout middle age, there is a corresponding fall in the thymic

production of naive T cells, leaving peripheral T cell expansion to play a greater role in protecting older subjects.

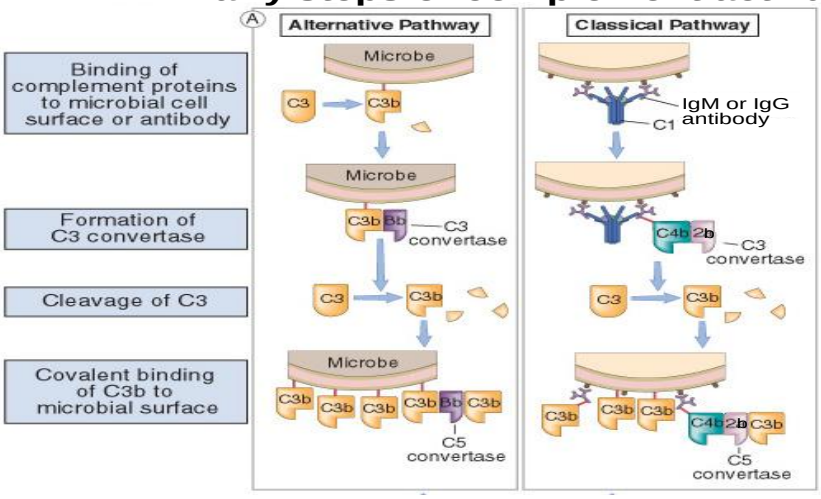
Q. 16 Write a note on Complement System.

Ans. Complement system opsonizes antigens for phagocytosis and can promote direct lysis of some bacteria. All the C' components pre-exist in an inactive form in the blood (mostly made in the liver). C3 is the most abundant. All the components act in cascade manner of the enzymatic actions. When a C' component is cleaved, the larger 'b' fragment is chemically labile and becomes covalently bound to nearby surfaces; the smaller 'a' fragment is soluble and can diffuse away.



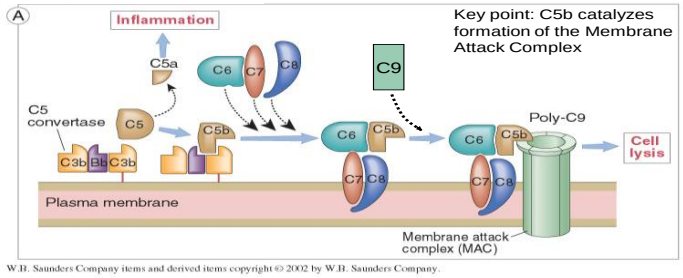
The membrane attack complex makes hole on the bacterial membrane which causes changes in cytoplasmic gradient and thus resulting into cell lysis.

Early steps of complement activation

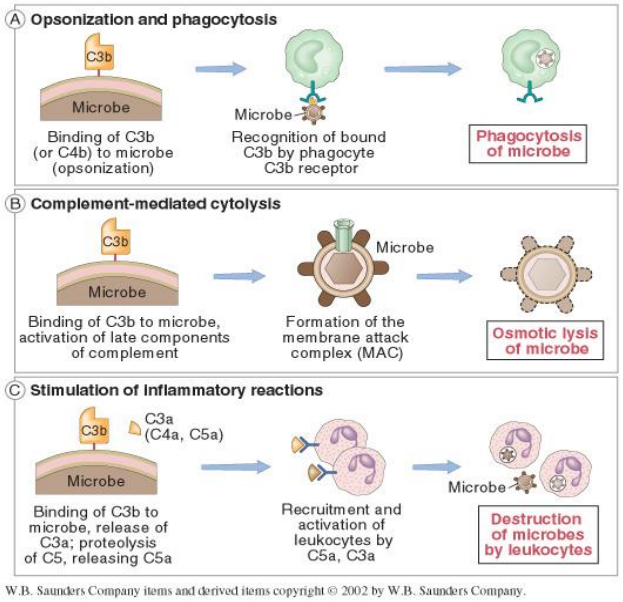


Note: because C3 is very abundant, once a C3 convertase forms, lots of C3b is generated and becomes covalently bound to nearby surfaces

The late steps of complement activation



The biological functions of complement



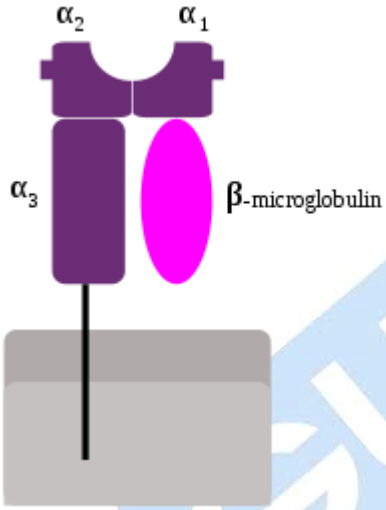
C5a, C3a and C4a also known as Anaphylatoxins
-> induce mast cell and basophil mediator release (when occurring systemically, can cause anaphylactic shock)

Q. 17 write a note on **MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)**

Ans. In humans also known as HUMAN LYMPHOCYTE ANTIGEN (HLA). MHC is really a group of genes that code for antigens. All cells contain antigens. Because the antigens on your cells are slightly different from the antigens on someone else's cells, you are distinctly different from everyone else in the world. The immune system has to be able to determine between you and someone else; it has to recognize self from non-self.

There are three classes of genes in the MHC. Class I and II genes code for antigens (proteins) that are displayed "present" on the surface of cells. In the immune response, the very first thing that has to be done is to present the antigen to cells of the immune system. Class III MHC codes for proteins important in inflammation and will not be discussed here.

MHC class I

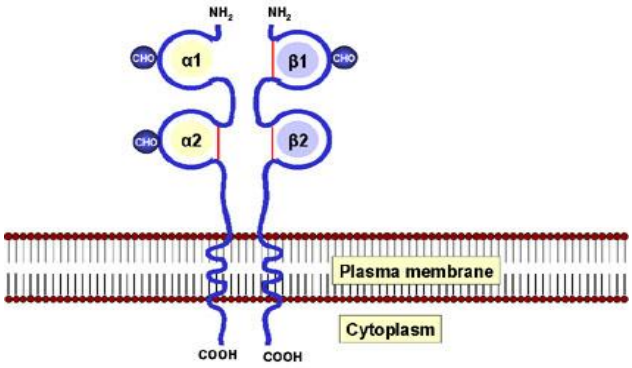


Class I MHC molecules are composed of two polypeptide chains, a long α chain and a short β chain called β2-microglobulin .The α chain has four regions.

- A cytoplasmic region, containing sites for phosphoylation and binding to cytoskeletal elements.
- A transmembrane region containing hydrophic amino acids by which the molecule is anchored in the cell membrane.
- A highly conserved α3 immunoglubilin-like domain to which CD8 binds.
- A highly polymorphic peptide binding region formed from the α1 and α2 domains. The β2- microglobulin associates with the chain and helps maintain the proper conformation of the molecule.

It is found on all nucleated cell types.These are usually endogenous antigens; intracellular antigens. Normal cells display samples of their intracellular proteins on their surface for surveillance by immune cells. When a cell changes (cancer) it will display different proteins on its surface to be hopefully recognized by immune cells. Viral antigens are a common source of foreign MHC classes I antigens. Cytotoxic T-cells can only recognize an antigen if it is physically bound to a MHC class I molecule. (Cytotoxic T-cells are class I restricted).

MHC Class II



Class II MHC molecules are composed of two polypeptide chains an α and a β chain of approximately equal length . Both chains have four regions:

- A cytoplasmic region containing sites for phosphoylation and binding to cytoskeletal elements
- A transmembrane region containing hydrophic amino acids by which the molecule is anchored in the cell membrane
- A highly conserved α2 domain and a highly conserved β2 domain to which CD4 binds
- A highly polymorphic peptide binding region formed from the α1 and β1 domains

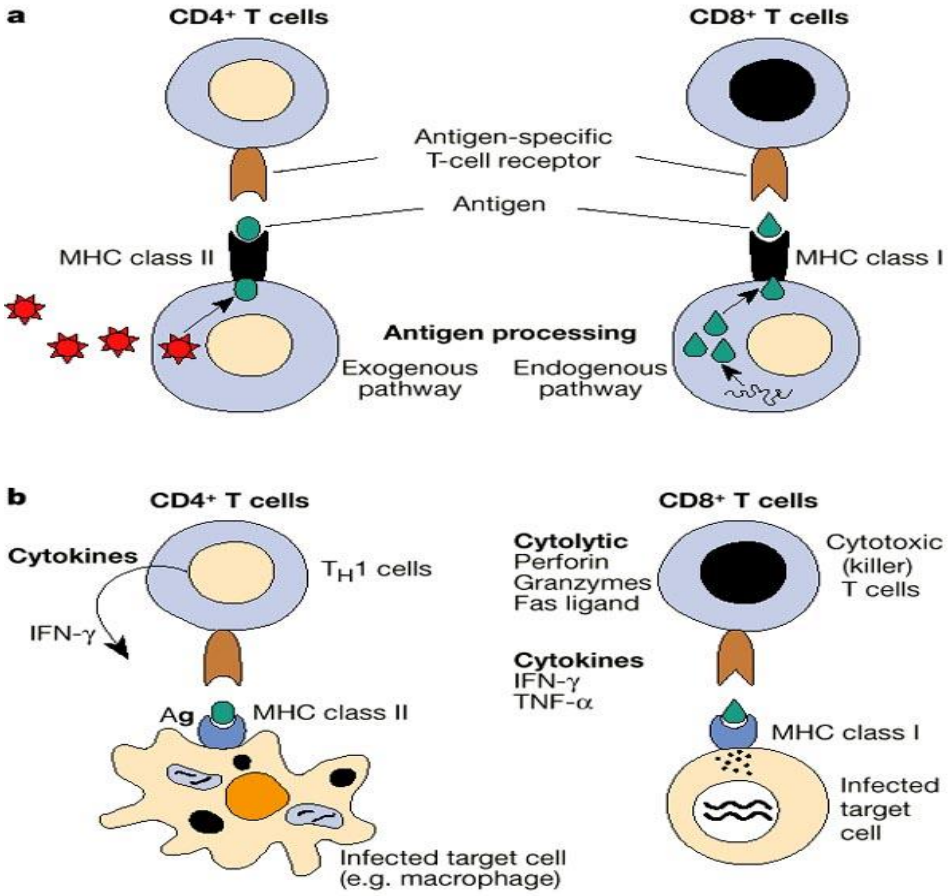
MHC class II is used to present antigens obtained from extracellular sources. These cells (class II MHC) have to have the ability to engulf the antigen. Therefore such cells would be macrophages, dendritic cells and B-cells. CD4 cells (helper cells) are class II restricted because they can only recognize antigens in the contents of class II molecules. Therefore the helper T-cell doesn't go around attacking your own cells.

Q 18. Describe Cell mediated immunity (CMI).

Ans. These develop gradually over a period of 24 to 48 hours after the second encounter with an antigen. T lymphocytes are primarily responsible for cellular immunity. Stem cells continuously migrate from the bone marrow to the thymus gland, where they develop into T cells.By spending time in the thymus, these cells are programmed to become T cells rather than antibody producing B lymphocytes. T cells attack foreign invaders directly.

Cellular reactions are initiated by the binding of an antigen with an antigen receptor . The T cells then carry the antigenic message, or blue print, to the lymph nodes, where the production of other T cells is stimulated. Some T cells remain in the lymph nodes and retain a memory for the antigen. Other T cells migrate from the lymph nodes into the general circulatory system and ultimately to the tissues.

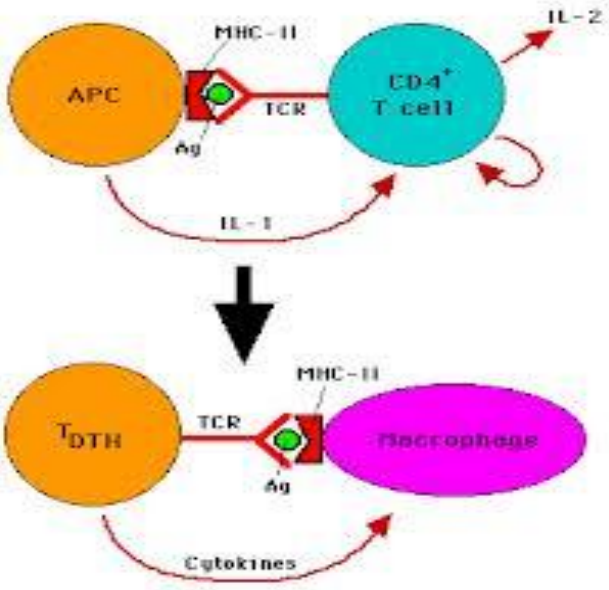
Types of T cell



When activated, **helper T** cells secrete cytokines that attract and activate B cells, cytotoxic T cells, natural killer cells, macrophages and other cells of the immune system. Helper T cells produce different types of cytokines and determine whether the immune response will be the production of antibodies or cell mediated immune response.

Cytotoxic T cells is a direct attack cell that is capable of killing micro-organisms and, at times, even some of the body's own cells.For this reason these are called killer cells.Cytotoxic attack the antigen directly by altering the cell membrane and causing cell lysis and releasing cytolytic enzymes and cytokines.

Supressor T cells are capable of suppressing the functions of both cytotoxic and helper T cells. It is believed that these suppressor functions serve the purpose of preventing the cytotoxic cells from causing excessive immune reactions that might be damaging to the body's own tissues.



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Multiple Choice questions (Immunology)

1. A child stung by a bee experiences respiratory distress within minutes and lapses into unconsciousness. This reaction is probably mediated by

A. IgE antibody.
B. IgG antibody.
C. sensitized T cells.
D. complement.
E. IgM antibody.

Ans: A

2. What is the role of class II MHC proteins on donor cells in graft rejection?

A. They are the receptors for interleukin-2, which is produced by macrophages when they attack the donor cells.
B. They are recognized by helper T cells, which then activate cytotoxic T cells to kill the donor cells.
C. They induce the production of blocking antibodies that protect the graft.
D. They induce IgE which mediates graft rejection.

Ans: B

3. AIDS is caused by a human retrovirus that kills

A. B lymphocytes.
B. lymphocyte stem cells.
C. CD-positive T lymphocytes.
D. CD8-positive T lymphocytes.
Ans: C

4. C is cleaved to form Ca and Cb by C convertase. Cb is involved in all of the following EXCEPT

A. altering vascular permeability.
B. promoting phagocytosis.
C. forming alternative-pathway C convertase.
D. forming C5 convertase.
Ans: A

5. After binding to its specific antigen, a B lymphocyte may switch its

A. immunoglobulin light-chain isotype.
B. immunoglobulin heavy-chain class.
C. variable region of the immunoglobulin heavy chain.
D. constant region of the immunoglobulin light chain.

Ans: B

6. Ca and C5a can cause

A. bacterial lysis.
B. vascular permeability.
C. phagocytosis of IgE-coated bacteria.
D. aggregation of C and C2.
Ans: B

7. Neutrophils are attracted to an infected area by

A. IgM.
B. vascular permeability.
C. phagocytosis of IgE-coated bacteria.
D. aggregation of C and C2.
Ans: C

8. Complement fixation refers to

A. the ingestion of Cb-coated bacteria by macrophages.
B. the destruction of complement in serum by heating at 56°C for 0 minutes.
C. the binding of complement components by antigen-antibody complexes.
D. the interaction of Cb with mast cells.
Ans: C

9. The classic complement pathway is initiated by interaction of C1 with
A. antigen.
B. factor B.
C. antigen-IgG complexes.
D. bacterial lipopolysaccharides.
Ans: C

10. Natural killer cells are
A. B cells that can kill without complement.
B. cytotoxic T cells.
C. increased by immunization.
D. able to kill virus-infected cells without prior sensitization.

Ans: D

11. A positive tuberculin skin test (a delayed hypersensitivity reaction) indicates that

A. a humoral immune response has occurred.
B. a cell-mediated immune response has occurred.
C. both the T and B cell systems are functional.
D. only the B cell system is functional.
Ans: B

12. Reaction to poison ivy or poison oak is
A. an IgG-mediated response.
B. an IgE-mediated response.
C. a cell-mediated response.
D. an Arthus reaction.
Ans: C

13. A primary immune response in an adult human requires approximately how much time to produce detectable antibody levels in the blood?

A. 12 hours
B. days
C. 1 week
D. weeks
Ans: C

14. The membrane IgM and IgD on the surface of an individual B cell

A. have identical heavy chains but different light chains
B. are identical except for their CH regions
C. are identical except for their VH regions
D. have different VH and VL regions
Ans: B

15. The BEST method to demonstrate IgG on the glomerular basement membrane in a kidney tissue section is the

A. precipitin test.
B. complement fixation test.
C. agglutination test.
D. indirect fluorescent-antibody test.
Ans: D

16. Which one of the following substances is NOT released by activated helper T cells?

A. interleukin-1
B. gamma interferon
C. interleukin-2
D. interleukin-
Ans: A

17. The minor histocompatibility antigens on cells

A. are detected by reaction with antibodies and complement.
B. are controlled by several genes in the major histocompatibility complex.
C. are unimportant in human transplantation.
D. induce reactions that can cumulatively lead to a strong rejection response.
Ans: D

18. Which one of the following is NOT true of class I MHC antigens?

A. They can be assayed by a cytotoxic test that uses antibody and complement.
B. They can usually be identified in the laboratory in a few hours.
C. They are controlled by at least three gene loci in the major histocompatibility complex.
D. They are found mainly on B cells, macrophages, and activated T cells.
Ans: D

19. An antigen found in relatively high concentration in the plasma of normal fetuses and a high proportion of patients with progressive carcinoma of the colon is
A. viral antigen.
B. carcinoembryonic antigen.
C. alpha-fetoprotein.
D. heterophil
Ans: . B

20. An antibody directed against the idiotypic determinants of a human IgG antibody would react with

A. the Fc part of the IgG.
B. an IgM antibody produced by the same plasma cell that produced the IgG.
C. all human kappa chains.
D. all human gamma chains.
Ans: . B

21. Which one of the following is NOT true of the gene segments that combine to make up a heavy-chain gene?

A. Many V region segments are available.
B. Several J segments and several D segments are available.
C. V, D, and J segments combine to encode the antigen-binding site.

D. A V segment and a J segment are preselected by an antigen to make up the variable-region portion of the gene.
Ans: D

22. When immune complexes from the serum are deposited on glomerular basement membrane, damage to the membrane is caused mainly by
A. gamma interferon.
B. phagocytosis.
C. cytotoxic T cells.
D. enzymes released by polymorphonuclear cells.
Ans: D

23. If an individual was genetically unable to make J chains, which immunoglobulin(s) would be affected?

A. IgG
B. IgM
C. IgA
D. IgG and IgM
E. IgM and IgA

Ans: E

24. The antibody-binding site is formed primarily by

A. the constant regions of H and L chains.
B. the hypervariable regions of H and L chains.
C. the hypervariable regions of H chains.
D. the variable regions of H chains.
E. the variable regions of L chains.
Ans: B

25. The class of immunoglobulin present in highest concentration in the blood of a human newborn is

A. IgG.
B. IgM.
C. IgA.
D. IgD.
E. IgE.
Ans: A

26. Individuals of blood group type AB

A. are Rh(d)-negative.
B. are "universal recipients" of transfusions.
C. have circulating anti-A and anti-B antibodies.
D. have the same haplotype.
Ans: B

27. Cytotoxic T cells induced by infection with virus A will kill target cells

A. from the same host infected with any virus.
B. infected by virus A and identical at class I MHC loci of the cytotoxic T cells.
C. infected by virus A and identical at class II MHC loci of the cytotoxic T cells.
D. infected with a different virus and identical at class I MHC loci of the cytotoxic cells.
E. infected with a different virus and identical at class II MHC loci of the cytotoxic cells.
Ans: B

28. Antigen-presenting cells that activate helper T cells must express which one of the following on their surfaces?

A. IgE
B. gamma interferon
C. class I MHC antigens
D. class II MHC antigens
Ans: D

29. Which one of the following does NOT contain Cb?

A. classic-pathway C5 convertase
B. alternative-pathway C5 convertase
C. classic-pathway C convertase
D. alternative-pathway C convertase

Ans: C

30. Complement lyses cells by
A. enzymatic digestion of the cell membrane.
B. activation of adenylate cyclase.
C. insertion of complement proteins into the cell membrane.
D. inhibition of elongation factor 2

Ans: C

31. Agglutination reaction is more sensitive than precipitation for the detection of
A. antigens
B. antibodies

C.complement
D.antigen-antibody complexes

Answer: A

32. Precipitation reaction is relatively less sensitive for the detection of
A.antigens
B.antigen-antibody complexes
C.antibodies
D.complement

Answer: C

33. In which of the following case a large lattice is formed?
A.Antibody is in excess
B.Antigens and antibodies are in optimal proportion
C.Antigen is in excess
D.None of these

Answer: B

Key Terms

Active immunity: usually long-lasting immunity that is acquired through the production of *antibodies* and memory T cells within the organism in response to the presence of *antigens*.

Adaptive immune system: also called the acquired immune system, this component of the immune system comprises *white blood cells*, particularly *lymphocytes.* When it is presented with a new microbe or vaccine, it may take days or weeks to respond or adapt, but the resultant improved immune readiness, or "memory," is sustained for long periods (years).

Allergy: a misguided reaction by the immune system to harmless foreign substances.

Antibody: a protein on the surface of *B cells* that is also secreted in large amounts into the blood or lymph in response to an *antigen,* a component within an invader such as a bacterium, virus, parasite, or transplanted organ. Antibodies neutralize the antigen, and thereby the invader, by binding to it, often directing it toward a macrophage for destruction. Also called an immunoglobulin.

Antigen: a foreign substance (usually a protein or carbohydrate) capable of triggering an immune response in an organism.

B cell: a type of *lymphocyte* that produces *antibodies*, which bind to free-floating microbes circulating in the blood so that they cannot infect other cells.

Biological barriers: the body's first layer of protection against harmful microbes; skin is a prime example.

CD4+ helper T cells:*T cells* with CD4 receptors that respond to *antigens* on the surface of specific molecules by secreting a certain type of cytokine that stimulates *B cells* and killer *T cells.* Helper T cells are infected and killed by *HIV;* people who develop *AIDS* have no more than one-fifth the normal number of helper T cells.

Cytokine: a class of substance secreted by cells of the immune system to regulate immune cells.

Dendritic cell: an *antigen*-presenting immune cell that initiates the immune response by activating *lymphocytes* and stimulating the secretion of *cytokines.* Dendritic cells also prevent autoimmune reactions by instructing the T lymphocytes to be silent or tolerant to the body itself.

DNA (deoxyribonucleic acid): nucleic acid that carries the cell's genetic information and is capable of self-*replication* and the synthesis of RNA.

E. coli: a bacterium that is used in public health as an indicator of fecal pollution (as of water or food) and in medicine and genetics as a research organism. *E. coli* occurs in various strains that may live as harmless inhabitants of the human lower intestine or may produce a toxin causing intestinal illness.

Enzymes: complex proteins produced by living cells and that catalyze specific reactions from *biochemicals.*

Epidemic: an outbreak of disease that simultaneously affects an atypically large number of individuals within a population, community, or region.

Genetic engineering: deliberate alteration of genetic material by intervention in genetic processes.

Highly active *antiretroviral therapy* (HAART): a treatment to combat AIDS using several antiretroviral drugs at the same time.

Histamine: a compound found in mammalian tissues that causes stretching of capillaries, contraction of smooth muscle, and stimulation of gastric acid secretions; released during allergic reactions.

Immune deficiency diseases (IDDs): diseases that result when one or more parts of the immune system are missing or defective.

Immunoglobulin E (IgE): a class of *antibodies* that function in allergic reactions.

Inactivated vaccines:*vaccines* made by growing and purifying large numbers of the target organism in the laboratory and then killing them with heat, radiation, or chemicals. The immune system reacts to the dead microorganisms, producing immunity.

Inflammation: a buildup of fluid and cells that occurs as the immune system fights a hostile invader.

Innate immune system: component of the immune system that consists of a set of genetically encoded responses to pathogens and does not change or adapt during the lifetime of the organism. Innate immunity involves quickly mobilized defenses triggered by receptors that recognize a broad spectrum of microbes; in contrast to adaptive immunity, it does not acquire memory for an improved response during a second exposure to infection.

Killer T cell: a type of *lymphocyte* that directly attacks and kills infected cells or other targets, including tumor cells and even one's own tissues. Killer cells are generated by the coordinated action of *dendritic cells* and *CD4+ helper T cells.*

Knockout: term used in genetic engineering when a specific gene is deliberately removed in order to create an organism unable to carry out the functions the gene codes for; knockouts are used by immunologists to determine the functions of specific genes that encode immune proteins.

Lymph nodes: small, rounded structures in the lymphatic system that contain disease-fighting *white blood cells*, especially *lymphocytes*, and filter out harmful microbes and toxins. Lymph nodes may become enlarged when they are actively fighting infection.

Lymphocyte: A type of white bloodcell, varieties of which include B cells and Tcells, or B lymphocytes and T lymphocytes.

Monocyte: A type of white blood cell that phagocytizes (engulfs and digests) foreign microorganisms.

Pathogen:A disease-carrying parasite, usually a microorganism.

Phagocyte:A cell that engulfs and digests another cell.

Penicillin: a mixture of nontoxic antibiotics produced by mold and used regularly to treat harmful bacteria.

Plasma cell: an *antibody*-producing l*ymphocyte* derived from a *B cell* upon reaction with a specific *antigen*.

Protease: an *enzyme* that catalyzes the splitting of proteins into smaller molecules. To treat AIDS, scientists have designed drugs that interfere with protease made by the HIV virus, which is essential to its replication.

Red blood cells: any of the hemoglobin-containing cells that carry oxygen to the tissues and are responsible for the red color of vertebrate blood..

Regulatory T cells (Treg cells): special *T cells* that regulate or suppress immune responses, preventing autoimmunity for example.

Replication: process by which an organism produces a copy of itself—for example, the way microbes reproduce.

Retrovirus: a type of *RNA* virus (such as HIV) that reproduces by transcribing itself into *DNA* (using *reverse transcriptase*). The resultant DNA inserts itself into a cell's DNA and is reproduced by the cell.

Reverse transcriptase (RT): an *enzyme* that catalyzes the formation of *DNA* using *RNA* as a template.

RNA (ribonucleic acid): a group of molecules similar in structure to a single strand of *DNA*. The function of RNA is to carry the information from the DNA in the cell's nucleus into the body of the cell to assemble proteins.

Rotavirus: a *retrovirus* with a double-layer protein shell and a wheel-like appearance. Rotaviruses cause diarrhea, especially in infants.

Stem cell transplants: a kind of *passive immune therapy* that transfers cells instead of antibodies. Stem cells have the capacity to give rise to all elements of the immune system, such as many types of *lymphocytes* and *phagocytes*.

Steroids: a large family of chemical substances, comprising many hormones, body constituents, and drugs; they are often *immunosuppressive*.

Subunit vaccines:*vaccines* that contain only a part of the target microorganism.

Synapses: specialized junctions at which cells of the nervous system signal to one another and to nonneuronal cells, such as those of muscles and glands.

T cell: A type of lymphocyte, also known as a T lymphocyte, that plays a key role in the immune response. T cells include cytotoxic T cells, which destroy virus-infected cells in the cell-mediated immune response; helper T cells, which are key participants in specific immune responses that bind to APCs, activating both the antibody and cell-mediated immune responses; and suppressor T cells, which deactivate T cells and B cells.

Vaccine:A preparation containing microorganisms, usually either weakened or dead, which are administered as a means of increasing immunity to the disease caused by those microorganisms.

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Microbiology

Q.1 Write contributions of the following scientists.

Ans.

(i) **Antony Van Lieu wenhoek**. (162-172)
He was the first person to observe and describe microorganisms accurately. He constructed simple microscopes that could magnify up to 50 to 00 times.

(ii) **Louis Pasteur** (1822-1895)
Resolved the controversy over the concept of the spontaneous generation of living organisms by 1861.

(iii) **John Tyndall** (1820-189)
Demonstrated that dust indeed carry germs and that if dust was absent broth remained sterile even if directly exposed to air.

Tyndall provided evidence for the existence of exceptionally heat-resistant forms of bacteria.

(iv) **Josef Lister** (1827-1912)
Developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds. That gave indirect

(v) **Robert Koch** (18-1910)

Gave direct demonstration of the role of bacteria in causing disease came from the study of anthrax. He gave some postulates known as Koch's postulates.

Q.2 Write down the Koch's postulates.

Ans. Koch's postulates are criteria for proving a microorganism and a specific disease.
These are –

(i) The microorganism must be present in every case of the disease but absent from healthy individuals.

(ii) The same disease must result when the isolated microorganism is inoculated in to a healthy host.

(iii) The same microorganism must be isolated again from the disease host.

Q. Who discovered agar?

Ans. A better alternation for gelatin 'Agar' was suggested by F.E. Hess, the wife of W. Hesse one of Koch's assistants.

Q. Who developed Petridish?

Ans. In 1887 Richard Petri developed the Petridish.

Q.5 Write differences between prokaryotic and eukaryotic plasma membranes.

Ans.

(i) Bacterial plasma membranes usually have a higher proportion of protein than do eukaryotic membranes.

(ii) Bacterial membrane lacks sterols. Although they contain pent acyclic sterol-like molecules called hope noids.

(iii) Many archeal membranes differ mention some functions of plasma from bacterial membranes in having a monolayer with lipid molecule spanning the whole membrane.

(iv) The cell wall of bacteria always have peptidogly can containing muramic acid. While arches & Eucarya have no muramic acid.

(v) Membrane lipids in bacteria have ester –linked, straight-chains fatty acids. While the arches have ether-linked, branched aliphatic chains.

Q.6 What are mesosomes?

Ans. Meso somes are invaginations of the plasma membrane in the shape of vesicles, tubules or lamellae.

Q.7 What are inclusion bodies in bacterial cells?

Ans. These are granules of organic and inorganic material that are often clearly visible in a light microscope.

These bodies are often used for storage and also reduce osmotic pressure by tying up molecules in particulate form.

These bodies may be enclosed by a monolayer or may not be enclosed.

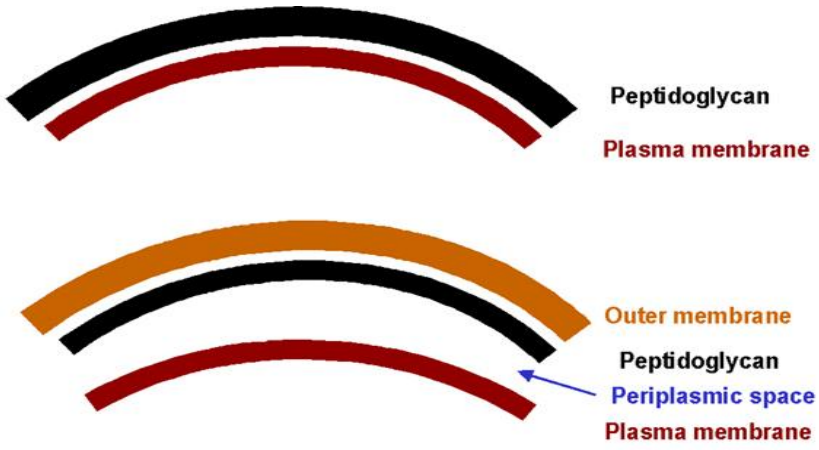
Free bodies are polyphosphate granules, cyanophycin granules and some glycogen granules.

Membrane enclosed bodies are –
Play – β-hydoxybutyrate granules
Sulfur granules

Carboxysomes
Gas vacuoles

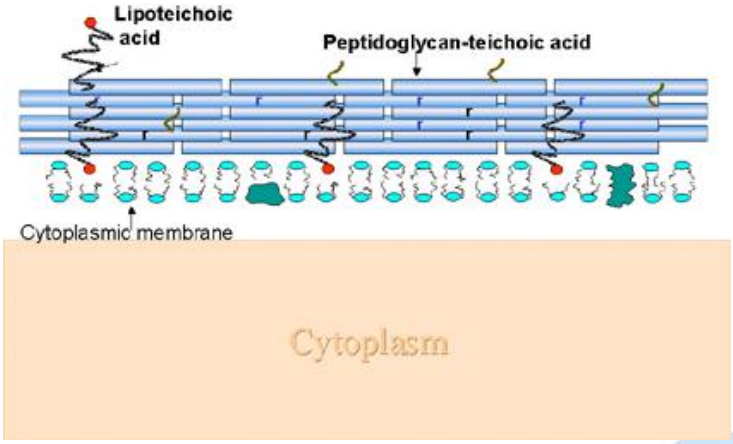
Q.8 What is cell envelope in a bacterial cell? Explain.

Ans. The cell envelope may be defined as the cell membrane and cell wall plus an outer membrane if one is present. The cell wall consists of the peptidoglycan layer and attached structures. Most bacterial cell envelopes fall into two major categories - Gram positive and Gram negative. This is based on Gram staining characteristics that reflect major structural differences between the two groups. Other types of cell wall are found in a few bacterial species (neither Gram positive nor Gram negative).



The Gram positive cell envelope

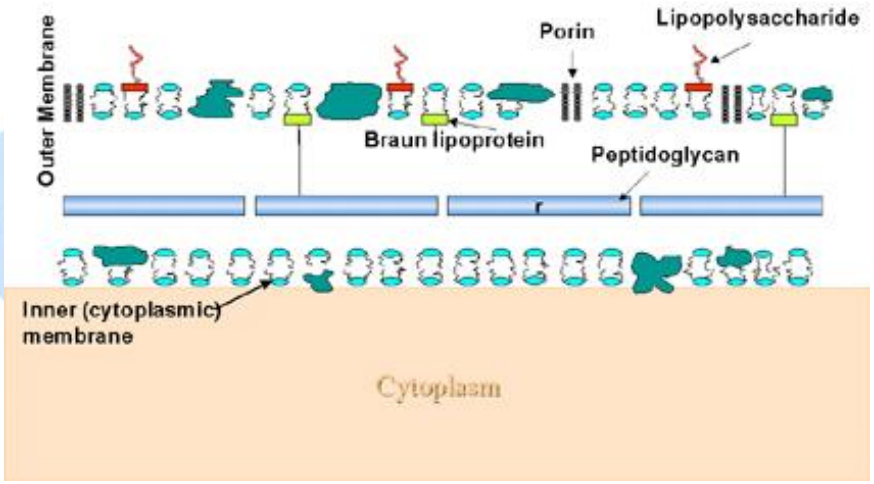
Gram positive bacteria have a outer thick layer of peptidoglycan having a very small periplasmic space. The peptidoglycan is a single bag-shaped, highly cross-linked macromolecule that surrounds the bacterial cell membrane and provides rigidity. It is huge (billions in molecular weight; compare proteins which are thousands in molecular weight). Peptidoglycan consists of a glycan (polysaccharide) backbone consisting of N-acetyl muramic acid and N-acetyl glucosamine with peptide side chains containing D- and L-amino acids and in some instances diaminopimelic acid. The side chains are cross-linked by peptide bridges. These peptide bridges vary in structure among bacterial species. Muramic acid, D-amino acids and diaminopimelic acid are not synthesized by mammals.



PG is found in all eubacteria except *Chlamydia* and*Mycoplasma*.

The Gram negative cell envelope

Covalently linked to the thin peptidoglycan is the Braun lipoprotein which binds the outer membrane to the cell wall. Like other membranes, the outer membrane contains proteins and phospholipids. Unlike other membranes, it contains additional molecules (lipopolysaccharide). The lipopolysaccharide is important to the bacterial cell since it helps to provide a permeability barrier to hydrophobic substances.



The lipopolysaccharide consists of three regions: an outer O antigen, a middle core and an inner lipid A region. The core contains several sugars (heptoses and ketodeoxyoctonic acid), not found elsewhere in nature, and lipid A contains β hydroxyfatty acids (uncommon in nature). The molecule displays endotoxin activity. Porins in the outer membrane help form channels to allow passage of small hydrophilic nutrients (such as sugars) through the outer membrane.

Acid fast and related bacteria (mycobacteria, nocardia and corynebacteria)

The cell envelopes of these organisms are considerably more complex than other bacteria. Mycolic acid (long, branch chained fatty acids) covalently bound via a polysaccharide to peptidoglycan. Other mycolic acid-containing compounds and other complex lipids form a thick waxy membranous layer outside the peptidoglycan layer.

Q.9 What bacterial slime layers? What is their significance?

Ans. A gelatinous polysaccharide or polypeptide outer covering of certain bacteria is called glycocalyx. These are the structures that surround the outside of the cell envelope. The glycocalyx is referred to as a capsule if it is firmly attached to the cell wall, or as a slime layer if loosely attached. The chemical nature of bacterial capsules is diverse but majority of them are polysaccharides. These polymers are composed of repeating oligosaccharide units. However, the capsule of *Bacillus anthracis* is composed of a polypeptide (polyglutamic acid). Yersinia pestis produces a capsule of mixed amino acids. Capsules may be weakly antigenic to strongly antigenic, depending on their chemical complexity. Capsules may be covalently linked to the underlying cell wall or just loosely bound to it. They have no net charge and will not bind charged dye particles, hence they can't be stained. Bacteria with capsules form smooth (S) colonies while those without capsules form rough (R) colonies. A given species may undergo a phenomenon called S-R variation whereby the cell loses the ability to form a capsule. Some capsules are very large and absorb water (e.g., *Klebsiella pneumoniae*) forming mucoid (M) colonies. Capsules are often lost during in vitro culture. They are not essential to cell viability and some strains within a species will produce a capsule, while others do not. Capsules are sometimes referred as K antigens (in Enterobacteriaceae) or as Vi antigen (in *Salmonella typhi*). Capsular antigens may either be specific to a species or may be shared by few different bacteria. For example, K2 antigen of *Klebsiella* cross-reacts with Pneumococcal type2 antigen.

Significance:

• Virulence factor. Capsules of pathogenic bacteria inhibit ingestion and killing by phagocytes. It can also prevent complement-mediated bacterial cell lysis. Capsules protect the cells from lysozyme. Mutant strains lacking capsule are avirulent.

• Permit bacteria to adhere to cell surfaces and structures such as medical implants and catheters. This is a first step in colonization and sometimes leads to disease.

• Capsules can be a source of nutrients and energy to microbes. Streptococcus mutans, which colonizes teeth, ferments the sugar in the capsule and acid byproducts contribute to tooth decay.

• Prevent cell from drying out (desiccation).

• Toxicity to the host cell; capsule of Bacteroides fragilis induces abscess formation.

• Capsules may protect cells from bacteriophages.

• Capsules play a role in antigenic mosaic.

• Capsules may trap ions.

Examples:

Streptococcus pneumoniae, Streptococcus mutans, Klebsiella pneumoniae, Bacillus anthracis, Neisseria meningitidi

Q.10 Describe the structure of cell wall of gram positive and gram negative bacteria? Write its significance.

Ans. The layers of cell envelope lying between the cytoplasmic membrane and the capsule are referred to collectively as cell wall. In gram positive bacteria, the cell wall mainly consists of peptidoglycan and teichoic acid while the cell wall in gram negative bacteria includes peptidoglycan, lipoprotein, outer membrane and lipopolysaccharide layers. Cell wall does not take up any stain and hence are not seen by light microscope. Most bacteria have a complex cell wall consisting of peptidoglycan (also called murein, mucopeptide). This complex polymer consists of three parts,

• A backbone consisting of alternating units of NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid).

• Tetrapeptide side chain attached to NAM

• Peptide cross-bridges, which are short chains of amino acids that crosslink the backbone.

Gram positive bacterial cell wall: In gram positive bacteria, there may be as many as 0 sheets of peptidoglycan, comprising up to 50% of cell wall material. Electron micrographs show the peptidoglycan of Gram positive cells to be 20-80 nm thick. Most gram positive cell walls contain additional substances such as teichoic acid and teichuronic acid. These are water soluble polymers of ribitol or glycerol. There are two types of teichoic acid, wall teichoic acid (linked to peptidoglycan) and lipoteichoic acid (linked to membrane). Some gram positive bacteria may lack wall teichoic acid but all contain lipoteichoic acid. The teichoic acid constitutes major antigens of cells that possess them. Teichoic acid binds to Magnesium ions and plays a role in supply of this ion to the cell. Teichuronic acids are produced in place of teichoic acid when phosphate is limiting. Teichoic acid in Streptococcus pneumoniae bears the Forssman antigen. Gram positive cells stain purple due to retention of the crystal violet dye during the Gram stain procedure. If peptidoglycan

is digested away from the cell, gram positive cells lose their cell walls and become protoplasts while the gram negative cells become spheroplasts.

Gram negative bacteria. The space between the inner and outer membranes is known as the periplasmic space, which contains digestive enzymes and other transport proteins. Gram negative cell walls contain three components that lie outside the peptidoglycan layer: lipoprotein, outer membrane and lipopolysaccharide. Lipoprotein stabilizes the outer membrane by anchoring it to peptidoglycan. Outer membrane is phospholipid bilayer in which the outer phospholipids are replaced by lipopolysaccharides. It is structurally similar to cytoplasmic membrane and serves to prevent leakage of periplasmic proteins and protects the cell from bile salts and protelolytic enzymes. The outer membranes contain several important porins, which specifically allow transport of solutes. Lipopolysaccharide consists of a polysaccharide core, a complex lipid called Lipid A and a terminal series of repeat units. The polysaccharide core is similar in all gram negative bacteria. Each species contains unique terminal repeat units. Lipopolysaccharide (LPS) is toxic in nature and is called endotoxin because it is firmly bound to the cell wall and released only when cell is lysed. If LPS is split into Lipid A and polysaccharide, all the toxicity is associated with Lipid A and polysaccharide represents the major surface antigen of bacterial cell. This antigen is designated as somatic "O" antigen and is used in serological typing of species. It was formerly known as Boivin antigen. Antigenic specificity is conferred by the terminal repeat units

Significance of cell wall:

• Maintains cell shape, any cell that loses its cell wall, loses its shape as well.

• Protects bacteria from osmotic lysis

• Acts as a barrier, protects cell contents from external environment

• Determines reactivity to Gram stain, cells become gram negative if they lose cell wall

• Attachment site for flagella

• Site of action of certain antimicrobial agents (e.g. Penicillins, Cephalosporins)

• Bacteria may attach to surface, produce slime, divide and produce microcolonies within the slime layer andconstruct a biofilm. E.g. formation of dental plaque mediated by the bacterium *Streptococcus mutans*.

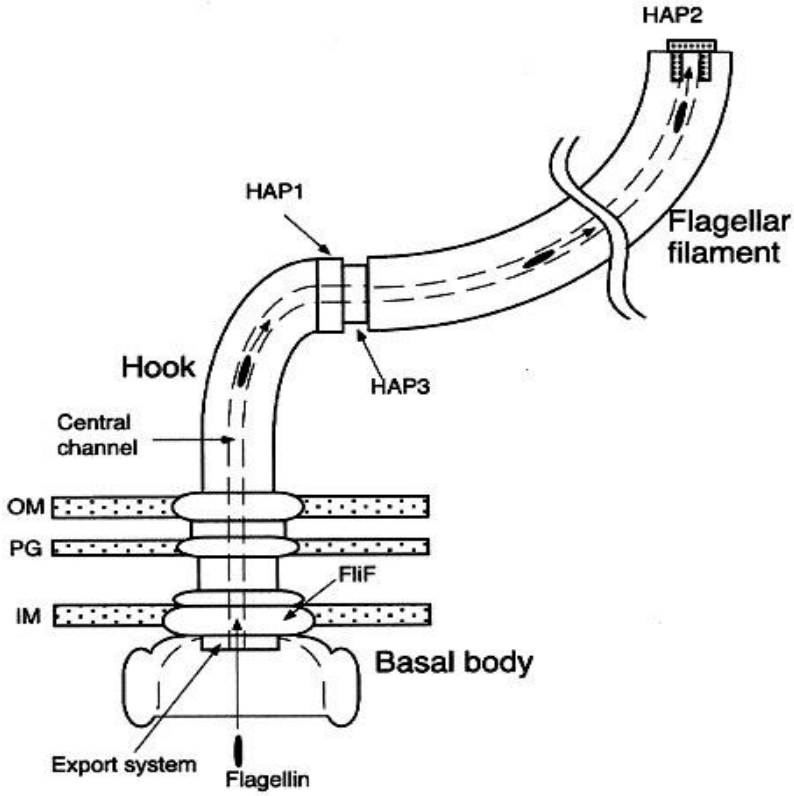
• Confer specific antigenicity to a strain/species that can be exploited to detect and identify an isolate.

Q.11 Write briefly about the structure and function of bacterial flagella and pilli.

Ans. Some bacteria are motile and some are not. Almost all motile bacteria possess flagella as the organ of locomotion. Such bacteria tend to move towards or away from the source of stimulus. These stimuli can be chemicals (chemotaxis), light (phototaxis), air (aerotaxis) or magnetism (magnetotaxis).

Structure: Procaryotic flagella are much thinner than eukaryotic flagella and they lack the typical 9 + 2 arrangement of microtubules. Over 0 genes are involved in its assembly and function. They are approximately -20µm long and end in a square tip. Since flagella are very thin (20-0 nm in diameter), they are below the resolution limits of a normal light microscope and cannot be seen. The bacterial flagellum is a non contractile, composed of single kind of protein subunit called flagellin. It is anchored to the bacterial cytoplasmic membrane and cell well by means of disklike structures. A flagellum comprises of three parts, filament, hook and basal body. The flagellum is attached to the cell body by hook and basal body. While the hook and basal body are embedded in the cell envelope, the filament is free. If a flagellum is cut off it will regenerate until reaches a maximum length. This is so because the growth is not from base, but from tip. The basal body bears a set of rings, one pair in gram positive bacteria and two pairs in gram negative bacteria. While the rings named S and M are common to both, the rings names P and L are found only in gram negative bacteria. Rings in the basal body rotate relative to each other causing the flagella to turn like a propeller. The energy to drive the basal body is obtained from the proton motive force. Bacteria move at average speed of 50µm/sec, the fastest being Vibrio cholerae that moves 200µm/sec.

The numbers of flagella, as well as their location on the cell surface are characteristic of a species.



Flagella arrangements are:

1. Monotrichous - a single flagellum at one pole (also called polar flagellum) E.g. Vibrio cholerae

2. Amphitrichous - single flagellum at both poles. Eg. Spirilla

. Lophotrichous - two or more flagella at one or both poles of the cell E.g. Spirillum undula

. Peritrichous - completely surrounded by flagella E.g. E.coli

Significance of flagella:

i. Primarily function is motility (chemotaxis, aerotaxis, phototaxis etc). Positive taxis is movement toward a favorable environment whereas negative taxis is movement away from a repellent.

ii. Flagella can help in identifying certain types of bacteria. For example, Proteus species show 'swarming' type of growth on solid media.

iii. Flagellar antigens are used to distinguish different species and strains of bacteria (serovars). Variations in the flagellar H antigen are used in serotyping.

Fimbriae and Pilli

Fimbriae are short, hair-like structures made up of protein pilin and are present in many gram negative bacteria. Even though pili arise from plasma membrane they are not considered part of the plasma membrane. They are anchored in the membrane and protrude through the cell wall to the outside of the cell. Fimbriae are shorter and straighter than flagella and are more numerous. They are 0.5µm long and 10 nm thick. Since they are made up of protein, they are antigenic. Bacteria from different genera may possess common fimbrial antigens. Fimbriae are usually seen in young cultures and lost on subcultures on solid media. While some authors use the two terms (fimbriae and pili) interchangeably, some restrict the term pili to denote sex pili. Sex pili acts to join bacterial cells for transfer of DNA from one cell to another by a process called conjugation.

Significance:

• They act as adhesins and allow bacteria to colonize cells. For example, Neisseria gonorrhoea uses its fimbriae to attach to the lining of the genital tract and initiate an infection.

• Fimbriae can also detect chemical signals and are important in bacterial cell communication and biofilm formation.

• Fimbriae also act as receptors for bacteriophages.

• Fimbriae of Streptococcus pyogenes are coated with M protein, which acts as an important virulence factor by adhering to host cells and resisting phagocytosis.

• Fimbriated bacteria form surface pellicle of liquid media.

• Some fimbriae can agglutinate RBC of guinea pigs, horses, pigs and fowls. This haemagglutination may or may not be inhibited by mannose.

Q.12 Mention the basic principles of bacterial DNA replication.

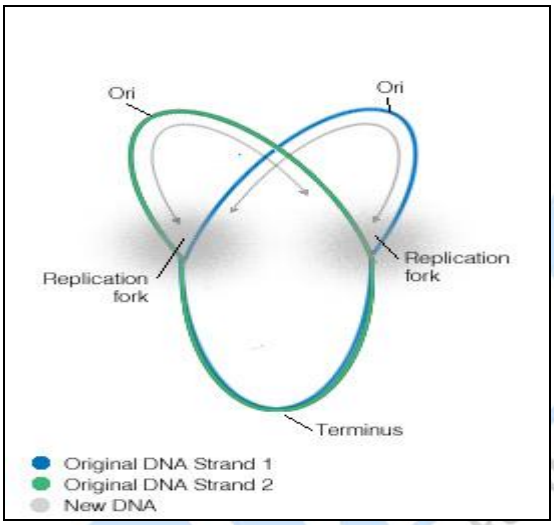
Ans. DNA replication is a fundamental process of all living cells. Same mechanisms are utilized from prokaryotes to higher eukaryotes though the proteins involved are different. Procaryotic DNA replication follows the following basic principles.

1. The replicon contains control elements needed for replication.
   a) The origin is a cis -acting site able to affect only the molecule of DNA on which it resides.

2. In prokaryotes the replicon contains both the origin of replication and the genes that code for proteins which regulate replication.
   a) The entire genome in bacteria is considered the replicon.
   b) Plasmids are considered different replicons.

. In eukaryotes the term replicon is only used to describe that unit of DNA that contains the origin of replication.

. The replication fork (or replication bubble) is the point at which replication is occurring (growing point). The replication fork moves sequentially along its DNA from its starting point at the origin.



5. The bacterial replicon support the following functions:
a) it initiates the replication cycle
b) it controls the frequency of initiation events
c) it segregates replicated chromosomes to daughter cells

6. The genome of bacteria only has a single origin of replication in the locus: OriC (25 bp).
Replication in bacteria starts at OriC and moves in both directions on the circular DNA molecule.

7. In bacteria both replication forks move around the DNA until they meet. At this point termination of replication occurs.

8. There are two termination regions ter D and ter C, B.
a) The ter sequences are short (~2 bp). The termination sequence function in only one orientation.

Q.1. Give a note on the proteins involved in procaryotic DNA replication.

Ans. Several enzymes are used in the process of bacterial DNA replication.

1. DNA gyrase (a type of DNA topoisomerase): used to relax supercoils.
2. A helicase is needed to separate the strands. It requires ATP.

a) A single stranded binding protein binds to single stranded DNA, preventing the re-formation of the double stranded molecule.

. Primase: specialized RNA polymerase used to synthesize 15-50 bp primers needed for DNA replication.

. DNA polymerase I: DNA repair and minor role in DNA replication.

5. DNA polymerase III: large protein complex enzymes used to synthesize complementary DNA strands. Each DNA polymerase can extend DNA chains by adding nucleotides one at a time to a free '-OH end. The base added depends on the template strand. Errors can occur every 100,000 bases/replication.

6. DNA polymerases II, IV, V: used for DNA repair.

7. DNA ligase: used to form covalent bonds between Okazaki fragments on lagging strand

Q.1 Write the steps involved in bacterial DNA replication?

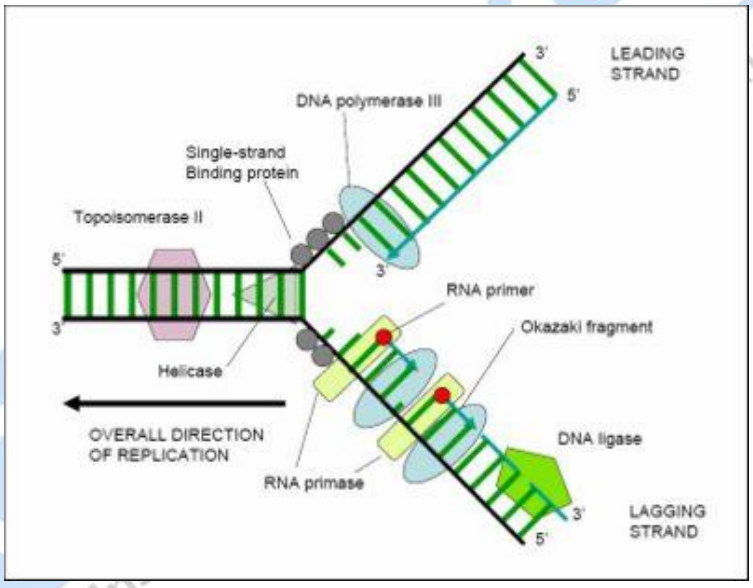
Ans. Steps in replication are:

1. DNA is nicked and supercoils are relaxed by DNA topoisomerase.

2. The DNA strands are separated by DNA helicase.

. Single stranded DNA binding proteins (SSB) prevent DNA from re-forming double stranded helix.

. A primer binds to the origin.



a) An RNA sequence is synthesized on the template so that the free '-OH can be extended by DNA pol.

b) An RNA base, pairs with the template allowing its '-OH end to be used to prime DNA synthesis.

c) A primer terminus is generated within duplex DNA by a nick.

d) A primer is first synthesized complementing the '--> 5' DNA template strand forming the leading strand. Then another primer is synthesized complementing the 5' --> ' DNA template strand forming the lagging strand.

e) There is only a single priming event in the leading strand but several in the lagging strand.

5. DNA polymerase III (in bacteria) or a (in eukaryotes) enters the replication complex (replisome)

6. A nucleotide enters the position at the end of the growing chain and a bond is formed.

7. The enzyme moves one pair forward ready for the next nucleotide. If a mistake is made then the enzyme moves backward removing the last base added and creating a site for replacement.

a) In some cases the synthesizing and proofreading functions are handled by the same polypeptide subunit, but in other polymerases they are contained in different subunits.

b) For example, DNA polymerase I can be cleaved into 2 fragments:

(A) Klenow: 68KDa, has both activities: polymerase (2/ carboxy end) and '--> 5' exonuclease (N-terminus). These active sites are 0 Amstrongs from each other.

(B) Small fragment : KDa, it has a 5'--> ' exonuclease activity which removes 10 bp at a time. This provides DNA polymerase I with the ability to start DNA synthesis from a nicked DNA molecule.

8. DNA synthesis is discontinuous on the lagging strand.

9. Basic problem: The replication fork moves from 5'--> ' in one strand and from '--> 5' on the other. The problem is that nucleotides are only added 5'--> '.

a) On the leading strand, DNA synthesis is continuous in the 5'--> ' direction.

b) On the lagging strand a stretch of single stranded parental DNA must be exposed and then a segment is synthesized in the reverse orientation (5'--> '). These segments are called Okazaki fragments

10. In bacteria DNA polymerase I (in eukaryotes DNA polymerase e) removes the primers, and fill in the gaps.

11. Then DNA ligase forms covalent bonds between lagging strand fragment (Okazaki) and filled-in spaces.

12. In bacteria, replication termination is signaled by termination sequences.

1. As soon as the new complementary strands are made, there will be two double stranded DNA molecules (from each old one), one strand corresponding to the old template strand and one strand corresponding to the newly synthesized one. This is called semi-conservative.

Q.15 What are the methods of sexual reproduction in bacteria? Describe 'Conjugation' in detail.

Ans. Sexual reproduction involves the combining genetic material between two individuals. In bacteria there three processes by which recombination may take place

i. Conjugation

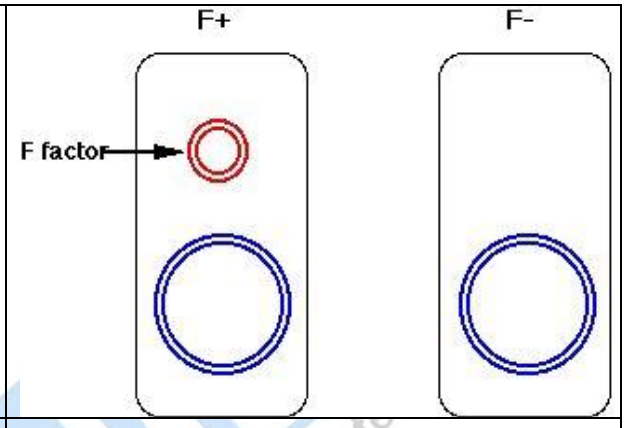
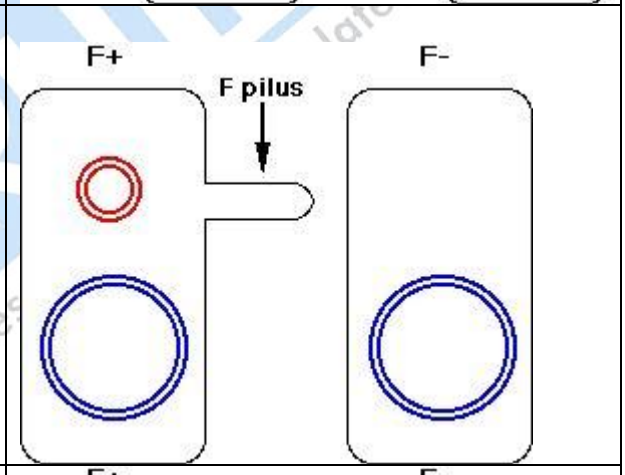
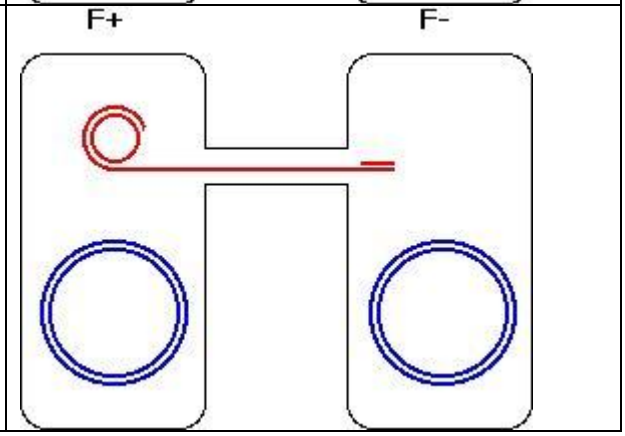
ii. Transformation

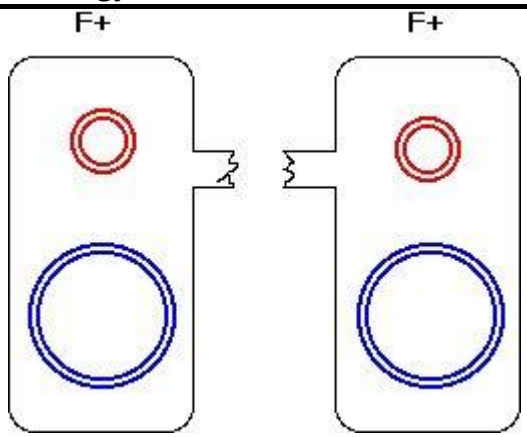
iii. Transduction

Conjugation - Conjugation is a mating process involving bacteria. It involves transfer of genetic information from one bacterial cell to another, and requires physical contact

between the two bacteria involved. The contact between the cells is via a protein tube called an **F** or **sex pilus**, which is also the conduit for the transfer of the genetic material.

Basic conjugation involves two strains of bacteria: **F+** and **F-**. The difference between these two strains is the presence of a **Fertility factor** (or F factor) in the F+ cells. The F factor is an episome that contains 19 genes and confers the ability to conjugate upon its host cell. Genetic transfer in conjugation is from an F+ cell to an F- cell, and the genetic material transferred is the F factor itself. Here is an overview of the process:

Basic conjugation occurs between an F+ cell and an F- cell. The difference between these two types of cells is the presence or absence of the F (fertility) factor, which is a circular DNA molecule independent of the bacterial chromosome (the larger circular molecule.	
The F+ cell initiates conjugation by extending an F pilus toward the F- cell. Among the genes present on the F factor are the genes encoding the proteins required for pulus construction.	
The F pilus, when finished, temporarily connects the two cells. On strand of the F factor is nicked, and begins unwinding from the other strand. The nicked strand begins to transfer through the F pilus to the F- cell. As it does so, this strand begins to be replicated, as does circular strand remaining behind in the F+ cell.	

Eventually, the nicked strand completely passes through to the recipient cell, and is completely replicated. This process produces a new F factor in the recipient cell. The pilus is broken, severing the connection between the two cells. Since both cells now contain an F factor, both cells are F+. The new F+ cell (which was the F- cell, can now initiate conjugation with another F- cell.	

Recombination rarely occurs with this kind of conjugation. This is because the F factor is not homologous to the DNA in the bacterial chromosome.

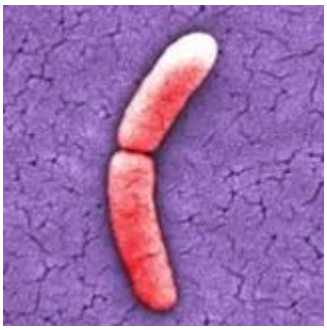
Q.16 What are different methods of asexual reproduction in bacteria? Describe the process of endospore formation and highlight its significance.

Ans. Asexual reproduction is very common in microorganisms. The following methods are used by bacteria to reproduce asexually

i. Binary Fission
ii. Budding
iii. Spore formation

Bacteria reproduce by binary fission. During binary fission, the cell divides into two daughter cells that are similar in size and shape.

Binary fission begins with the single DNA molecule replicating and both copies attaching to the cell membrane. Next, the cell membrane begins to grow between the two DNA molecules. Once the bacterium just about doubles its original size, the cell membrane begins to pinch inward. A cell wall then forms between the two DNA molecules dividing the original cell into two identical daughter cells.



Bacterial Reproduction: This salmonella bacterium is undergoing the process of binary fission. The cell divides resulting in the formation of two identical cells.

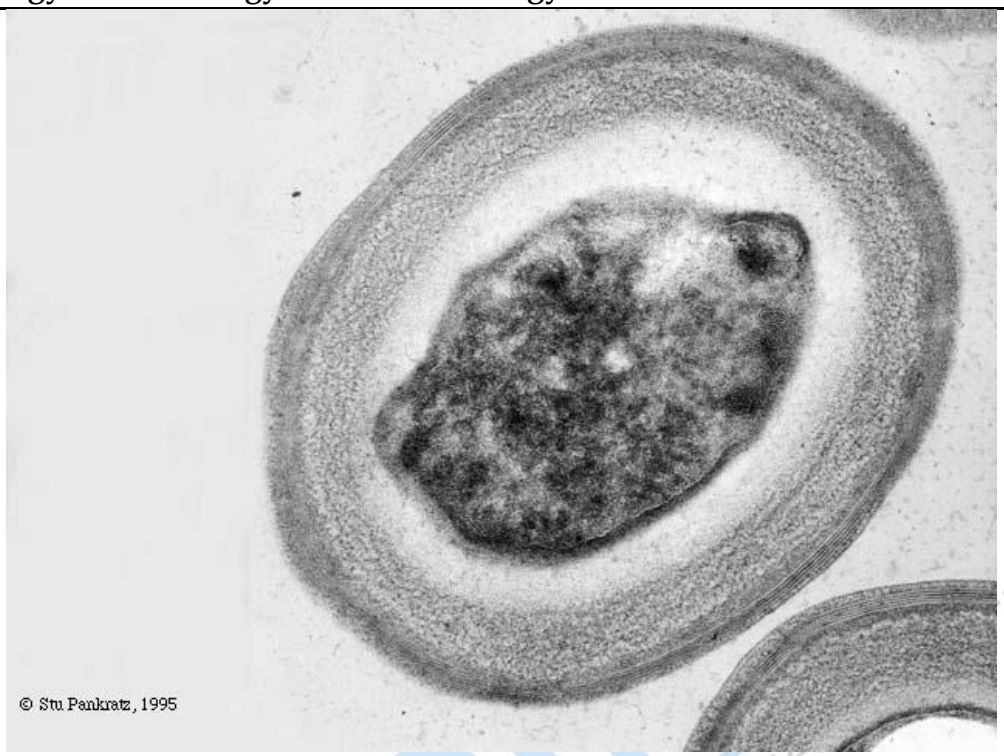
Spore Formation

Sporulation is the formation of nearly dormant forms of bacteria. In a limited number of bacteria, spores can preserve the genetic material of the bacteria when conditions are inhospitable and lethal for the normal (vegetative) form of the bacteria. The commitment of a bacterium to the sporulation process sets in motion a series of events that transform the cell.

Structure of Endospores

Bacteria produce a single endospore internally. The spore is sometimes surrounded by a thin covering known as the *exosporium*, which overlies the *spore coat*. The spore coat, which acts like a sieve that excludes large toxic molecules like lysozyme, is resistant to many toxic molecules and may also contain enzymes that are involved ingermination. The *cortex* lies beneath the spore coat and consists of peptidoglycan. The *core wall* lies beneath the cortex and surrounds the protoplast or *core* of the endospore. The core contains the spore chromosomal DNA is encased in chromatin-like proteins known as SASPs, that protect the spore DNA from UV radiation and heat. The core also contains normal cell structures, such as ribosomes and otherenzymes, but is not metabolically active.

Up to 20% of the dry weight of the endospore consists of calcium dipicolinate within the core, which is thought to stabilize the DNA.Dipicolinic acid could be responsible for the heat resistance of the spore, and calcium may aid in resistance to heat and oxidizing agents. However, mutants resistant to heat but lacking dipicolinic acid have been isolated, suggesting other mechanisms contributing to heat resistance are at work.



Formation of Endospore

Sporulation begins with the duplication of the bacterial genome. The second copy and some of the cytoplasm is then enveloped in an in-growth of the membrane that surrounds the bacterium. The result is essentially a little spherical cell inside the larger bacterium. The little cell is referred to as the "daughter cell" and the original bacterium is now called the "mother cell." Another membrane layer is laid down around the daughter cell. Between these two membranes lies a layer of peptidoglycan material, the same rigid material that forms the stress-bearing network in the bacterial cell wall. Finally, a coat of proteins is layered around the outside of the daughter cell. The result is a nearly impregnable sphere.

The above spore is technically termed an **endospore,** because the formation of the membrane-enclosed daughter cell occurs inside the mother cell.

In a so-called **exospore**, the duplicated DNA migrates next to a region on the inner surface of the cell membrane and then a bud forms. As the bud protrudes further outward, the DNA is drawn inside the bud.

Examples of endospore forming bacteria

These include those in the genera *Bacillus* and *Clostridium*. Endospore forming bacteria include *Methylosinus*, *Cyanobacteria*, and *Microsporidia*.

When still in the mother cell, the location of the spore (e.g., in the center, near one end or at one pole) is often a distinctive feature for a particular species of bacteria, and can be used as a feature to identify the bacteria.

Genetics of Sporulation

The genetic grounding for the commitment to form a spore is a protein called SpoA. This protein functions to promote the transcription of genes that are required for the conversion of the actively growing bacterium to a spore. The formation of an active SpoA protein is controlled by a series of reactions that are themselves responsive to the environmental conditions. Thus, the activation of SpoA comes only after a number of checkpoints have been passed. In this way a bacterium has a number of opportunities to opt out of the sporulation process. Once committed to sporulation, the process is irreversible.

Significance of Sporulation

Sporulation ultimately provides for a multilayered structure can be maintained for a very long time. Relative to the norm life span of the microorganism, spores are designed to protect a bacterium from heat, dryness, and excess radiation for a long time. Endospores of *Bacillus subtilis* have been recovered from objects that are thousands of years old. Furthermore, these are capable of resuscitation into an actively growing and dividing cell. Spores have been recovered from amber that is more than 250 million years old.The sporulation process has been well studied in *Bacillus subtilis*. The process is stimulated by starvation. Typically, sporulation is a "last resort," when other s fail (e.g., movement to seek new food, production of enzymes to degrade surrounding material, production of antimicrobial agents to wipe out other microbes competing for the food source, etc.).

Q.17 What are main components of bacterial nutrition? Explain the role of carbon source and growth factors.

Ans. Microorganisms are extraordinarily diverse in their requirements for growth. As you have learned in the lectures, microorganisms are greatly affected by environmental conditions and will grow in accordance to how these environmental niches support their individual needs. Factors that affect microbial growth include but are not limited to, **pH**, **osmolarity**, **water activity**, **temperature** and **oxygen levels**. There is a great deal of nutritional diversity among microorganisms; therefore, microbial growth is greatly affected by the nutrients that are available in their environment.

Nutritional Type	**Energy Source**	**Carbon Source**	**Examples**
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs or Lithotrophs	Inorganic compounds, e.g. H_2, NH, NO_2,	CO_2	A few Bacteria and many Archaea

(Lithoautotrophs)	H_2S		
Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Major nutritional types of procaryotes.

The specific nutritional requirements of heterotrophic bacteria can also be quite diverse. All microorganisms are made up of four biochemical molecules: proteins, lipids, carbohydrates and nucleic acids; however, these organisms may differ in their individual ability to synthesize these molecules.

The media that one uses in a laboratory setting may be classified as **defined** or **complex (undefined)**. Complex media are composed of extracts from plants, animals or yeast and therefore are rich in nutrients. Such media is complex because the precise individual components of these media are unknown; however, as these media contain a wide range of nutrients that are well above the minimal nutritional requirements of the organism being cultured, these media support the growth of a wide range of organisms. A defined media is a media in which all of the constituents and the amounts of these constituents are known. Defined media typically supports a narrower range of heterotrophic microorganisms. Defined media typically consist of salts and a carbon source in the form of glucose. Depending on the fastidiousness of an organism, these media can be supplemented with vitamins, nucleic acids, cofactors and amino acids.

Selective media are used to select for the growth of some bacteria while inhibiting the growth of others. Such media take advantage of the differences in the nutritional needs of individual bacteria or exploit the ability of some organisms to grow in the presence of a noxious compound. For example, *Pseudomonas aeruginosa* is able to grow in the presence of a variety of antibiotics (compounds that inhibit bacterial growth or kill bacteria) while organisms like *Staphylococcus aureus* or *Escherichia coli* are **sensitive** to these antibiotics.

Differential media allow a variety of organism to grow but contain substances that allow the student to distinguish between the different types of bacteria growing on the media.

Selective-Differential Media have characteristics of **both** selective media and differential media. These media only allow a subset of bacteria to grow and allow the student to distinguish between the different types of bacteria that are able to grow on these media. For example, one can distinguish between bacteria that ferment lactose and those that do not in MacConkey Agar by adding a carbon source (lactose) and a pH indicator (methyl red)—bacteria that ferment lactose produce acids that imparts a color change to the media surrounding the individual bacterial colony.

Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	1	NH, NO, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus		inorganic phosphates (PO)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO, H_2S, So, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Carbon and Energy Sources for Bacterial Growth

The carbon requirements of organisms must be met by organic carbon (a chemical compound with a carbon-hydrogen bond) or by CO_2. Organisms that use organic carbon are **heterotrophs** and organisms that use CO_2 as a sole source of carbon for growth are called **autotrophs**.

Growth factors

These are required in small amounts by cells because they fulfill specific roles in metabolism. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells. Growth factors are organized into three categories.

1. **purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)
2. **amino acids**: required for the synthesis of proteins
3. **vitamins**: needed as coenzymes and functional groups of certain enzymes

Some bacteria (e.g *E. coli*) do not require any growth factors: they can synthesize all essential purines, pyrimidines, amino acids and vitamins, starting with their carbon source, as part of their own intermediary metabolism. Certain other bacteria (e.g. *Lactobacillus*) require purines, pyrimidines, vitamins and several amino acids in order to grow. These compounds must be added in advance to culture media that are used to grow these bacteria. The growth factors are not metabolized directly as sources of carbon or energy, rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotrophs**.

Q.18 What are the environmental factors which affect the growth of bacteria?

Ans. The procaryotes exist in nature under an enormous range of physical conditions such as O_2 concentration, Hydrogen ion concentration (pH) and temperature. The exclusion limits of life on the planet, with regard to environmental parameters, are always set by some microorganism, most often a procaryote, and frequently an Archaeon.

The Effect of Oxygen

Oxygen is a universal component of cells and is always provided in large amounts by H_2O. However, procaryotes display a wide range of responses to molecular oxygen O_2 .

Obligate aerobes require O_2 for growth; they use O_2 as a final electron acceptor in aerobic respiration.

Obligate anaerobes (occasionally called **aerophobes**) do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance, which either kills or inhibits their growth. Obligate anaerobic procaryotes may live by fermentation, anaerobic respiration, bacterial photosynthesis, or the novel process of methanogenesis.

Facultative anaerobes (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism. Under anaerobic conditions (no O_2) they grow by fermentation or anaerobic respiration, but in the presence of O_2 they switch to aerobic respiration.

Aerotolerant anaerobes are bacteria with an exclusively anaerobic (fermentative) type of metabolism but they are insensitive to the presence of O_2. They live by fermentation alone whether or not O_2 is present in their environment.

Terms used to describe O_2 Relations of Microorganisms.

Environment			
Group	**Aerobic**	**Anaerobic**	**O_2 Effect**
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth Toxic	
Facultative Anaerobe (Facultative Aerobe)	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

The response of an organism to O_2 in its environment depends upon the occurrence and distribution of various enzymes which react with O_2 and various oxygen radicals that are invariably generated by cells in the presence of O_2. All cells contain enzymes capable of reacting with O_2. For example, oxidations of flavoproteins by O_2 invariably result in the formation of H_20_2 (peroxide) as one major product and small quantities of an even more toxic free radical, superoxide or O_2.-. Also, chlorophyll and other pigments in cells can react with O_2 in the presence of light and generate singlet oxygen, another radical form of oxygen which is a potent oxidizing agent in biological systems.

The Effect of pH on Growth

The pH, or hydrogen ion concentration, $[H^+]$, of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. Appreciating that pH is measured on a logarithmic scale, the $[H^+]$ of natural environments varies over a billion-fold and some microorganisms are living at the extremes, as well as every point between the extremes! Most free-living procaryotes can grow over a range of pH units, about a thousand fold change in $[H^+]$. The range of pH over which an organism grows is defined by **three cardinal points**: the **minimum pH**, below which the organism cannot grow, the **maximum pH**, above which the organism cannot grow, and the **optimum pH**, at which the organism grows best. For most bacteria there is an orderly increase in growth rate between the minimum and the optimum and a corresponding orderly decrease in growth rate between the optimum and the maximum pH, reflecting the general effect of changing $[H^+]$ on the rates of enzymatic reaction.

Microorganisms which grow at an optimum pH well below neutrality (7.0) are called **acidophiles**. Those which grow best at neutral pH are called **neutrophiles** and those that grow best under alkaline conditions are called **alkaliphiles**. Obligate acidophiles, such as some *Thiobacillus* species, actually require a low pH for growth since their membranes dissolve and the cells lyse at neutrality. Several genera of Archaea, including *Sulfolobus* and *Thermoplasma*, are obligate acidophiles. Among eukaryotes, many fungi are acidophiles, and the champion of growth at low pH is the eukaryotic alga *Cyanidium* which can grow at a pH of 0.

The Effect of Temperature on Growth

A particular microorganism will exhibit a range of temperature over which it can grow, defined by three cardinal points in the same manner as pH. Considering the total span of temperature where liquid water exists, the procaryotes may be subdivided into several subclasses on the basis of one or another of their cardinal points for growth. For example, organisms with an optimum temperature (T) near 7°C (the body temperature of warm-blooded animals) are called **mesophiles**. Organisms with an optimum T between about 5°C and 70°C are **thermophiles**. Some Archaea with an optimum T of 80°C or higher and a maximum T as high as 115°C, are now referred to as **extreme thermophiles** or **hyperthermophile**s. The cold-loving organisms are**psychrophiles** defined by their ability to grow at 0°C. A variant of a psychrophile (which usually has an optimum T of 10-15°C) is a**psychrotroph**, which grows at 0°C but displays an optimum T in the mesophile range, nearer room temperature. Psychrotrophs are the scourge of food storage in refrigerators since they are invariably brought in from their mesophilic habitats and continue to grow in the refrigerated environment where they spoil the food. Of course, they grow slower at 2°C than at 25°C.

Terms used to describe microorganisms in relation to temperature requirements for growth.

Temperature for growth (°C)

Group	Minimum	Optimum	Maximum	Comments
Psychrophile	Below 0	10-15	Below 20	Grow best at relatively low T
Psychrotroph	0	15-0	Above 25	Able to grow at low T but prefer moderate T
Mesophile	10-15	0-0	Below 5	Most bacteria esp. those living in association with warm-blooded animals
Thermophile	5	50-85	Above 100 (boiling)	Among all thermophiles is wide variation in optimum and maximum T

Water Availability

Water is the solvent in which the molecules of life are dissolved, and the availability of water is therefore a critical factor that affects the growth of all cells. The availability of water for a cell depends upon its presence in the atmosphere (relative humidity) or its presence in solution or a substance (**water activity**). The water activity (Aw) of pure H_2O

is 1.0 (100% water). Water activity is affected by the presence of solutes such as salts or sugars, that are dissolved in the water. The higher the solute concentration of a substance, the lower is the water activity and vice-versa. Microorganisms live over a range of Aw from 1.0 to 0.7. The Aw of human blood is 0.99; seawater = 0.98; maple syrup = 0.90; Great Salt Lake = 0.75. Water activities in agricultural soils range between 0.9 and 1.0.

Q.19 Write short note on the following-

- **Streptococci**
- **Tetanus**
- **Meningitis**
- **Diarrhea**

Ans.

Streptococci:-*Streptococcus* is a genus of spherical Gram-positive bacteria belonging to thephylum Firmicutes and the lactic acid bacteria group. Cellular division occurs along a single axis in these bacteria, and thus they grow in chains or pairs, hence the name — from Greek meaning easily bent or twisted, like a chain (twisted chain). In Contrast with this with staphylococci, which divide along multiple axes and generate grape-like clusters of cells. Most streptococci are oxidase- andcatalase-negative, and many are facultative anaerobes. In addition to streptococcal pharyngitis (strep throat), certain *Streptococcus* species are responsible for many cases of meningitis, bacterial pneumonia, endocarditis,erysipelas and necrotizing fasciitis (the 'flesh-eating' bacterial infections). However, many streptococcal species are nonpathogenic. Indeed, streptococci are a necessary ingredient in Emmentaler ("Swiss") cheese. Streptococci are also part of the normal commensal microbiome of the mouth, skin, intestine, and upper respiratory tract of humans.

As a rule, individual species of *Streptococcus* are classified based on their hemolyticproperties. Alpha hemolysis is caused by an oxidation of iron in hemoglobin, giving it a greenish color on blood agar. Beta hemolysis is complete rupture of red blood cells, giving distinct, wide, clear areas around bacterial colonies on blood agar. Other streptococci are labeled as gamma-hemolytic, actually a misnomer, as no hemolysis takes place.

In the medical setting, the most important groups are the alpha-hemolytic streptococci, *S. pneumoniae* and *Streptococcus* Viridans-group, and the beta-hemolytic streptococci of Lancefield groups A and B (also known as "Group A strep" and "Group B strep"). Beta-hemolytic streptococci are further characterised via the Lancefield serotyping – based on specific carbohydrates in the bacterial cell wall. These are named Lancefield groups A to V (except I and J).

ii. Tetanus :- Tetanus is infection of the nervous system with the potentially deadly bacteria *Clostridium tetani (C. tetani).*

Causes, incidence, and risk factors

Spores of the bacteria *C. tetani* live in the soil and are found around the world. In the spore form, *C. tetani*may remain inactive in the soil, but it can remain infectious for more than 0 years.

Infection begins when the spores enter the body through an injury or wound. The spores release bacteria that spread and make a poison called tetanospasmin. This poison blocks nerve signals from the spinal cord to the muscles, causing severe muscle spasms. The spasms can be so powerful that they tear the muscles or cause fractures of the spine.

The time between infection and the first sign of symptoms is typically 7 to 21 days. Most cases of tetanus in the United States occur in those who have not been properly vaccinated against the disease.

Symptoms

Tetanus often begins with mild spasms in the jaw muscles (lockjaw). The spasms can also affect the chest, neck, back, and abdominal muscles. Back muscle spasms often cause arching, called opisthotonos.

Sometimes the spasms affect muscles that help with breathing, which can lead to breathing problems.

Prolonged muscular action causes sudden, powerful, and painful contractions of muscle groups. This is called tetany. These episodes can cause fractures and muscle tears.

Other symptoms include:

- Drooling
- Excessive sweating
- Fever
- Hand or foot spasms
- Irritability
- Swallowing difficulty
- Uncontrolled urination or defecation

Treatment

Treatment may include:

- Antibiotics, including penicillin, clindamycin, erythromycin, or metronidazole (metronidazole has been most successful)
- Bedrest with a nonstimulating environment (dim light, reduced noise, and stable temperature)
- Medicine to reverse the poison (tetanus immune globulin)
- Muscle relaxers such as diazepam

- Sedatives
- Surgery to clean the wound and remove the source of the poison (debridement)

Breathing support with oxygen, a breathing tube, and a breathing machine may be necessary.

iii. Meningitis :- Meningitis is a bacterial infection of the membranes covering the brain and spinal cord (meninges).

Causes, incidence, and risk factors

The most common causes of meningitis are viral infections that usually get better without treatment. However, bacterial meningitis infections are extremely serious, and may result in death or brain damage, even if treated.

Meningitis may also be caused by:

- Chemical irritation
- Drug allergies
- Fungi
- Tumors

Acute bacterial meningitis is a medical emergency, and requires immediate treatment in a hospital.

Viral meningitis is milder and occurs more often than bacterial meningitis. It usually develops in the late summer and early fall, and often affects children and adults under age 0. Most infections occur in children under age 5. Most viral meningitis is due to enteroviruses, which are viruses that also can cause intestinal illness.

Many other types of viruses can cause meningitis. For example, viral meningitis can be caused by herpes viruses, the same virus that can cause cold sores and genital herpes (although people with cold sores or genital herpes are not at a greater risk of developing herpes meningitis).
Recently, West Nile virus, spread by mosquito bites, has become a cause of viral meningitis in most of the United States.
Symptoms

Symptoms usually come on quickly, and may include:

- Fever and chills
- Mental status changes
- Nausea and vomiting

- Sensitivity to light (photophobia)
- Severe headache
- Stiff neck (meningismus)

Other symptoms that can occur with this disease:

- Agitation
- Bulging fontanelles
- Decreased consciousness
- Poor feeding or irritability in children

Meningitis is an important cause of fever in children and newborns.

People cannot tell if they have bacterial or viral meningitis by how they feel, so they should seek prompt medical attention.

Treatment

Doctors prescribe antibiotics for bacterial meningitis. The type will vary depending on the bacteria causing the infection. Antibiotics are not effective in viral meningitis.

Other medications and intravenous fluids will be used to treat symptoms such as brain swelling, shock, andseizures. Some people may need to stay in the hospital, depending on the severity of the illness and the treatment needed.

iv. Diarrhea :-

Diarrhea is the frequent passage of loose, watery, soft stools with or without abdominal bloating, pressure, and cramps commonly referred to as gas. Diarrhea can come on suddenly, run its course, and be helped with home care to prevent complications such as dehydration.

Diarrhea is one of the most common illnesses in all age groups and is second only to the common cold as a cause of lost days of work or school.

In the United States, each child will have experienced seven to 15 episodes of diarrhea by age 5.

People of all ages can suffer from diarrhea, and the average adult has approximately four episodes of acute diarrhea per year.

Diarrhea and related complications can cause severe illness. The most significant cause of severe illness is loss of water and electrolytes. In diarrhea, fluid passes out of the body before it can be absorbed by the intestines. When the ability to drink fluids fast enough to

compensate for the water loss because of diarrhea is impaired, dehydration can result. Most deaths from diarrhea occur in the very young and the elderly whose health may be put at risk from a moderate amount of dehydration.

Diarrhea Causes

Viral infections cause most cases of diarrhea and are typically associated with mild-to-moderate symptoms with frequent, watery bowel movements, abdominal cramps, and a low-grade fever. Diarrhea generally lasts approximately to 7 days.

The following are the common causes of diarrhea caused by viral infections:

- rotavirus is a common cause of diarrhea in infants;
- norovirus (for example, Norwalk virus, caliciviruses) is the most common cause of epidemics of diarrhea among adults and schoolage children (for example, cruise ship infection, schools, nursing homes, day care facilities, and restaurants); and
- adenovirus infections are common in all age groups.

Bacterial infections cause the more serious cases of diarrhea. Typically, infection with bacteria occurs from contaminated food or drinks (food poisoning). Bacterial infections also cause severe symptoms, often with vomiting, fever, and severe abdominal cramps or abdominal pain. Bowel movements occur frequently and may be watery.

The following are examples of causes of diarrhea caused by bacterial infections:

- *Campylobacter*, *salmonellae*, and *shigella* organisms are the most common causes of bacterial diarrhea.
- Less common causes are *Escherichia coli* (commonly called *E coli*) *Yersinia*, and *listeria*.
- Use of antibiotics can lead to an overgrowth of *Clostridium difficile* (*C diff*) bacteria in the intestines.

Diarrhea Symptoms

- Watery, liquid stools
- Abdominal cramps
- Fever: A high fever is not common. If present, the affected person may have a more severe illness than acute diarrhea.

- Dehydration: If diarrhea leads to dehydration, it is a sign of potentially serious disease.

Q.22. Describe the causes, transmission and treatment of Tuberculosis.

Ans. Pulmonary tuberculosis (TB) is a contagious bacterial infection that involves the lungs, but may spread to other organs.

Causes, incidence, and risk factors

Pulmonary tuberculosis (TB) is caused by the bacteria *Mycobacterium tuberculosis (M. tuberculosis)*. You can get TB by breathing in air droplets from a cough or sneeze of an infected person. This is called primary TB.

In the United States, most people will recover from primary TB infection without further evidence of the disease. The infection may stay asleep or inactive (dormant) for years. However, in some people it can reactivate.

Most people who develop symptoms of a TB infection first became infected in the past. However, in some cases, the disease may become active within weeks after the primary infection.

The following people are at higher risk for active TB:

- Elderly
- Infants
- People with weakened immune systems, for example due to AIDS, chemotherapy, diabetes, or certain medications

The following factors may increase the rate of TB infection in a population:

- Increase in HIV infections
- Increase in number of homeless people (poor environment and nutrition)
- The appearance of drug-resistant strains of TB

In the United States, there are approximately 10 cases of TB per 100,000 people. However, rates vary dramatically by area of residence and socioeconomic status.

Symptoms

The primary stage of TB usually doesn't cause symptoms. When symptoms of pulmonary TB occur, they may include:

- Cough (usually cough up mucus)
- Coughing up blood

- Excessive sweating, especially at night
- Fatigue
- Fever
- Unintentional weight loss

Other symptoms that may occur with this disease:

- Breathing difficulty
- Chest pain
- Wheezing

Signs

Examination may show:

- Clubbing of the fingers or toes (in people with advanced disease)
- Enlarged or tender lymph nodes in the neck or other areas
- Fluid around a lung (pleural effusion)
- Unusual breath sounds (crackles)

Treatment

The goal of treatment is to cure the infection with drugs that fight the TB bacteria. Treatment of active pulmonary TB will always involve a combination of many drugs (usually four drugs). All of the drugs are continued until lab tests show which medicines work best.

The most commonly used drugs include:

- Isoniazid
- Rifampin
- Pyrazinamide
- Ethambutol

Other drugs that may be used to treat TB include:

- Amikacin
- Ethionamide
- Moxifloxacin
- Para-aminosalicylic acid
- Streptomycin

Q.2. Write notes on the following

i. AIDS

ii. Hepatitis-B

Ans.

i. **AIDS**: - AIDS (acquired immune deficiency syndrome) is the final stage of HIV disease, which causes severe damage to the immune system.

Causes, incidence, and risk factors

Important facts about the spread of AIDS include:

- AIDS is the sixth leading cause of death among people ages 25 - in the United States, down from number one in 1995.
- The World Health Organization estimates that more than 25 million people worldwide have died from this infection since the start of the epidemic.
- In 2008, there were approximately . million people around the world living with HIV/AIDS, including 2.1 million children under age 15.

Human immunodeficiency virus (HIV) causes AIDS. The virus attacks the immune system and leaves the body vulnerable to a variety of life-threatening infections and cancers.

Common bacteria, yeast, parasites, and viruses that usually do not cause serious disease in people with healthy immune systems can cause fatal illnesses in people with AIDS.

HIV has been found in saliva, tears, nervous system tissue and spinal fluid, blood, semen (including pre-seminal fluid, which is the liquid that comes out before ejaculation), vaginal fluid, and breast milk. However, only blood, semen, vaginal secretions, and breast milk have been shown to transmit infection to others.

The virus can be spread (transmitted):

- Through sexual contact -- including oral, vaginal, and anal sex
- Through blood -- via blood transfusions (now extremely rare in the U.S.) or needle sharing
- From mother to child -- a pregnant woman can transmit the virus to her fetus through their shared blood circulation, or a nursing mother can transmit it to her baby in her breast milk

Other methods of spreading the virus are rare and include accidental needle injury, artificial insemination with infected donated semen, and organ transplantation with infected organs.

AIDS and blood or organ donation:

- AIDS is NOT transmitted to a person who DONATES blood or organs. People who donate organs are never in direct contact with people who receive them. Likewise, a person who donates blood is never in contact with the person receiving it. In all these procedures, sterile needles and instruments are used.
- However, HIV can be transmitted to a person RECEIVING blood or organs from an infected donor. To reduce this risk, blood banks and organ donor programs screen donors, blood, and tissues thoroughly.

People at highest risk for getting HIV include:

- Injection drug users who share needles
- Infants born to mothers with HIV who didn't receive HIV therapy during pregnancy
- People engaging in unprotected sex, especially with people who have other high-risk behaviors, are HIV-positive, or have AIDS
- People who received blood transfusions or clotting products between 1977 and 1985 (before screening for the virus became standard practice)
- Sexual partners of those who participate in high-risk activities (such as injection drug use or anal sex)

Symptoms

AIDS begins with HIV infection. People who are infected with HIV may have no symptoms for 10 years or longer, but they can still transmit the infection to others during this symptom-free period. If the infection is not detected and treated, the immune system gradually weakens and AIDS develops.

Acute HIV infection progresses over time (usually a few weeks to months) to asymptomatic HIV infection (no symptoms) and then to early symptomatic HIV infection. Later, it progresses to AIDS (advanced HIV infection with CD T-cell count below 200 cells/mm).

Almost all people infected with HIV, if they are not treated, will develop AIDS. There is a small group of patients who develop AIDS very slowly, or never at all. These patients are called nonprogressors, and many seem to have a genetic difference that prevents the virus from significantly damaging their immune system.

The symptoms of AIDS are mainly the result of infections that do not normally develop in people with a healthy immune system. These are called opportunistic infections.

People with AIDS have had their immune system damaged by HIV and are very susceptible to these opportunistic infections. Common symptoms are:

- Chills

- Fever
- Sweats (particularly at night)
- Swollen lymph glands
- Weakness
- Weight loss

Note: At first, infection with HIV may produce no symptoms. Some people, however, do experience flu-like symptoms with fever, rash, sore throat, and swollen lymph nodes, usually 2 - weeks after contracting the virus. Some people with HIV infection stay symptom-free for years between the time when they are exposed to the virus and when they develop AIDS.

Treatment

There is no cure for AIDS at this time. However, a variety of treatments are available that can help keep symptoms at bay and improve the quality of life for those who have already developed symptoms.

Antiretroviral therapy suppresses the replication of the HIV virus in the body. A combination of several antiretroviral drugs, called highly active antiretroviral therapy (HAART), has been very effective in reducing the number of HIV particles in the bloodstream. This is measured by the viral load (how much free virus is found in the blood). Preventing the virus from replicating can improve T-cell counts and help the immune system recover from the HIV infection.

HAART is not a cure for HIV, but it has been very effective for the past 12 years. People on HAART with suppressed levels of HIV can still transmit the virus to others through sex or by sharing needles. There is good evidence that if the levels of HIV remain suppressed and the CD count remains high (above 200 cells/mm), life can be significantly prolonged and improved.

However, HIV may become resistant to one combination of HAART, especially in patients who do not take their medications on schedule every day. Genetic tests are now available to determine whether an HIV strain is resistant to a particular drug. This information may be useful in determining the best drug combination for each person, and adjusting the drug regimen if it starts to fail. These tests should be performed any time a treatment strategy begins to fail, and before starting therapy.

When HIV becomes resistant to HAART, other drug combinations must be used to try to suppress the resistant strain of HIV. There are a variety of new drugs on the market for treating drug-resistant HIV.

Treatment with HAART has complications. HAART is a collection of different medications, each with its own side effects. Some common side effects are:

- Collection of fat on the back ("buffalo hump") and abdomen

- General sick feeling (malaise)
- Headache
- Nausea
- Weakness

When used for a long time, these medications increase the risk of heart attack, perhaps by increasing the levels of cholesterol and glucose (sugar) in the blood.

Any doctor prescribing HAART should carefully watch the patient for possible side effects. In addition, blood tests measuring CD counts and HIV viral load should be taken every months. The goal is to get the CD count as close to normal as possible, and to suppress the amount of HIV virus in the blood to a level where it cannot be detected.

Other antiviral medications are being investigated. In addition, growth factors that stimulate cell growth, such as erthythropoetin (Epogen, Procrit, and Recomon) and filgrastim (G-CSF or Neupogen) are sometimes used to treat AIDS-associated anemia and low white blood cell counts.
Medications are also used to prevent opportunistic infections (such as *Pneumocystis jiroveci* pneumonia) if the CD count is low enough. This keeps AIDS patients healthier for longer periods of time. Opportunistic infections are treated when they happen.

ii. **Hepatitis-B**:- Hepatitis B is irritation and swelling (inflammation) of the liver due to infection with the hepatitis B virus (HBV).

Other types of viral hepatitis include:

- Hepatitis A
- Hepatitis C
- Hepatitis D

Causes, incidence, and risk factors

Hepatitis B infection can be spread through having contact with the blood, semen, vaginal fluids, and other body fluids of someone who already has a hepatitis B infection.

Infection can be spread through:

- Blood transfusions (not common in the United States)
- Direct contact with blood in health care settings
- Sexual contact with an infected person
- Tattoo or acupuncture with unclean needles or instruments
- Shared needles during drug use

- Shared personal items (such as toothbrushes, razors, and nail clippers) with an infected person

The hepatitis B virus can be passed to an infant during childbirth if the mother is infected.

Risk factors for hepatitis B infection include:

- Being born, or having parents who were born in regions with high infection rates (including Asia, Africa, and the Caribbean)
- Being infected with HIV
- Being on hemodialysis
- Having multiple sex partners
- Men having sex with men

Most of the damage from the hepatitis B virus occurs because of the way the body responds to the infection. When the body's immune system detects the infection, it sends out special cells to fight it off. However, these disease-fighting cells can lead to liver inflammation.

Symptoms

Symptoms may not appear for up to 6 months after the time of infection. Early symptoms may include:

- Appetite loss
- Fatigue
- Fever, low-grade
- Muscle and joint aches
- Nausea and vomiting
- Yellow skin and dark urine due to jaundice

People with chronic hepatitis may have no symptoms, even though gradual liver damage may be occurring. Over time, some people may develop symptoms of chronic liver damage and cirrhosis of the liver.

Signs and tests

The following tests are done to identify and monitor liver damage from hepatitis B:

- Albumin level
- Liver function tests
- Prothrombin time

The following tests are done to help diagnose and monitor people with hepatitis B:

- Antibody to HBsAg (Anti-HBs) -- a positive result means you have either had hepatitis B in the past, or have received a hepatitis B vaccine
- Antibody to hepatitis B core antigen (Anti-HBc) -- a positive result means you had a recent infection or an infection in the past
- Hepatitis B surface antigen (HBsAg) -- a positive result means you have an active infection
- Hepatitis E surface antigen (HBeAg) -- a positive result means you have a hepatitis B infection and are more likely to spread the infection to others through sexual contact or sharing needles

Treatment

Acute hepatitis needs no treatment other than careful monitoring of liver and other body functions with blood tests. You should get plenty of bed rest, drink plenty of fluids, and eat healthy foods.

In the rare case that you develop liver failure, you may need a liver transplant. A liver transplant is the only cure in some cases of liver failure.

Some patients with chronic hepatitis may be treated with antiviral medications or a medication called peginterferon. These medications can decrease or remove hepatitis B from the blood and reduce the risk of cirrhosis and liver cancer.

Liver transplantation is used to treat severe, chronic hepatitis B liver disease.

Patients with chronic hepatitis should avoid alcohol and should always check with their doctor or nurse before taking any over-the-counter medications or herbal supplements. This even includes medications such asacetaminophen, aspirin, or ibuprofen.

Multiple choice questions

Structural Organisation

1. Which of the following is/are not a gram-positive bacterium?

A.Streptococci
B.*Pseudomonas*
C.Mycobacteria
D.None of these

Answer: B

2. Gram-positive bacteria, responsible for food poisoning, is/are
   A.*Mycoplasmas*
   B.*Pseudomonas*
   C.*Clostridia*
   D.all of these

   Answer: C

4. Which of the following gram-positive bacteria causes pharyngitis (sore throat)?
   A.*Neisseria* B.*Streptococcus*
   C.*Staphylococcus* D.*Mycobacterium*

   Answer: B

5. Which of the following gram-negative bacteria is/are not aerobic?
   A.*Pseudomonas*
   B.*Neisseria*
   C.*Escherichia*
   D.None of these

   Answer: C

6. The enzyme, which hydrolyses the murein is
   A.perxoidase
   B.tannase
   C.lysozyme
   D.none of these

   Answer: C

7. Bacterial spoilage is identified by which of the following morphological characteristics?
   A.Encapsulation
   B.Endospores
   C.Cell aggregation
   D.All of these

   Answer: D

9. Gram-negative bacteria, responsible for food poisoning, is/are
   A.*Salmonella*
   B.*Pseudomonas*
   C.*Clostridia*
   D.None of these

   Answer: A

10. Which of the following is a gram-positive bacteria?
   A.*Lactobacillus*
   B.*Staphylococci*
   C.*Streptococci*
   D.All of these

   Answer: D

11. Which of the following bacteria lack a cell wall and are therefore resistant to penicillin?
   A.Cyanobacteria B.Mycoplasmas
   C.Bdellovibrios D.Spirochetes
   Answer: B

12. A cluster of polar flagella is called
   A.lophotrichous B.amphitrichous
   C.monotrichous D.petritrichous
   Answer: A

13. The protein from which hook and filaments of flagella are composed of, is
   A.keratin B.flagellin
   C.gelatin D.casein
   Answer: B

14. The cooci which mostly occur in single or pairs are
   A.Streptococci
   B.Diplococci
   C.Tetracocci
   D.None of these
   Answer: B

20. Which of the following may contain fimbriae?
    A.Gram-positive bacteria
    B.Gram-negative bacteria
    C.Both (a) and (b)
    D.None of these
    Answer: B

21. Peptidoglycan accounts for _____ of the dry weight of cell wall in many gram positive bacteria
    A.50% or more
    B.About 10%
    C.11%+ 0.22%
    D.About 20%
    Answer: A

22. Bacteria having no flagella are unable to
    A.move
    B.reproduce
    C.stick to tissue surfaces
    D.grow in nutrient agar
    Answer: A

23. Which of the following is true about cell wall of gram-positive bacteria?
    A.It consists of multiple layers
    B.It is thicker than that associated with gram-negative bacteria
    C.It contains teichoic acids
    D.All of these
    Answer: D

24. The cell walls of many gram positive bacteria can be easily destroyed by the enzyme known as
    A.lipase B.lysozyme
    C.pectinase D.peroxidase
    Answer: B

25. The cell wall of
    A.gram-positive bacteria are thicker than gram-negative bacteria
    B.gram-negative bacteria are thicker than gram-positive bacteria
    C.both have same thickness but composition is different

D.none of these
Answer: A

26. Peptidoglycan is also known as
A.N-acetyl muramic acid
B.murein mucopeptide
C.N acetylglucosamine
D.mesodiaminopimetic acid
Answer: B

27. Genetic system is located in the prokaryotes in
A.nucleoid
B.chromatin
C.nuclear material
D.all of these

Answer: D

28. Which is most likely to be exposed on the surface of a gram-negative bacterium?
A.Pore protein (porin)
B.Protein involved in energy generation
C.Lipoteichoic acid
D.Phospholipids

Answer: A

Microbiology: Growth and Nutrition of Bacteria

1. Which of the following is not the characteristic of a growth curve?
A. Shows development of microbial population under relatively stable environmental conditions
B.Plotted with logarithmic numbers
C.Graphs numbers of microbes versus time
D.Each growth curve consists of four distinct phases
Answer: D

2. Generation time of *Escherichia coli* is
   A.20 minutes
   B.20 hours
   C.20 days
   D.200 hours
   Answer: A

3. The organism which obtain their energy from chemicals are designated as
   A.prototrophs B.chemotrophs
   C.organotrophs D.autotrophs
   Answer: B

4. Nutrient content and biological structures are considered as
   A.implicit factor for microbial growth
   B.intrinsic factor for microbial growth
   C.processing factor
   D.none of the above
   Answer: B

5. The organism which grows best above 45°C are called
   A.psychrophilic
   B.mesosphilic
   C.thermophilic
   D.any of these
   Answer: C

6. The straightforward method of binary fission explains how bacteria
   A.grow in nutrient agar
   B.evolve
   C.move
   D.reproduce
   Answer: D

7. Bacteria of genus *Nitrosomonas* use _____ as their electron source.
   A.ammonia B.H_2S
   C.succinate D.light
   Answer: A

8. All organisms require at least small amounts of carbondioxide, However, some can use CO_2 as

their sole source of carbon. Such organisms are termed as

A.autotrophs B.phototrophs
C.chemotrophs D.photo-organotrophs
Answer: A.

9. The generation time of a culture that produces two generations per hour is
A.greater than that produces three generations per hour
B.lesser than that produces three generations per hour
C.equal to that produces three generations per hour
D.none of the above
Answer: A

10. Which of the following organisms typically get their carbon for biosynthesis from organic compounds?
A.Aerobic, glucose-respiring bacteria (aerobic respiration)
B.Ammonia-oxidizing bacteria (chemolithotrophic bacteria)
C.Photosynthetic cyanobacteria (phototrophic metabolism)
D.None of the above
Answer: A

Hepatitis virus

11. The viruses, which is/are transmitted by parenteral and sexual routes is/are
A.Hepatitis B virus
B.Hepatitis C virus
C.Hepatitis G virus
D.All of these
Answer: D

12. Which of the following antigenic types of hepatitis B virus is present in the envelope?
A.HbsAg B.HBcAg
C.HBeAg D.HBxAg
Answer: A

13. Which of the following antigenic types of hepatitis B virus is prevalent in India?

A.Adw
B.Adr
C.Ayw
D.Ayr
Answer: C

Replication

14. Both strands of DNA serve as templates concurrently in
A.replication
B.excision repair
C.mismatch repair
D.none of these
Answer: A

15. Which of the following repairs nicked DNA by forming a phosphodiester bond between adjacent nucleotides?
A.Helicase
B.DNA gyrase
C.Topoisomerases
D.DNA ligase
Answer: D

16. The enzyme that catalyzes the synthesis of DNA is called
A.DNA polymerase
B.DNA gyrase
C.DNA ligase
D.helicase
Answer: A

17. Which of the following possesses both 5'-3' and 3'-5' exonuclease activity?
A.Kornberg enzyme
B.DNA polymerase III
C.Taq DNA polymerase
D.None of these
Answer: A

Key terms of Microbiology

Acidophile A microorganism that has its growth optimum between about pH 0 and 5.5.

Anthrax	An infectious disease of animals caused by ingesting Bacillus anthracis spores. Can also occur in humans and is sometimes called woolsorter's disease.
Antibiotic	A microbial product or its derivative that kills susceptible microorganisms or inhibits their growth.
Antitoxin	An antibody to a microbial toxin, usually a bacterial exotoxin, that combines specifically with the toxin, in vivo and in vitro, neutralizing the toxin.
Autotroph	An organism that uses CO2 as its sole or principal source of carbon.
Auxotroph	A mutated prototroph that lacks the ability to synthesize an essential nutrient and therefore must obtain it or a precursor from its surroundings.
Axial filament	The organ of motility in spirochetes. It is made of axial fibrils or periplasmic flagella that extend from each end of the protoplasmic cylinder and overlap in the middle of the cell. The outer sheath lies outside the axial filament.
Bacillus	A rod-shaped bacterium.
Bactericide	An agent that kills bacteria.
Bacteriocin	A protein produced by a bacterial strain that kills other closely related strains.
Bacteriophage	A virus that uses bacteria as its host; often called a phage.
Balanced growth	Microbial growth in which all cellular constituents are synthesized at constant rates relative to each other.
Binary fission	Asexual reproduction in which a cell or an organism separates into two cells.
Biochemical oxygen demand (BOD)	The amount of oxygen used by organisms in water under certain standard conditions; it provides an index of the amount of microbially oxidizable organic matter present.
Biodegradation	The breakdown of a complex chemical through biological processes that can result in minor loss of functional groups, fragmentation into larger constitutents, or complete breakdown to carbon dioxide and minerals. Often the term refers to the undesired microbial-mediated destruction of materials such as paper, paint, and textiles.
Bioinsecticide	A pathogen that is used to kill or disable unwanted insect pests. Bacteria, fungi, or viruses are used, either directly or after manipulation, to control insect populations.

Bioluminescence	The production of light by living cells, often through the oxidation of molecules by the enzyme luciferase.
Biomagnification	The increase in concentration of a substance in higher-level consumer organisms.
Biopesticide	The use of a microorganism or another biological agent to control a specific pest.
Bioremediation	The use of biologically mediated processes to remove or degrade pollutants from specific environments. Bioremediation can be carried out by modification of the environment to accelerate biological processes, either with or without the addition of specific microorganisms.
Biosensor	The coupling of a biological process with production of an electrical signal or light to detect the presence of particular substances.
Bioterrorism	The intentional or threatened use of viruses, bacteria, fungi, or toxins from living organisms to produce death or disease in humans, animals, and plants.
Biotransformation or microbial transformation	The use of living organisms to modify substances that are not normally used for growth.
Botulism	A form of food poisoning caused by a neurotoxin (botulin) produced by Clostridium botulinum serotypes A-G; sometimes found in improperly canned or preserved food.
Broad-spectrum drugs	Chemotherapeutic agents that are effective against many different kinds of pathogens.
Budding	A vegetative outgrowth of yeast and some bacteria as a means of asexual reproduction; the daughter cell is smaller than the parent.
Bulking sludge	Sludges produced in sewage treatment that do not settle properly, usually due to the development of filamentous microorganisms.

Bursa of Fabricius	Found in birds; the blind saclike structure located on the posterior wall of the cloaca; it performs a thymus like function. A primary lymphoid organ where B-cell maturation occurs. Bone marrow is the equivalent in mammals.
Burst size	The number of phages released by a host cell during the lytic life cycle.
Cancer	A malignant tumor that expands locally by invasion of surrounding tissues, and systemically by metastasis.
Capsid	The protein coat or shell that surrounds a virion's nucleic acid.
Capsule	A layer of well-organized material, not easily washed off, lying outside the bacterial cell wall.
Carrier	An infected individual who is a potential source of infection for others and plays an important role in the epidemiology of a disease.
Chemolithotrophic autotrophs	Microorganisms that oxidize reduced inorganic compounds to derive both energy and electrons; CO2 is their carbon source. Also called chemolithoautotrophs.
Chemoorganotrophic heterotrophs	Organisms that use organic compounds as sources of energy, hydrogen, electrons, and carbon for biosynthesis.
	Organisms that obtain energy from the oxidation of chemical compounds.
Concatemer	A long DNA molecule consisting of several genomes linked together in a row.
	Complementary mating types that participate in a form of protozoan sexual reproduction called conjugation.
Conjugation	The form of gene transfer and recombination in bacteria that requires direct cell-to-cell contact. 2. A complex form of sexual reproduction commonly employed by protozoa.
Conjugative plasmid	A plasmid that carries the genes for sex pili and can transfer copies of itself to other bacteria during conjugation.
Cosmid	A plasmid vector with lambda phage cos sites that can be packaged in a phage capsid; it is useful for cloning large DNA fragments.

Death phase	The decrease in viable microorganisms that occurs after the completion of growth in a batch culture.
Disease	A deviation or interruption of the normal structure or function of any part of the body that is manifested by a characteristic set of symptoms and signs.
Disinfectant	An agent, usually chemical, that disinfects; normally, it is employed only with inanimate objects.
Disinfection	The killing, inhibition, or removal of microorganisms that may cause disease. It usually refers to the treatment of inanimate objects with chemicals.
Endospore	An extremely heat- and chemical-resistant, dormant, thick-walled spore that develops within bacteria.
Enzyme-linked immunosorbent assay (ELISA)	A technique used for detecting and quantifying specific antibodies and antigens.
Episome	A plasmid that can exist either independently of the host cell's chromosome or be integrated into it.
Epitope	An area of the antigen molecule that stimulates the production of, and combines with, specific antibodies; also known as the antigenic determinant site.
F_ plasmid	An F plasmid that carries some bacterial genes and transmits them to recipient cells when the F_ cell carries out conjugation; the transfer of bacterial genes in this way is often called sexduction.
Sulfur bacteria	A diverse group of nonphotosynthetic proteobacteria that can oxidize reduced sulfur compounds such as hydrogen sulfide. Many are lithotrophs and derive energy from sulfur oxidation. Some are unicellular, whereas others are filamentous gliding bacteria.

Biotechnology

Q.1. Enlist major landmarks in the development of biotechnology.

Ans. The major landmarks can be conveniently summarised in a tabulated form as follows-

186:	Louis Pasteur	Proved existence of microorganisms Showed that all living things are produced by other living things
1869:	Johann Meischer	Isolated DNA from the nuclei of white blood cells
189:	Koch, Pasteur	Fermentation process patented
	Lister	Institutes Diphtheria antitoxin isolated
1902:	Walter Sutton	Coined the term "gene" Proposed that chromosomes carry genes (factors which Mendel said that could be passed from generation to generation)
1910:	Thomas H. Morgan	Proved that genes are carried on chromosomes "Biotechnology" term coined
1918:		Germans Use acetone produced by plants to make bombs
		Yeast grown in large quantities for animal and glycerol
		Made activated sludge for sewage treatment process
1927:	Herman Mueller	Increased mutation rate in fruit flies by exposing them to x-rays
1928:	Alexander Fleming	Discovered antibiotic properties of certain molds
198:		Proteins and DNA studied by x-ray crystallography
		Term 'molecular biology" coined
191:	George Beadle	Proposed "one gene, one enzyme" hypothesis
	Edward Tatum	
Mid-190's:		Penicillin produced
195:	James Watson	Determined the double helix structure of DNA
	Francis Crick	
1956:	Dangr	Sequenced insulin (protein) from pork

1958:	Coenberg	Discovered DNA polymerase
1966:	Marshall Nirenberg	Determined that a sequence of three nucleotide
1970:		Isolation of reverse transcriptase
1971:		Discovery of restriction enzymes
1972:	Paul Berg	Cut sections of viral DNA and bacterial DNA with same restriction enzyme
197:	Stanley Cohen	Produced first recombinant DNA organism
	Herbert Boyer	Beginning of genetic engineering
1975:		Moratorium on recombinant DNA techniques
1976:		National Institute of Health guidelines developed for study of recombinant DNA
1977:		First practical application of genetic engineering
		human growth hormone produced by bacterial cells
1978:	Genentech, Inc.	Genetic engineering techniques used to produce human insulin in *E. coli*
		First biotech company on NY stock exchange
	Stanford University	First successful transplantation of mammalian gene
		Discoverers of restriction enzymes receive Nobel Prize in medicine
1979:	Genentech, Inc.	Produce human growth hormone and two kinds of interferon DNA from malignant cells transformed a strain of cultured mouse cells - new tool for analyzing cancer genes
1980:		US. Supreme Court decided that manmade microbes could be patented
198:	Genetech, Inc.	Licensed Eli Lily to make insulin
		First transfer of foreign gene in plants
1985:		Plants can be patented
1986:		First field trials of DNA recombinant plants resistant to insects, viruses, bacteria
1988:		First living mammal was patented
199:		Flavr savr tomatoes sold to public

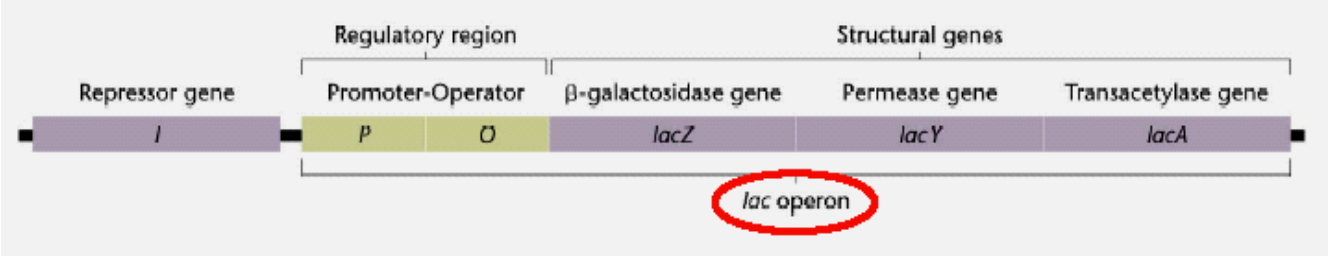
Q.2. Define Gene.

Ans. A locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions, and or other functional sequence regions.

Q.. What is 'lac operon'? Explain.

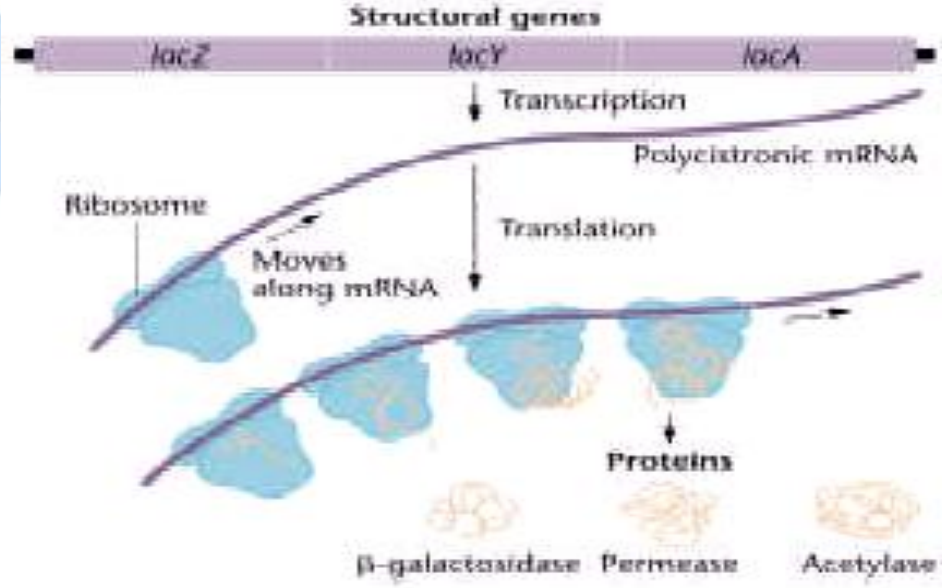
Ans. The Operon Model

An operon is a coordinately regulated unit of transcription in bacteria. The operon model was proposed by Jacob, Monod, and Wollman based on their genetic and biochemical studies on lactose-requiring mutations of E. coli. Some genes are always"on" i.e. transcribed and translated are termed as constitutive genes.Some genes are "off" but can be turned on are referred as inducible genes. They are in positive control. Some genes are usually "on" but can be turned off. They are referred as repressible genes. They are in negative control.An operon is a unit of the bacterial chromosome consisting of the following components:



Operon generally are of two types namely anabolic operon and catabolic operon. Those operon directing the synthesis of enzymes responsible for catabolic reactions then they are known as catabolic operon where as operon coding enzymes for synthetic reactions are known as anabolic operons. Lac operon is an fine example for catabolic operon because it codes for the enzymes which are responsible for degradation of lactose.

Lac operon consists of lacZ,lacY,lacA genes as structural genes and operator,promoter and suppressor genes as regulatory genes.



1. A regulatory gene

The regulatory gene codes for a regulatory protein. The lac repressor, encoded by the lacI gene, is the regulatory protein of the lac operon.

2. An operator

The operator is the region of DNA of the operon that is the binding site for the regulatory protein.

. A promoter

The promoter is the DNA sequence of the operon recognized by DNA-dependent RNA polymerase. The initiation site for RNA synthesis is immediately downstream of the promoter. **The gene for DNA-dependent RNA polymerase is not part of the operon**, since the RNA polymerase enzyme transcribes all bacterial operons.

. Structural Genes

The operon encodes one or more genes for inducible enzymes. The lac operon encodes enzymes necessary for lactose metabolism, including ß-galactosidase, ß-galactoside permease, and ß-galactoside transacetylase.

REGULATION:

Lactose as an energy source

E. coli prefers to use glucose as an energy source when both glucose and lactose are available. Lactose is an alternative energy source that can be used if glucose is absent. The overall rate of messenger RNA synthesis from the lac operon, and from other operons for alternate catabolic energy sources, is indirectly regulated by the concentration of glucose in the cell.The following table is a summary of the concepts on the regulation of lac mRNA synthesis by intracellular levels of lactose and glucose. The relative concentration of cAMP and the relative levels of lac mRNA synthesis in the presence or absence of glucose and lactose are shown.

Glucose	cAMP	Lactose	lac mRNA
present	low concentration	absent	no mRNA
present	low concentration	present	low level of mRNA
absent	high concentration	absent	no mRNA
absent	high concentration	present	high level of mRNA

Cyclic-AMP as regulator and catabolite repression

cAMP is the second messenger used directly in the regulation of lac operon expression in response to changing levels of glucose. Control of the rate of transcription of an operons for catabolic enzymes such as the lac operon is regulated through intracellular levels of '-5-cyclic adenosine monophosphate, or cAMP. cAMP is synthesized from ATP by the enzyme adenyl cyclase. The intracellular level of cAMP is in turn regulated by the

intracellular concentration of glucose. cAMP is a co-activator of the CRP protein, a transcriptional enhancer for operons that encode genes for catabolism of energy sources other than glucose, including the lac operon. As glucose levels fall, cAMP levels rise and transcription of an induced lac operon is enhanced. As the levels of glucose rise, cAMP levels fall, and lac operon mRNA synthesis is not enhanced. The mRNA synthesis from the E. coli lac operon is inducible. mRNA synthesis does not occur unless lactose is available for catabolism. The level of mRNA synthesis from the lac operon is also regulated by the intracellular concentration of glucose via a mechanism called catabolite repression.

Briefly, maximum rates of lac mRNA synthesis occur only at low levels of glucose. As suggested by the diagram, cAMP present in cells in response to low glucose levels binds to the CRP protein and the cAMP-CRP complex enhances the level of mRNA synthesis from the lac operon as much as 50 fold. CRP alone cannot activate transcription.

Q.. How the gene expression is regulated in eukaryotic cells?

Ans. The latest estimates are that a human cell, a eukaryotic cell, contains some 21,000 genes.

- Some of these are expressed in all cells all the time. These so-called **housekeeping genes** are responsible for the routine metabolic functions (e.g. respiration) common to all cells.
- Some are expressed as a cell enters a particular pathway of differentiation.
- Some are expressed only as conditions around and in the cell change. For example, the arrival of a hormone may turn on (or off) certain genes in that cell.

There are several methods used by eukaryotes.

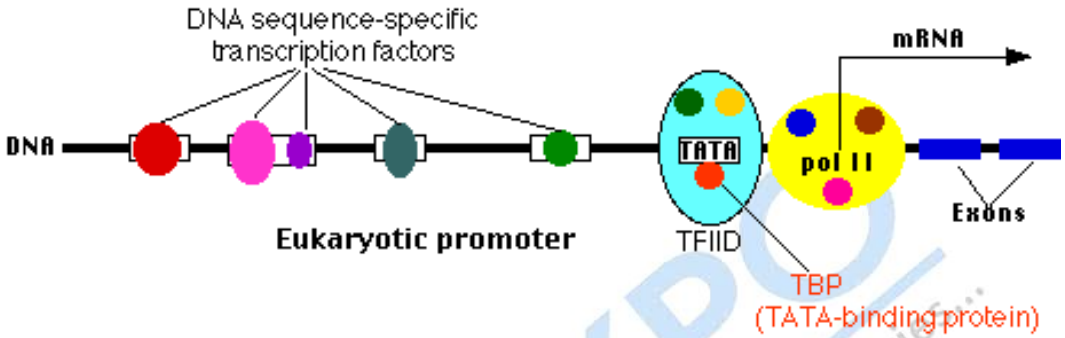
- Altering the **rate of transcription** of the gene. This is the most important and widely-used strategy and the one we shall examine here.
- However, eukaryotes supplement transcriptional regulation with several other methods:
    - Altering the rate at which RNA transcripts are processed while still within the nucleus. [
    - Altering the stability of messenger RNA (mRNA) molecules; that is, the rate at which they are degraded.
    - Altering the efficiency with which ribosomes translate the mRNA into a polypeptide.

Protein-coding genes have **exons** whose sequence encodes the polypeptide; **introns** that will be removed from the mRNA before it is translated; **a transcription start site** a **promoter**, the **basal or core promoter** located within about 0 base pairs (bp) of the start site, an **"upstream" promoter**, which may extend over as many as 200 bp farther upstream, **enhancers** and **silencers**. **Adjacent genes** are often separated by an **insulator**

which helps them avoid cross-talk between each other's promoters and enhancers (and/or silencers).

Transcription start site

This is where a molecule of **RNA polymerase II** (**pol II**, also known as **RNAP II**) binds. Pol II is a complex of 12 different proteins (shown in the figure in yellow with small colored circles superimposed on it).



The start site is where transcription of the gene into RNA begins.

The basal promoter

The basal promoter contains a sequence of 7 bases (TATAAAA) called the **TATA box**. It is bound by a large complex of some 50 different proteins, including

- **Transcription Factor IID** (**TFIID**) which is a complex of
  - **TATA-binding protein** (**TBP**), which recognizes and binds to the TATA box
  - 1 other protein factors which bind to TBP — and each other — but not to the DNA.
- **Transcription Factor IIB** (**TFIIB**) which binds both the DNA and pol II.

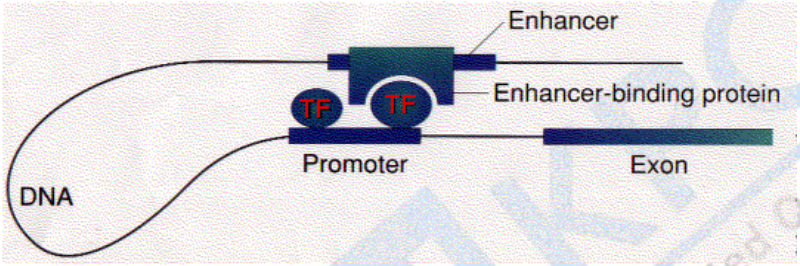
The basal or core promoter is found in all protein-coding genes. This is in sharp contrast to the upstream promoter whose structure and associated binding factors differ from gene to gene.

Many different genes and many different types of cells share the same transcription factors — not only those that bind at the basal promoter but even some of those that bind upstream. What turns on a particular gene in a particular cell is probably the unique **combination** of promoter sites and the transcription factors that are chosen. **Hormones exert many of their effects by forming transcription factors.**The complexes of **hormones with their receptor** represent one class of transcription factor. Hormone

"response elements", to which the complex binds, are **promoter sites**. **Embryonic development requires the coordinated production and distribution of transcription factors.**

Enhancers

Some transcription factors ("Enhancer-binding protein") bind to regions of DNA that are thousands of base pairs away from the gene they control. Binding increases the rate of transcription of the gene. Enhancers can be located upstream, downstream, or even within the gene they control.



Silencers

Silencers are control regions of DNA that, like enhancers, may be located thousands of base pairs away from the gene they control. However, when transcription factors bind to them, expression of the gene they control is repressed.

Insulators

Insulators are

- stretches of DNA (as few as 2 base pairs may do the trick)
- located between the
  - enhancer(s) and promoter or
  - silencer(s) and promoter

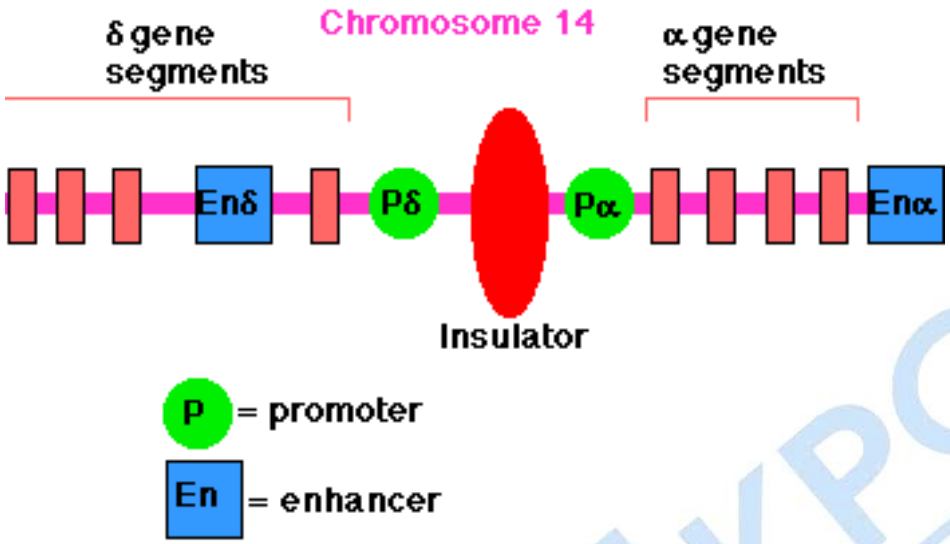
  of **adjacent** genes or clusters of adjacent genes.

Their function is to prevent a gene from being influenced by the activation (or repression) of its neighbors.

Example:

The enhancer for the promoter of the gene for the delta chain of the **gamma/delta** T-cell receptor for antigen (**TCR**) is located close to the promoter for the alpha chain of the

alpha/beta TCR (on chromosome 1 in humans). A T cell must choose between one or the other.



There is an insulator between the alpha gene promoter and the delta gene promoter that ensures that activation of one does not spread over to the other. All insulators discovered so far in vertebrates work only when bound by a protein designated **CTCF** ("CCCTC binding factor"; named for a nucleotide sequence found in all insulators). CTCF has 11 zinc fingers.

Q.5. How a gene is cloned? Write basic steps.

Ans. Gene cloning refers to a set of experimental methods in molecular biology that their replication within host organisms. The use of the word *cloning* refers to the fact that the method involves the replication of a single DNA molecule starting from a single living cell to generate a large population of cells containing identical DNA molecules. There are five steps of gene cloning-

1. A piece of DNA that contains the gene to be cloned is put into a DNA molecule called a vector to produce a recombined DNA molecule.

2. The vector transports the gene to the host cell.

. In the host cell, the vector multiples and makes many identical copies of itself and also of the gene.

. When the host divides, copies of the recombined DNA molecules and the gene are now said to be cloned.

5. After many cell divisions, a clone of host cells is produced. Each cell has at least one copy of the recombined DNA, and the genes are cloned.

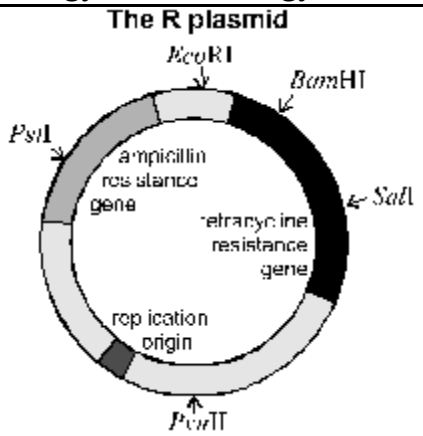
Q.6. What are Vectors?

Ans. In biology a vector is something that carries things between species. E.g. the mosquito is a vector that carries the malaria parasite into humans. In genetic engineering a vector is a length of DNA that carries the gene we want into a host cell. A vector is needed because a length of DNA containing a gene on its own won't actually do anything inside a host cell. Since it is not part of the cell's normal genome it won't be replicated when the cell divides, it won't be expressed, and in fact it will probably be broken down pretty quickly. A vector gets round these problems by having these properties:

- It is big enough to hold the gene we want
- It is circular (or more accurately a closed loop), so that it is less likely to be broken down
- It contains control sequences, such as a transcription promoter, so that the gene will be replicated or expressed.
- It contain marker genes, so that cells containing the vector can be identified.
-

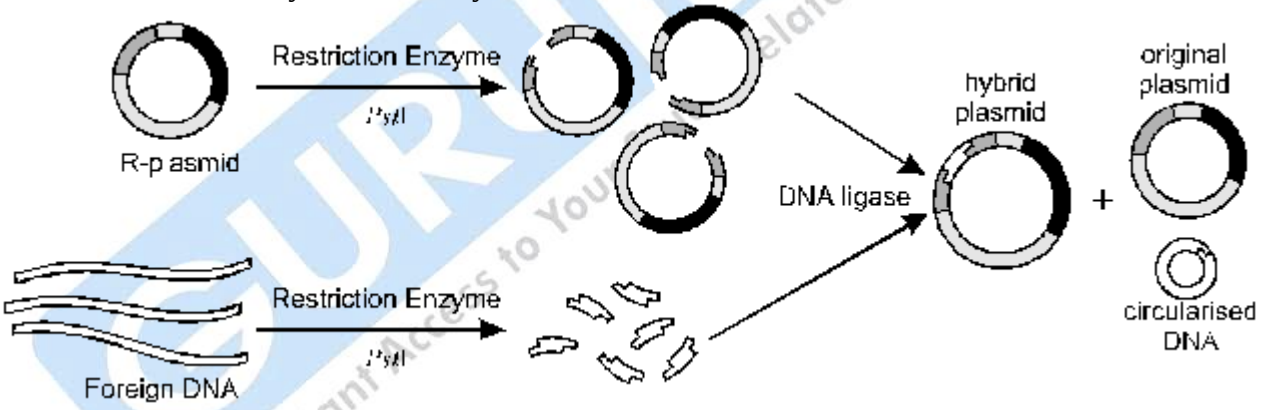
TYPE OF VECTOR	MAX LENGTH OF DNA INSERT
Plasmid	10 kbp
Virus or phage	0 kbp

Plasmids are by far the most common kind of vector, so we shall look at how they are used in some
Plasmids are short circular bits of DNA found naturally in bacterial cells. A typical plasmid conta
genes and there are around 10 copies of a plasmid in a bacterial cell. Plasmids are copied when th
divides, so the plasmid genes are passed on to all daughter cells. They are also used naturally for exc
of genes between bacterial cells (the nearest they get to sex), so bacterial cells will take up a pla
Because they are so small, they are easy to handle in a test tube, and foreign genes can quite eas
incorporated into them using restriction enzymes and DNA ligase.



One of the most common plasmids is the R-plasmid (or pBR22). plasmid contains a replication o several recognition sequences different restriction enzymes (with r like *Eco*RI), and two marker g which confer resistance to dif antibiotics (ampicillin and tetracycli

The diagram below shows how DNA fragments can be incorporated into a plasmid using restrictio ligase enzymes. The restriction enzyme used here (*Pst*I) cuts the plasmid in the middle of one o marker genes (we'll see why this is useful later). The foreign DNA anneals with the plasmid and is j covalently by DNA ligase to form a hybrid vector (in other words a mixture or hybrid of bacteria foreign DNA). Several other products are also formed: some plasmids will simply re-anneal themselves to re-form the original plasmid, and some DNA fragments will join together to form cha circles. Theses different products cannot easily be separated, but it doesn't matter, as the marker gene be used later to identify the correct hybrid vector.



Q. 7. How is the DNA is transferred into cells?

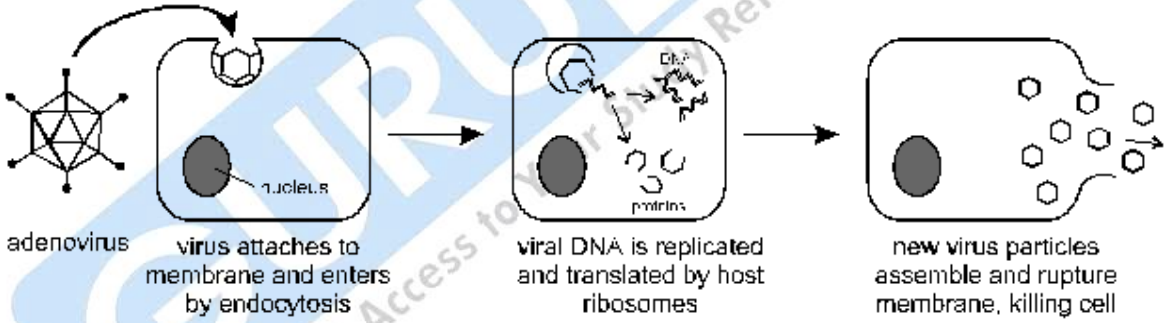
Ans. Vectors containing the genes we want must be incorporated into living cells so that they can be replicated or expressed. The cells receiving the vector are called host cells, and once they have successfully incorporated the vector they are said to be transformed. Vectors are large molecules which do not readily cross cell membranes, so the membranes must be made permeable in some way. There are different ways of doing this depending on the type of host cell.

- Heat Shock. Cells are incubated with the vector in a solution containing calcium ions at 0°C. The temperature is then suddenly raised to about 0°C. This heat shock causes some of the cells to take up the vector, though no one knows why. This works well for bacterial and animal cells.
- Electroporation. Cells are subjected to a high-voltage pulse, which temporarily disrupts

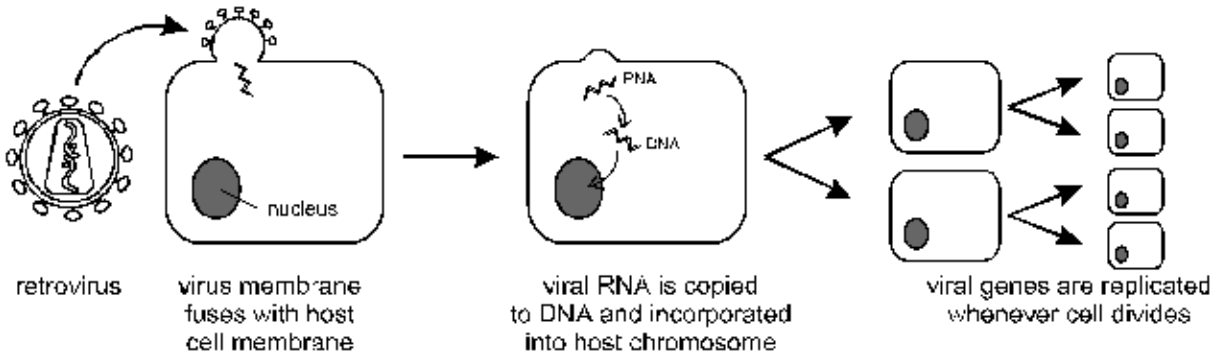
the membrane and allows the vector to enter the cell. This is the most efficient method of delivering genes to bacterial cells.

- Viruses. The vector is first incorporated into a virus, which is then used to infect cells, carrying the foreign gene along with its own genetic material. Since viruses rely on getting their DNA into host cells for their survival they have evolved many successful methods, and so are an obvious choice for gene delivery. The virus must first be genetically engineered to make it safe, so that it can't reproduce itself or make toxins. Three viruses are commonly used:

TYPE OF VIRUS	DETAILS
Bacteriophages	(also called phages) are viruses that infect bacteria. They are an effective way of delivering genes into bacteria cells in culture.
Adenoviruses	are human viruses that causes respiratory diseases including the common cold. Their genetic mate double-stranded DNA, and they are ideal for delivering genes to living patients in gene therapy. DNA is not incorporated into the host's chromosomes, so it is not replicated, but their gen expressed. The adenovirus is genetically altered so that its coat proteins are not synthesised, so new particles cannot be assembled and the host cell is not killed..



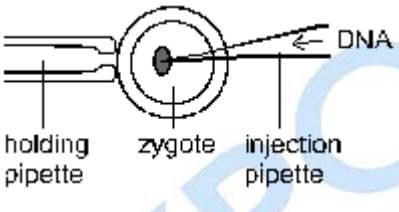
Retroviruses	are a group of human viruses that include HIV. They are enclosed in a lipid membrane and their g material is double-stranded RNA. On infection this RNA is copied to DNA and the DNA is incorp into the host's chromosome. This means that the foreign genes are replicated into every daughte After a certain time, the dormant DNA is switched on, and the genes are expressed in the host cells.

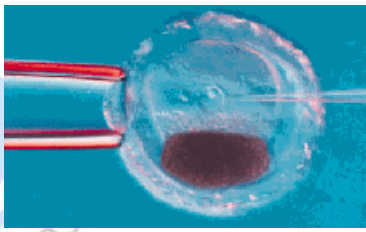


- Plant Tumours. This method has been used successfully to transform plant cells, which are perhaps the hardes The gene is first inserted into the plasmid of a soil bacterium, and then plants are infected with the bacteriu bacterium inserts the plasmid into the plant cells' chromosomal DNA and causes a "crown gall" tumour tumour cells can be cultured in the laboratory.

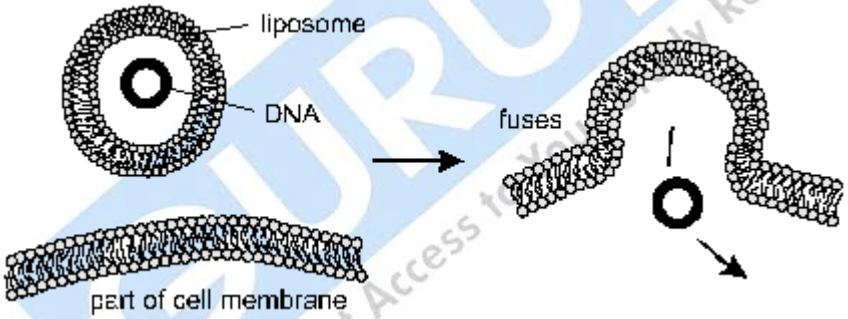
- Gene Gun. This technique fires microscopic gold particles coated with the foreign DNA at the cells compressed air gun. It is designed to overcome the problem of the strong cell wall in plant tissue.

- Micro-Injection. A cell is held on a pipette under a microscope and the foreign DNA is injected directly into the nucleus using an incredibly fine micro-pipette. Used where there are only a very few cells available, such as fertilised animal egg cells.





- Liposomes. Vectors can be encased in liposomes, which are small membrane vesicles. The liposomes fuse with the cell membrane (and sometimes the nuclear membrane too), delivering the DNA into the cell. This works for many types of cell, but is particularly useful for delivering genes to cell *in vivo* (such as in gene therapy).



Q.8. What are the applications of genetic engineering?

Ans. By far the most numerous applications are still as research tools, and the techniques above are helping geneticists to understand complex genetic systems. Despite all the hype, genetic engineering still has very few successful commercial applications, although these are increasing each year. The applications so far can usefully be considered in three groups.

• Gene Products	using genetically modified organisms (usually microbes) to produce chemicals, usually for medical or industrial applications.
• New Phenotypes	using gene technology to alter the characteristics of organisms (usually farm animals or crops)

• Gene Therapy	using gene technology on humans to treat a disease

Gene Products

The biggest and most successful kind of genetic engineering is the production of gene products. These products are of medical, agricultural or commercial value. This table shows a few of the examples of genetically engineered products that are already available.

PRODUCT	USE	HOST ORGANISM
Insulin	human hormone used to treat diabetes	bacteria /yeast
Factor VIII	human blood clotting factor, used to treat haemophiliacs	bacteria
AAT	enzyme used to treat cystic fibrosis and emphysema	sheep
rennin	enzyme used in manufacture of cheese	bacteria /yeast

The products are mostly proteins, which are produced directly when a gene is expressed, but they can also be non-protein products produced by genetically-engineered enzymes. The basic idea is to transfer a gene (often human) to another host organism (usually a microbe) so that it will make the gene product quickly, cheaply and ethically. It is also possible to make "designer proteins" by altering gene sequences, but while this is a useful research tool, there are no commercial applications yet.

Since the end-product is just a chemical, in principle any kind of organism could be used to produce it. By far the most common group of host organisms used to make gene products are the bacteria, since they can be grown quickly and the product can be purified from their cells. Unfortunately bacteria cannot not always make human proteins, and recently animals and even plants have also been used to make gene products. In neither case is it appropriate to extract the product from their cells, so in animals the product must be secreted in milk or urine, while in plants the product must be secreted from the roots. This table shows some of the advantages and disadvantages of using different organisms for the production of genetically-engineered gene products.

TYPE OF ORGANISM	ADVANTAGES	DISADVANTAGES

Prokaryotes (i.e.Bacteria)	no nucleus so DNA easy to modify; have plasmids; small genome; genetics well understood; asexual so can be cloned; small and fast growing; easy to grow commercially in fermenters; will use cheap carbohydrate; few ethical problems.	can't splice introns; no post-translational modification; small gene size
Eukaryotes	can do post-translational modifications; can accept large genes	Do not have plasmids (except yeast); often diploid so two copies of genes may need to be inserted; control of expression not well understood.
Fungi (yeast, mould)	asexual so can be cloned; haploid, so only one copy needed; can be grown in vats	can't always make animals gene products
Plants	photosynthetic so don't need much feeding; can be cloned from single cells; products can be secreted from roots or in sap.	cell walls difficult to penetrate by vector; slow growing; must be grown in fields; multicellular
Animals (pharming)	most likely to be able to make human proteins; products can be secreted in milk or urine	multicellular; slow growing

Human Insulin

Insulin is a small protein hormone produced by the pancreas to regulate the blood sugar concentration. In the disease insulin-dependent diabetes the pancreas cells don't produce enough insulin, causing wasting symptoms and eventually death. The disease can be successfully treated by injection of insulin extracted from the pancreases of slaughtered cows and pigs. However the insulin from these species has a slightly different amino acid sequence from human insulin and this can lead to immune rejection and side effects.

The human insulin gene was isolated, cloned and sequenced in the 1970s, and so it became possible to insert this gene into bacteria, who could then produce human insulin in large amounts. Unfortunately it wasn't that simple. In humans, pancreatic cells first make pro-insulin, which then

undergoes post-translational modification to make the final, functional insulin. Bacterial cells cannot do post-translational modification. Eventually a synthetic cDNA gene was made and inserted into the bacterium *E. coli*, which made pro-insulin, and the post-translational conversion to insulin was carried out chemically. This technique was developed by Eli Lilly and Company in 1982 and the product, "humulin" became the first genetically-engineered product approved for medical use.

In the 1990s the procedure was improved by using the yeast *Saccharomyces cerevisiae* instead of *E. coli*. Yeast, as a eukaryote, is capable of post-translational modification, so this simplifies the production of human insulin. However another company has developed a method of converting pig insulin into human insulin by chemically changing a few amino acids, and this turns out to be cheaper than the genetic engineering methods. This all goes to show that genetic engineers still have a lot to learn.

Bovine Somatotropin

This is a growth hormone produced by cattle. The gene has been cloned in bacteria by the company Monsanto, who can produce large quantities of BST. in the USA cattle are often injected with BST every 2 weeks, resulting in a 10% increase in mass in beef cattle and a 25% increase in milk production in dairy cows. BST was tested in the UK in 1985, but it was not approved and its use is currently banned in the EU. This is partly due to public concerns and partly because there is already overproduction of milk and beef in the EU, so greater production is not necessary.

Renin

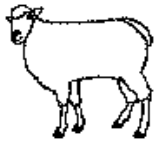
Rennin is an enzyme used in the production of cheese. It is produced in the stomach of juvenile mammals (including humans) and it helps the digestion of the milk protein caesin by solidifying it so that is remains longer in the stomach. The cheese industry used to obtain its rennin from the stomach of young calves when they were slaughtered for veal, but there are moral and practical objections to this source. Now an artificial cDNA gene for rennin has been made from mRNA extracted from calf stomach cells, and this gene has been inserted into a variety of microbes. The rennin extracted from these microbes has been very successful and 90% of all hard cheeses in the UK are made using microbial rennin. Sometimes (though not always) these products are labelled as "vegetarian cheese".

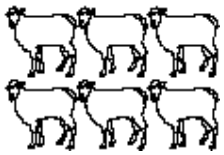
AAT(alpha-1-antitrypsin)

AAT is a human protein made in the liver and found in the blood. As the name suggests it is an inhibitor of protease enzymes like trypsin and elastase. There is a rare mutation of the AAT gene (a single base substitution) that causes AAT to be inactive, and so the protease enzymes to be uninhibited. The most noticeable effect of this in the lungs, where elastase digests the elastic

tissue of the alveoli, leading to the lung disease emphysema. This condition can be treated by inhaling an aerosol spray containing AAT so that it reaches the alveoli and inhibits the elastase there.

AAT for this treatment can be extracted from blood donations, but only in very small amounts. The gene for AAT has been found and cloned, but AAT cannot be produced in bacteria because AAT is a glycoprotein, which means it needs to have sugars added by post translational modification. This kind of modification can only be carried out by animals (because they have a golgi body), and AAT is now produced by genetically-modified sheep. In order to make the AAT easy to extract, the gene was coupled to a promoter for the milk protein b-lactoglubulin. Since this promoter is only activated in mammary gland cells, the AAT gene will only be expressed in mammary gland cells, and so will be secreted into the sheep's milk. This makes it very easy to harvest and purify without harming the sheep. The first transgenic sheep to produce AAT was called Tracy, and she was produced in Edinburgh in 199. This is how Tracy was made:

A female sheep is given a fertility drug to stimulate her egg production, and several mature eggs are collected from her ovaries.	
The eggs are fertilised *in vitro*.	
A plasmid is prepared containing the gene for human AAT and the promoter sequence for b-lactoglobulin. Hundreds of copies of this plasmid are microinjected into the nucleus of the fertilised zygotes. Only a few of the zygotes will be transformed, but at this stage you can't tell which.	
The zygotes divide *in vitro* until the embryos are at the 16-cell stage.	
The 16-cell embryos are implanted into the uterus of surrogate mother ewes. Only a few implantations result in a successful pregnancy.	

Test all the offspring from the surrogate mothers for AAT production in their milk. This is the only way to find if the zygote took up the AAT gene so that it can be expressed. About 1 in 20 eggs are successful.	
Collect milk from the transgenic sheep for the rest of their lives. Their milk contains about 5 g of AAT per litre of milk. Also breed from them in order to build up a herd of transgenic sheep.	
Purify the AAT, which is worth about £50 000 per mg.	

New phenotypes

This means altering the characteristics of organisms by genetic engineering. The organisms are usually commercially-important crops or farm animals. It can be seen as a high-tech version of selective breeding, which has been used by humans to alter and improve their crops and animals for at least 10 000 years. Nevertheless GMOs have turned out to be a highly controversial development. We don't study any of these in detail, but this table gives an idea of what is being done.

ORGANISM	MODIFICATION
long life tomatoes	There are two well-known projects, both affecting the gene for the enzyme (PG) which softens the fruits as they ripen. Tomatoes that make less PG ripen more slowly and retain more flavour. The American "Flavr Savr" tomato used antisense technology to silence the gene, while the British Zeneca tomato disrupted the gene. Both were successful and were on sale for a few years, but neither is produced any more.
Insect-resistant crops	Genes for various powerful protein toxins have been transferred from the bacterium *Bacillus thuringiensis* to crop plants including maize, rice and potatoes. These Bt toxins are thousands of times more powerful than chemical insecticides, and since they are built-in to the crops, insecticide spraying (which is non-specific and damages the environment) is unnecessary.
Nitrogen-	This is a huge project, which aims to transfer the 15-or-so genes required for

fixing crops	nitrogen fixation from the nitrogen-fixing bacteria *Rhizobium* into cereals and other crop plants. These crops would then be able to fix their own atmospheric nitrogen and would not need any fertiliser. However, the process is extremely complex, and the project is nowhere near success.
Tick-resistant sheep	The gene for the enzyme chitinase, which kills ticks by digesting their exoskeletons, has bee transferred from plants to sheep. These sheep should be immune to tick parasites, and may not need sheep dip.

Gene Therapy

This is perhaps the most significant, and most controversial kind of genetic engineering. It is also the least well-developed. The idea of gene therapy is to genetically alter humans in order to treat a disease. This could represent the first opportunity to cure incurable diseases. Note that this is quite different from using genetically-engineered microbes to produce a drug, vaccine or hormone to treat a disease by conventional means. Gene therapy means altering the genotype of a tissue or even a whole human.

For Example, Cystic fibrosis (CF) is the most common genetic disease in the UK, affecting about 1 in 2500. It is caused by a mutation in the gene for protein called CFTR (Cystic Fibrosis Transmembrane Regulator). The gene is located on chromosome 7, and there are actually over 00 different mutations known, although the most common mutation is a deletion of three bases, removing one amino acid out of 180 amino acids in the protein. CFTR is a chloride ion channel protein found in the cell membrane of epithelial (lining) tissue cells, and the mutation stops the protein working, so chloride ions cannot cross the cell membrane.

Chloride ions build up inside these cells, which cause sodium ions to enter to balance the charge, and the increased concentration of the both these ions inside the epithelial cells decreases the osmotic potential. Water is therefore retained inside the cells, which means that the mucus secreted by these cells is drier and more sticky than normal. This sticky mucus block the tubes into which it is secreted, such as the small intestine, pancreatic duct, bile duct, sperm duct, bronchioles and alveoli.

These blockages lead to the symptoms of CF: breathlessness, lung infections such as bronchitis and pneumonia, poor digestion and absorption, and infertility. Of these symptoms the lung effects are the most serious causing 95% of deaths. CF is always fatal, though life expectancy has increased from 1 year to about 20 years due to modern treatments. These treatments include physiotherapy many times each day to dislodge mucus from the lungs, antibiotics to fight infections, DNAse drugs to loosen the mucus, enzymes to help food digestion and even a heart-lung transplant.

Given these complicated (and ultimately unsuccessful) treatments, CF is a good candidate for gene therapy, and was one of the first diseases to be tackled this way. The gene for CFTR was

identified in 1989 and a cDNA clone was made soon after. The idea is to deliver copies of this good gene to the epithelial cells of the lung, where they can be incorporated into the nuclear DNA and make functional CFTR chloride channels. If about 10% of the cells could be corrected, this would cure the disease.

Two methods of delivery are being tried: liposomes and adenoviruses, both delivered with an aerosol inhaler, like those used by asthmatics. Clinical trials are currently underway, but as yet no therapy has been shown to be successful.

Gene therapy is in its infancy, and is still very much an area of research rather than application. No one has yet been cured by gene therapy, but the potential remains enticing. Gene therapy need not even be limited to treating genetic diseases, but could also help in treating infections and environmental diseases:

- White blood cells have be genetically modified to produce tumour necrosis factor (TNF), a protein that kills cancer cells, making these cells more effecting against tumours.
- Genes could be targeted directly at cancer cells, causing them to die, or to revert to normal cell division.
- White blood cells could be given antisense genes for HIV proteins, so that if the virus infected these cells it couldn't reproduce.

Q.9. What is the difference between animal cell culture and tissue culture?

Ans. The term "**tissue culture**" refers to the culture of whole of organs, tissue fragments as well as dispersed cells on a suitable nutrient medium.

It can be further subdivided into:

- Organ Culture
- Cell Culture

The culture of whole organs or intact organ fragments with the intent of studying thei continued function or development is called **Organ culture**.

When the cells are removed from the organ fragments prior to or during cultivation, thus disrupting their normal relationships with neighboring cells, it is called **Cell culture**.

Q.10. What are the salient features of animal cell culture?

Ans. Salient Features of Animal cell culture

a) Animal cells can grow in simple glass or plastic containers in nutritive media but they grow only to limited generations.

b) Animal cells exhibit contact inhibition. In culture the cancer cells apparently differ from the normal cells. Due to uncontrolled growth and more rounded shape, they loose contact inhibition and pile over each other.

c) There is a difference in the in vitro and in vivo growth pattern of cells. For example

(i) there is an absence of cell-cell interaction and cell matrix interaction,
(ii) there is a lack of three-dimensional architectural appearance, and
(iii) changed hormonal and nutritional environment. They way of adherence to glass or plastic container in which they grow, cell proliferation and shape of cell results in alterations.

d) The maintenance of growth of cells under laboratory conditions in suitable culture medium is known as primary cell culture.

e) Cells are dissociated form tissues by mechanical means and by enzymatic digestion using proteolytic enzymes.

f) Cells can grow as adherent cells (anchorage dependent) or as suspension cultures (anchorage independent).

g) The primary culture is subcultured in fresh media to establish secondary cultures.

h) The various types of cell lines are categorized into two types as Finite cell line and Continuous cell line. Finite cell lines are those cell lines which have a limited life span and grow through a limited number of cell generations. The cells normally divide 20 to 100 times (i.e. is 20-100 population doublings) before extinction. Cell lines transformed under in vitro conditions give rise to continuous cell lines. The continuous cell lines are transformed, immortal and tumorigenic.

i) The physical environment includes the optimum pH, temperature, osmolality and gaseous environment, supporting surface and protecting the cells from chemical, physical, and mechanical stresses.

j) Nutrient media is the mixture of inorganic salts and other nutrients capable of sustaining cell survival in vitro

k) Serum is essential for animal cell culture and contains growth factors which promote cell proliferation. It is obtained as exuded liquid from blood undergoing coagulation and filtered using Millipore filters.

l) Cryo preservation is storing of cells at very low temperature (1800C to -196_0C) using liquid nitrogen. DMSO is a cryopreservative molecule which prevents damage to cells.

m) In order to maintain the aseptic conditions in a cell culture, a LAF hood is used. Based on the nature of cells and organism the tissue culture hoods are grouped into three types: Class I, Class II, and Class III.

n) CO_2 incubators are used and designed to mimic the environmental conditions of the living cells.

o) An inverted microscope is used for visualizing cell cultures in situ

p) For most animal cell cultures low speed centrifuges are needed.

q) Neuronal cells constitute the nervous system. In culture the neuronal cells cannot divide and grow.

r) The cells that form connective tissue (skin) is called fibroblast. The fibroblast can divide and grow in culture to some generations after which they die. All normal animal cells are mortal.

s) Organ culture- The culture of native tissue that retains most of the in vivo histological features is regarded as organ culture.

t) Histotypic culture- The culturing of the cells for their reaggregation to form a tissue-like structure represents histotypic culture.

u) Organotypic culture- This culture technique involves the recombination of different cell types to form a more defined tissue or an organ.

There are certain terms that are associated with the cell lines. These are as follows:

(i) Split ratio- The divisor of the dilution ratio of a cell culture at subculture.
(ii) Passage number- It is the number of times that the culture has been cultured,
(iii) Generation number- It refers to the number of doublings that a cell population has undergone.

In fact these parameters help us to distinguish the cancer cells in culture from the normal cells because the cancer cells in culture, change shape (more rounded), loose contact inhibition, pile on each other due to overgrowth and uncontrolled growth.

Q.11. What are different kinds of animal cell culture?

Ans. Primary cell culture

The maintenance of growth of cells dissociated from the parental tissue (such as kidney, liver) using the mechanical or enzymatic methods, in culture medium using suitable glass or plastic containers is called Primary Cell Culture.

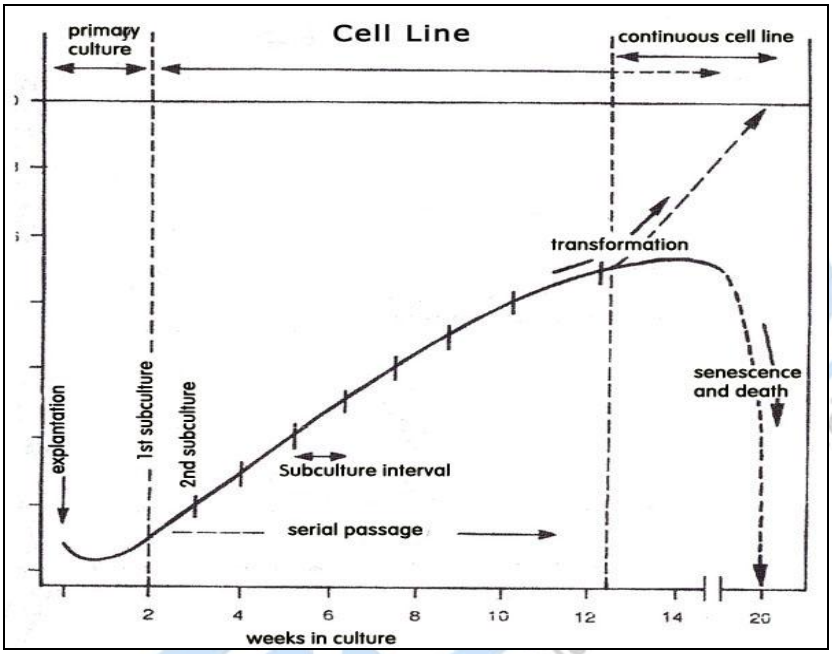


Fig. Showing the salient features of cell culture with evolution of a cell line

The primary cell culture could be of two types depending upon the kind of cells in culture.

a) **Anchorage Dependent /Adherent cells**- Cells shown to require attachment for growth are set to be Anchorage Dependent cells. The Adherent cells are usually derived from tissues of organs such as kidney where they are immobile and embedded in connective tissue. They grow adhering to the cell culture.

b) **Suspension Culture/Anchorage Independent cells** - Cells which do not require attachment for growth or do not attach to the surface of the culture vessels are anchorage independent cells/suspension cells. All suspension cultures are derived from cells of the blood system because these cells are also suspended in plasma in vitro e.g. lymphocytes.

Secondary cell cultures

When a primary culture is sub-cultured, it becomes known as secondary culture or cell line. Subculture (or passage) refers to the transfer of cells from one culture vessel to another culture vessel.

Subculturing- Subculturing or splitting cells is required to periodically provide fresh nutrients and growing space for continuously growing cell lines. The process involves removing the growth media, washing the plate, disassociating the adhered cells, usually enzymatically. Such cultures may be called secondary cultures.

Cell Line

A Cell Line or Cell Strain may be finite or continuous depending upon whether it has limited culture life span or it is immortal in culture. On the basis of the life span of culture, the cell lines are categorized into two types:

a) **Finite cell Lines** - The cell lines which have a limited life span and go through a limited number of cell generations (usually 20-80 population doublings) are known as Finite cell lines. These cell lines exhibit the property of contact inhibition, density limitation and anchorage dependence. The growth rate is slow and doubling time is around 2-96 hours.

b) **Continuous Cell Lines** - Cell lines transformed under laboratory conditions or in vitro culture conditions give rise to continuous cell lines. The cell lines show the property of ploidy (aneupliody or heteroploidy), absence of contact inhibition and anchorage dependence. They grow in monolayer or suspension form. The growth rate is rapid and doubling time is 12-2 hours.

c) **Monolayer cultures** - When the bottom of the culture vessel is covered with a continuous layer of cells, usually one cell in thickness, they are referred to as monolayer cultures.

d) **Suspension cultures** - Majority of continuous cell lines grow as monolayers. Some of the cells which are non-adhesive e.g. cells of leukemia or certain cells which can be mechanically kept in suspension, can be propagated in suspension. There are certain advantages in propagation of cells by suspension culture method. These advantages are:
(a) The process of propagation is much faster.,
(b) The frequent replacement of the medium is not required.,
(c) Suspension cultures have a short lag period,
(d) treatment with trypsin is not required,
(e) a homogenous suspension of cells is obtained,
(f) the maintenance of suspension cultures is easy and bulk production of the cells is easily achieved.,
(g) scale-up is also very convenient.

The cell lines are known by:

a) A code e.g. NHB for Normal Human Brain.
b) A cell line number- This is applicable when several cell lines are derived from the same cell culture source e.g. NHB1, NHB2.

c) Number of population doublings, the cell line has already undergone e.g. NHB2/2 means two doublings.

Q.12. How are cell lines characterized?

Ans. The cell lines are characterized by their a) growth rate and b) karyotyping.

a) **Growth Rate** - A growth curve of a particular cell line is established taking into consideration the population doubling time, a lag time, and a saturation density of a particular cell line. A growth curve consist of:

1) Lag Phase: The time the cell population takes to recover from such sub culture, attach to the culture vessel and spread.

2) Log Phase: In this phase the cell number begins to increase exponentially.

) Plateau Phase: During this phase, the growth rate slows or stops due to exhaustion of growth medium or confluency.

b) Karyotyping - Karyotyping is important as it determines the species of origin and determine the extent of gross chromosomal changes in the line. The cell lines with abnormal karyotype are also used if they continue to perform normal function. Karyotype is affected by the growth conditions used, the way in which the cells are subcultured and whether or not the cells are frozen.

c) There are certain terms that are associated with the cell lines.

These are as follows:

(i) Split ratio- The divisor of the dilution ratio of a cell culture at subculture. (ii) Passage number- It is the number of times that the culture has been cultured., (iii) Generation number- It refers to the number of doublings that a cell population has undergone.

Cell line	Product
Human tumour	Angiogenic factor
Human leucocytes	Interferon
Mouse fibroblasts	Interferon
Human Kidney	Urokinase
Transformed human kidney cell line, TCL-598	Single chain urokinase-type plasminogen activator (scu-PA)
Human kidney cell (29)	Human protein (HPC)
Dog kidney	Canine distemper vaccine
Cow kidney	Foot and Mouth disease (FMD) vaccine
Chick embryo fluid	Vaccines for influenza, measles and mumps
Duck embryo fluid	Vaccines for rabies and rubella

Chinese hamster ovary (CHO) cells	1. Tissue-type plasminogen activator (t-PA) 2. B-and gamma interferons 3. Factor VIII

Table-Some animal cell lines and the products obtained from them

Q.13 What are the general requirements for animal cell and tissue culture?

Ans. Among the essential requirements for animal cell culture are special incubators to maintain the levels of oxygen, carbon dioxide, temperature, humidity as present in the animal's body. The synthetic media with vitamins, amino acids and fetal calf serum. Following parameters are essential for successful animal cell culture:

a) Temperature- In most of the mammalian cell cultures, the temperature is maintained at 70C in the incubators as the body temperature of Homo sapiens is 70C.

b) Culture media- The culture media is prepared in such a way that it provides-

1) The optimum conditions of factors like pH, osmotic pressure, etc.

2) It should contain chemical constituents which the cells or tissues are incapable of synthesizing. Generally the media is the mixture of inorganic salts and other nutrients capable of sustaining cells in culture such as amino acids, fatty acids, sugars, ions, trace elements, vitamins, cofactors, and ions. Glucose is added as energy source-it's concentration varying depending on the requirement. Phenol Red is added as a pH indicator of the medium.There are two types of media used for culture of animal cells and tissues- the natural media and the synthesized media.

) Natural Media - The natural media are the natural sources of nutrient sufficient for growth and proliferation of animal cells and tissues. The Natural Media used to promote cell growth fall in three categories.

i) Coagulant, such as plasma clots. It is now commercially available in the form of liquid plasma kept in silicon ampoules or lyophilized plasma. Plasma can also be prepared in the laboratory taking out blood from male fowl and adding heparin to prevent blood coagulation.

ii) Biological fluids such as serum. Serum is one of the very important components of animal cell culture which is the source of various amino acids, hormones, lipids, vitamins, polyamines, and salts containing ions such as calcium, ferrous, ferric, potassium etc. It also contains the growth factors which promotes cell proliferation, cell attachment and adhesion factors. Serum is obtained from human adult blood, placental, cord blood, horse blood, calf blood. The other forms of biological fluids used are coconut water, amniotic

fluid, pleural fluid, insect haemolymph serum, culture filtrate, aqueous humour, from eyes etc.

iii) Tissue extracts for example Embryo extracts- Extracts from tissues such as embryo, liver, spleen, leukocytes, tumour, bone marrow etc are also used for culture of animal cells.

Synthetic media

Syntheic media are prepared artificially by adding several organic and inorganic nutrients, vitamins, salts, serum proteins, carbohydrates, cofactors etc. Different types of synthetic media can be prepared for a variety of cells and tissues to be cultured. Synthetic media are of two types- Serum containing media (media containing serum) and serum- free media (media with out serum). Examples of some media are: minimal essential medium (MEM), RPMI 160 medium, CMRL 1066, F12 etc.

Advantages of serum in culture medium are:
(i) serum binds and neutralizes toxins,
(ii) serum contains a complete set of essential growth factors, hormones, attachment and spreading factors, binding and transport proteins,
(iii) it contains the protease inhibitors,
(iv) it increases the buffering capacity,
(v) it provides trace elements.

Disadvantages of serum in culture medium are:
(i) it is not chemically defined and therefore it's composition varies a lot,
(ii) it is sometimes source of contamination by viruses, mycoplasma, prions etc,
(iii) it increases the difficulties and cost of down stream processing,
(iv) it is the most expensive component of the culture medium.

) pH- Most media maintain the pH between 7 and 7.. A pH below 6.8 inhibits cell growth. The optimum pH is essential to maintain the proper ion balance, optimal functioning of cellular enzymes and binding of hormones and growth factors to cell surface receptors in the cell cultures. The regulation of pH is done using a variety of buffering systems. Most media use a bicarbonate-CO2 system as its major component.

5) Osmolality- A change in osmolality can affect cell growth and function. Salt, Glucose and Amino acids in the growth media determine the osmolality of the medium. All commercial media are formulated in such a way that their final osmolality is around 00 mOsm.

Q.14 Describe the applications of animal cell culture?

Ans. The animal cell cultures are used for a diverse range of research and development. **These areas are:**

a) production of antiviral vaccines, which requires the standardization of cell lines for

the multiplication and assay of viruses.

b) Cancer research, which requires the study of uncontrolled cell division in cultures.

c) Cell fusion techniques.

d) Genetic manipulation, which is easy to carry out in cells or organ cultures.

e) Production of monoclonal antibodies requires cell lines in culture.

f) Production of pharmaceutical drugs using cell lines.

g) Chromosome analysis of cells derived from womb.

h) Study of the effects of toxins and pollutants using cell lines.

i) Use of artificial skin.

j) Study the function of the nerve cells.

Somatic Cell Fusion

One of the applications of animal cell culture is the production of hybrid cells by the fusion of different cell types. These hybrid cells are used for a the following purposes:
(i) study of the control of gene expression and differentiation,
(ii) study of the problem of ' malignancy',
(iii) viral application,
(iv) gene mapping,
(v) production of hybridomas for antibody production.

In 1960s, in France for the first time, the hybrid cells were successfully produced from mixed cultures of two different cell lines of mouse. Cells growing in culture are induced by some of the viruses such as 'Sendai virus' to fuse and form hybrids. This virus induces two different cells first to form heterokaryons. During mitosis, chromosomes of heterokaryon move towards the two poles and later on fuse to form hybrids. It is important to remove the surface carbohydrates to bring about cell fusion. Some other chemicals like polyethylene glycol also induce somatic cell fusion.

Many commercial proteins have been produced by animal cell culture and there medical application is being evaluated.

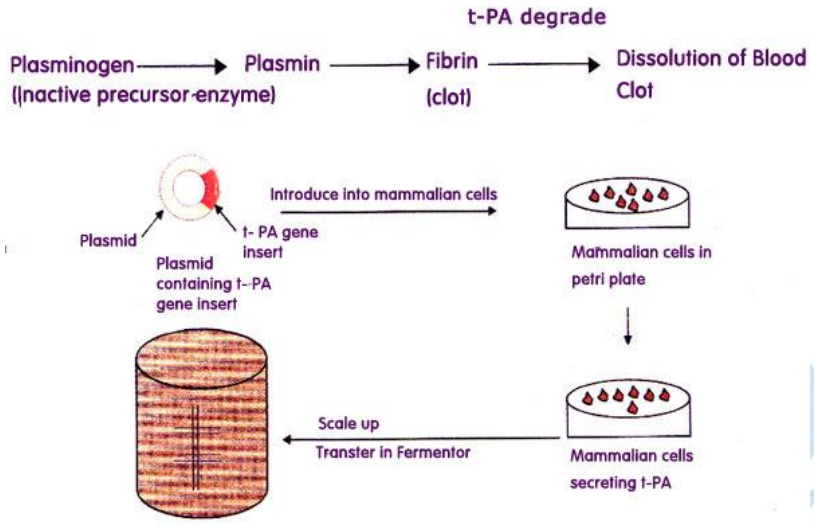


Fig showing the production of t-PA

Tissue Plasminogen activator (t-PA) was the first drug that was produced by the mammalian cell culture by using rDNA technology. The recombinant t-PA is safe and effective for dissolving blood clots in patients with heart diseases and thrombotic disorders.

Blood Factor VIII

Haemophilia A is a blood disorder which is a sex-linked genetic disease in humans. The patients suffering from Haemophilia A lack factor VIII, which plays an important role in the clotting of blood. This factor VIII is secreted by a gene present on X-chromosome but this gene undergoes mutations in people suffering from Haemophilia. Current therapy for this disease is the transfusion of blood factor VIII into patients. Using rDNA technology, Factor VIII has been produced from mammalian cell culture e.g. Hamster kidney cell.

Erythropoietin (EPO)

The EPO is a glycoprotein consisting of 165 amino acids and is formed in the foetal liver and kidneys of the adults. It causes proliferation and differentiation of progenitor cells into the erythrocytes (erythroblasts) in the bone marrow. Erythropoietin is hormone-like in nature and is released by the kidney under hypoxic or anoxic conditions caused by anaemia.

Amgen Inc. holds US patent for preparation of, eErythropoietin, by recombinant method using Chinese Hamster Ovary cell lines. Erythropoietin (EPO) is a hormone-like substance released by the kidney under hypoxic or anoxic conditions caused by anaemia.

r-HUEPO- recombinant human erythro- protein has been effectively used to treat anemia associated with AIDS, renal failure etc.

The production of Monoclonal Antibodies using hybridoma technology

Antibodies are proteins synthesized in blood against antigens and are collected from the blood serum. The antibodies, which are heterogenous and non specific in action are called polyclonal antibodies. If a specific lymphocyte, after isolation and culture in vitro becomes capable of producing a single type of antibody bearing specificity against specific antigen, it is known as monoclonal antibody. The monoclonal antibodies are used in the diagnosis of diseases because of the presence of desired immunity. However, these antibody secreting cells cannot be maintained in culture. It was observed that the myeloma cells (bone marrow tumour cells due to cancer) grow indefinitely and also produce immunoglobulins which are infact monoclonal antibodies.

In 197, George Kohler and Milstein isolated clones of cells from the fusion of two parental cell lines – lymphocytes from spleen of mice immunized with red blood cells from sheep and myeloma cells. These cells were maintained in vitro and produced antibodies. The hybrid cells maintained the character of lymphocytes to secrete the antibodies, and of myeloma cells to multiply in culture. These hybrid cell lines are called "Hybridoma" and are capable of producing unlimited supply of antibodies. Hybridoma are obtained by using an antibody producing lymphocytes cell and a single myeloma cell. Monoclonal antibodies bind very specifically to an epitope (specific domains) on an antigen and by using them it is possible to detect the presence of specific antigens.

The Monoclonal antibodies are used for the treatment of patients with malignant leukaemia cells, B cell lymphomas and allograft rejection after transplantation. CD is an antigen present on the surface of mature T- cells lymphocytes. If T- cell population is depleted or controlled, the transplanted organ will not be rejected. An antibody that acts against CD surface antigen of T-cells is called OKT i.e. anti-CD Moab. OKT is a monoclonal antibody which has been licensed for clinical use for the treatment of acute renal allograft rejection. OKT removes antigen bearing cells from circulation thereby helps in accepting the graft.

When Monoclonal antibodies are used as enzymes using the technique of enzyme engineering, then they are called **abzymes**.Using animal cell cultures, it is also possible to produce **Polyclonal Antibodies**. Polyclonal antisera are derived from many cells therefore contains heterogeneous antibodies that are specific for several epitopes or an antigen.

Q.15. Write a note on 'Ethics in animal biotechnology'.

Ans. There are some serious issues related to genetic modification of animals using animal genetic engineering techniques. One is not sure of the consequences of these genetic modifications and the further interaction with the environment. Proper clinical trials are also necessary before one can use it for commercial purposes. In the recent past people have raised objections on some of the methods used e.g. the transfer of a human genes

into food animals, use of organisms containing human genes as animal feed. Some religious groups have expressed their concern about the transfer of genes from animals whose flesh is forbidden for use as food into the animals that they normally eat. Transfer of animal genes into food plants that may be objectionable to the vegetarians.

Besides this, there are several other aspects of this issue have to be sorted out.

a) What will be the consequences, if a modified animal will breed with other domestic or wild animals thereby transferring the introduced genes to these populations?

b) What are the health risks to human on consumption of genetically modified animals and their products?

c) With the production of disease resistant animals, what will be the effect on ecology?

d) There is also wide spread concern about the risks of human recipients getting infected with animal viral diseases after a xenotransplantation., which might infect the population at large.

e) There are also concerns about the risk that drug resistance gene markers used in genetic engineering procedures might inadvertently be transferred and expressed.

The need of the hour is to formulate clear guidelines which should be followed while using genetic engineering techniques in bio-medical research. e.g. products from transgenic organisms should be clearly marked to give choice to people who follow dietary restrictions due to religious beliefs. In fact all the ethical and moral issues raised by some aspects of biotechnology should be addressed by open discussion and dialogue.

Q.16. How is genetic counseling relevant in Biotechnology?

Ans. Biotechnology has also contributed in the field of genetic counseling especially to the couples who may be at high risk of giving birth to a child with congenital defects or diseases. These parents can decide voluntarily not to have a child or may undergo selective abortions after investigations during antenatal care.

The hereditary or congenital defects are tested through the process of amniocentesis. Around the fifteen week of pregnancy, a sample of amniotic fluid containing fetal cells is removed and cultured in vitro. The cultured cells are then subjected to biochemical enzyme test and karyotype analysis. The results are particularly relevant to the situation where one or both parents have a family history of a particular hereditary disease and one has to evaluate the possible chances of the children having the same disease. This type of test followed by genetic counseling is also done when a couple may have an abnormal child and would like to know the chances of having abnormal child on the next pregnancy. In some cases, if the defect is controlled by single recessive gene and both

parents are normal, in three out of four cases the child would be normal. At least 5 diseases which can be identified by this technique are known. The number of disease specific DNA probes is also increasing at a fast rate, so that antenatal diagnosis by DNA analysis or 'enzyme linked immunosorbent assay'(ELISA) should be possible for all single gene defects e.g. the incidence of Thallassemia in Cypriot community in U.K. has decreased to 20 per year following the use of antenatal diagnosis.

Making a choice of baby's sex

It is always interesting for a married couple to know the sex of the unborn baby. The chromosome techniques have made it possible to know the sex of the developing fetus by drawing amniotic fluid and preparing karyotypes from cells derived from the fetus which are floating in this fluid.

Using certain procedures it has been possible to have a child of a preferred sex. The technique involves "sephadex gel column" in which sperms with Y chromosome are trapped in the gel as they are lighter and the X bearing sperms reach the bottom as they are heavier. After separation these sperms are used to fertilize the ova by artificial insemination.

Q.17. What is hybridoma technology and how it is used to produce monoclonal antibodies?

Ans. Fusion of cultured animal cells can yield interspecific hybrids useful in somatic-cell genetics.Cultured animal cells infrequently undergo cell fusion spontaneously. The fusion rate, however, increases greatly in the presence of certain viruses that have a lipoprotein envelope similar to the plasma membrane of animal cells. A mutant viral glycoprotein in the envelope promotes cell fusion. Cell fusion also is promoted by polyethylene glycol, which causes the plasma membranes of adjacent cells to adhere to each other and to fuse .As most fused animal cells undergo cell division, the nuclei eventually fuse, producing viable cells with a single nucleus that contains chromosomes from both "parents." The fusion of two cells that are genetically different yields a hybrid cell called a heterokaryon.

Fusion of cultured animal cells. (a) Unfused growing mouse cells with a single nucleus per cell. (b) Fused mouse cells with $2-5$ nuclei per cell. Fusion was induced.

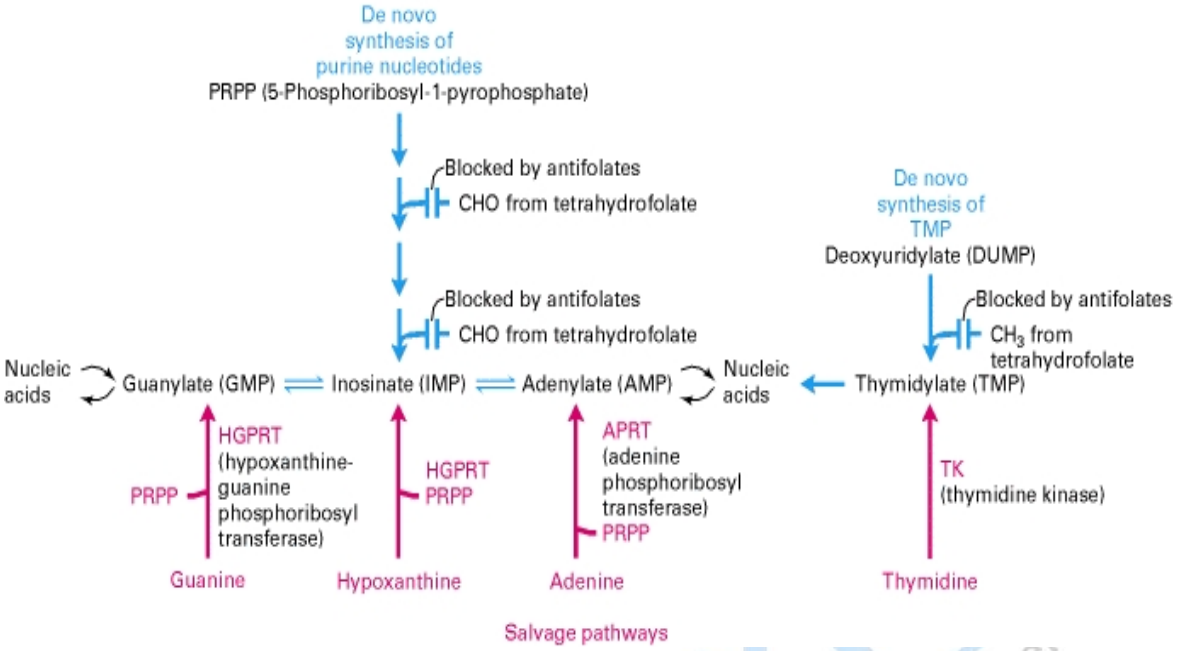
Because some **somatic cells** from animals can be cultured from single cells in a well-defined medium, it is possible to select for genetically distinct cultured animal cells, just as is done with bacterial and yeast cells. Moreover, during mitosis the chromosomes in an animal cell are large and highly visible after staining, making it easy to distinguish individual chromosomes. Genetic studies of cultured animal cells are called *somatic-cell genetics* to distinguish them from *classical genetics,* which deals with whole organisms derived from **germ cells** (sperm and eggs).

Cultured cells from different mammals can be fused to produce interspecific hybrids, which have been widely used in somatic-cell genetics. For instance, hybrids can be prepared from human cells and mutant mouse cells that lack an enzyme required for synthesis of a particular essential metabolite. As the human-mouse hybrid cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. In a medium that can support growth of both the human cells and mutant mouse cells, the hybrids eventually lose all human chromosomes. However, in a medium lacking the essential metabolite that the mouse cells cannot produce, the one human chromosome that contains the gene encoding the needed enzyme will be retained, because any hybrid cells that lose it following mitosis will die. All other human chromosomes eventually are lost.

By using different mutant mouse cells and media in which they cannot grow, researchers have prepared various panels of hybrid cell lines. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes. Because each chromosome can be identified visually under a light microscope, such hybrid cells provide a means for assigning, or "mapping," individual genes to specific chromosomes. For example, suppose a hybrid cell line is shown microscopically to contain a particular human chromosome. That hybrid cell line can then be tested biochemically for the presence of various human enzymes, exposed to specific antibodies to detect human surface antigens, or subjected to DNA hybridization and cloning techniques to locate particular human DNA sequences. The genes encoding a human protein or containing a human DNA sequence detected in such tests must be located on the particular human chromosome carried by the cell line being tested. Panels of hybrids between normal mouse and mutant hamster cells also have been established; in these hybrid cells, the majority of mouse chromosomes are lost, allowing mouse genes to be mapped to specific mouse chromosomes.

Hybrid Cells Often Are Selected on HAT Medium

One metabolic pathway has been particularly useful in cell-fusion experiments. Most animal cells can synthesize the purine and pyrimidine nucleotides de novo from simpler carbon and nitrogen compounds, rather than from already formed purines and pyrimidines. The folic acid antagonists amethopterin and aminopterin interfere with the donation of methyl and formyl groups by tetrahydrofolic acid in the early stages of de novo synthesis of glycine, purine nucleoside monophosphates, and thymidine monophosphate. These drugs are called *antifolates,* since they block reactions involving tetrahydrofolate, an active form of folic acid. Many cells, however, contain enzymes that can synthesize the necessary nucleotides from purine bases and thymidine if they are provided in the medium; these *salvage pathways* bypass the metabolic blocks imposed by antifolates.



De novo and salvage pathways for nucleotide synthesis

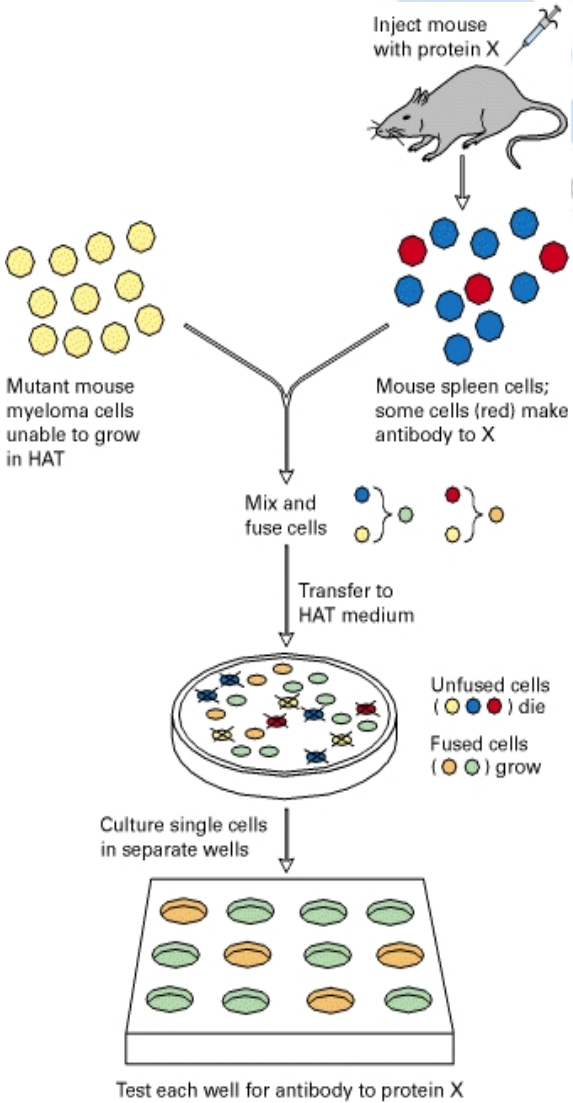
In a normal medium, cultured animal cells synthesize purine nucleotides (AMP, GMP, IMP) and thymidylate (TMP) by de novo pathways (blue). These require the transfer of a methyl or formyl group from an activated form of tetrahydrofolate (e.g., N^5,N^{10}-methylenetetrahydrofolate), as shown in the upper portion of the diagram. Antifolates, such as aminopterin and amethopterin, block the reactivation of tetrahydrofolate, preventing purine and thymidylate synthesis. Normal cells can also use salvage pathways (red) to incorporate purine bases or nucleosides and thymidine added to the medium. Cultured cells lacking one of the enzymes of the salvage pathways—HGPRT, APRT, or TK—will not survive in media containing antifolates

De novo and salvage pathways for nucleotide synthesis. In a normal medium, cultured animal cells synthesize purine nucleotides (AMP, GMP, IMP) and thymidylate (TMP) by de novo pathways.

A number of mutant cell lines lacking the enzyme needed to catalyze one of the steps in a salvage pathway have been isolated. For example, cell lines lacking thymidine kinase (TK) can be selected because such cells are resistant to the otherwise toxic thymidine analog 5-bromodeoxyuridine. Cells containing TK convert 5-bromodeoxyuridine into 5-bromodeoxyuridine monophosphate. This nucleoside mono- phosphate is then converted into a nucleoside triphosphate by other enzymes and is incorporated by DNA polymerase into DNA, where it exerts its toxic effects. This pathway is blocked in cells with a *TK* mutation that prevents production of functional TK enzyme. Hence, TK^- mutants are

resis- tant to the toxic effects of 5-bromodeoxyuridine. Similarly, cells lacking the HGPRT enzyme have been selected because they are resistant to the otherwise toxic guanine analog 6-thioguanine. As we will see next, $HGPRT^-$ cells and TK^- cells are useful partners in cell fusions with one another or with cells that have salvage-pathway enzymes but that are differentiated and cannot grow in culture by themselves.

The medium most often used to select hybrid cells is called *HAT medium*, because it contains hypoxanthine (a purine), aminopterin, and thymidine. Normal cells can grow in HAT medium because even though aminopterin blocks de novo synthesis of purines and TMP, the thymidine in the media is transported into the cell and converted to TMP by TK and the hypoxanthine is transported and converted into usable purines by HGPRT. On the other hand, neither TK^- nor $HGPRT^-$ cells can grow in HAT medium because each lacks an enzyme of the salvage pathway. However, hybrids formed by fusion of these two mutants will carry a normal *TK* gene from the $HGPRT^-$ parent and a normal *HGPRT* gene from the TK^-parent. The hybrids thus will produce both functional salvage-pathway enzymes and grow on HAT medium. Likewise, hybrids formed by fusion of mutant cells and normal cells can grow in HAT medium.



Hybridomas Are Used to Produce Monoclonal Antibodies

Each normal B **lymphocyte** in an animal is capable of producing a single type of antibody directed against a specific determinant, or epitope, on an antigen molecule. If an animal is injected with an antigen, B lymphocytes that make antibody recognizing the antigen are stimulated to grow and proliferate. Each antigen-activated B lymphocyte forms a clone of cells in the spleen or lymph nodes, with each cell of the clone producing identical antibody, termed monoclonal antibody. Because most natural antigens contain multiple epitopes, exposure of an animal to an antigen usually stimulates formation of several different B-lymphocyte clones, each producing a different antibody;

a mixture of antibodies that recognize different epitopes on the same antigen is said to be *polyclonal.*

Immortal myeloma cells that lack HGPRT, an enzyme of the purine-salvage pathway are fused with normal antibody-producing spleen cells from an animal that was immunized with protein X. The spleen cells can make HGPRT. When plated in HAT medium, the unfused cells do not grow: the mutant myeloma cells because they cannot make purines via the salvage pathway, and the spleen cells because they have a limited life span in culture. Thus only fused cells, formed from a myeloma cell and a spleen cell, survive on HAT medium, proliferating into clones called *hybridomas.* Each hybridoma produces a single antibody. Once a hybridoma that produces a desired antibody is identified, the clone can be cultured to yield large amounts of that antibody.

For many types of studies involving antibodies, monoclonal antibody is preferable to polyclonal antibody. However, biochemical purification of monoclonal antibody from serum is not feasible, in part because the concentration of any given antibody is quite low. For this reason, researchers looked to culture techniques in order to obtain usable quantities of monoclonal antibody. Because primary cultures of normal B lymphocytes do not grow indefinitely, such cultures have limited usefulness for production of monoclonal antibody. This limitation can be avoided by fusing normal B lymphocytes with oncogenically transformed lymphocytes called *myeloma cells,* which are immortal.

Fusion of a myeloma cell with a normal antibody-producing cell from a rat or mouse spleen yields a hybrid that proliferates into a clone called a hybridoma. Like myeloma cells, hybridoma cells are immortal. Each hybridoma produces the monoclonal antibody encoded by its B-lymphocyte partner. Many different myeloma cell lines from mice and rats have been established; from these, $HGPRT^-$ lines have been selected based on their resistance to 6-thioguanine as described above. If such mutant myeloma cells are fused with normal B lymphocytes, any fused cells that result can grow in HAT medium, but the parental cells cannot .Each selected hybridoma then is tested for production of the desired antibody; any clone producing that antibody then is grown in large cultures, from which a substantial quantity of pure monoclonal antibody can be obtained.

Procedure for producing a monoclonal antibody to protein X. Immortal myeloma cells that lack HGPRT, an enzyme of the purine-salvage pathway are fused with normal

Such pure antibodies are very valuable research reagents. For example, a monoclonal antibody that interacts with protein X can be used to label, and thus locate, protein X in specific cells of an organ or in specific cell fractions. Once identified, even very scarce proteins can be isolated by affinity chromatography in columns to which the monoclonal antibody is bound. Monoclonal antibodies also have become important diagnostic and therapeutic tools in medicine. Monoclonal antibodies that bind to and inactivate toxic proteins (toxins) secreted by bacterial pathogens are used to treat diseases caused by these pathogens. Other monoclonal antibodies are specific for cell-surface proteins expressed by

certain types of tumor cells; chemical complexes of such monoclonal antibodies with toxic drugs are being developed for cancer chemotherapy.

Q.18. What are transgenic animals? How are they produced? Write their applications in brief.

Ans. The ability to produce transgenic mice and particularly the ability to perform specific changes in a predetermined gene by gene targeting has permitted the design of many new animal models of human disease .Another experimental approach involving genetic manipulation of animals has had a major impact recently. In 1997 a new era in mammalian genetics was heralded when the procedure of cell nuclear transfer permitted 'cloning' of an adult mammal for the first time. This involved transfer of the nucleus from an adult cell into an enucleated oocyte and the technology has subsequently been applied as an alternative route to generating transgenic animals.

The creation and applications of transgenic animals

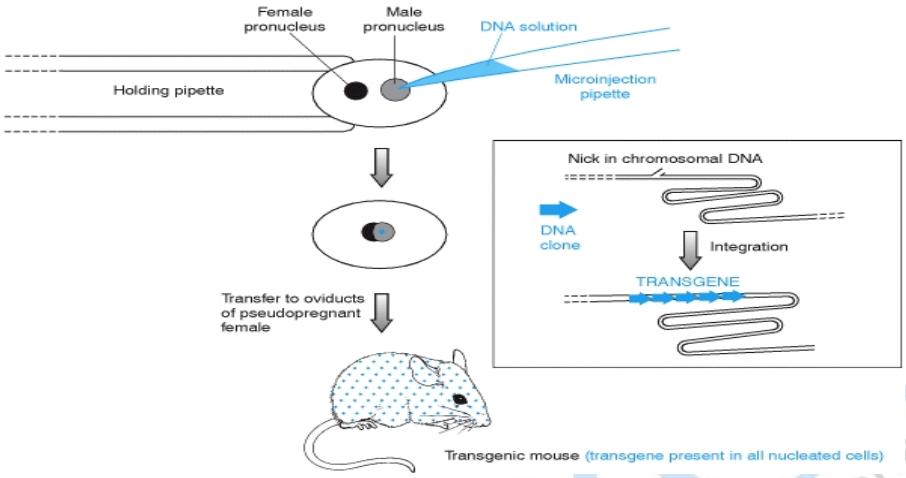
Of the different transgenic animals that have been made thus far, transgenic *Drosophila*, transgenic frogs, and transgenic fish have been very important for understanding aspects of gene function and development in these species. Transgenic sheep and other transgenic livestock animals have been produced largely to serve as bioreactors, whole-animal expression cloning systems in which introduced genes are expressed to give large amounts of therapeutic or commercially valuable gene products. But it has been transgenic mice that have been the most useful to biomedical research, both in providing animal models of disease and in permitting the most useful analyses of mammalian gene function.

Transgenic animals can be produced following transfer of cloned DNA into fertilized oocytes and cells from very early stage embryos

Transgenesis involves transfer of foreign DNA into totipotent or pluripotent embryo cells (either fertilized oocytes, cells of the very early embryo or cultured embryonic stem cells) followed by insertion of the transferred DNA into host chromosomes. If the foreign DNA integrates into the chromosomes of a fertilized oocyte, the developing animal will be *fully transgenic* since all nucleated cells in the animal should contain the transgene. If chromosomal integration occurs later, at a postzygotic stage, the animal will be a mosaic, with some cells containing the transgene and some others lacking it. If the transgene is present in germline cells it can be passed through sperm or egg cells into some of the animal's progeny, and PCR-based tests can be used to quickly screen for the presence of the transgene. Progeny that are transgene positive can be expected to be fully transgenic; all their cells should have developed from a fertilized oocyte containing the transgene.

Pronuclear microinjection

To obtain transgenic mice by this route, females are superovulated, mated to fertile males and sacrificed the next day. Fertilized oocytes are recovered from excised oviducts.



The DNA of interest is then microinjected using a micromanipulator into the male pronucleus of individual oocytes. Surviving oocytes are reimplanted into the oviducts of foster females and allowed to develop into mature animals.

Very fine glass pipettes are constructed using specialized equipment: one, a holding pipette, has a bore which can accommodate part of a fertilized oocyte, and thereby hold it in place, while the microinjection pipette has a very fine point which is used to pierce the oocyte and thence the *male* pronucleus (because it is bigger). An aqueous solution of the desired DNA is then pipetted directly into the pronucleus. The introduced DNA clones can integrate into chromosomal DNA at nicks, forming transgenes, usually containing multiple head-to-tail copies. Following withdrawal of the micropipette, surviving oocytes are reimplanted into the oviducts of *pseudopregnant* foster females (which have been mated with a vasectomized male; the mating act can initiate physiological changes in the female which stimulate the development of the implanted embryos). Newborn mice resulting from development of the implanted embryos are checked by PCR for the presence of the desired DNA sequence.

During this procedure, the microinjected DNA (transgene) randomly integrates into chromosomal DNA, usually at a single site, although rarely two sites of integration are found in a single animal. Individual insertion sites typically contain multiple copies of the transgenes integrated into chromosomal DNA as head-to-tail concatemers (it is not unusual to find 50 or more copies at a single insertion site). As a result of chromosomal integration, the transgenes can be passed on to subsequent generations in mendelian fashion: if the foreign DNA has integrated at the one-cell stage, it should be transmitted to 50% of the offspring.

Transfer into pre- or postimplantation embryos

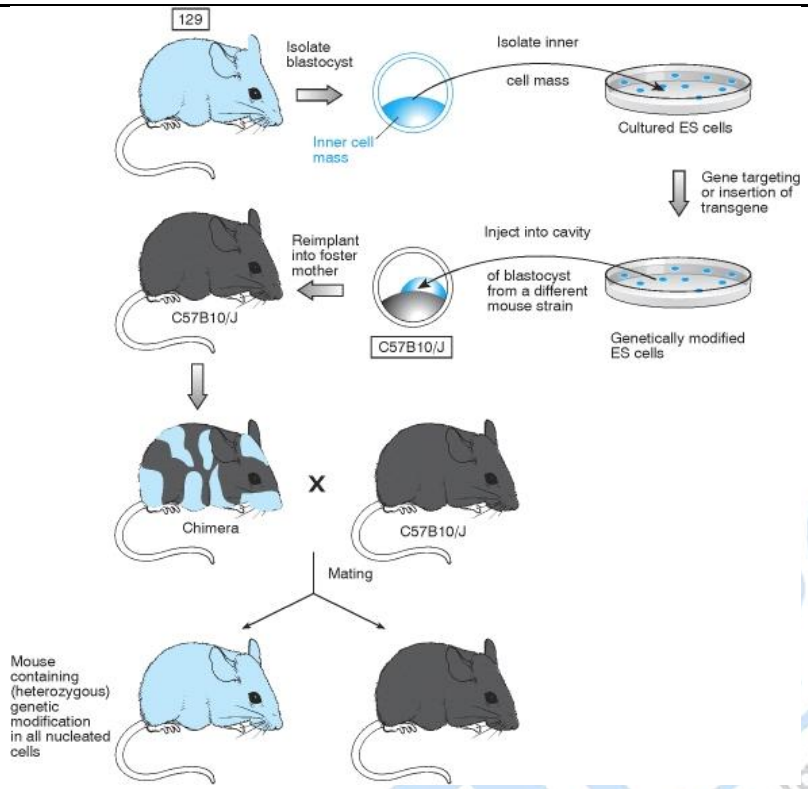
Cells from very early stage embryos may be totipotent or at least pluripotent and can provide a route for foreign DNA to enter the germline. DNA can be transferred into unselected cells of very early embryos, as described in this section or into cell lines derived from embryonic stem cells.

One method that allows foreign DNA to integrate into the chromosomes of the target cells uses retroviruses, RNA viruses which naturally undergo an intermediate DNA form prior to integrating into cellular genomes. Infection of preimplantation mouse embryos with a retrovirus such as Moloney murine leukemia virus or injection of the retrovirus into early postimplantation mouse embryos results in mosaic offspring. Retroviruses should integrate rarely and at random into accessible cells, and the use of replication- defective retroviruses provides heritable markers for clonal descendants of the target cell (unlike wild-type viruses which spread from cell to cell). This approach has been used, therefore, for studying cell lineage using reporter genes.

In *Drosophila*, efficient chromosomal integration is possible by using sequences from a *Drosophila* transposable element known as the P element to permit insertion of single copies of a gene at random in the genome. The gene or DNA fragment to be inserted is first manipulated so as to be flanked by the two terminal sequences of the P element. The modified DNA is then microinjected into a very young *Drosophila* embryo along with a separate plasmid containing a gene encoding a transposase. In the presence of the transposase the terminal P element sequences allow the intervening DNA fragment to transpose and as a result of the ensuing transposition events, the injected DNA often enters the germline in a single copy.

Cultured embryonic stem (ES) cells provide a powerful route to genetic modification of the germline

The microinjection of foreign DNA into fertilized oocytes is technically difficult and not suited to large-scale production of transgenic animals or to sophisticated genetic manipulation. A popular alternative, but one which has so far been restricted to the construction of genetically modified mice, involves transferring the foreign DNA initially into cultured **embryonic stem** (**ES**) **cells**. Mouse ES cells are derived from .5–.5 day postcoitum embryos and arise from the *inner cell mass* of the blastocyst. The ES cells can be cultured *in vitro* and retain the potential to contribute extensively to all of the tissues of a mouse, *including the germline*, when injected back into a host blastocyst and reimplanted in a pseudopregnant mouse.



Cells from the *inner cell mass* were cultured following excision of oviducts and isolation of blastocysts from a suitable mouse strain. Such embryonic stem (ES) cells retain the capacity to differentiate into, ultimately, the different types of tissue in the adult mouse. ES cells can be genetically modified while in culture by insertion of foreign DNA or by introducing a subtle mutation. The modified ES cells can then be injected into isolated blastocysts of another mouse strain (e.g. C57B10/J which has a black coat color that is recessive to the agouti color of the 129 strain) and then implanted into a pseudopregnant foster mother of the same strain as the blastocyst. Subsequent development of the introduced blastocyst results in a chimera containing two populations of cells (including germline cells) which ultimately derive from different zygotes (normally evident by the presence of differently colored coat patches). Backcrossing of chimeras can produce mice that are heterozygous for the genetic modification. Subsequent interbreeding of heterozygous mutants generates homozygotes.

Transgenic animals have been used for a variety of studies investigating mammalian gene expression and function

Transgenic animals have been extremely important for analyzing human genes and have helped greatly in our understanding of a variety of fundamental biological processes, notably in immunology, neurobiology, cancer and developmental studies. The following list is far from exhaustive but is intended to illustrate some major types of application:

- ***Investigating gene expression and its regulation***. Although evidence for *cis*-acting regulatory elements is often inferred initially from studies using cultured cells, they need to be validated in whole animal studies. Transgenes consisting of the presumptive regulatory sequence(s) coupled to a reporter gene, such as *lacZ*, provide a sensitive method of detecting gene expression and a powerful way of investigating regulation of gene expression. Long-range control of gene expression is often investigated using YAC transgenes.
- ***Investigating gene function by targeted gene inactivation***. Specific genes can be inactivated by a gene targeting procedure to introduce a transgene into the target gene (insertional inactivation). The effect on the phenotype of creating a null mutation in the gene of interest can provide powerful clues to gene function.
- *Investigating dosage effects and ectopic expression.* In some cases, valuable information can be gained by over-expressing a transgen or by expressing it *ectopically* (the transgene is coupled to a tissue-specific promoter which causes expression in cells; the phenotypic consequence may provide valuable clues to function).
- ***Cell lineage ablation***. Transgenes can be designed consisting of a tissue-specific promoter coupled to a sequence encoding a toxin, for example diphtheria toxin subunit A or ricin. When the promoter becomes active at the appropriate stage of tissue differentiation, the toxin is produced and kills the cells. Thus, certain cell lineages in the animal can be eliminated (cell ablation) and the phenotypic consequences monitored.
- ***Investigating gain of function***. In principle, any mammalian gene that produces a dominant negative effect or gain of function can be investigated by introduction of an appropriate transgene. In some cases, this can provide proof of a suspected biological function. A classical example concerns the *Sry* gene. A variety of different genetic analyses had implicated this gene as a major male-determining gene but convincing proof was obtained using a transgenic mouse approach. The experiment consisted of transferring a cloned *Sry* gene into a fertilized 6,XX mouse oocyte. As a result of this artificial intervention, the resulting mouse, which nature had intended to be female, turned out to be male.
- ***Modeling human disease***. Insertional inactivation is often used to model loss of function mutations whilst gain of function mutations can often be modeled by inserting a mutant transgene.

The use of YAC transgenes and inducible promoters has greatly extended the applications of transgenic animals.

YAC transgenics have permitted study of large genes such as the 00 kb human *APP* gene, a gene known to contribute to Alzheimer's disease, encoding the amyloid protein precursor. A YAC transgene containing *APP* showed tissue- and cell type-specific expression patterns closely mirroring that of the endogenous mouse gene.

Q.19. Write a note on 'Biotechnology and society' emphasizing the impact of genomic research on society.

Ans. The field of biotechnology has had a lot of beneficial contribution in the area of healthcare, agriculture, food production, manufacture of industrial enzymes, and appropriate environmental management. However, the advancement in this field has also lead to some concerns and controversies raised by a number of groups, NGOs etc. ELSI is the short form to represent the ethical, legal, and social implications of biotechnology. ELSI broadly covers the relationship between BIOTECHNOLOGY and SOCIETY with particular reference to ethical and legal aspects.

Concerns about the Genetically modified organisms (GMOs)

There are concerns regarding the biosafety, ethics and issues related to the release of GMOs in the environment. Many contries and NGOs have opposed the release of the GMOs due to these reasons. In order to address theses issues, the UNIDO/WHO/FAO/UNEP has built up an Informal Working Group on Biosafety. In 1991, this group prepared the " Voluntary Code of Conduct for the release of Organisms into the Environment". The ICGEB organizes annual workshops on biosafety and on risk assessment for the release of GMOs. It collaborates with the management of UNIDO's BINAS (Biosafety Information Network and Advisory Service), whose aim is to monitor the global development in regulatory issues in biotechnology. An on-line bibliographic data-base on biosafety and risk assessment has also been created by ICGEB to evaluate the environmental release of GMOs.

Besides this, the ICGEB also assists its member states in developing the national biosafety framework.

The main areas of consideration for safety aspects in biotechnology are the following:

a) How to dispose off spent microbial biomass and purify the effluents from biotechnological processes?

b) The toxicity of the allergy associated with microbial production.

c) How to deal with the increase in the number of antibiotic resistant pathogenic microorganisms?

d) How to evaluate the pathogenicity of the genetically engineered microorganisms to infect humans, plants and animals?

e) How to prevent contamination, infection or mutation of the processed strains?

f) The evaluation of the interaction of the genetically engineered microbes with the elements of natural environment.

In the past, time and again, there has been public outrage against the use of genetically modified or transgenic plants and other organisms. In 1999, a British medical journal published the adverse effect of genetically modified (GM) potato (which was produced by

Rowett Research Insitute). This potato was found to contain snow drop lectin which affected the small intestine of the rats and stunted the growth and damaged their immune system. This led to worldwide public concern about this issue and created a lot of controversy about the safety of GM foods.

The transgenic Bt-plants such as cotton, corn, soybean, and potato were approved for cultivation in USA. However, some countries did not allow Bt-plants in their fields e.g. Br-rice was not allowed in Philippines, Bt-cotton in France. Many Governments are also suspicious of the use of GMOs due to various reason-risks, societal beliefs, and economic concerns.

Biological Warfare

Most of the countries of the world are signatories to the Biological Weapons Conventions of 1972. As a signatory, it is a voluntary pledge by a nation "never to produce microbial or other biological agents or toxins, whatever may be their method of production, for use in wars. However, many people have expressed their concerns about the possible use of genetic manipulations for military purposes in the near future.

Intellectual Property

With the fast pace development in the field of biotechnology, the issues related to legal characterization and the treatment of trade related biotechnological processes and products are of immense importance. These are popularly known as Intellectual Property. Intellectual Property includes Patents, trade secrets, copyrights, and trademarks. Intellectual Property Rights (IPR) is a collective term applied to a number of different types of legal rights granted by each country. The rights to protect this property prohibits others from making, copying, using or selling the proprietary subject matter.

In biotechnology, the intellectual property covers the processes and products which result from the development of genetic engineering techniques through the use of restriction enzymes to create recombinant DNA.

Another example of intellectual property is the development of crop varieties which are protected through "plant breeder's rights or PBRs. The PBRs ensures that the plant breeder who developed a particular variety gets the exclusive rights for marketing the variety.

Agriculture for the first time was included in the trade related intellectual property rights (TRIPS) and TRIPS is a major concern for developing countries. The following two major steps were taken in consideration of PBRs:
(a) The Food and Agriculture Organisation (FAO) has an International treaty on plant genetic resources for food and agriculture. This treaty consists of a particular classes which refers to operation of farmer's rights.

(b) The 'Plant Varietal Protection and Farmer's Rights Act 2001 agrees for the right of farmers, breeders, and researchers. The protection is provided by making compulsory licensing of rights, and inhibiting the import of plant varieties consisting of 'genetic use of restriction technology' (GURT) e.g. terminator technology of Monsanto.

Following conditions should be fulfilled to grant protection to the new varieties:

a) the new variety must always be new i.e. it should not have ever been exploited commercially.
b) It should be biologically distinct and possess different characters.
c) The new variety of the plant must have uniform characters.
d) The distinguishing character of new variety must be stable for generations.
e) The new variety should have taxonomic validity i.e. systematic position, generic and species names etc.

Recently Utility patents for both plant and animal genetic materials, have been allowed in some countries. This forbids the use of patented material for further breeding. The farmers are allowed to use and save the seeds for cultivation only after paying a fee to the patent holder.

Some concerns have been voiced regarding the implications of IPR on the genetic diversity and the conservation of genetic resources. IPRs will directly or indirectly affect the food security and distribution around the globe, biological diversity and ecological balance, employment avenues in the poor and developing countries, and the use of new and effective agricultural practices.

Q.20. How Biotechnology is important in medicine?

Ans. Biotechnology has wide range of applications in animal and human health care both directly as well as indirectly. It's direct use involves controlling the spread of a variety of diseases using gene manipulation at different levels. The indirect use is in manufacturing of nutritional food through single cell proteins and in detecting food contaminants through molecular probes.

Following approaches are used to control the diseases and thereby taking care of the human and animal health.

a) Providing immunity against the disease through the development of vaccine.
b) Early diagnosis of a disease.
c) Successful treatment of the disease.

Q.21. How vaccines are produced? Write their significance.

Ans. Biotechnology is used in three different ways in the development of vaccine:

a) Separation of a pure antigen using a specific monoclonal antibody.
b) Synthesis of an antigen with the help of a cloned gene.
c) Synthesis of peptides to be used as vaccines.

Use of monoclonal antibodies for immunopurification of antigens

The method of immunopurification using monoclonal antibodies is used, to separate specific antigen from a mixture of very similar antigens. Once purified, the antigen is used for developing vaccine against a pathogen. Individual interferons (which have the property of inhibiting viral infection and cell proliferation) have been purified using this technique. These interferons were later used for clinical trials and then commercially used.

Use of cloned genes for the synthesis of antigens

Hundreds of genes in eukaryotes have been cloned from genomic DNA or from cDNA. These clones genes included a number of genes for specific antigens and some have been used for the synthesis of antigens leading to the preparation of vaccines. A very good example of this is cloning of Hepatitis B virus (HBV) genome. The HBV genome was cloned in the plasmid pBR22 followed by it's propagation in ***E.coli***. The antigens produced from this clone reacted with hepatitis B core antibody (HBAb) which has been used to produce hepatitis B vaccine.

Efforts are on to use this method to produce an Anti- malarial vaccine. Malarial parasite *Plasmodium falciparum* is the malarial parasite which has become a major threat to human health which spreads by the mosquito bite. In human body, the malarial parasite passes through several antigenically distinct phases viz. sporozoite, merozoite, gametocytes etc. The vaccines can be developed to control any of these phases hence we can have antisporozoite vaccine, antimerozoite vaccine etc. Out of all these, considerable progress has been made in the making of antisporozoite vaccine due to cloning of gene meant for circumsporozoite (CS) protein. This protein was obtained directly from DNA of erythrocytic form of parasite, rather than as cDNA from mRNA.This cloned gene may, in course of time, lead to the synthesis of vaccine by synthesizing CS protein by clone gene.

Synthetic peptides as vaccines

Vaccines can also be prepared through short synthetic peptide chains. There are several ways by which these can be used as vaccines.

As it is the three dimensional structure (not the amino acid sequence) of the protein which is responsible for the immunogenic response, it is essential to find out the protein region involved in immunogenic response. E.g. in Foot and Mouth Disease virus (FMDV), the amino acid 11-160 of virus polypeptide can produce antibodies which can neutralize FMDV and provide protection. The region of 201-21 amino acids of the same protein also

could neutralize FMDV hence it has been shown that small synthetic peptides representing these regions of proteins can show immunogenic response and can be used for the development of vaccine.

The immunogenic region of protein can also be located by gene coding for the protein. E.g. in Feline leukaemia virus, the clone gene of an immunogenic protein was cut into fragments by DNAase I and then cloned in lambda phage. Phage colonies (plaques) with different cloned fragments are screened with a specific monoclonal antibody that neutralizes the pathogen. The fragments which react with antibody must be synthesizing the immunogenic peptide fragments which can be sequenced. Using this method it was possible to identify a 1 amino acid immunogen of the envelope protein of Feline leukaemia virus (FLV). The corresponding synthetic peptide was also found to compete with the virus for antibody.

The immunogenic region of a protein in a pathogen can also be identified by eluting it from purified major histocompatibility complex (MHC) molecules. Different MHC allelic variants bind with different proteins and purified using specific T cells. Peptides can be eluted from these purified MHC molecules and later on sequenced. The sequenced peptides are used to make synthetic peptides which are used as vaccines.

Q.21. What is artificial blood?

Ans. It should be clarified that the term "artificial blood" is really a misnomer. The complexity of blood is far too great to allow for absolute duplication in a laboratory. Instead, researchers have focused their efforts on creating artificial substitutes for 2 important functions of blood: oxygen transport by red blood cells and hemostasis by platelets.

Red Blood Cell Substitutes

Two major types of red cell substitutes are under development: hemoglobin based and perfluorocarbon (PFC) based. PFCs are completely synthetic hydrocarbon-based compounds and will be discussed later. The hemoglobin-based substitutes use hemoglobin from several different sources: human, animal, and recombinant. Human hemoglobin is obtained from donated blood that has reached its expiration date and from the small amount of red cells collected as a by-product during plasma donation. One unit of hemoglobin solution can be produced for every 2 units of discarded blood. There is a concern that the worsening shortage of blood donors will eventually limit the availability of human hemoglobin for processing. The companies that use human hemoglobin are confident in their supply, especially from the plasma centers that use paid donors.

Animal hemoglobin is obtained from cows. This source creates some apprehension regarding the possible transmission of animal pathogens, specifically bovine spongiform encephalopathy. The Biopure Corporation, which uses bovine hemoglobin, has an affiliation with a local breeding farm, allowing close monitoring of the health and diet of the animals. The company is very confident about the safety of its product. Forty units of hemoglobin solution can be obtained per slaughtered cow.

Surface-modified hemoglobin

Surface-modified hemoglobin is created by attaching large molecules, such as polyethylene glycol, to surface lysine groups. This modification also increases the viscosity and oncotic pres-sure of the solution. Two companies, Enzon and Apex Bioscience, have developed surface-modified hemoglobin solutions. Cross-linked hemoglobin

Polymerized hemoglobin

To polymerize hemoglobin, surface amino acid groups are linked by reagents such as glutaraldehyde. Polymerized hemoglobin is the only product to date that has not triggered significant vasoconstriction after infusion. Three companies currently have products in phase III clinical trials; all products could receive approval from the Food and Drug Administration (FDA) in 2002.

Perfluorocarbon-based substitutes

PFC-based solutions have been in development for several decades. An article by Clark and Gollan in the 1960s contained the famous photo of a mouse submerged in a container and "breathing" liquid.

Adverse reactions and limitations in the use of oxygen-carrying solutions

Adverse reactions associated with hemoglobin-based products include elevations in blood pressure, gastrointestinal dysmotility, and mild, temporary increases in pancreatic enzymes. Patients also develop jaundice due to the infusion of free hemoglobin. Treatment with PFC-based products can cause mild thrombocytopenia (10% to 15% decrease) and a flulike syndrome. Because patients need to be on high concentrations of oxygen when PFCs are used, the risk of oxygen toxicity exists with prolonged administration. Since both types of products are taken up by human macrophages, there is also the theoretical risk that macrophage function will be altered.

All current red cell substitutes have a short duration of action—lasting only about 2 hours in the circulation —and are very expensive, with estimates at $500 per unit. Finally, use of these products can interfere with clinical laboratory testing (11–1). Hemoglobin solutions will make the patient's blood specimens appear hemolyzed, and PFC solutions can produce lipemia. Both factors can affect the results obtained by some test systems. Close communication between the clinicians using the products and the laboratory will have to occur if reliable test results are to be reported.

Platelet Substitutes

The greatest progress in the field of blood substitutes has been with the oxygen-carrying solutions. However, research on platelet substitutes has been under way since the 1950s. One of the biggest factors pushing the need for platelet alternatives is the 5-day shelf life of the current blood product. This rapid outdate adds additional constraints to an already limited supply. The platelets are also stored at room temperature, thus increasing the risk of bacterial overgrowth. The risk of bacterial contamination of random donor platelets has

been estimated to be 1:1500. Ideally, a platelet substitute would have the following properties: effective hemostasis with a significant duration of action, no associated thrombogenicity, no immunogenicity, sterility, long shelf life with simple storage requirements, and easy preparation and administration. Several different forms of platelet substitute are now under development: infusible platelet membranes (IPM), thrombospheres, and lyophilized human platelets. Only one product, IPM, is currently in clinical trials in the USA.

Infusible platelet membranes

Infusible platelet membranes are produced from outdated human platelets. The source platelets are fragmented, virally inactivated, and lyophilized; they can then be stored up to 2 years. Although the platelet membranes still express some blood group and platelet antigens, they appear to be resistant to immune destruction.

Thrombospheres

Thrombospheres (Hemosphere, Irvine, Calif) are not platelets; they are composed of cross-linked human albumin with human fibrinogen bound to the surface. The mechanism of action has not yet been elucidated. Experimentally, the thrombospheres appear to enhance platelet aggregation but do not themselves activate platelets.

Lyophilized human platelets

A lyophilized platelet product has been under development since the late 1950s. The current process involves briefly fixing human platelets in paraformaldehyde prior to freeze-drying in an albumin solution .The fixation step kills microbial organisms, and the freeze-drying greatly increases the shelf life.

Q .22. Write short notes on the following
i. Antibiotics
ii.PCR

Ans. **Antibiotics :-** An **antibacterial** is a compound or substance that kills or slows down the growth of bacteria. The term is often used synonymously with the term *antibiotic(s)*; today, however, with increased knowledge of the causative agents of various infectious diseases, *antibiotic(s)* has come to denote a broader range of antimicrobial compounds, including antifungal and other compounds. Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics. Other antibiotics simply lack advantage over those already in use, or have no other practical applications.Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly modified. A more modern version of this approach is a rational design program.

This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

Industrial production techniques

Antibiotics are produced industrially by a process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is simpler to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

Strains used for production

Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more. Another technique used to increase yields is gene amplification, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids. This process must be closely linked with retesting of antibiotic production and effectiveness.

PCR :- The **polymerase chain reaction** (**PCR**) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.Developed in 198 by Kary Mullis, PCR is now a common and often indispensable technique used in medical, chemical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases. In 199, Mullis was awarded the Nobel Prize in Chemistry along with Michael Smith for his work on PCR.

The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase (after which the method is named) are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA

template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium Thermus aquaticus. This DNA polymerase enzymatically assembles a new DNA strand from DNA building-blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides (also called DNA primers), which are required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary first to physically separate the two strands in a DNA double helix at a high temperature in a process called DNA melting. At a lower temperature, each strand is then used as the template in DNA synthesis by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions.

PCR is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 0 kb in size.

A basic PCR set up requires several components and reagents. These components include:

- *DNA template* that contains the DNA region (target) to be amplified.
- Two primers that are complementary to the ' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
- Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C.
- *Deoxynucleoside triphosphates* (dNTPs; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand.
- Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- Divalent cations, magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis
- *Monovalent cation* potassium ions.

The PCR is commonly carried out in a reaction volume of 10–200 µl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction. Many modern thermal cyclers make use of the Peltier effect, which permits both heating and cooling of the block holding the PCR tubes simply by reversing the electric current. Thin-walled reaction tubes permit favorable thermal conductivity to allow for rapid thermal equilibration. Most thermal cyclers have heated lids to prevent condensation at the top of

the reaction tube. Older thermocyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

Q.23 How Biotechnology can be useful in waste-water treatment?

Ans. The sewage is defined as the waste water resulting from the various human activities, agriculture and industries and mainly contains organic and inorganic compounds, toxic substances, heavy metals and pathogenic organisms. The sewage is treated to get rid of these undesirable substances by subjecting the organic matter to biodegradation by microorganisms. The biodegradation involves the degradation of organic matter to smaller molecules (CO2, NH, PO etc.) and requires constant supply of oxygen. The process of supplying oxygen is expensive, tedious, and requires a lot of expertise and manpower. These problems are overcome by growing microalgae in the ponds and tanks where sewage treatment is carried out. The algae release the O2 while carrying out the photosynthesis which ensures a continuous supply of oxygen for biodegradation. The algae are also capable of adsorbing certain heavy toxic metals due to the negative charges on the algal cell surface which can take up the positively charged metals. The algal treatment of sewage also supports fish growth as algae is a good source of food for fishes. The algae used for sewage treatment are Chlorella, Euglene, Chlamydomnas, Scenedesmus, Ulothrix, Thribonima etc.

Q.24 What do you understand by Biomining?

Ans. Biomining is the extraction of specific metals from their ores through biological means usually bacteria. Although it is a new technique used by the mining industry to extract minerals such as copper, uranium and gold from their ores but nowadays biomining occupies an increasingly important place among the available mining technologies. Today biomining is no longer a promising technology but an actual economical alternative for treating specific mineral ores.Traditional extractions involve many expensive steps such as roasting and smelting, which requires sufficient concentrations of elements in ores while low concentrations are not a problem for bacteria because they simply ignore the waste which surrounds the metals, attaining extraction yields of over 90% in some cases.

Biological Tools: A variety of mineral oxidizing bacteriareadily found can easily oxidize iron and sulfurcontaining minerals. These include the iron- and sulfuroxidizing *Acidithiobacillus ferrooxidans* (previously,*Thiobacillus ferrooxidans*), the sulfur-oxidizing *Acidithiobacillus thiooxidans* (previously *Thiobacillus thiooxidans*) and *Acidithiobacillus caldus* (previously, *Thiobacillus caldus*) and the iron-oxidizing*Leptospirillum ferrooxidans* and *Leptospirillumferriphilum*. Several species of fungi can be used for biomining. Experiments have shown, that two fungal strains *Aspergillus niger* and *Penicillium simplicissimum* were able to mobilize Cu and Sn by 65% and Al, Ni, Pb and Zn by more than 95% . Similiarly, 'Phytomining' is based on the tendency of some plant species to bioaccumulate excessive amounts of metals from their host rock. The plants, called *hyperaccumulators* are grown on highly mineralized soils or post-mine lands and their yield (bio-ore) is used as a pure metal source . Compared to the

bacterial mining, these technologies are not so popular primarily because of the longevity of these processes and so their unprofitability.

Q.25. Write an essay on 'Fermentation technology'?

Ans. The process of fermentation is defined as "a biological process that occurs in the absence of oxygen or under anaerobic conditions. The word "Fermentation" originates from verb of Latin origin "fervere" which means to boil. However in the modern times the Industrial fermentation is used for large- scale cultivation of microorganisms which are largely aerobic in nature. Instead of fermentation technology, the term Bioprocess technology is more in use as bioprocessing involves a variety of enzyme-catalysed reactions carried out by living cells (or cell free systems) in industrial setups. The most important equipment in use for bioprocessing is a Fermenter or a Bioreactor.

Bioreactor

A bioreactor is a device in which the microorganisms are cultivated and motivated to form the desired products by maintaining optimum conditions for growth and metabolic activity. A fermenter refers to the device used for the cultivation of prokaryotic cells e.g. bacteria, fungi etc. whereas a bioreactor is used for growing eukaryotic cells. A typical conventional bioreactor has cylindrical vessel with domed top and bottom generally made up of stainless steel. The reaction vessel which is surrounded by a jacket, is provided with a sparger at the bottom through which air (or other gases such as CO2 and NH (for pH maintenance) can be introduced. The reaction vessel also has side ports for pH, temperature and dissolved O2 sensors. The agitator shaft is connected to a motor at the bottom. Above the liquid level of the reaction vessel, connections for acid, alkali, antifoam chemicals and inoculum are located. The bioreactor is designed to work at very high temperatures (150-1800C), high pressure (77-12 kPa) and also to withstand vacuum which prevents its collapse while cooling.

Types of Bioreactors

Depending on the design of the reactor, the bioreactors are of following types:

a) **Continuous stirred tank bioreactors** - These bioreactors have a cylindrical vessel with motor driven central shaft which gives support to one or more agitators (impellers). The shaft is fitted at the bottom of the bioreactor. The diameter of the impeller is usually one third of the vessel diameter. The impellers are available in different designs like- Rustom disc, concave bladded, marine propeller etc. In stirred tank reactors, the air is added to the culture medium under pressure through a device called sparger. The sparger along with the impellers (agitators) enables better and efficient gas distribution through out in the vessel. The advantages of using stirred tank reactors are: the efficient transfer of gas to growing cells which keeps the growth of cells in healthy limits, stirring ensures

good mixing of the contents, the operating conditions are flexible and the bioreactors are easily available which makes them commercially viable products.

b) **Bubble column bioreactors** - In these bioreactors, the gas or air is introduced at the base of the column through perforated pipes or plates, or metal microporous spargers. The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio (height o diameter ratio) of -6. The rate of flow of gas affects the O2 transfer and mixing.

c) **Airlift bioreactors** - Airlift bioreactors are commonly used for aerobic bioprocessing technology. In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of baffle or draft tube. The air/gas is pumped into one of the two zones referred to as 'riser' and the other zone that receives no gas is known as 'downcomer'. The dispersion flows up the riser zone while the down flow occurs in the downcomer. Further there are two types of bioreactors:

(1) **Internal loop bioreactor** - These bioreactors have a single container with a central draft tube that creates interior liquid circulation channels which keeps the volume and circulation at a fixed rate for fermentation.

(2) **External loop airlift bioreactor**-These have an external loop to keep the liquid in circulation through separate independent channels. The modifications can be made in these bioreactors depending on the requirements of different fermentation processes.

() **Two stage airlift bioreactors** -These bioreactors have two bioreactors which are basically used for the temperature dependent formation of products. The growing cells from one bioreactor (maintained at temperature 00C are pumped into another bioreactor (at temperature 20C). This is done because it is very difficult to increase the temperature quickly from 00C to 20C in the same vessel. The cells are grown in the first bioreactor and with the help of the fitted valves and a transfer tube and pump, they are transferred into the second bioreactor, where the actual bioprocessing takes place.

() **Tower bioreactors** - In this type of bioreactor, a high hydrostatic pressure is generated at the bottom of the reactor which increases the solubility of O2 in the medium. Since the top is expanded, the pressure is reduced which helps in the expulsion of CO2. The cycle completes with the medium flowing back into the downcomer. The advantage with Tower bioreactor is that it has high aeration capacities without having moving parts.

(d) **Fluidized bed bioreactors** - These bioreactors are mainly suitable to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, microbial flocs etc. The design of the bioreactors is such that the top is extended and the reaction column is narrow which retains the solids in the reactor and allows the liquids to flow out. To maintain an efficient operation of fluidized beds, gas is sparged to create a suitable gas-liquid-solid fluid bed. The recycling of the liquid ensures continuous contact between the reaction contents and biocatalysts which increases the efficiency of bioprocessing.

(e) **Packed bed bioreactors**- A packed bed bioreactor consists of a bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column. The solids are generally porous or non-porous gels which maybe compressible or rigid in nature. The nutrient broth continuously flows over the immobilized biocatalyst and the products are released into the fluid from where they are removed. However, due to poor mixing, it is difficult to control the pH of packed bioreactors by the addition of acid or alkali.

(f) **Photobioreactors** - These bioreactors are specialized for fermentation that can be carried out either by exposing to sunlight or artificial illumination. The photobioreactors are made up of glass or transparent plastic which are the solar receivers. The cell cultures are circulated through the solar receivers by using centrifugal pumps or airlift pumps. These bioreactors work in the temperature which ranges from 25-00C. In these bioreactors, the microorganisms e.g. microalgae, cyanobacteria etc. grow during the day time while the products (e.g. beta-carotene, asthaxanthin) are produced during the night.

Solid substrate Fermentation (SSF)

In some biotechnological processes, the growth of the microorganisms is carried out on solid substrates more or less in the absence of free water. Only approximately 15% of moisture is present which is essential for solid-substrate fermentation. Cereal grains, wheat bran, sawdust, wood shavings etc are some of the commonly used solid substrates for SSF. The technique of SSF is used for the production of edible mushrooms, cheese, soy sauce, and many enzymes and organic acids. It is carried out as non-aspetic process and therefore saves sterilization costs. The bioreactors used in this type of fermentation process have simple designs with simple aeration process and effluent treatment. The yield of the products is very high, at low energy expenditure. However, in this process only microorganisms, that can tolerate only low moisture content can be used. It also difficult to monitor O2 and CO2 levels in SSF. The slow growth of microorganisms, also become a limiting factor for product formation.

Working of a bioreactor

In the operation of a bioreactor, there are a few steps that are followed.

a) Sterilization - The most important requirement is to maintain aseptic or sterile conditions for aseptic fermentation. In order to achieve this, the air supplied during fermentation, he growth medium and the bioreactor it self and all it's accessories are sterilized. There are two methods of sterilization that are followed:

(1) In situ sterilization- In this, the bioreactor is filled with the required medium followed by injection of pressurized steam into the jacket or coil surrounding the reaction vessel. The whole system is heated to about 1200C and maintained at this temperature for about 20 minutes. However, this method is not energy efficient and prolonged heating destroys the vitamins and precipitates the components of the medium.

(2) Continuous heat sterilization - In this, the empty bioreactor is first sterilized by injecting pressurized steam and the medium is rapidly heated to 100C for a short period again by injecting the pressurized steam. This is an energy efficient method and also does not precipitate the medium components.

b) Aeration - Oxygen is stored in compressed tanks and is introduced at the bottom of the bioreactor through a 'sparger'. Aeration of the fermentation medium supplies oxygen to the production microorganisms and remove carbon dioxide from the bioreactor. The gases released during the fermentation accumulate in the headspace and then pass out through an air outlet. The headspace is a vacant space on the upper part of the bioreactor and is generally about 20% of it's volume. The air-lift system of aeration involves sparging of air done at the bottom of the fermenter with an upward flow of air bubbles. The aeration capacity of the system depends on the air-flow rate and the internal pressure. The stirred system of aeration involves increasing the aeration capacity by stirring using impellers driven by motor. The aeration capacity of the fermenter depends on the rate of stirring, rate of air flow and internal pressure.

c) **Inoculation and sampling** - The sterilized bioreactors with growth medium are inoculated with the production organisms. The size of the inoculum is generally 1-10% of the total volume of the medium. During the fermentation process, the samples are regularly withdrawn to check contamination and to measure the amount and quantity of product formed.

d) **Control systems** - Various factors like- pH, temperature, dissolved oxygen, adequate mixing, concentration of the nutrients, foam formation etc are continuously monitored to maintain optimal growth environment in the bioreactor. Very sensitive sensors are available which carry out automated monitoring of these variables. The ideal pH range for optimal growth of microorganisms is between 5.5- 8.5. The pH changes due to the release of metabolites into the medium by the growing microorganisms. The required pH level is maintained by adding acid or alkali followed by thorough mixing of the medium components. The optimal temperature is maintained by using the heating and cooling systems fitted in the bioreactor. Continuous monitoring of dissolved oxygen concentration is also a must for the optimal bioreactions. The oxygen is sparingly soluble in water (0.008g/l at 250C) and is introduced into the bioreactor as the air bubbles.

The concentration of the nutrients is also important because the limiting concentrations of nutrients helps in the optimal product formation and the high nutrient concentrations have inhibitory effect on the microbial growth. Another important factor to control is **the "foam formation**". The protein rich media is used in industrial fermentation which leads to 'froth' or **'foam formation**' on agitation during aeration. Some antifoam chemicals lower the surface tension of the medium and causes the foam bubbles to collapse. Mineral oils with silicone or vegetable oils are also used as antifoam agents. The bioreactors can also be fitted with mechanical foam control devices which break the foam bubbles and throw back into the fermentation medium. The proper and continuous mixing of the

microbial culture is very important to maintain optimal levels of oxygen in the nutrient medium and to prevent the accumulation of toxic metabolic products.

e) **Cleaning** - After the completion of the fermentation process, the products are 'harvested' (removal of contents for processing) and the bioreactor is prepared for the next round of fermentation after cleaning technically referred to as 'turn around'. The cleaning of the bioreactors is carried out by using high-pressure water jets from the nozzles fitted into the reaction vessel. In order to maintain the cost effectiveness of the bioreactor, the time taken for turn around which is known as 'down time', is kept as short as possible.

Downstream processing (DSP)

The extraction and purification of a biotechnological product from fermentation is referred to as downstream processing. The methods adopted for downstream processing depends on the nature of the end products, it's concentration, stability, and the degree of purification required. Both intracellular metabolites as well as extracellular metabolites are isolated by DSP. The intracellular metabolites are products located within the cells e.g. vitamins, enzymes etc. The **extracellular metabolites** are the products present outside the cells e.g. most antibiotics, amino acids, alcohol, citric acid, enzymes like amylases, proteases etc. some products like vitamin B12, flavomycin etc are present both as intracellular as well as extracellular products.

Downstream processing involves a number of steps which are as follows:

a) **Solid-liquid separation** - The first step is to separate whole cells and other insoluble substances from the culture broth. This is done by using several methods:

1) **Flotation**- The process of aeration involves the bubbling of gas in to the liquid broth. The cells and other solid particles get adsorbed on gas bubbles which form a foamy layer which is collected and removed.

2) **Flocculation** - The cells or cellular debris form large aggregates and settle down which can be easily removed. Some flocculating agents like inorganic salt, organic poly-electrolyte, mineral hydrocolloid etc are often used to achieve appropriate flocculation.

) **Filtration** - this is the most commonly used technique to separate the biomass and culture filtrate. The rate of filtration depends on many factors such as the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters like depth filters, absolute filters, rotary drum vacuum filters, membrane filters etc. are used. There are three major types of filtration processes used depending on the size of the particles- microfiltration, ultrafiltration, and reverse osmosis.

) **Centrifugation** - The centrifugation is mostly used for separating solid particles from liquid phase. The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. In the bioreactors, the

continuous flow industrial centrifuges are used where there is a continuous feeding of the slurry and collection of clarified fluid. The solid deposits are intermittently removed. Various models of centrifuges used are: Tubular bowl centrifuge, Disc centrifuge, Multichamber centrifuge, Scroll centrifuge or decanter etc.

b) **Release of intracellular products** - The biotechnological products that are located with in the cells like vitamins, enzymes, etc are released in an active form for their further processing and isolation. The microorganisms or cells can de disintegrated or disrupted by physical, chemical, or enzymatic methods depending on the nature of the cells.

1) **Physical methods of cell disruption** are (i) **Ultrasonication**, (ii) **Osmotic shock** (used for releasing hydrolytic enzymes and binding proteins from gram-negative bacteria), (iii) **Heat Shock treatment**, (iv) **High pressure homogenization**, (v) **Impingement** which involves hitting a stationary surface or a second stream of suspended particles with a stream of suspended cells at high velocity and pressure. **Microfluidizer** is a device developed on the basis of the principle of impingement. (vi) **Grinding with glass beads** where the cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads.

2) **Chemical methods**- Treatment with alkalies, organic solvents, and detergents lyse the cells to release the content. Alkali treatment is used for the extraction of some bacterial proteins. The organic solvents like methanol, ethanol, isopropanol, butanol etc also disrupt the cells. The organic solvent, toluene, which is commonly used, dissolves membrane phospholipids and creates the membrane pores to release intracellular contents. The ionic detergents denature the membrane proteins and lyse the cells e.g. cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate. Non-ionic detergents are less reactive and also affect the purification steps.

) **Enzymatic methods** - Lysozyme is the most commonly used enzyme which hydrolyses beta-1,-glycosidic bonds of the mucopepide in bacterial cell walls e.g. gram positive bacteria. This enzyme is commercially available produced from hen egg white. For gram-negative bacteria, lysozymes are in use with EDTA to break the cells. When the cell wall gets digested by lysozyme, the periplasmic membrane breaks due to osmotic pressure, which releases the intracellular contents. Glucanase and mannanase along with proteases lyse the cell wall of yeast.

c) **Concentration** - The biological products are concentrated by getting rid of water which is present in the filtrate. Depending on the nature of the desired products and the cost effectiveness, the techniques used to concentrate the biotechnological products are evaporation, liquid-liquid extraction, membrane filtration, precipitation, and adsorption.

(i) **Evaporation** - The water from the broth is removed by the process of evaporation using evaporators. The evaporators use a heating device which supplies the steam. There is a unit for the separation of concentrated product and vapour and a condenser for

condensing vapour. The commonly used evaporators are Plate evaporator, Falling film evaporator, Forced film evaporator, Centrifugal forced film evaporator.

(ii) **Liquid-Liquid extraction** - In liquid-liquid extraction, the biological products are concentrated by transferring the desired product from one liquid phase to another liquid phase. The process of liquid-liquid extraction is categorized as extraction of low molecular weight products and extraction of high molecular weight products.

(iii) **Membrane filtration** - This technique involves the use of a semipermeable membrane that selectively retains the particles/molecules that are bigger than the pore size while the smaller molecules pass through the membrane pores. The membranes are made up of polymeric materials such as polyethersulfone and polyvinyl difluoride. Now a days, microfilters and ultrafilters made up of ceramics and steel are being used which are easy to clean and sterilize. Pervaporation is a technique in which the volatile products are separated by a process of permeation through a membrane coupled with evaporation and is used to extract and concentrate volatile products. Perstraction- This technique is used to recover and concentrate hydrophobic compoundsvand is based on the principle of membrane filtration coupled with solvent extraction.

(iv) **Precipitation** - This is the most common method used to concentrate proteins and polysaccharides in industrial processes. The alteration in temperature and in pH, neutral salts, organic solvents, high molecular weight polymers (ionic and non ionic) etc are used in precipitation. Neutral salts like commonly used ammonium sulphate increases the hydrophobic interactions between protein molecules which leads to their precipitation. Ethanol, acetone and propanol are the commonly used organic solvents for protein precipitation which reduce the dielectric constant of the medium and increase the electrostatic interaction between the protein molecules. The precipitation process is carried out below 00C because the proteins get denatured by organic solvents. Polyethylene glycol (PEG) is a high weight non-ionic polymer that also precipitates the proteins by reducing the quantity of water available for protein salvation. In the category of ionic polymers e.g. polyacrylic acid, polyethyleneimine are used which form complexes with oppositely charged protein molecules and neutralize the charges. This leads to the precipitation of proteins. Besides this, the physical factors like increase in temperature, increase in pH, etc also leads to the precipitation of proteins. Besides these, the affinity precipitation (affinity interaction e.g. between antigen and antibody)and precipitation using ligands is also used.

d) **Purification** – The products of fermentation are purified by using the technique of chromatography. Chromatography consists of a stationary phase and a mobile phase. The porous solid matrix packed in a column constitutes the stationary phase and the mixture of the compound to be separated is loaded on this. The compounds are eluted by a mobile phase. A large number of matrices are commercially available for the purification of proteins e.g. agarose, cellulose, porous silica, cross linked dextran etc. Some of the commonly used chromatography techniques used are: Gel-filtration chromatography- In this technique, the separation of molecules is based on the size, shape and molecular weight using sponge-like gel beads with pores serving as molecular sieves for separation of smaller and bigger molecules. The Ion-exchange chromatography involves the

separation of molecules based on their surface charges. It is useful for the purification of antibiotics, besides the purification of proteins. In ion exchange chromatography, the pH of the medium is very crucial because the net charge varies with pH. The ionic bound molecules are eluted from the matrix by changing the pH of the eluant or by increasing the salt concentration. The ion-exchangers are of two types- Cation exchangers- having negatively charged groups like carboxymethyl and sulfonate e.g. Dowex, HCR, Amberlite IR etc. Anion exchangers have positively charged groups like DEAE (diethylaminoethyl) e.g. Dowex SAR, Amberlite IRA etc. The Affinity chromatography is based on an interaction of a protein with an immobilized ligand. The ligand can be a specific antibody, substrate, substrate analogue or an inhibitor. The protein bound to the ligand is eluted out by changing the pH of the buffer, altering the ionic strength or by using another free ligand molecule.

e) **Formulation**- The maintenance of activity and stability of biotechnological products during storage and distribution is known as 'formulation'. As proteins are highly sensitive and lose their biological activity, hence their formulations requires special care. In order to prolong their shelf life, certain stabilizing additives are added e.g. sugars (sucrose, lactose), salts (sodium chloride, ammonium sulphate), polymers (polyethylene glycol) and polyhydric alcohols (glycerol). Proteins are formulated in the form of solutions, suspensions or dry powder. For some small molecules (antibiotics, citric acid), formulation is done by crystallization using salts. 'Drying' is an important component of product formulation which involves the transfer of heat to a wet product for removal of moisture. The commercially available dryers in use are- contact, convection and radiation dryers. Spray drying is used for drying large volumes of liquids where small droplets of liquid containing the product are passed through a nozzle directing over a stream of hot gas. After the evaporation of water, the solid particles are left behind.

Freeze drying or lyophilization is the most preferred method for drying and formulation of a wide range of products- pharmaceuticals, food stuff, bacteria, viruses etc. Freeze drying does not cause loss of biological activity of the desired product. Lyophilization is caused due to the sublimation of a liquid from a frozen state. The product with the liquid is frozen and then freeze dried under vacuum. After releasing the vacuum, the product containing vials are sealed e.g. penicillin is freeze dried directly in ampules.

It is ideal to integrate the fermentation and downstream processing to finally get the desired product. The efforts are on the integrate these steps and a limited success has also been achieved.

Q.26. Write short note on Enzyme Biotechnology?

Ans. Enzymes are biological catalysts which initiate and accelerate biochemical reactions in the living cells. They are proteinaceous in nature and can be extracted from living tissues. They are purified and crystallized. The purified enzyme can carry out a specific biochemical reaction outside the cell. This property of the enzyme has been exploited in it's use in the area of biotechnology. The enzymes are also modified through enzyme

modeling and can be used for gene manipulations. The enzymes are purified by following three steps: dialysis, centrifugation and electrophoresis. The commercially prepared enzymes are purified and then concentrated and sterile filtered. This reduces the volume and the microbial contamination of the sample enzyme. Often, before storage and transport, the sample are freeze dried with additives as sugar substrates and dextrans.

The enzymes are being used commercially and in Industries. In textile Industry, 'Amylases' are extracted from bacteria, fungi etc and used as softening agents for starched clothes. Proteolytic enzyme 'alcalase' is added in many detergents to remove proteinaceous stains from the clothes. Enzymes isolated from certain bacteria are used commercially to produce acetone, butanol, lactic acid, citric acid etc. In meat and beer processing, the proteases 'Papain' (from papaya) and bromelain (from pineapple) are used to tenderize meat. These enzymes hydrolyze the peptide bonds. Similarly a number of enzymes like, 'Glucose isomerase' in soft drink industry,''lactase' in ice-cream industry, 'pectinases' in juice and wine processing etc are being commercially produced and widely used.

Using biotechnology, especially the technique of immobilized enzyme systems, it was possible to produce a variety of substances e.g. high-fructose corn syrup was produced using immobilized enzyme glucose isomerase.

These immobilized enzymes are worth billions of dollars in the market and support multibillion dollar industries. It is especially a profitable venture because the cost involved in the production of these enzymes is only a fraction of the value of the products manufactured.

Immobilization of enzymes is the imprisonment of a biocatalyst in a distinct phase that allows exchange with, but is separated from bulk phase in which substrate, effector or inhibitor molecules are dispersed and monitored. An immobilized enzyme is physically entrapped or covalently bonded by chemicals means to an inert and usually insoluble matrix, where it acts upon it's natural substrate.

Multiple choice questions

Environment Biotechnology

1. The biogas production process takes place at the temperature
   A.lesser than 25°C
   B.25-40°C
   C.45-60°C
   D.all of these
   Answer: D
2. Advanced treatment is generally used to treat waste water to

A.remove coarse solids
B.remove settleable solids
C.reduce BOD
D.remove additional objectionable substances
Answer: D

3. Treatment of municipal water supplies is based upon
A.coagulation, filtration, chlorination
B.chlorination, filtration, coagulation
C.filtration, coagulation, chlorination
D.coagulation, chlorination, filtration
Answer: A

4. What is an anaerobic digester?
A.New diet drink
B.Microbe that eats hazardous waste
C.Method to convert agricultural waste into a biogas
D.All of the above
Answer: C

5. The use of microbes to break down synthetic waste products such as polychlorinated biphenyls is called
A.bioinformatics
B.biolistics
C.biotechnology
D.bioremediation
Answer: D

6. Activated sludge contains large number of
A.bacteria
B.yeasts and molds
C.protozoa
D.all of these
Answer: D

7. Which of the following is generally not referred to the sewerage system?
A.Sanitary sewers
B.Storm sewers
C.Combined sewers
D.Solid sewers
Answer: D

8. The magnitude of BOD of wastewater is related to
A.bacterial count

B.amount of organic material
C.amount of inorganic material
D.all of the above
Answer: B

9. Biogas production is
A.a temperature-dependent process
B.a temperature independent process
C.an oxygen dependent process
D.none of the above
Answer: A

General Biotechnology

1. A molecular technique in which DNA sequences between two oligonucleotide primers can be amplified is known as
A.southern blotting
B.northern blotting
C.polymerase chain reaction
D.DNA replication

Answer: **C**

2. *Agrobacterium tumefaciens* is
A.a disease in humans that causes loss of sight
B.a bacterium that can be used to introduce DNA into plants
C.a fungi that is used to produce antibiotics in large amounts
D.a disease in humans that causes loss of weight

Answer: **B**

3. In genetic engineering, a chimera is
A.an enzyme that links DNA molecules
B.a plasmid that contains foreign DNA
C.a virus that infects bacteria
D.a fungi

Answer: **B**

4. Which of the following is obtained using processed mRNA molecules as a template?
   A.rDNA B.mDNA
   C.cDNA D.tDNA
   Answer: **C**

5. Vectors are
   A.molecules that degrade nucleic acids
   B.molecules that help in replication
   C.molecules that are able to covalently bond to and carry foreign DNA into cells
   D.molecules that protect host cells from invasion by foreign DNA
   Answer: **C**
6. Which of the following is not commonly used as vector?
   A.Artificial chromosome
   B.Cosmid
   C.Fungi
   D.Plasmid

   Answer: **C**

7. Which of the following vector can maintain the largest fragment of foreign DNA?
   A.YAC B.Cosmid
   C.Plasmid D.Phage
   Answer: **A**

8. Which of the following enzyme is used to covalently bond foreign DNA to a vector plasmid?
   A.DNA polymerase
   B.Restriction endonuclease
   C.DNA ligase
   D.DNA helicase
   Answer: **C**

9. Bacterial cells protect their own DNA from degradation by restriction endonucleases by
   A.methylating the DNA at the sites that the enzyme recognizes
   B.deleting all recognition sites from the genome
   C.not producing any restriction endonucleases
   D.having anti restriction endonucleases

Answer: **A**

10. Which type of restriction endonuclease cuts the DNA within the recognition site?
A.Type I
B.Type II
C.Type III
D.All of the these
Answer: **B**

11. Restriction endonucleases which recognize and cut same recognition sequences are known as

(A) isoschizomers
(C) isoaccepting endonucleases
(B) isozymes
(D) abzymes

Ans : A

12. The study of evolutionary relationships is known as
(A) genomics
(B) proteomics
(C) phylogenetics
(D) genetics

Ans: C

13. The number of replicons in a typical mammalian cell is
a) 40-200
b) 400
c) 1000-2000
d) 50000-100000
Ans: a) 40-200

14. Zinc fingers are characteristic of
(a) Blood clotting Proteins
(b) RNA Chaperones
(c) DNA binding proteins
(d) Lysosomal hydrolyses
Ans: c (DNA binding proteins)

- *Chaperons: proteins that assist in proper folding of proteins*.

15. Which of the following process require energy
a) ligation

b) restriction digestion
c) hybridization
d) transformation
Ans: a

16. Viral replication within cells is inhibited by
a) IL-4
b) IL-1
c) IFN alpha
d) TNF alpha
Ans:c
Interferons: *are antiviral agents (proteins) secreted by virus infected cells and induces a virus resistant state to the surrounding cells by inhibiting its replication.*

17. Highest capacity vector is
a) Cosmid
b) YAC
c) Yeast integrative vector
d) Bacteriophage vector
Ans: b

Note:

- *Cosmid: A plasmid with a cos site of lambda phage.Insert size: 30-45 kb*
- *YAC: yeast artificial chromosome. Insert size: 1 Mb*
- *Bacteriophage vectors: refers to lambda and M13 phage vectors*
- *Lambda phage vectors: Insert size: 8-23 kb*
- *M13 vectors used for obtaining single stranded copies of cloned DNA that are suited for DNA sequencing*
- *BAC: Bacterial Artificial Chromosome. Insert size: 300 kb*

18. Deoxy position of deoxyribose in DNA is a

(a) 1st Carbon
(b) 3rd Carbon
(c) 2nd Carbon
(d) 5th Carbon
Ans: c (2nd position)

19. Which of the following transgenic crops occupies the largest area in the world?
(A) Herbicide tolerant soybean
(B) Herbicide tolerant maize
(C) Insect resistant cotton
(D) Insect resistant potato
Ans: a (Herbicide tolerant soybean)

20. Rifampicin is a specific inhibitor of
(A) Bacterial RNA polymerase
(B) RNA polymerase II
(C) RNA polymerase I
(D) RNA polymerase III
Ans: Bacterial RNA polymerase

- *Rifampin specifically inhibits bacterial RNA polymerase, the enzyme responsible for DNA transcription*

21. The Philadelphia chromosome is
(A) an example of gene amplification
(B) a product of a reciprocal translocation
(C) a characteristic of Burkitt's lymphoma
(D) an example of duplication
Ans: b (a product of a reciprocal translocation)

- *Deletion of one arm of chromosome 21.*
- *Clinical symptoms: Chronic granulocytic leukemia*

22. Most predominant antibody in serum is
(A) IgG
(B) IgD
(C) IgE
(D) IgA
Ans: a (IgG)

- *IgG: Most abundant immunoglobulin, produce in the late primary response after IgM production, predominant in secondary immune response, the one and only Ig that cross placenta.*
- *IgA: Secretory antibody, Heaviest antibody and second most abundant*
- *IgM: Macroimmunoglobulin, pentamer, does not cross placenta, also known as natural antibody, first produced antibody upon infection.*
- *IgD: Present on surface of B-cells and in serum.*
- *IgE: which is responsible for allergic reactions, mediate hyper sensitivity. These are concentrated in mucous membrane, skin and lungs.*

Key terms of Biotechnology

Agrobacterium tumefaciens: A gram-negative, rod-shaped flagellated bacterium responsible for crown gall tumor in plants. This bacterium is now used to deliberately transfer genetic material into plants through biotechnology.

Biobased products: Fuels, chemicals, building materials, or electric power or heat produced from biological material(s). The term may include any energy, commercial or industrial products, other than food or feed, that uses biological products or renewable domestic agricultural (plant, animal and marine), or forestry materials.

Biopharming: The production of biopharmaceuticals in plants or domestic animals.

Biotechnology: A set of biological techniques developed through basic research and now applied to research and product development. Biotechnology refers to the use of recombinant DNA, cell fusion, and new bioprocessing techniques.

Biotechnology-derived: The use of molecular biology and/or recombinant DNA technology, or in vitro gene transfer, to develop products or to impart specific capabilities in plants or other living organisms.

Bt corn: A corn plant that has been developed though biotechnology so that the plant tissues express a protein derived from a bacterium, Bacillus thuringiensis, which is toxic to some insects but non-toxic to humans and other mammals.

Cry1A: A protein derived from the bacterium Bacillus thuringiensis that is toxic to some insects when ingested. This bacterium occurs widely in nature and has been used for decades as an insecticide although it constitutes less than 2 percent of the overall insecticides used.

Embryonic stem (ES) cells: Cell lines derived from early embryos that have the potential to differentiate into all types of somatic cells as well as to form germ line cells, and hence whole animals, when injected into early embryos.

Enucleated oocyte (cytoplast): An egg cell from which the nucleus has been removed mechanically.

Fibroblast: A type of relatively undifferentiated cell found in many parts of the body involved primarily in wound healing. Fibroblasts are relatively easy to grow in cell culture and often are used for this purpose.

Gene gun: A device invented at Cornell University that allows genetic material to be introduced into a new organism. The genetic material from the donor is "shot" into cells of the recipient, and the material is incorporated into its DNA.

Gene splicing: The isolation of a gene from one organism and then the introduction of that gene into another organism using techniques of biotechnology.

Genetic engineering: The technique of removing, modifying, or adding genes to a DNA molecule to change the information it contains. By changing this information, genetic engineering changes the type or amount of proteins an organism is capable of producing, thus enabling it to make new substances or perform new functions.

Genetically modified organism (GMO): Often, the label GMO and the term "transgenic" are used to refer to organisms that have acquired novel genes from other organisms by laboratory "gene transfer" methods.

Genome: All the genetic material in the chromosomes of a particular organism; its size is generally given as its total number of base pairs.

Genomics: is the mapping and sequencing of all the genetic material in the **DNA** of a particular organism as well as the use of information derived from genome sequence data to further elucidate what genes do, how they are controlled, and how they work together.

Genotype: The genetic identity of an individual. Genotype often is evident by outward characteristics.

Germline cells: Cells that contain inherited material that comes from the eggs and sperm, and that are passed on to offspring.

Hybrid: Seed or plants produced as the result of controlled cross-pollination as opposed to seed produced as the result of natural pollination. Hybrid seeds are selected to have higher quality traits (for example, yield or pest tolerance).

Knock In: Replacement of a gene by a mutant version of the same gene using homologous

Knock Out: Inactivation of a gene by homologous recombination following transfection with a suitable DNA construct.

Microinjection: The introduction of DNA into the nucleus of an oocyte, embryo, or other cell by injection through a very fine needle.

Mutation: Any inheritable change in DNA sequence.

Nuclear reprogramming: Restoration of the correct embryonic pattern of gene expression in a nucleus derived from a somatic cell and introduced into an oocyte.

Nuclear transfer (NT): The generation of a new animal nearly identical to another one by injection of the nucleus from a cell of the donor animal into an enucleated oocyte of the recipient.

Organic agriculture: A concept and practice of agricultural production that focuses on production without the use of synthetic pesticides.

Pesticide resistance: A genetic change in response to selection by a pesticide, resulting in the development of strains capable of surviving a dose lethal to most individuals in a normal population. Resistance may develop in insects, weeds, or pathogens.

Plasmid: A circular DNA molecule capable of replication in bacteria. Plasmids are the usual means of propagation of DNA for transfection or other purposes.

Pleiotropy: A phenomenon whereby a particular gene affects multiple traits.

Pronuclear injection: The use of a fine needle to inject DNA into the nucleus of an unfertilized egg.

Recombinant DNA molecules (rDNA): A combination of DNA molecules of different origin that are joined using recombinant DNA technologies.

Recombinant DNA technology: Procedure used to join together DNA segments in a cell-free system (an environment outside a cell or organism). Under appropriate conditions, a recombinant DNA molecule can enter a cell and replicate there, either autonomously or after it has become integrated into a cellular chromosome.

Recombination: The process by which progeny derive a combination of genes different from that of either parent.

Selectable marker: A gene, usually encoding resistance to an antibiotic, added to a vector construct to allow easy selection of cells that contain the construct from the large majority of cells that do not.

Selective breeding: Making deliberate crosses or matings of organisms so the offspring will have a desired characteristic derived from one of the parents.

Somatic cells: Cells of body tissues other than the germline.

Tissue culture: A process of growing a plant in the laboratory from cells rather than seeds. This technique is used in traditional plant breeding as well as when using techniques of agricultural biotechnology.

Traditional breeding: Modification of plants and animals through selective breeding. Practices used in traditional plant breeding may include aspects of biotechnology such as tissue culture and mutation breeding.

Transfection: Alteration of the genome of a cell by direct introduction of DNA, a small portion of which becomes covalently associated with the host cell DNA.

Transgenic: Containing genes altered by insertion of DNA from an unrelated organism. Taking genes from one species and inserting them into another species to get that trait expressed in the offspring.

Vector: A type of DNA, such as a plasmid or phage that is self-replicating and that can be used to transfer DNA segments among host cells. Also, an insect or other organism that provides a means of dispersal for a disease or parasite.

Vertical transmission: Inheritance of a gene from parent to offspring.

Virus: A noncellular biological entity that can reproduce only within a host cell. Viruses consist of nucleic acid covered by protein; some animal viruses also are surrounded by a membrane. Inside the infected cell, the virus uses the synthetic capability of the host to produce progeny viruses.

Vitamins: Various substances those are essential in minute quantities to the nutrition of animals and plants.

Xenotransplantation: Transplantation of cells, tissues, or organs from one species to another.

Zygote: A fertilized oocyte.

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