COURSE CODE:	*VBB 202*
COURSE TITLE:	*Biochemistry of Proteins & Molecular Biology*
NUMBER OF UNITS:	*3 Units*
COURSE DURATION:	*Three hours per week*

COURSE DETAILS:

Course Coordinator:	**Dr (Mrs) Funmilola Clara Thomas** *DVM., M.Sc.*
Email:	**eraforosi@yahoo.com**
Office Location:	**Glass house, COLVET**
Other Lecturers:	**Dr Babatunde Samuel Okediran** B.Sc., DVM., M.Sc

COURSE CONTENT:

Protein; classification, structure and functions. Amino acids, Peptides and peptide bonds; essential and non-essential amino acids. Metabolism of amino acids: transamination, oxidative deamination, decarboxylation etc. urea cycle. Interconversions of threonine. In born errors of metabolism. Chemistry asnd structure of nucleic acids. Catabolism of purines, pyrimidines and nucleotides; disorders of purine and pyrimidine metabolism. Plasma proteins, structure and fucntions; blood clotting mechanisms, iron; sources absorbtion, distribution in the body and excretion; anemia; hemochromatosis. Heamoglobin/; structure, propreties and functiioons. Metabolism of porphyrin. Porphyrinurias and jaundice. Heamoglobinopathies. HbS thalassemias, haemophilia etc. Gene cloning and application of recombinant DNA technology in veterinary medicine. Immunoglobulins: typoes structures and functions. Antigens and antigenis determinants. Retroviruss, molecular bases and involvement in cancer; proto-oncogenes and oncogenes. Translocation and gene arrangement in disease state of animals.

COURSE REQUIREMENTS:

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

READING LIST:

1. V.K Malhotra. Biochemistry for students tenth edition Jaypee brothers medical publishers (p) ltd.
2. J.Jerry Kaneko., John W. Harvey., Michael L. Bruss. Clinical Biochemistry of Domestic Animals Sixth edition. Academic Press.
3. Harvey Lodish., David Baltimore., Arnold Berk., S. Lawrence Zipursky., Paul Matsudaira and James Darnell. Molecular Cell Biology. Third Edition. By Scientific American Books, Inc.
4. David A. Bender., Kathleen M. Botham., Daryl K. Granner., Frederick W. Keeley., Peter A. Mayers., Robert K. Murray., Victor W. Rodwell., P. Anthony Weil.(2006). Harpers Illustrated Biochemistry. 27th Edition. Published By Mc Graw Hill

LECTURE NOTES

IMMUNOGLOBULINS: TYPES, STRUCTURES, FUNCTIONS AND BIOMEDICAL IMPORTANCE.

Immunoglobulins (Igs) are glycoprotein molecules also called antibodies(Abs) , that are produced in response to foreign substances entering the living body- antigens or immunogens(viruses, bacteria, or toxins etc), binding to them and forming antigen-antibody complexes resulting in Ag elimination and protection of the body of the host). Igs are produced by the lymphocytes and are found in fraction of blood called gamma globulin. Gerald M. Edelman and Rodney Robert Porter ere the notable researchers who worked extensively on purification and structural analysis of Igs, particularly the IgG type.

Igs are synthesized with a molecular arrangement that fits the shape of molecules on the antigens or immunogens, in order to allow effective binding of the Abs. Igs binding to Ags basically help to inactivate, weaken or enhance phagocytosis of Ags.

GENERAL FUNCTIONS

1. Antigens binding- Igs bind to specific Antigenic determinants (AD) on an antigen. They bind to at least 2 or in a few cases more Ads which are closely related and the number of ADs an Ab can bind to is referred to as its valency.

2. Most Igs mediate several effector functions which include fixation of complement that results to lyses of cells and release of biologically active molecules, binding of various cells to facilitate specific functions by bound cells e.g. phagocytic cells, lymphocytes, platelets etc.

   Most effector functions of Abs are carried out after the Ab binds to Ags. Different Igs molecules can have different Ag binding properties because of different V_H and V_L regions.

BASIC STRUCTURE OF IMMUNOGLOBULINS

All Igs have the same basic structural units of 2 identical light chains and 2 identical heavy chains, the heavy and light chains are joined together by interchain disulphide bonds and non-covalent interactions. The number of interchain disulphide bonds varies among different Igs. Within the polypeptide chains i.e. the heavy and light chains there are also present intra-chain disulphide bonds. Amino acid sequence of both heavy and light chains of an Ig characterizes two distinct regions of the chains based on variability of the amino acid sequence, known as VARIABLE (V) and CONSTANT (C) regions .Light and heavy chains are composed of both a variable and constant region designated V_L and C_L (light chains) and V_H and C_H (heavy chains).The amino acid sequence of the variable region form the N-terminal ends of the chains and determine antigenic specificity of the Igs. Constant regions are the same for each specific class of Ig and carry the effector sites.

Light chain- V_L-about 100-110amino acids, C_L-100-110 amino acids. There are two types of light chains, kappa and lambda,(κ and λ) the κ are twice as much as λ. There are also four classes of the λ chains. These chains weigh about 23KDa. Differences in the type of light chains also form a basis for grouping of Igs into various types. The variable region makes up half of the entire light chain and the constant region the remaining half.

Heavy chains- V_H-110 amino acids, C_H-330-440 amino acids. There are 5 types of heavy chains which defines the class of Igs, namely, Alpha, Gamma, Miu, Delta and Epsilon (α, γ,μ,δ,ε).the heavy chains are between 53-75KDa.the variable region makes up a quarter of the entire heavy chain while ¾ of the remaining chain is the constant region.

The hinge region is the area of the Ig where the arms of the Abs form a 'Y',it is a flexible region. Igs also have domains formed from folds of the globular region containing the intrachain disulphide bonds and they are V_L and C_L (light chain domains) and V_H and C_H (heavy chain

domains), seen in the three dimensional images of the Ig. The constant region of light chain and the appropriate heavy chain form globular constant domains while the variable regions of light chain 1 and corresponding heavy chain interact to form globular variable domain.

Ig s also have attached to their C_H oligosaccharides and in other cases these carbohydrates are attached to other areas.

The variable regions of an Ig are also further divided into hypervariable or complementarity determining regions (CDRs) which distinguishes Abs with different specificities and is found on both light and heavy chains and the frame work regions lie between the CDRs. There are about 3 hypervariable regions on the V_L and 4 on the $V_{H,}$ and these contribute to uniqueness of each antibody.

Proteolytic digestion of Igs have produced fragments which have been found useful in elucidating the structure-function relationship of the Ig.

Fab- also referred to as the antigen binding fragment, is gotten upon digestion of Ig with papain and its cleavage at the hinge region. It contains the antigen binding site synonymous to V_H and V_L which is particular to the kind of antigenic determinant the Ab will bind.

Fc- this is also called fragment crystallizable because it is readily crystallized and it contains the remainder of the two heavy chains. It contains different domains ands which mediate effector functions of an Ig. Variations in the Fc determines the different classes of Igs.

The hinge region is between the Fab and the Fc portion and controls interactions between these portions.

$F(ab)_2$- treatment of Igs with pepsin results in cleavage of the heavy chain, resulting in a fragment that contains both antigen binding sites, it is called $F(ab)_2$ because it is divalent. Fc portion is digested into small peptides by pepsin. The $F(ab)_2$ binds to Ag but does not mediate effector functions.

IMMUNOGLOBIULINS TYPES AND CLASSES.

Based on differences in the amino acid sequences in the constant region of the heavy chains there are five classes of Igs.

1. IgG- gamma heavy chain
2. IgM-miu heavy chain
3. IgA- alpha heavy chain
4. IgD- delta heavy chain
5. IgE- epsilon heavy chain.

In each class of Ig small differences in the constant regions of the heavy chain still occur, leading to subclasses of the Igs e.g. IgG1,IgG2,IgG3 etc.

IgG

All IgG are monomers, subtypes and subclasses differ in number of disulphide bonds and lengths of hinge region.

Properties.

1. It is the most versatile Ig and can carry out all functions of Ig molecules.
2. It is the major Ig in serum
3. It is also found/ the major Ig in extravascular spaces.
4. It is the only Ig that crosses the placenta.
5. It fixes complement although not all subclasses do this well.
6. It binds to cells and is a good poisoning(substance that enhances phagocytosis).

IgM

It normally exists as a pen tamer in serum but can also occur as a monomer. It has an extra domain on the mui chain (C_{H4}) and another protein covalently bound via S-S . called J-chain. This chain helps it to polymerize to the pentamer form.

Properties

1. It is the first Ig to be made by fetus in most species and new B cells when stimulated by Ags.
2. It is the 3^{rd} most abundant Ig in serum.
3. It is a good complement fixing Ig leading to lyses of microorganisms
4. It is also a good agglutinating Ig, hence clumping microorganisms for eventual elimination from the body.
5. It is also able to bind some cells via Fc receptors.
6. B cells have surface IgMs , which exists as monomers and lacks J chain but have an extra 20 amino acid at the C-terminal that anchors it to the cell membrane.

IgA

Serum IgA is monomeric, but IgA found in secretions is a dimer having a J chain. Secretory IgA also contains a protein called secretory piece or T- piece, this is made in epithelial cells and added to the IgA as it passes into secretions helping the IgA to move across mucosa without degradation in secretions

Properties

1. It is the second most abundant Ig in serum
2. It is the major class of Ig in secretions- tears, saliva, colostrums,mucus,and is important in mucosal immunity.
3. It binds to some cells- PMN cells and lymphocytes
4. It does not normally fix complement.

IgD

It exists as monomers.

Properties

1. It is found in low levels in serum and its role in serum is uncertain
2. It is found primarily on B cells surface and serves as a receptor for Ag.
3. It does not fix complement.

IgE

It occurs as a monomer and has an extra domain in the constant region.

Properties

1. It is the least common serum Ig, but it binds very tightly to Fc receptors on basophils and mast cells even before interacting with Ags.
2. It is involved in allergic reactions because it binds to basophils and mast cells.
3. It plays a role in parasitic helminthic diseases. Serum levels rise in these diseases. Eosinophils have Fc receptors for IgEs and when eosinophoils bind to IgEs coated helminthes death of the parasite results.

BIOMEDICAL IMPORTANCE OF IGS

IgG- Increases occur in:-chronic granulomatous infections and infections of all types, hyperimmnunization, liver disease, severe malnutrition, dysproteinemia, rheumatoid arthritis etc.

Decreases occur in:-aggammaglobulinemia, lymphoid aplasia, selective IgG, IgA deficiency, IgA myeloma and chronic lymphoblastic leukemia.

IgM. Increases occur in: - Waldenstrom's macroglobulinemia, Trypanosomosis, Actinomycosis, Bartonellosis, Malaria, Lupus erythromatosis, Rheumatoid arthritis, Dysgammaglobulinemia etc

Decreases occur in: - Aggammaglobulinemia, lymphoproliferative disorders, lymphoid aplasia, IgG and IgA myeloma and chronic lymphoblastic leukemia.

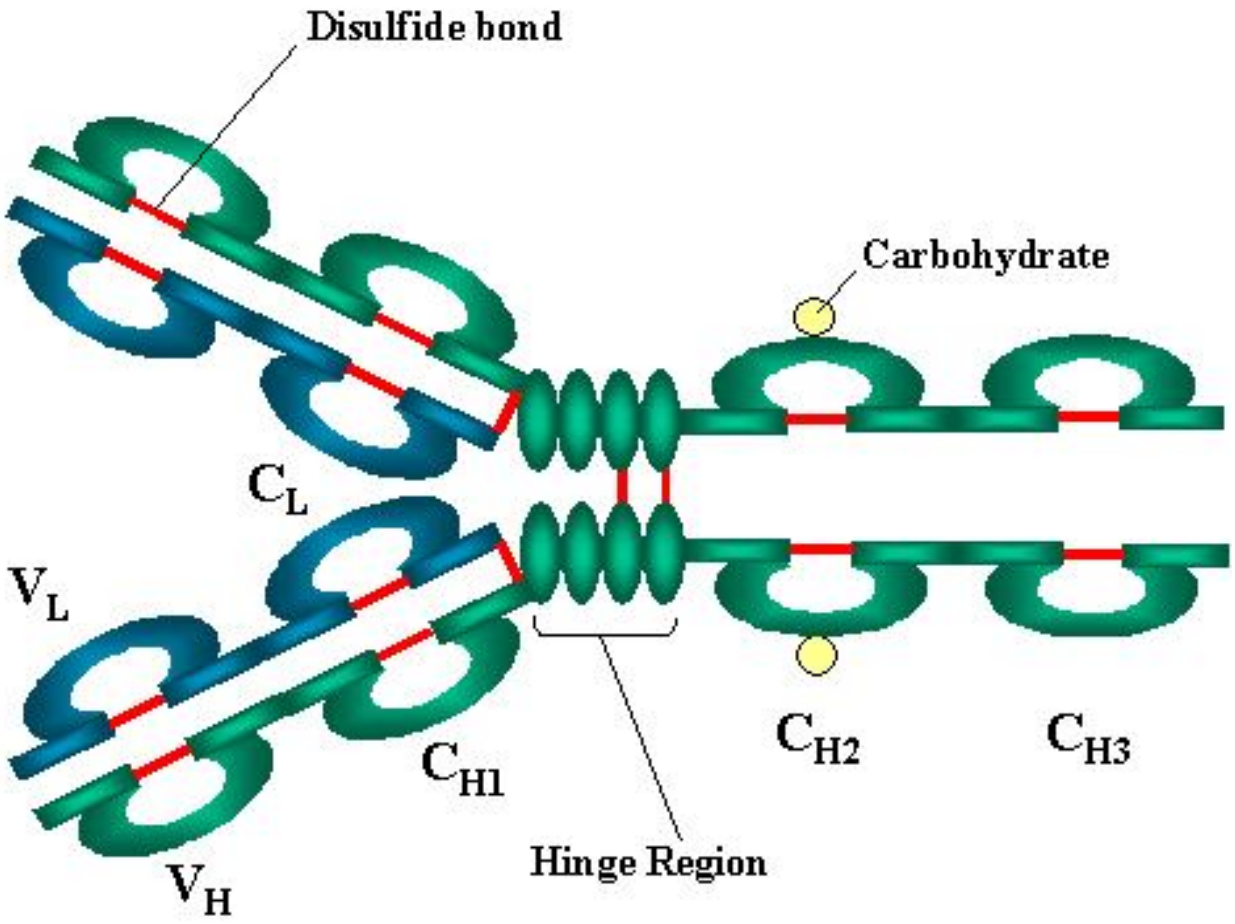
IgA Increases occur in:-Wiskott-Aldrich syndrome, cirrhosis of the liver, IgA myeloma, autoimmune disorders, rheumatoid arthritis, lupus erythromatosis etc

Decreases occur in: - hereditary ataxia Telangectasia, Ig deficiency states, malabsorbtion syndromes, lymphoid aplasia, IgG myeloma, chronic lymphoblastic leukemia etc.

IgD Increases occur in: - chronic infections, IgD myelomas

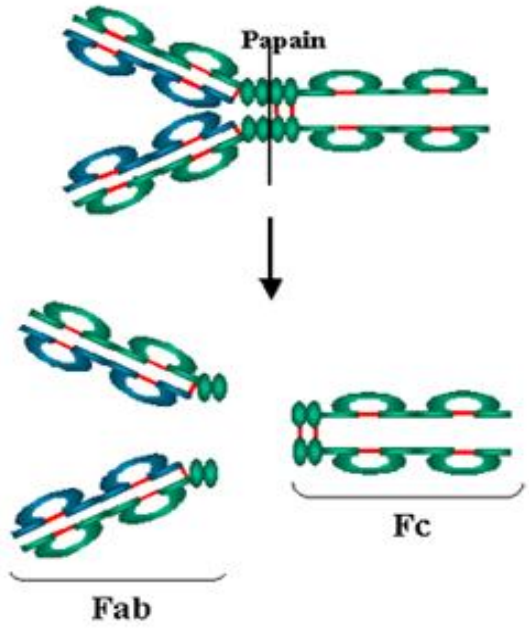
IgE Increases occur in:-atopic skin diseases e.g. eczema, hay fever, asthma, anaphylactic shock and IgE myelomas.

Decreases occur in:-congenital Aggammaglobulinemia, Hypogammaglobinemia etc


Disulfide bond
Carbohydrate
C_L
V_L
C_{H1}
C_{H2}
C_{H3}
Hinge Region
V_H

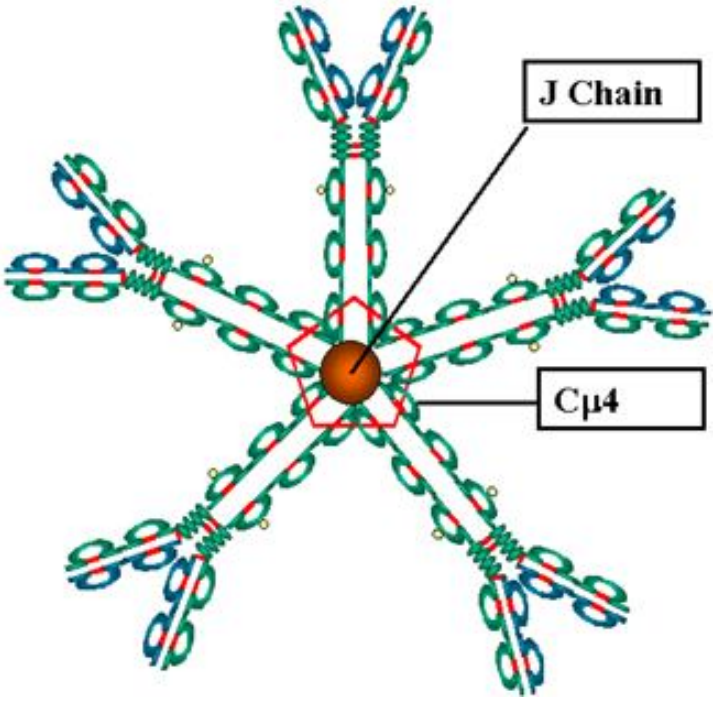
Immunoglobulin Fragments: Structure/Function Relationships

- Fab
  - Ag binding
  - Valence = 1
  - Specificty determined by V_H and V_L
- Fc
  - Effector functions



IgM

- Structure
  - Pentamer (19S)
  - Extra domain (C_{H4})
  - J chain



ANTIGENS AND ANTIGENIC DETERMINANTS; APPLICATIONS IN IMMUNOTHERAPY, HYBIDOMA TECHNOLOGY AND MONOCLONAL ANTIBODIES IN MEDICINE AND VETERINARY MEDICINE.

ANTIGENS AND ANTIGENIC DETERMINANTS

Antigens: - these are compounds which are capable of reacting with an antibody i.e. substance that reacts with the products of a specific immune response without necessarily being capable of inducing antibody formation.

Immunogen is used to describe substances that induces a specific immune response i.e. substances capable of eliciting Ab formation when injected into a host.

Generally however antigens or immunogens can be used interchangeably to refer to is a molecule which is capable of stimulating specific immunity.

Antigens could be proteins (syntheticpolypeptides, lipoproteins, and glycoproteins), polysaccharides (including lipopolysaccharides), nucleic acids or lipids. This includes parts (coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, fungi and other microorganisms, as well as non-microbial substances such as pollen, egg white, dust, bee sting, certain foods, morphine etc which are called **Allergens**, that bring on an allergic reaction (a type of immunologic reaction), (hence allergens are a class of antigens that produce allergic reactions).

All cells of the body possess Ags on their surface which acts as markers to help cells recognize each other however "Self" antigens are usually tolerated by the immune system; whereas "Non-self" antigens are identified as intruders and attacked by the immune system.

Immunogenicity is the ability to induce a humoral (antibody production) and/or cell-mediated immune response.
Antigenicity is the ability to combine specifically with the final products of the immune response.

Hapten:-is a chemically defined determinant that when conjugated to an immunogenic carrier stimulates synthesis of antibodies specific for that hapten. Free haptens, however, can react with products of the immune response after such products have been elicited. Haptens have the property of antigenicity but not immunogenicity.

Epitope:-is the unique region on an Ag that will bind a complementary Ab i.e. that portion of an antigen that combines with the products of a specific immune response. It is also called antigenic determinant. Epitopes generally are significantly smaller than the antigens which contain them (and much smaller than the size of an antibody).A single antigen often contains numerous Epitopes. Antigenic determinants react with Abs in a lock and key fashion based on structural

complementarity. Forces characteristic of Ag-Ab binding include Van Der Waal-London dipole interactions, hydrophobic interaction and ionic Columbic bonding, these cooperate together between the Epitopes of the Ags and the variable Fab regions of the Abs to form immune complex. The other [portion of the Ag other than the Epitope are termed immunogenic carrier.

Antigens enter the body through any of the following routes- ingestion, inhalation or injection.

PROPERTIES OF ANTIGENS OR IMMUNOGENS

1. Foreignness- the body can distinguish its own antigen from foreign ones, (having gotten used to overstimulation by the body's antigen) it does not produce antigens against itself but does readily to a new Ag that is introduced into the body. It has been observed that excess stimulation of the immune system by an Ag can lead to an immunologic paralysis where no Ab is mounted and this can be used to explain why the body does not mount Abs against its own Ags which are constantly present to stimulate it.
2. There are areas of structural stability within the molecule.
3. Size-a minimal molecular wt of 4000 to 5000Da, although there is no absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.
4. The compound should have ability to be metabolized or degradability, Ags that are easily phagocytosed are generally more immunogenic.
5. Randomness of structure-
6. **Physical form-**In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.
7. 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.
8. Accessibility to the immunogenic configuration of the Ab forming mechanism.
9. Affinity is a property of an Ag that refers to the energy of interaction between a single Ab combining site and corresponding Epitope on the Ag.

TYPES OF ANTIGENS

Ags can be broadly classified into exogenous and endogenous Ags.
Exogenous Ags are those that have entered the body from the outside, for example by inhalation, ingestion, or injection.

While endogenous antigens are antigens that have been generated within previously normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection. Endogenous antigens include xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens.
Ags may also classified as i) T-independent Ags which can directly stimulate the B cells to produce antibody without the requirement for T cell help, in general, polysaccharides are T-independent antigens and ii) T-dependent Ags that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens.
Other classes of Ags include autoantigens and tumor antigens.

Nature of antigens

Antigenic determinants can either be immunogenic or haptenic, the 3 dimensional structures of Ags are important in Ab specificity. It is believed that an immunogen must possess at least two determinants to stimulate Abs formation. In general antigenic determinants are small and are limited to approximately 4-8 residues. (Amino acids and/or sugars). The combining site of an antibody will accommodate an antigenic determinant of approximately 4-8 residues (ADs recognized by T-cells have 8-15 amino acids). Optical configuration and physical conformation contribute antigenic determinant immunochemical specificity.

Vaccination

The medical practice of immunization began at the end of the eighteenth century, when English physician Edward Jenner (1749–1823) successfully used extracts of body fluid from a dairymaid (a woman employed in a dairy) infected with cowpox (a mild disease) to inoculate a young boy against smallpox, a then-common and often fatal viral disease. Jenner called his method "vaccination," using the Latin words *vacca*, meaning "cow," and *vaccinia*, meaning "cowpox." Because the two diseases are caused by similar viruses that have the same antigens, antibodies that work against cowpox will also fight smallpox. In 1885, a rabies vaccine developed by French scientist Louis Pasteur (1822–1895) from the spinal fluid of infected rabbits proved to be successful. Since that time, vaccines have been developed for many diseases, including diphtheria, polio, pertussis (whooping cough), measles, mumps, rubella (German measles), hepatitis, and influenza. Vaccines are made from either weakened live or killed microorganisms. When introduced into the body, they stimulate the production of antibodies, providing active immunity against bacterial and viral diseases

IMMUNOTHERAPY

Immunotherapy is also called biologic therapy or biotherapy, it is treatment of disease by inducing, enhancing, or suppressing an immune response, and it incorporates an array of strategies of treatment based upon the concept of modulating the immune system to achieve a prophylactic (preventive) and or therapeutic (treatment) goal. Immunotherapy involves the

functions of the immune system which includes lymphocytes- B lymphocytes and T lymphocytes that include killer cells, T-helper cells and regulatory (suppressor) cells; and natural killer cells.

There are two main types of immunotherapies;

1. Active- here the body's own immune system is stimulated to fight the disease e.g. cancer vaccines, lymphokine activated killer cell therapy,tumour infiltrating lymphocyte vaccine, interleukine-2 etc

Vaccines are weakened, killed or live viruses, bacteria and other microorganisms and toxin administered to start an immune response in the body.
Passive-use of immune system components created outside the body such as antibodies e.g. monoclonal antibodies(antibodies are identical antibodies produced by clones (exact copies) of a single cell) antiserum (*polyclonal antibodies*), or the use of T-cell therapy to target and destroy harmful cancer cells and transplants that use donor immune cells to fight cancer or other diseases etc.

An additional form of immunotherapy is non-specific immunotherapies and adjuvants (immune stimulants) given to boost immune functions and improve how well another therapy works.

MONOCLONAL ANTIBODIES (MAbs)

These are antibodies produced by a single clone of plasma cells having identical structure and specificity and predictability (they bind to only a single Epitope on an Ag). They maybe polymers or monomers or fragments and are also called paraproteins. Normally pure, large quantities of any individual antibody are difficult to produce within an animal, the occurrence of MAbs in serum within the body is mainly due to pathological states called multiple myeloma (a malignant neoplasm of a single clone of plasma cells of the bone marrow that sometimes forms a solitary tumor called plasmacytoma, this often affects synthesis of other clones or plasma cells) or plasmacytoma.

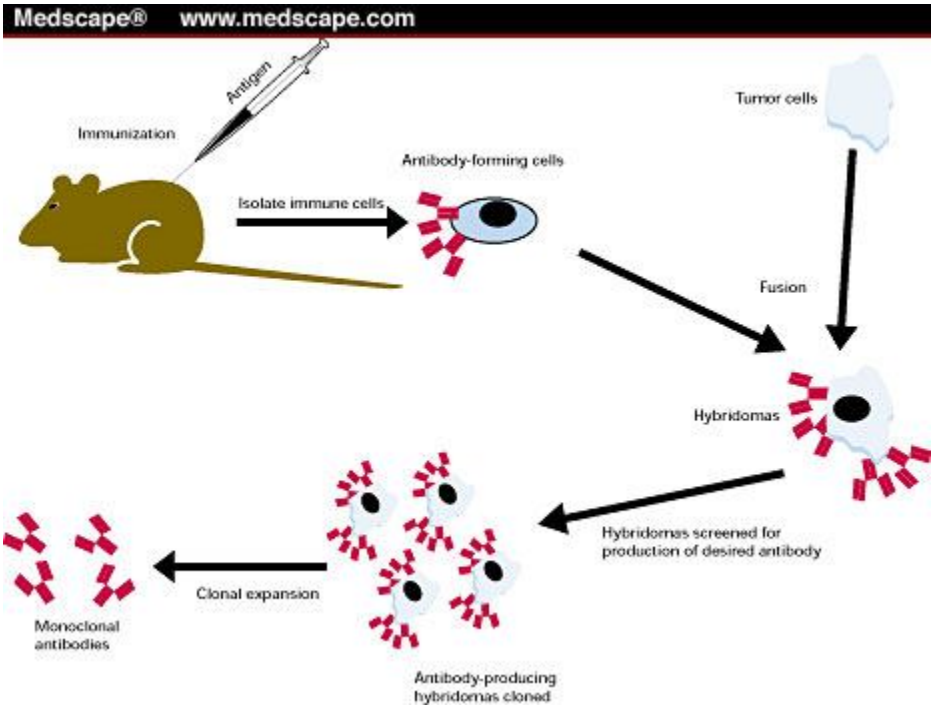
Monoclonal antibodies are the most widely used form of cancer immunotherapy at this time, they also widely used as reagents in immunoassay techniques (diagnosis of diseases).

The method for developing MAbs was developed by Milstein and Köhler and generally referred to as hybridoma technology.

HYBRIDOMA TECHNOLOGY

This involves a series of processes carried out to obtain large amounts of a single clone of Abs specific for one Epitope or antigenic determinant.

- B cells are obtained from the spleen of a laboratory animal that was initially injected with an Ag of interest or mixture of Ags.
- These B cells are fused with mouse myeloma (cancer) cells to make them immortal (otherwise they would eventually die out during propagation in tissue culture) by mixing the two types of cells in the presence of polyethylene glycol (PEG) which causes the cells to fuse by ununderstood mechanisms. The resulting cell is called a *hybridoma*.
- The mouse myeloma cells are cancer cells of the RES, specifically immortal B cells which are deficient in hypoxanthine guanine Phosphoribosyl Transferase (HGPRT) enzyme so cannot synthesize purine bases necessary for production of Abs.
- The cells are then placed in a selective medium- Hypoxanthine Aminopterin Thymine (HAT) medium to grow the fused hybrid cells selectively. The hybridoma cells can survive HAT medium while all unfused cells cannot be maintained in the medium and die.
- The hybrid cells continue to multiply as well as produce Abs. The antibody produced by individual *hybridomas* is characterized (screened, and cell lines detected).Desirable *hybridomas* (i.e., those making antibodies with desirable properties) may be grown and antibody produced via standard tissue culture techniques i.e. cloned in subcultures.
- The hybridoma cells can be frozen and stored and subsequently thawed when more Abs is required. They may also be grown in abdomen of mice and provide large supplies of Abs.



Application of monoclonal bodies (immunotherapy) in medicine and veterinary medicine.

1) MAbs are mainly used in the treatment of cancers. They have important clinical applications in the detection and early diagnosis of cancer
2) They can be used find or identify their specific antigens and this mainly applied for diagnostic purposes e.g. pregnancy diagnosis, disease diagnosis, tentative diagnosis of conditions such as cancer
3) They can be used to measure amounts of individual proteins (measuring protein and drug levels in serum).
4) Determine nature of infectious agents (identifying infectious agents)
5) Subclassify both normal and tumor cells
6) Accelerate the removal of drugs from the circulation when they reach toxic levels.
7) Used for typing of T and B cells
8) Detecting serological differences in viruses
9) Experimental treatment of lymphoid malignancies.etc

Autoimmune Disease

Autoimmune diseases occur when the body's immune system loses the ability to recognize the difference between self and foreign molecules. This results in the body producing antibodies, called autoantibodies, against its own tissues. Normally, antibodies are only produced against microorganisms that invade the body. The inability to make a distinction between self and nonself may lead to the destruction of body tissue and result in a number of chronic, debilitating diseases.

The cause of autoimmune reactions is not known. It is thought that infection by viruses and bacteria may trigger an autoimmune response. In addition, exposure to certain chemicals and ultraviolet light may alter proteins in the skin; the body may then become sensitive to these proteins and produce autoantibodies against them. Certain individuals seem to be genetically predisposed to have autoimmune responses. Some diseases that are associated with autoimmune responses are rheumatoid arthritis, lupus erythematosis, and pernicious anemia.

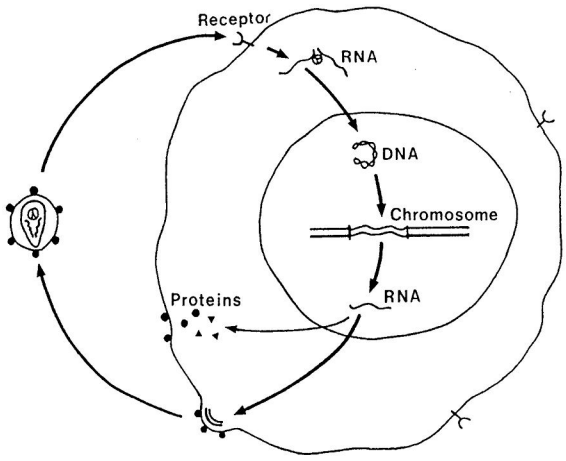
RETROVIRUSES, MOLECULAR BASES AND INVOLVEMENT IN CANCER, PROTONCOGENES AND ONCOGENES

RETROVIRUSES.

- Viruses are the smallest infectious agent known and can only replicate inside the cells of another organisms.
- There are two broad classes of viruses; namely DNA and RNA viruses.
- Oncogenic or tumor viruses are viruses capable of inducing the formation of cancer. Tumor viruses are of two distinct types, there are viruses with DNA genomes (e.g. papilloma and adenoviruses) and those with RNA genomes (termed retroviruses).
- Retroviruses are oncogenic viruses having RNA genomes. They were first associated with malignant diseases in animals more than hundred years ago and have been shown to cause leukemia, lymphoma and other forms of cancer in a wide variety of vertebrate animals ranging from fish to apes. They have been identified in virtually all organisms including invertebrates. The first oncogenic human retrovirus was isolated in1980.

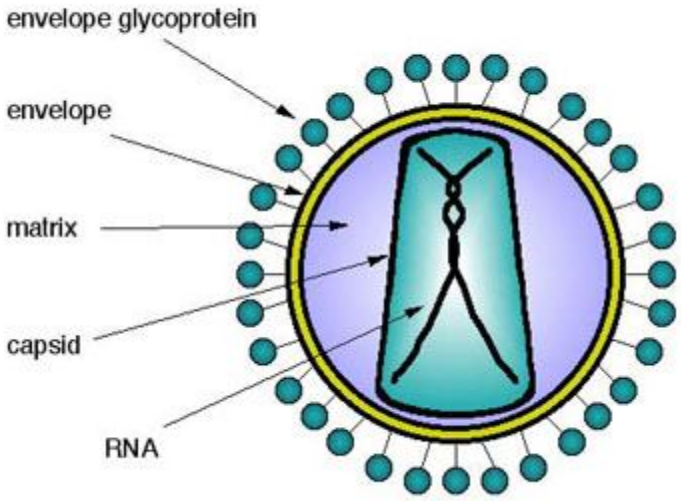
Retroviruses carry diploid, single-stranded RNA genomes in the virion and replicate by forming one double stranded DNA copy in the infected cell, by means of the viral enzyme, reverse transcriptase. This viral genome becomes integrated as a DNA called the 'provirus', into the chromosomal DNA of the host cell and thus persists for the lifetime of the infected cell and its progeny. The proviral genome carries its own promoter and enhancer elements in sequences duplicated at each end of the genome, known as long terminal repeats (LTR).
Expression of the provirus yields full length RNA transcripts that are packaged to become the genomes of progeny virus particles, and mRNA that is translated to provide the viral proteins. (They are transcribed by an RNA dependent DNA polymerase (reverse transcriptase) to produce a double stranded DNA copy of their RNA genome and subsequently serves as a template for gene expression).

Retroviruses are classified into groups including oncovirinae, (Rous Sarcoma Virus ((RSV) - which causes a slow neoplasm in chickens, the first retrovirus to be discovered), Lentivirinae **(**visna virus) and spumavirinae (spumaviruses).



Basic structure of the retrovirus includes an outer envelope which comes from the host cell plasma membrane, coat proteins (surface antigens) ,inside the membrane is an icosahedral capsid containing proteins, that also coat the genomic RNA. There are two molecules of genomic

RNA per virus particle with a 5' cap and a 3' poly A sequence. Thus, the virus is diploid. The RNA is plus sense (same sense as mRNA).About 10 copies of reverse transcriptase are present within the mature virus(a polymerase that copies RNA to DNA), Integrase (integrates the viral genome into the host genome), RNase H (cleaves the RNA as the DNA is transcribed so that reverse transcriptase can make the second complementary strand of DNA) and Protease (cleaves the polyproteins).



CANCER

Neoplasm is an abnormal mass of tissue with growth that exceeds and is uncoordinated with that of the surrounding normal tissues and persists in the same excessive manner even after cessation of the stimulus which evoked the change. Cancer is a common term used to refer to malignant neoplasm, they are characterized by diminished control of division, spread or metastasis of dividing cells to other parts of the body and invasion of local tissues. Cancers are the result of a disruption of the normal restraints on cellular proliferation. Three principal groups of agents have been known to cause cancer and these include radiant energy, chemical compounds and biological agents such as viruses. The central feature involved in the occurrence of cancer is damage to cellular DNA which subsequently affects regulatory processes in cells. Approximately 20% of human cancer incidence worldwide is attributable to virus infection.

PROTOONCOGENES AND ONCOGENES

A proto-oncogene is a gene whose protein product has the capacity to induce cellular transformation given it sustains some genetic insult (genes that cause normal cells to become cancerous when they are mutated).
Mutations in proto-oncogenes are typically dominant in nature, and the mutated version of a proto-oncogene is called an oncogene. Often, proto-oncogenes encode proteins that function to stimulate cell division, inhibit cell differentiation, and halt cell death. These activities of proto-oncogene are typically turned off once the developmental processes they regulate are completed. However, if the activity remains high, or if proto-oncogenes are inappropriately reactivated later in life, cancer may occur.

An oncogene is a gene that has sustained some genetic damage and, therefore, produces a protein capable of cellular transformation thus leading to increased cell division, decreased cell differentiation, and inhibition of cell death (taken together, these phenotypes define cancer cells) in other words, an oncogene is a gene that codes for a protein that potentially can transform a normal cell into a malignant cell. It may be transmitted by a virus in which case we refer to it as a viral oncogene.

Oncogenes arise as a result of mutations that increase the expression level or activity of a proto-oncogene. Underlying genetic mechanisms associated with oncogene activation include the following:

- Point mutations, deletions, or insertions that lead to a hyperactive gene product
- Point mutations, deletions, or insertions in the promoter region of a proto-oncogene that lead to increased transcription
- Gene amplification events leading to extra chromosomal copies of a proto-oncogene
- Chromosomal translocation events that relocate a proto-oncogene to a new chromosomal site that leads to higher expression
- Chromosomal translocations that lead to a fusion between a proto-oncogene and a second gene, which produces a fusion protein with oncogenic activity.

There are two classes of these genes in which altered expression can lead to loss of growth control:

(a) Those genes that are stimulatory for growth and which cause cancer when hyperactive. Mutations in these genes will be dominant these are classically referred to as oncogenes. Proto-oncogene's encodes growth factors such as epidermal growth factor (EGF), intracellular proteins to stimulate cell growth and division, Signaling of hormone , GTP-binding proteins involved in signal transduction from a surface receptor to the nucleus etc.

(b) Those genes that inhibit cell growth and which cause cancer when they are turned off. Mutations in these genes will be recessive. These are the anti-oncogenes or tumor-suppressor genes growth suppressors or recessive oncogenes.

MOLECULAR INVOLVEMENT OF RETROVIRUSES IN CANCER.

Viruses are involved in cancers because they can either carry a copy of one of the proto-oncogene's or can alter expression of the cell's copy of one of these genes.

The following stages occur in the infection process:

1) Binding to a specific cell surface receptor

2) Uptake by endocytosis or by direct fusion to the plasma membrane. The virus may require entry into a low pH endosome before fusion can occur, although some (e.g. HIV) can fuse directly with the plasma membrane

3) RNA (plus sense) is copied by reverse transcriptase to minus sense DNA. Here, the polymerase is acting as an RNA-dependent DNA polymerase. Since reverse transcriptase is a DNA polymerase, it needs a primer. This is a tRNA that is incorporated into the virus particle from the previous host cell.

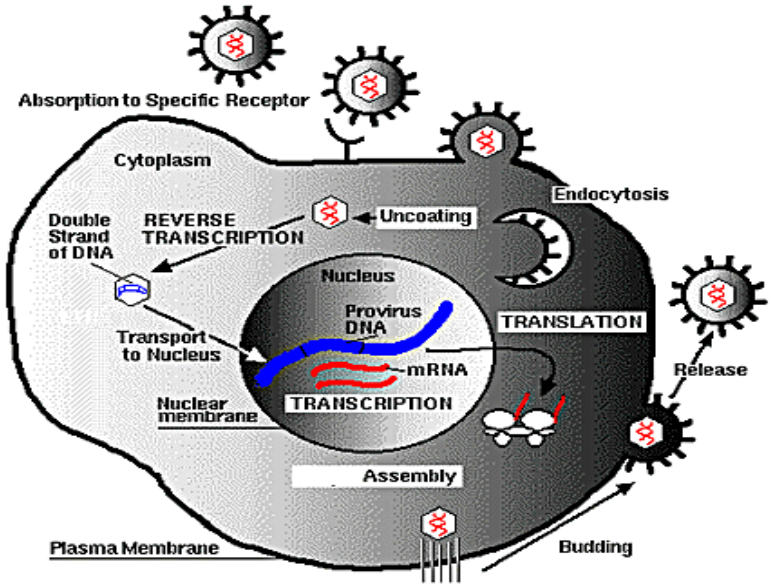
4) RNA is displaced and degraded by a virus-encoded RNase H activity. Reverse transcriptase now acts as a DNA-dependent DNA polymerase and copies the new DNA into a double strand DNA. This DNA form of the virus is known as a provirus.

5) Double strand DNA is *circularized* and *integrated* into host cell DNA (see below) using a virally encoded integrase enzyme. This DNA is copied every time cellular DNA is copied. Thus, at this stage the provirus is just like a normal cellular gene.

6) Full length, genomic RNA (plus sense) is copied from the integrated DNA by host RNA polymerase II which normally copies a gene to mRNA. The genomic RNA is capped and poly adenylated, just as an mRNA would be.

At some frequency, the viral DNA (provirus) integration process into the host genome leads to rearrangement of the viral genome and the consequent incorporation of a portion of the host genome into the viral genome. This process is termed transduction. Occasionally this transduction process leads to the virus acquiring a gene from the host that is normally involved in cellular growth control. Because of the alteration of the host gene during the transduction process as well as the gene being transcribed at a higher rate due to its association with the retroviral LTRs the transduced gene confers a growth advantage to the infected cell. The end result of this process is unrestricted cellular proliferation leading to tumorigenesis. The transduced genes are termed oncogenes. The normal cellular gene in its unmodified, non-transduced form is termed a proto-oncogene since it has the capacity to transform cells if altered in some way or expressed in an uncontrolled manner. Numerous oncogenes have been discovered in the genomes of transforming retroviruses.

The second mechanism by which retroviruses can transform cells relates to the powerful transcription promoting effect of the LTRs. When a retrovirus genome integrates into a host genome it does so randomly. At some frequency this integration process leads to the placement of the LTRs close to a gene that encodes a growth regulating protein. If the protein is expressed at an abnormally elevated level it can result in cellular transformation. This is termed retroviral integration induced transformation. It has recently been shown that HIV induces certain forms of cancers in infected individuals by this integration induced transformation process.



Retrovirus replication

TRANSLOCATION AND GENE ARRANGEMENT IN DISEASE STATE OF ANIMALS.

GENETIC DEFECTS

Genetic defects are caused by abnormalities in genes or chromosomes. There are three main types of gene diseases including gene mutation, chromosomal mutations and multifactorial problems.

Gene mutations refer to changes in gene structure as a result of change in the sequence of nucleotides of the DNA molecule in a particular region of the chromosome (alterations in DNA sequences), which is transferred to the mRNA (during transcription) and results in amino acid or protein alteration (during translation) and is subsequently seen as spontaneous changes in the phenotype as against that which as originally genotypically typed. These changes include deletion, inversions, substitution and insertion.

Mutations can also occur at the level of the chromosomes, and maybe structural or numerical. Structural chromosome aberrations include translocations, inversion, deletion, transpositions and duplication. Types of changes in the number of chromosomes in a cell maybe grouped as aneuploidy, polyploidy and abnormal euploidy. Chromosomal defects usually have more profound effects on the phenotype than gene mutation and these changes occur during meiosis.

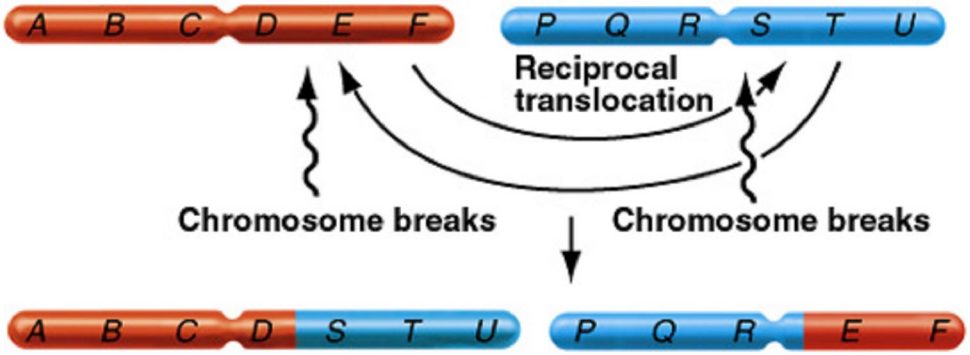
Mutations can be caused by copying errors in the genetic material during cell division, by exposure to ultraviolet or ionizing radiation, chemical mutagens, or viruses, or can be induced by the organism itself, by cellular processes such as hypermutation. In multicellular organisms with reproductive cells, mutations can be subdivided into germ line mutations, which can be passed on to progeny through the reproductive cells (during meiosis), and somatic mutations, which involve cells outside the reproductive group and which are not usually transmitted to offspring (during mitotic division).

CHROMOSOMAL MUTATIONS

TRANSLOCATION

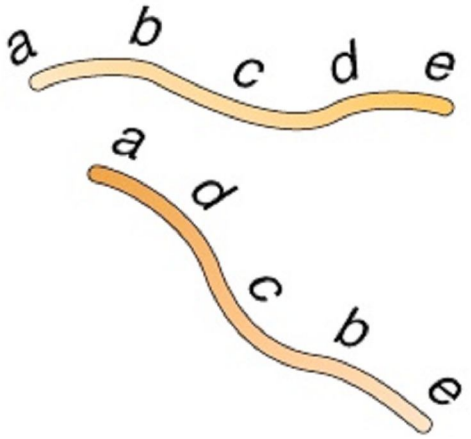
This occurs when a segment breaks off and rejoins of another end of the chromosome (reciprocal or balanced translocation) or another chromosome entirely (a non-homologous pairs; non-reciprocal or unbalanced translocation). Where there is translocation between non-homologous pairs, new pairs of homologous chromosomes can be produced (lead to duplications and deletions in progeny).

Translocations can often alter or abolish expression of the gene and gene products and maybe lethal. There are usually no consequences of translocation in homozygotes; genetic material is neither lost nor gained but in heterozygotes with non-reciprocal translocation genetically imbalanced gametes result with deletions or duplications; zygotes produced by these gametes are not viable.



INVERSIONS

This occurs when the order of a particular gene is reversed and result from insertion of a chromosome fragment in reverse orientation after breaking off the parent chromosome, there are usually no phenotypic consequences. However it can sometimes lead to a mutant phenotype i.e. the sequence may not be viable to produce an organism depending on which genes are affected. Advantageous characteristics from these mutations are also possible.



DELETIONS

Deletion (loss of segment); In these conditions genes of a chromosome are permanently lost as they become unattached to the centromere and are lost forever, hence the new chromosome after meiotic division, lacks certain genes which may prove fatal depending on how important these genes are.

Deletions maybe intragenic deletion; where small deletion within gene occurs and inactivates gene and has the same effect as a other null mutations of that gene, or multigene deletion in which case many genes are deleted, often with severe consequences such as gene imbalance.

Pseudodominance is a phenomenon that can also result from deletion where it seems as if the recessive alleles are showing dominance because the dominants have been deleted and possible expression of deleterious recessive mutation.

TRANSPOSITION

This refers to movement of DNA elements or segment from one site in the genome to another. Certain mobile genetic elements exists and can be found in all organisms, they have no known functions and are also known as transposons (transposable elements). There are two main classes of transposons- retrotransposons (related to retroviruses) and DNA-only transposons.

DUPLICATION

This is the gain of a segment. It is usually a source of new genes and gene families.
It can result into tandem duplication where segment is attached adjacent to its duplicate (adjacent duplications) in same or reverse order or non-tandem/ insertional duplication, here duplicate gene inserted elsewhere in the genome (same or reverse order). It may be a consequence of unequal crossing-over.
Most duplications have no phenotypic consequence but sometimes the effects can be seen due to increased gene dosage. Duplication plays a very important role in evolution through increase gene number and evolution of new genes (paralogs).
The mutant genes are displayed twice and the duplicate is usually harmless.

BLOOD: GENERAL PROPERTIES AND FUNCTIONS

Blood is a specialized body fluid that delivers necessary substances to the body's cells — such as nutrients and oxygen — and transports waste products away from those same cells. It can be referred to as a liquid connective tissue.

It consists of solid elements made up of RBCs, WBCs and platelets (commonly referred to as the formed elements of the blood),suspended in a fluid medium, plasma which contains, water (about92%),dissolved proteins, lipids, glucose, mineral ions, hormones, organic acids, urea and other wastes, carbon dioxide and circulates in a closed system of blood vessels

Vertebrate blood is bright red when its hemoglobin is oxygenated. By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%.

PROPERTIES OF BLOOD.

1. It is a viscous liquid with its flow properties adapted to flow effectively through tiny capillary blood vessels with as little resistance as possible.
2. Blood plasma, a fluid that is the blood's liquid medium, is straw-yellow in color, which comprises 55% of blood fluid, is mostly water (90% by volume)
3. The white blood cells consist of lymphocytes and monocytes with relatively clear cytoplasm, and three types of granulocytes, whose cytoplasm is filled with granules.
4. The normal pH of blood is in the range of 7.35–7.45
5. It has an average density of approximately 1060 $kg/m^{3.}$.
6. The various cells of blood are made in the bone marrow in a process called hematopoiesis.
7. The proteinaceous component of blood (including clotting proteins) is produced predominantly by the liver, while hormones are produced by the endocrine glands and the watery fraction is regulated by the hypothalamus and maintained by the kidney.

FUNCTIONS OF BLOOD

Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)

Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids)

Removal of waste such as carbon dioxide, urea, and lactic acid

Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies

Coagulation, which is one part of the body's self-repair mechanism

Messenger functions, including the transport of hormones and the signaling of tissue damage

Regulation of body pH and temperature.

THE RED CELL AND ITS METABOLISM

RBCs in mammals are non-nucleated biconcave shaped cells, highly flexible and lacking intracellular organelles. They are flattened and depressed in the center. Erythrocyte content consists mainly of hemoglobin. The precursors (Pronormoblast) of erythrocytes mature in the bone marrow, in a process called erythropoiesis, closely attached to a macrophage, these precursor cells manufacture hemoglobin until it accounts for some 90% of the dry weight of the cell, and as it matures the nucleus is squeezed out of the cell and is ingested by the macrophage. In addition the no-longer-needed proteins are expelled from the cell in vesicles called exosomes.

RBCs are terminally differentiated, that is, they can never divide, and live for about 120 days after which they and engulfed and phagocytosed by cells of the RES predominantly in the spleen, bone marrow and liver. They are responsible for the transport of oxygen and carbon dioxide.
In addition to their major function of O_2 and CO_2 transport RBCs also play some role in immune response by release of free radicals from damaged cells to destroy invading pathogens and also release S-nitrothiols that facilitate vasodilation when they (RBCs) are deoxygenated.

In many domestic animals such as dogs and horses the spleen acts as a reservoir of erythrocytes and sequesters large numbers of red blood cells which are dumped into the blood during times of exertion stress, yielding a higher oxygen transport capacity.

METABOLISM

As a result of not containing mitochondria matured RBCs do not utilize the oxygen they carry for energy unlike other cells, instead they use glucose to produce ATP by glycolytic pathway that ends with lactic acid production. Glucose enters the red cell via specified system of transport that is not influenced by insulin.

The pentose phosphate pathway is also for energy production. Reduced glutathione is very essential in the RBC as it helps to counteract the actions of potentially toxic peroxides produced in the course of metabolism. Ron in RBC is usually maintained in the ferrous form by NADH-dependent methemoglobin reductase

BLOOD CLOTTING MECHANISMS

IRON: SOURCES, ABSORBTION, DISTRIBUTION IN THE BODY, BIOMEDICAL FUNCTIONS AND EXCRETION

IRON AND ITS METABOLISM

(SOURCES, ABSORBTION, DISTRIBUTION IN THE BODY, BIOMEDICAL FUNCTIONS AND EXCRETION)

Iron is the 26^{th} atom in the periodic table with a molecular weight of approximately 56. It is the most abundant trace element as it is present in most cells of the body, plasma and the ECF.

Iron is an absolute requirement for most forms of life ,it serves numerous functions in the body especially relating to the transport of O_2 in Hb. Its unique ability to serve as both an electron donor and acceptor (and bind electronegative elements like nitrogen, oxygen and sulphur) makes it important in many life processes.

It exists in two states of oxidation in the body which are the ferric form (Fe^{3+}) and the ferrous form (Fe^{2+}). Fe^{3+} is favored at neutral PH while Fe^{2+} is favored in more acidic PH. When in the Fe^{3+} state, iron will form large complexes with anions, water and peroxides.

SOURCES

Hemoglobin , myoglobin and other heme proteins in meat, liver, blood meal and other animal protein as well in lima, soy and kidney beans, spinach, tuna, wheat, millet and oats and so on.

ABSORBTION

Heme iron, contained mainly in animal products, is absorbed much better than non-heme iron (vegetable iron) which accounts for over 85% of iron in the average diet. However, absorption of non-heme iron is increased when it is consumed with animal protein and vitamin C.

Most intestinal iron absorption occurs in the duodenum and jejunum (the first two sections of the small intestine). Iron uptake is tightly controlled to prevent iron overload, so only 6-12% percent

of dietary iron is absorbed by the intestines. Free iron in the intestines is reduced from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state on the luminal surface of intestinal enterocytes and transported into the cells through the action of the divalent metal transporter, DMT1, intestinal uptake of heme iron occurs through the interaction of dietary heme with the heme carrier protein (HCP1). The iron in the heme is then released within the enterocytes via the action the heme catabolizing enzyme heme oxygenase.

Iron is transported across the basolateral membrane of intestinal enterocytes into the circulation, through the action of the transport protein ferroportin, another enzyme hephaestin (a copper-containing ferroxidase with homology similar to ceruloplasmin), oxidizes the ferrous form back to the ferric form. Once in the circulation, ferric form of iron is bound to transferrin and passes through the portal circulation of the liver.

BODY DISTRIBUTION OF IRON

Iron is distributed in several compartments in the body, they are;

1. Hemoglobin ; which contains 0.34% of Fe by weight found within the RBCs.
2. Tissue iron; this is in the form of cellular enzymes and coenzymes either as part of the molecule or as a cofactor e.g. peroxides and cytochromes. All the iron within nucleated cells are referred as tissue iron.
3. Myoglobin; is a muscle protein containing iron similar to hemoglobin but does not occur as tetrameres.
4. Labile pool; this is iron found in no clear anatomical locations within the body.

Transferrin, synthesized in the liver, is the serum protein responsible for the transport of iron. Although several metals can bind to transferrin, the highest affinity is for the ferric (Fe^{3+}) form of iron. The ferrous form of iron does not bind to transferrin. Transferrin can bind two moles of iron. It can also serve as intracellular transporter o iron within the cell.

Ferritin is the major protein used for intracellular storage of iron. Ferritin without bound iron is referred to as apo-ferritin. Apo-ferritin is a large polymer of 24 polypeptide subunits. This multimeric structure of apo-ferritin is able to bind up to 2,000 iron atoms in the form of ferric-phosphate. The majority of intracellularly stored iron is found in the liver, skeletal muscle and reticuloendothelial cells.

Excess iron is toxic and may damage the intestines and other organs, as well as cause vomiting and diarrhea hence need for strict regulation of its absorption, the body's complex system of iron regulation and ferritin recycling ensures that as little iron is excreted as possible.

EXCRETION

Excess dietary iron is not absorbed or stored in intestinal enterocytes but is excreted in feces. As little iron is excreted as possible normally, most being recycled or stored in the body for later use. However losses do occur through the intestines, skin cell exfoliation, sweat and urine. Bleeding can also deplete iron reserves, necessitating enhanced activation of iron absorption machinery.

ANEMIA AND HEAMACHROMATOSIS

ANEMIA

This refers to shortage of RBCs or the content of Hb in them. This insufficient red cell mass can be the result of excessive destruction of RBCs (hemolysis i.e. hemolytic anemia), bleeding, blood disorders like thalassemia, or nutritional deficiencies e.g. iron, vitamin B12 (needed for the synthesis of Hb) deficiencies etc.

Hemolytic anemia occurs when red blood cells are being destroyed prematurely, due to a variety of reasons such as infections or certain medications — such as antibiotics or antiseizure drugs etc in autoimmune hemolytic anemia, the immune system mistakes RBCs for foreign invaders and begins destroying them. Blood disorders such as thalassemias, hemoglobinopathies can also result in rapid destruction of RBCs.

Bleeding or blood loss can also cause anemia and maybe because of excessive bleeding due to injury, surgery, cancers or a problem with the blood's clotting ability.

Inadequate production of RBCs is also another major cause of anemia and this could possibly be due to nutritional deficienciese.g.iron deficiency anemia, the most common cause of anemia in piglets. Or it maybe due to problem with the bone marrow due to a viral infection, or exposure to certain toxic chemicals, radiation, or medications (such as antibiotics, antiseizure drugs, or cancer treatments),or as a result of kidney failure (produces erythropoietin).

SIGNS

The first symptoms might be mild skin paleness and decreased pinkness of the mucous membranes. Irritability, fatigue, weakness and a rapid heartbeat. If the anemia is caused by excessive destruction of RBCs, symptoms also may include jaundice, a yellow discoloration of the mucous membranes. Decreased appetite, blood in the urine or feces, an enlarged spleen, abdominal distension and dark tea-colored urine may also be seen.

HEMACHROMATOSIS

This is a disorder of iron metabolism as a result of excess iron absorption, saturation of iron binding proteins and deposition of hemosiderin (amorphous iron deposits in cells, composed of ferritin, denatured ferritin, and other materials with its molecular structure poorly defined in tissues). Primarily affected are liver, pancreas, skin and can lead to cirrhosis of the liver and diabetes (when the pancreas is affected) and bronze pigmentation of the organs and skin.

The bronze pigmentation and resulting diabetes warrants the designation of the disease as bronze diabetes.

The condition is primarily genetic due to inheritance of an autosomal recessive allele. HFE gene (a histocompatibility complex gene) regulates iron transfer into cells via its formation of complex with transferrin hence a mutation in this gene results in abnormal iron intake and

storage. Secondary hemachromatosis which is not genetic can result from excess oral intake of iron or in patients receiving blood transfusion.

HAEMOGLOBIN: STRUCTURE, PROPERTIES AND BIOMEDICAL FUNCTIONS

Hemoglobin is the iron-containing oxygen-transport metalloproteins in the red cells of the blood in mammals and other animals. A spheroidal heme protein having four subunits each consisting of a globular protein non-covalently bound, with an embedded heme group. Hb has a molecular weight of about 64456. The globular protein units of Hb is made up of two identical pairs of polypeptide chains, i.e. two identical alpha (α)chains containing 141 amino acids and two identical non- α chains (beta(β),gamma(γ),delta(δ) or epsilon (ε) chains. In adult humans the non- α chains are beta (β), containing 146 amino acids. This is denoted as $\alpha 2\beta 2$ and termed hemoglobin A. The combination of two alpha chains and two gamma chains form fetal hemoglobin, termed hemoglobin F. The product of the delta globin gene is called hemoglobin A2

The different kinds of chains are encoded for by different genes. The genes that encode the alpha globin chains are on chromosome 16 (Figure 2). Those that encode the non-alpha globin chains are on chromosome 11 in humans.

The pairing of one alpha chain and one non-alpha chain produces a hemoglobin dimer (two chains). The hemoglobin dimer does not efficiently deliver oxygen, however. Two dimers combine to form a hemoglobin tetramer, which is the functional form of hemoglobin.

The heme group consists of an iron atom held in a heterocyclic ring, known as a porphyrin. This iron atom is the site of oxygen binding. The iron atom binds equally to all four nitrogen atoms in the center of the ring, which lie in one plane. Oxygen is then able to bind to the iron centre perpendicular to the plane of the porphyrin ring while the last position is used to form a coordinate covalent bond with the side chain of a single histidine amino acid of the protein, called the proximal histidine.

STRUCTURE

Hb is largely alpha-helical; each chain contains helical segments between which are short non coiled segments. The chains are wound round itself to form a pocket in which the heme group nestles and this pocket is usually formed by hydrophobic amino acids.

FUNCTIONS

1. Hb binds and transports oxygen from the lungs to tissues.
2. It also transports CO_2 from tissues to lungs.
3. It acts as a buffer, by transporting protons as $Hb.2H^+$.

HAEMOGLOBINOPATHIES: HBS, THALASSAMIEAS, HEMOPHILIA

HEMOGLOBINOPATHIES.

These are disorders in the structure of Hb resulting in altered biologic function as a result of defects in the genes that code for one or more of the globin chains. More than 700 structural variants of Hb have in described in man and animals .

Hbpathies can occur as a result of point mutations in the DNA code for globin chains, others as a result of deletions of extensive portion of the globin genomes or as a result of insertion of single or double nucleotides, inversions or substitution.

Defects in Hb can occur in one of three circumstances;

1. Structural defects in the Hb molecule as a result mutations in the globin gene
2. Diminished production of one of the globin subunits- this mutational changes result in a condition called thalasemias.
3. Abnormal association of the otherwise normal subunits e.g. all four subunits being solely α or β chains.

HbS

Is a classical example of Hbpathies that occurs in humans where the two α chains are normal but one of the β chains has a mutation which is a single base substitution reflected at the level of the sixth amino acid, where an adenine nucleotide is replaced by thymine giving a GTG codon (for valine) instead of a GAG(for glutamic acid) which is found in HbA the normal hemoglobin.

This single amino acid substitutions causes a considerable change in the structure of the entire Hb molecule by causing a protrusion that accidentally fits into a complememtary site on the β chain of the next Hb molecule hence the Hb molecules hook together, are collapsed and result in a sickle shaped, rigid RBC i.e. the Hb lies along like fibres in the RBC instead of being globular, Valine is less polar than glutamic acid, and most hydrophobic amino acids aggregate together internally and expose the hydrophilic (polar) ones to react with water.

Thus the RBCs with such sickle shape are unable to carry oxygen adequately especially if the oxygen tension is low, but when the oxygen tension is high, the Hb molecules depolymerize and return to a normal state but this only for a short duration.

This Hb defect results in a condition known as sickle cell disease and is mainly characterized by anemia, weakness etc.

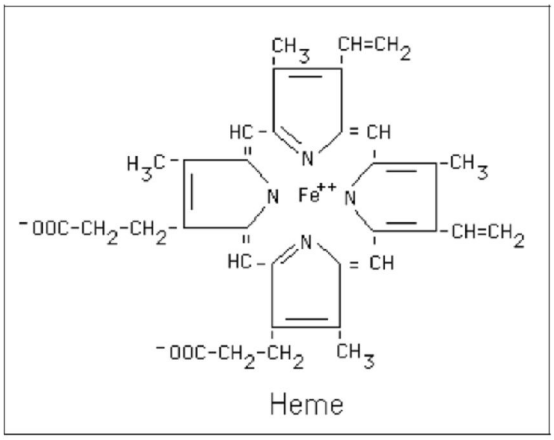
HAEMOGLOBINOPATHIES: HBS, THALASSAMIEAS, HEMOPHILIA

METABOLISM OF PORPHYRINE AND PORPHINURIAS, FORMATION OF BILE PIGMENTS AND JAUNDICE.

Porphyrins are cyclic compounds composed of four pyrole rings linked through methyne bridges i.e. (-HC=). Heme is a member of the family of porphyrins. The parent porphyrin is porphine, and substituted porphines are called porphyrins. Many important proteins contain heme as a prosthetic group for example Hemoglobin (oxygen transport), Myoglobin (oxygen transport), Cytochromes (electron transport) and Catalase (H_2O_2 utilization).

Porphyrins are generally known to form complexes with metal ions such as iron, magnesium, copper etc at the nitrogen atom. When an iron complex is formed the resulting compound is Heme, while chlorophyll is formed with a magnesium porphyrin complex. In nature the hydrogen atoms of the pyrole rings are substituted by chemical groups or substituents such as A = acetic acid (-CH2COOH), P = propionic acid (-CH2CH2COOH), M = methyl (-CH3), V = vinyl (-CH=CH2) groups and depending on which substituent groups are attached the porphyrins are named differently for example Coproporphyrin contains M and P only, protoporphyrin contains M and P and V, uroporphyrin contains A and P only etc.
Asymmetrically arranged chemical groups in a porphine (another name for porphyrin) are termed type III porphins while those which are symmetrical in arrangement of substituents are called type I. Types II and IV do not occur in natural systems. Heme is an example of type III porphine.
General properties of porphins

1. Solubility depends on number of carboxylate groups, -COO- e.g. uroporphyrins, 8 carboxylates (more soluble) and protoporphyrins, 2 carboxylates (less soluble).
2. Color: dark red/purple
3. Fluorescent
4. Chelate metal ions.

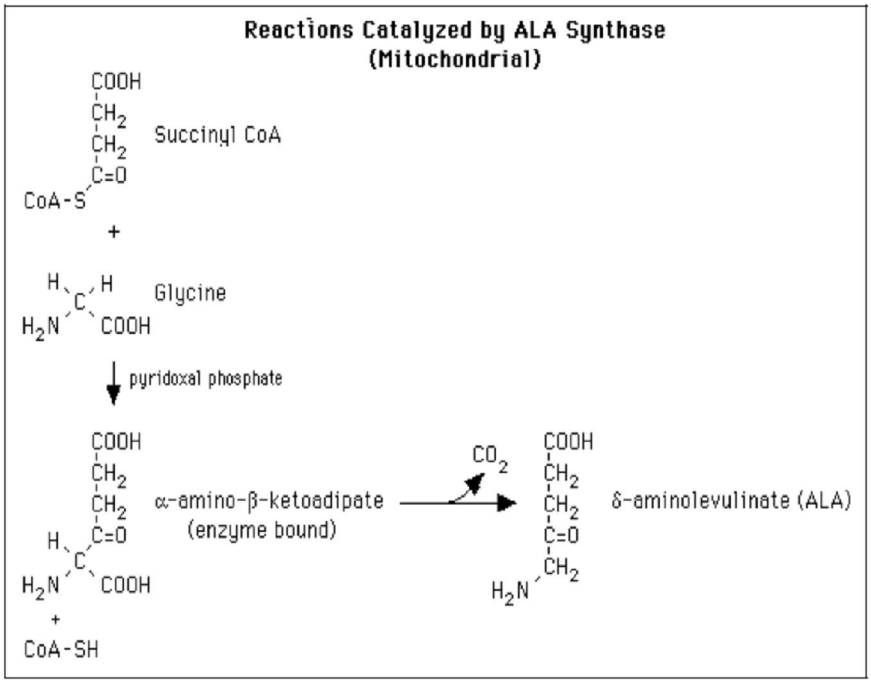


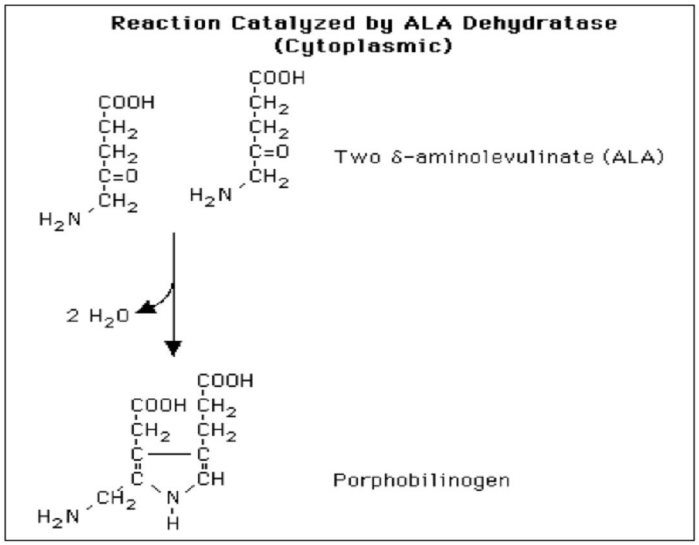
Heme

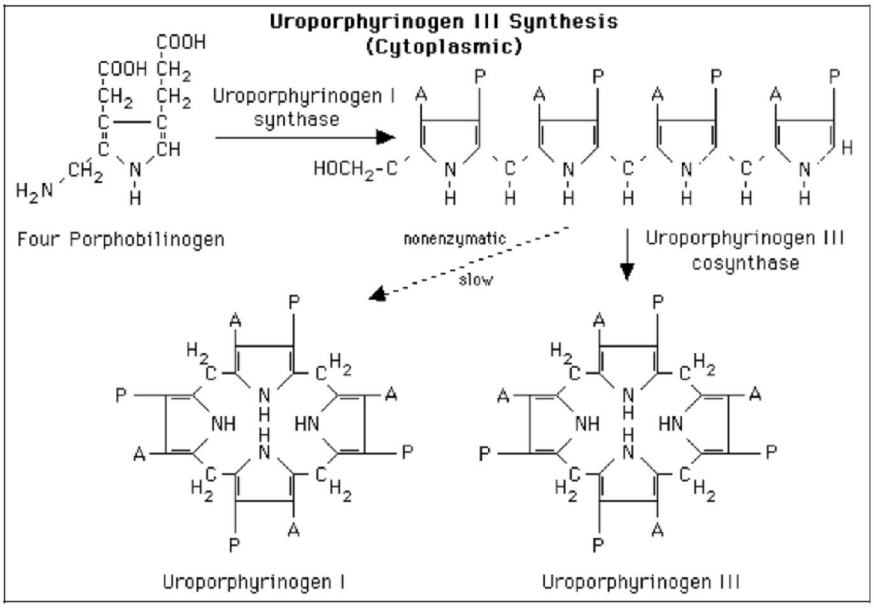
Porphyrinogens are a closely related compounds that have extra hydrogen atoms and also differ in pattern of double bonds available, hence are linked by methenyl bridges. They are colorless, do not fluoresce and are easily auto oxidized to porphyrins e.g. urobilinogen.

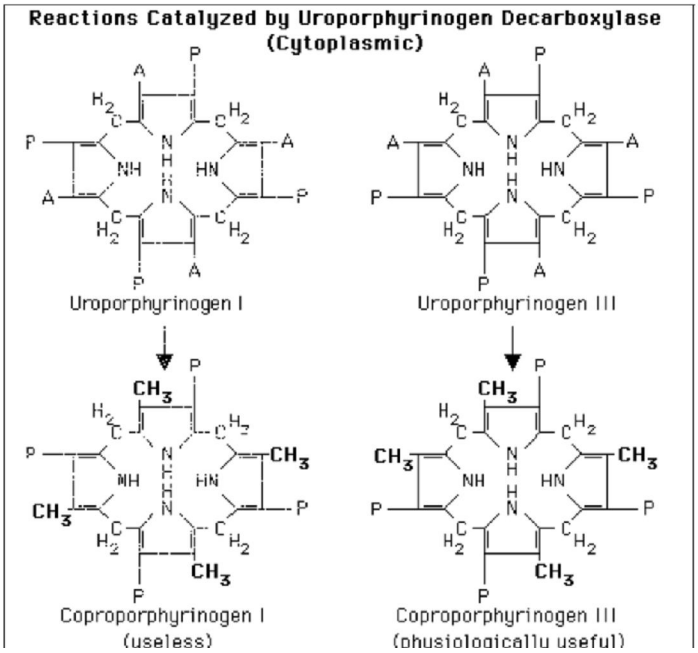
BIOSYNTHESIS OF HEME

The synthesis of porphyrins is an essential pathway to the synthesis of heme for hemoglobin in the RBC. Site of reaction is partly in the mitochondria and partly in the cytoplasm. Heme is synthesized mainly in the erythropoietic and liver cells.

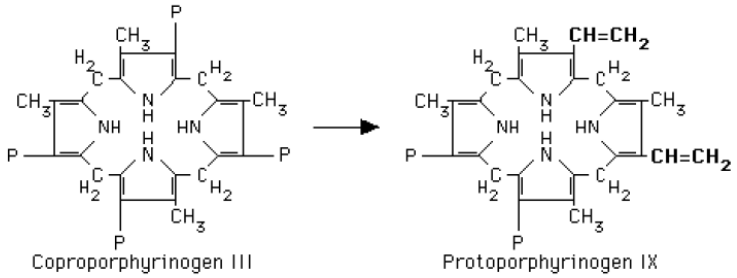

Reactions Catalyzed by ALA Synthase
(Mitochondrial)
Succinyl CoA
Glycine
pyridoxal phosphate
α-amino-β-ketoadipate
(enzyme bound)
δ-aminolevulinate (ALA)
CO2
CoA-SH


Reaction Catalyzed by ALA Dehydratase
(Cytoplasmic)
Two δ-aminolevulinate (ALA)
2 H2O
Porphobilinogen

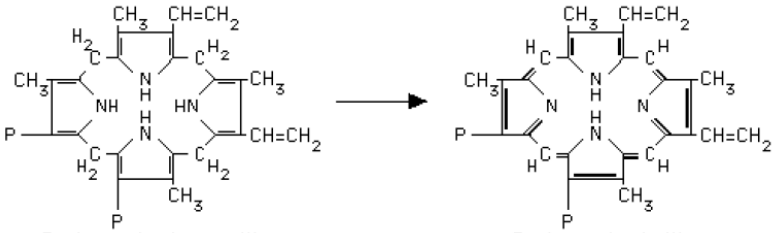

Uroporphyrinogen III Synthesis
(Cytoplasmic)
Uroporphyrinogen I synthase
Four Porphobilinogen
nonenzymatic
slow
Uroporphyrinogen III cosynthase
Uroporphyrinogen I
Uroporphyrinogen III

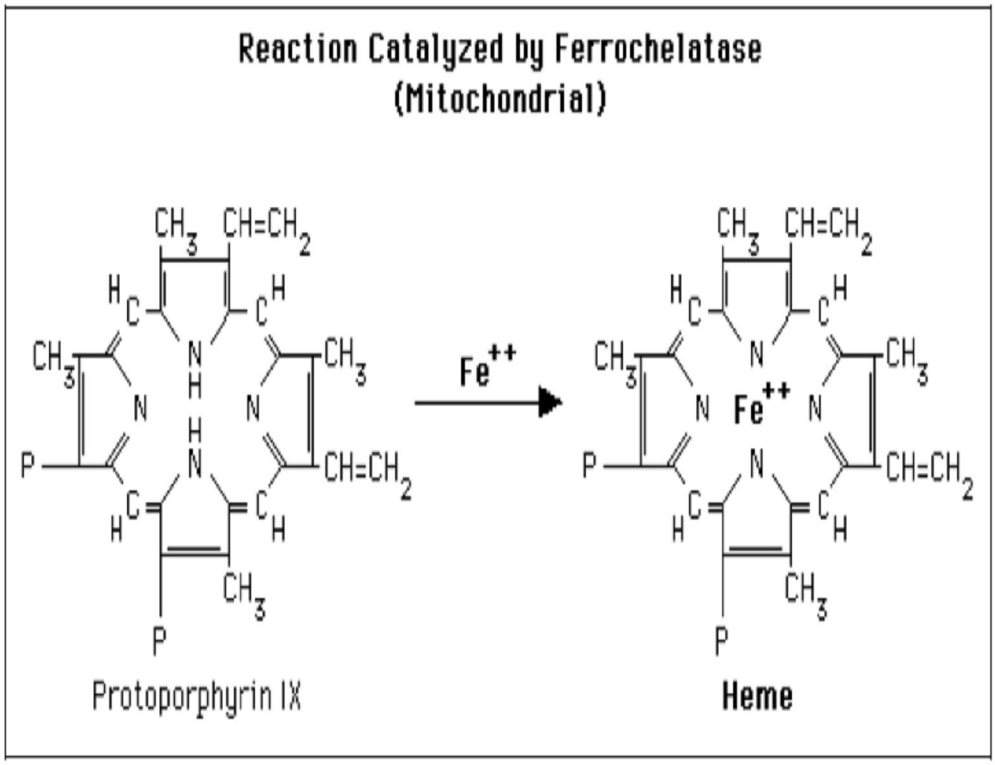

Reactions Catalyzed by Uroporphyrinogen Decarboxylase
(Cytoplasmic)
Uroporphyrinogen I
Uroporphyrinogen III
Coproporphyrinogen I
(useless)
Coproporphyrinogen III
(physiologically useful)

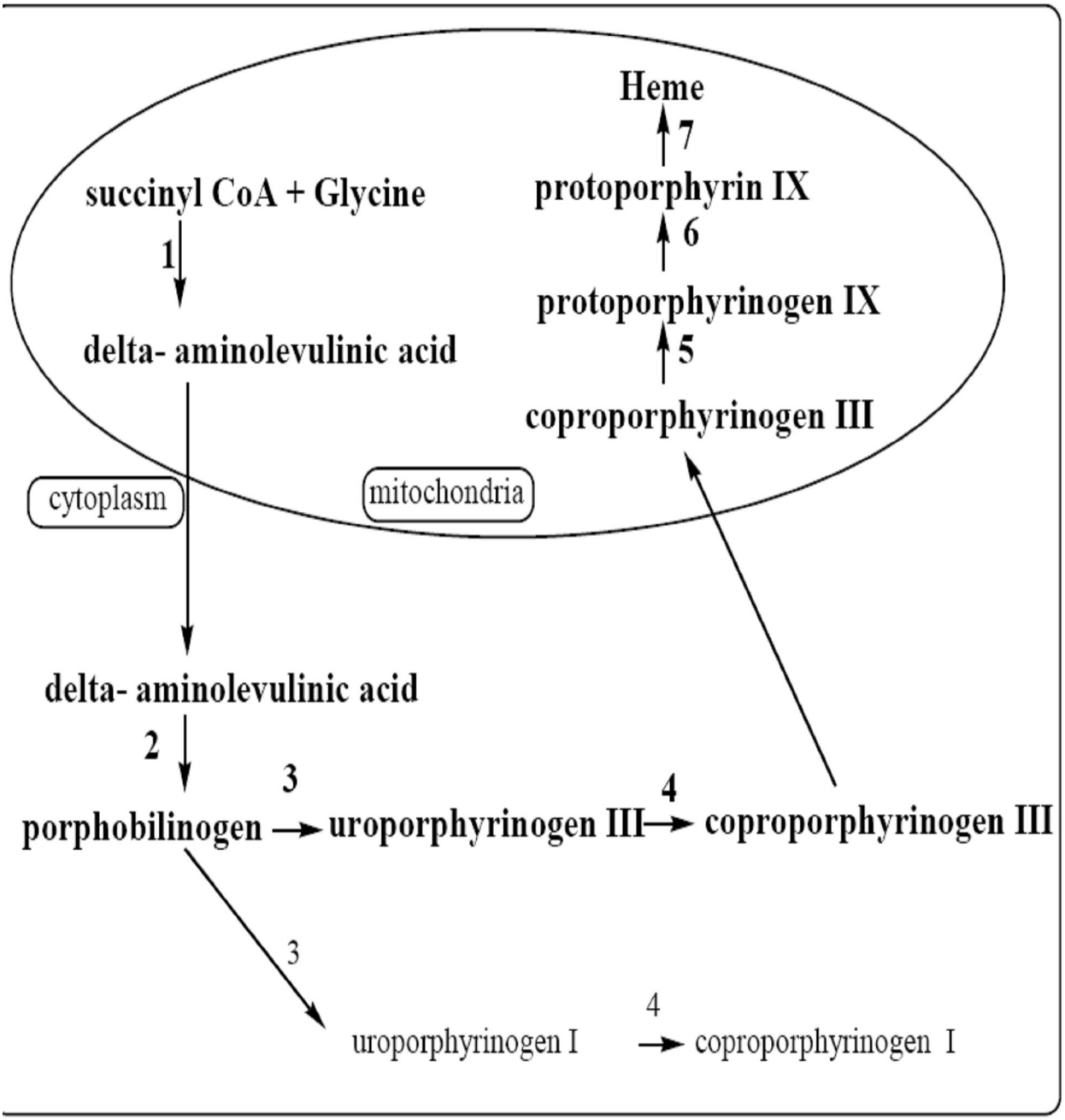
Reaction Catalyzed by Coproporphyrinogen III Oxidase (Mitochondrial)



Reaction Catalyzed by Protoporphyrinogen IX Oxidase (Mitochondrial)




Reaction Catalyzed by Ferrochelatase
(Mitochondrial)
Fe++
Protoporphyrin IX
Heme



Site and reactions of heme synthesis

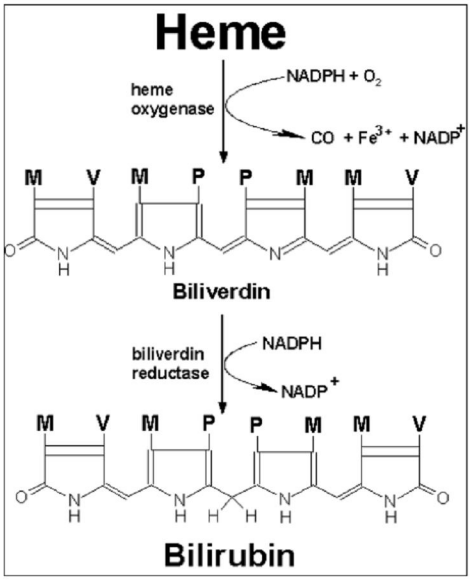
REGULATION OF HEME SYNTHESIS

1. Substrate availability: Fe++ must be available for ferrochelatase.
2. Feedback regulation: heme is a feedback inhibitor of ALA synthase. The Fe^{3+} oxidation product of heme is termed hemin. Hemin acts as a feed-back inhibitor on ALA synthase. Hemin also inhibits transport of ALA synthase from the cytosol (its' site of synthesis) into the mitochondria (its' site of action) as well as represses synthesis of the enzyme.
3. Effects of drugs and steroids: Certain drugs and steroids can increase heme synthesis via increased production of the rate limiting enzyme, ALA synthase.

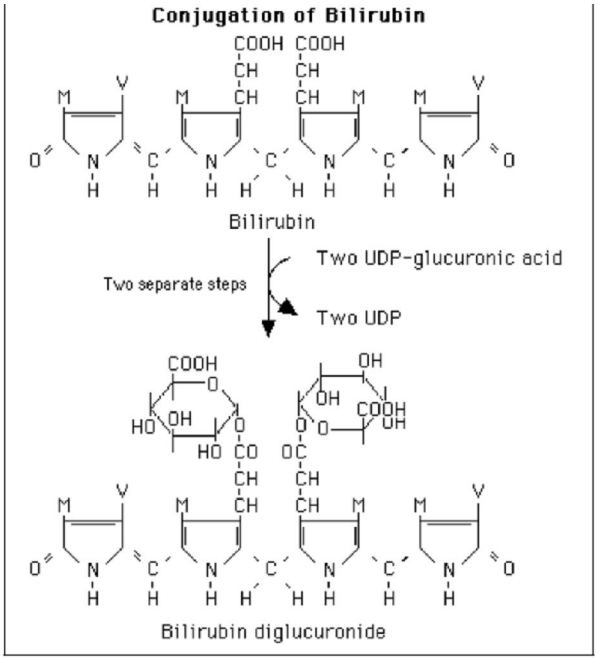
CATABOLISM OF HEME

Cells of the reticuloendothelial system in spleen, liver and bone marrow engulf aged RBCs to remove them from circulation releasing its contents of hemoglobin. The porphin portion of heme is degraded after the globin fragment is degraded to constituent amino acids and iron is recycled for use.

The catabolism of heme starts with its oxidation. The heme ring is opened by heme oxygenase (found in the endoplasmic reticulum); this oxidation produces a linear tetrapyrole called biliverdin, ferric iron and carbon monoxide. Subsequent reduction of biliverdin produces bilirubin.



Bilirubin is highly non-polar (lipid soluble) hence is not easily excreted from the body and has to be converted to a more polar –water soluble compound. Within the blood bilirubin is transported by a carrier the physiological carrier is serum albumin. Conjugation of bilirubin with glucuronic acid in the liver by hepatocytes increases its water solubility and eases its excretion. Conjugation is accomplished by attaching two molecules of glucuronic acid to it in a two step process by UDP glucuronyl transferase. The reaction is a transfer of two glucuronic acid groups sequentially to the propionic acid groups of the bilirubin. The major product is bilirubin diglucuronide which is excreted in the bile. It is subject to subsequent transformations to other species by the intestinal bacteria.



BILE PIGMENTS

These consist of bilirubin and its catabolic products they range from yellow red to orange yellow in color and give feces its characteristic brownish color. In the intestine (after conjugation of bilirubin by the hepatocytes) bacteria act on the compound to produce the final porphyrin products, urobilinogens and urobilins, that are found in the feces. A small fraction of urobilinogen is reabsorbed into the blood, extracted by the kidney, and excreted in the urine. Another portion of the reabsorbed urobilinogens are taken up by the liver and further reexcreted in bile what is known as undergoing enterohepatic circulation. In the distal portion of the GIT urobilinogens are oxidized to produce stercobilin, mesobilin and urobilin (the major pigments in feces).

JAUNDICE

Jaundice or hyperbilirubinemia also called icterus is the accumulation of bilirubin or bile pigments above normal levels in the plasma leading to the yellowish discoloration of skin, mucous membrane and tissues. Bilirubin has been shown to inhibit DNA synthesis, uncouple

oxidative phosphorylation, and inhibit ATPase activity in brain and mitochondria. Bilirubin also inhibits a variety of different classes of enzymes including dehydrogenases, electron transport proteins, hydrolyases, and enzymes of RNA synthesis, protein synthesis and carbohydrate metabolism, hence very toxic in the system.

There are three major types of jaundice;

1. Prehepatic jaundice this occurs as a result of increased production of bilirubin as a result of more rapid breakdown of RBCs than normal, more bilirubin is conjugated and excreted than normally, but the conjugation mechanism is overwhelmed, and an abnormally large amount of unconjugated bilirubin is found in the blood. This may occur as a consequence of a hemolytic disease causing massive destruction of RBCs.
2. Hepatic jaundice occurs because of an inability of the hepatocytes to adequately conjugate bilirubin either as a result of inability to take up bilirubin from the blood (As a result, unconjugated bilirubin accumulates), or an impairment of the conjugation pathway (also unconjugated bilirubin accumulates) or inability of the hepatocytes to secrete the already conjugated bilirubin after it is formed hence conjugated bilirubin returns to the blood.
3. Post hepatic jaundice is caused by an obstruction distal to the liver e.g. biliary obstruction like a calculi that interferes with secretion or passage of the conjugated bilirubin into the intestine hence the is reabsorbtion of conjugated bilirubin back into the system (a proportion normally exchanges back into the blood plasma but in health this is very small).

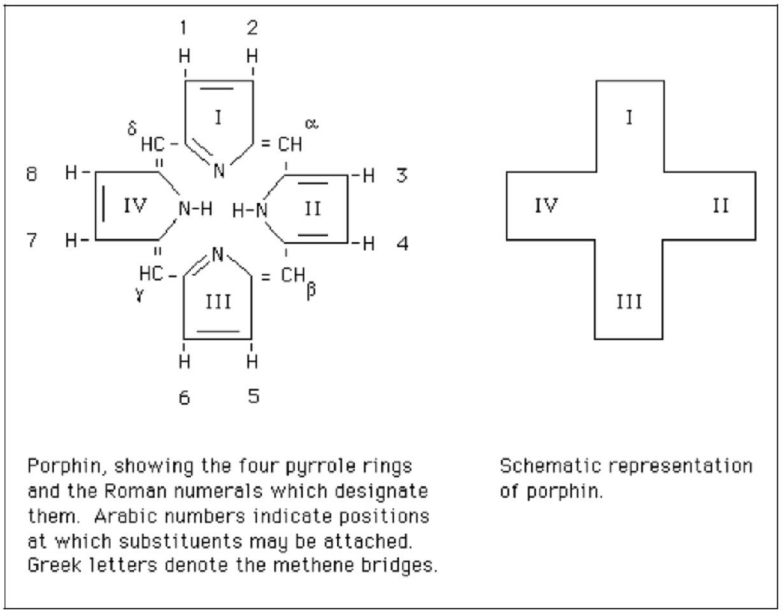
PORPHYRIAS

These are disorders that arise in heme biosynthesis as a result of defects in enzymes that catalyze the various reactions. Typically there is an increase in levels of intermediates of heme synthesis within the blood, urine and other body tissues and fluids and these can cause toxic effects.

Porphyrias maybe either acquired (as a results of poisonous or drug effects on enzymes) or hereditary (caused by a gene defect). Porphyrias may also be classified as erythroid or hepatic depending on site of enzyme defect.

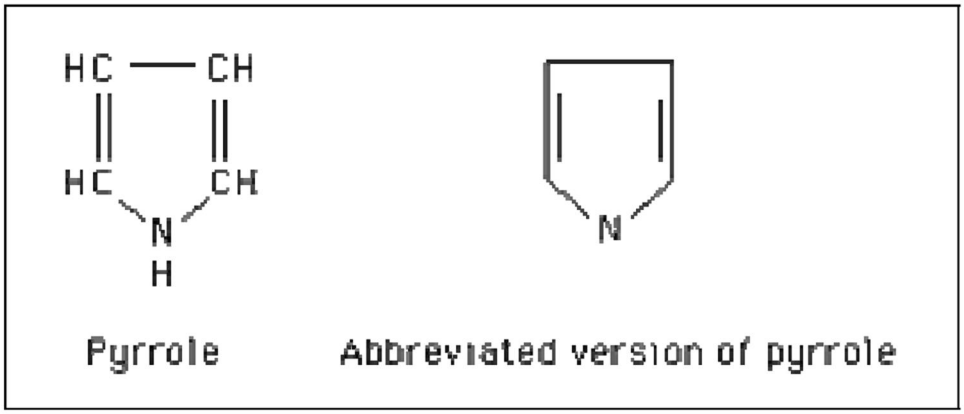
The most common porphyrias known is that caused by a defect in the enzyme porphobilinogen deaminase (PBG deaminase) called acute intermittent porphyrias.

Porphyrias generally lead to excretion of deposits in urine that color it red or reddish brown; they may also be deposited in teeth. Ulcerative and photosensitive systems on the skin may also results when the porphobilinogens are oxidized to porphins. There may also be neurological symptoms, which cannot be explained.



Porphin, showing the four pyrrole rings and the Roman numerals which designate them. Arabic numbers indicate positions at which substituents may be attached. Greek letters denote the methene bridges.

Schematic representation of porphin.



AMINO ACIDS AND PEPTIDES

OCCURRENCE

Amino acids and peptides are present in humans, animals, tissues, blood, microorganisms and plants.

MEDICAL AND BIOLOGICAL IMPORTANCE

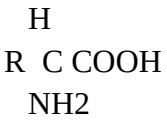
1. Amino acids serve as building blocks of proteins. Some amino acids are found in free form in human blood.
2. They also serve as precursors of hormones, purines, pyrimidines, porphyrins, vitamins and biologically important amines like histamine.
3. Peptides have many important biological functions. Some of them are hormones. They are used as anti-biotics and antitumor agents.
4. Some peptides are required for detoxification reactions. Some peptides serve as neurotransmitters.
5. Amino acid proline protects living organisms against free radical induced damage.
6. Some peptides are involved in regulation of cell cycle and apoptosis.
7. Peptides of vertebrates and invertebrates act as antimicrobial agents. They are part of innate immunity. Bacterial infections at epithelial surface induce production of antimicrobial peptides, which cause lysis of microbes.
8. Peptides are enzyme inhibitors. Natural and synthetic peptide inhibitors of angiotensin converting enzyme (ACE) act as a anti hypertensives. Peptide inhibitors of ACE present

in physiological foods, lowers blood pressure after they are absorbed from intestine. Lisinopril, Enalapril etc. are synthetic peptide inhibitors of ACE that are used as drugs in the treatment of hypertension.

9. Some synthetic peptides are used as enzyme substrates.

CHEMICAL NATURE OF AMINO ACIDS

Amino acids are carboxylic acids containing an amino group. In most of the amino acids, an amino group is attached to α-carbon atom next to the carboxyl group hence they are α-amino acids. The general formula is shown in Figure 2.1.



α - Carbon atom

Where 'R' is called as side chain and it represents variety of structures

COMMON AMINO ACIDS

Though more than 200 amino acids are identified in nature, only 20 amino acids serve as building blocks of body proteins. They are known as common amino acids. In addition to the common amino acids, derived amino acids are also found in proteins.

CLASSIFICATION OF AMINO ACIDS

Amino acids have been classified in various ways.

I. Based on side chain and ring structure present, amino acids are classified into 7 major classes.

1. Amino acids with aliphatic side chain. They are also called as *aliphatic amino acids*. They are glycine, alanine, valine, leucine and isoleucine . Valine, leucine and isoleucine are called as branched chain amino acids.

Aliphatic amino acids

2. Amino acids with side chain containing hydroxyl groups. They are also called as hydroxy amino acids. They are serine and threonine.

3. Amino acids with side chain containing sulfur atoms. They are also called as sulfur containing amino acids. They are cysteine, methionine and cystine.

4. Amino acids with side chain containing acidic groups or their amides. They are also called as *acidic amino acids*. They are aspartic acid, aspargine, glutamic acid and glutamine.

5. Amino acids with side chain containing basic groups. They are also called as *basic amino acids*. They are arginine, lysine, hydroxy lysine and histidine

6. Amino acids containing aromatic rings. They are also called as *aromatic amino acids*. They are phenylalanine, tyrosine and tryptophan.

7. Imino acids. They are proline and hydroxy proline.

II. Amino acids are also classified according to the reaction in solution or charge. They are categorized in 3 classes, acidic, basic and neutral amino acids. Acidic amino acids are aspartic acid, glutamic acid. Basic amino acids are arginine, lysine and histidine. Rest

of the amino acids are neutral amino acids.
III. Another classification of amino acids is based on the number of amino and carboxyl groups present in the molecule.
Example. Mono-amino mono-carboxylic acid (Glycine), Mono-amino dicarboxylic acid (Glutamate).
IV. Amino acids are also classified according to their nutritional importance. Nutritionally amino acids are classified into
(*a*) *Essential amino acids:* These amino acids are not synthesized in the body and hence they have to be obtained from the diet. They are also referred as indispensable amino acids. They are methionine (M), arginine (A), tryptophan (T), threonine (T), valine (V), isoleucine (IL), leucine (L), phenyl alanine (P), histidine (H) and Lysine (L). Together they are remembered as (MATTVILLPHLY). Sometimes histidine and arginine are referred as semi-essential because body synthesizes these amino acids to some extent. Lack of essential amino acids in the diet gives rise to growth failure.
(*b*) *Non-essential amino acids*: These amino acids are synthesized in the body. They are alanine, glycine, serine, tyrosine, glutamate, glutamine, aspartate, aspargine, cysteine and proline. They need not be present in the diet.

Rare Amino Acids or Unusual Amino Acids

These are the amino acids that are not found in proteins but play important roles in metabolism.

Examples

1. Ornithine, citrulline and arginino succinic acid of urea cycle.
2. β-alanine is part of co-enzyme A
3. Taurine is part of bile acids.
4. γ-aminobutyric acid is a neurotransmitter.
5. Mono- and di-iodotyrosine are precursors of thyroxine.
6. Pantothenic acid is a water-soluble vitamin.
7. Homoserine is an intermediate of methionine catabolis
8. **Homocysteine.** It is also an intermediate of methionine catabolism. It is a atherothrombogenic agent. It triggers platelet adhesion. Hence, it is considered as a risk factor for development of coronary artery disease (CAD).
9. **S-allylcysteine sulfoxide.** It is an amino acid obtained from garlic. It has many therapeutic effects. It is commonly called as alli

PROPERTIES OF AMINO ACIDS

1. *Optical isomerism:* All the amino acids except glycine have at least one asymmetric carbon atom because of this they exhibit optical isomerism. Presence of single asymmetric carbon atom gives rise to two optical isomers. One isomer is the mirror image of the other isomer. If a carbon atom is linked to four different groups through covalent bonds then it is called as *asymmetric carbon*. The two mirror images of amino acid serine are L-serine and D-serine.Further, the optical isomers of

amino acids are optically active. They are capable of rotating plane polarized light. Some amino acids rotate plane polarized to left and some rotate the plane polarized light to right. All the amino acids present in human proteins are L-isomers. D-isomers are usually absent but they are found in some peptide antibiotics.

Optical isomers of serine (*b*) Asymmetric carbon atom

2. *Acid-base or charge properties of amino acids:* Amino acids act as acids and bases. So they are called as *ampholytes* or *amphoteric substances*. Acids are those compounds that give protons on dissociation. Bases are those compounds that combine with protons. Bases are also called as alkalies. Proton concentration is quantitatively expressed as pH. It is defined as negative logerithem of proton or H+ or hydrogen ion concentration.

pH = – log [H+]

The pH scale extends from 1 to 14, which corresponds to hydrogen ion concentration of 1M to 1 × 10–14 M. The pH 7.0 represents neutrality pH values less than 7 represents acidity or acids and pH values above 7 refers to bases or alkalinity. Some common acids are hydrochloric acid (HCl), sulphuric acid (H_2SO4) and bases are sodium hydroxide (NaOH) and potassium hydroxide (KOH). Further acid is neutralized by base and vice versa.

Function of an amino acid as acid:

As base: R COO Addition of acid R COOH

So, amino acids have two ionizable groups (–COOH, NH_3^+). The –COOH is several times more easily dissociates than - NH_3^+).

At neutral pH both groups are ionized, i.e., the carboxyl group exist in dissociated form where as amino group exist as associated form.

This doubly charged molecule of amino acid containing positive and negative charges is called as zwitter ion. It is electrically neutral so it does not move in an electrical field.

The charge of an amino acid always depends on the pH of its surroundings. In other words, the charge of amino acid is altered by changing pH of its surroundings. This property is exploited for the separation of amino acids. In strong acidic conditions (pH < 2) the –COOH remains undissociated. When the pH is raised at pH of about 3 the proton from the –COOH is lost –COO– is generated. This is called pK of acid group because at this pH dissociated (–COO–) and undissociated (–COOH) species are found in equal amounts. Similarly, if the pH is increased to 10, the amino group ($–NH_3^+$) dissociates to $–NH_2$ group. This pH is called the pK of amino group of amino acid because at this pH associated ($–NH_3^+$) and dissociated ($–NH_2$) species are present in equal amounts.

Therefore, an amino acid has two pK values corresponding to the two ionizable groups. pK values indicates strength of each group. Further an amino acid exist as zwitter ion at neutral pH and as cation at acidic pH and as anion at basic pH.

Example: For alanine, pKa is 2.4 and pKam is 9.7 (K is dissociation constant), the low pK value of –COOH indicates more ionizing power.

Isoelectric pH: It is the pH at which the net charge of an amino acid is zero or when the number of positive charges are equal to number of negative charges. At isoelectric

pH amino acids have minimum solubility. The isoelectric pH of an amino acid having one amino group and one carboxyl group is equal to the arithamatic mean of pKa and pKam values.

For most amino acids pI is close to 6.0. The situation differs for amino acids having more than two ionizable groups. For example, glutamate is dicarboxylic acid so it can have three pK values (two for carboxyl groups and one for amino group). Similarly, the basic amino acid lysine can have three pK values (two for amino groups and one for carboxyl group). In these cases, a different formula is used to obtain isoelectric pH. For acidic amino acid like glutamate the isoelectric pH is equal to the half of sum of two pK values of acidic groups.

For basic amino acid like lysine the isoelectric pH is equal to the half of sum of two pK values of amino groups.

3. *Buffering action of amino acids:* Buffers are salts of weak acids and they resist change in pH when acid or alkali is added. Since amino acids are ampholytes they act as buffers. However, the buffering action of amino acids in the blood is insignificant because of their low concentration.

4. *Ultra violet light (UV) absorption of amino acids.* Amino acids do not absorb visible light. Aromatic amino acids absorb ultraviolet light. Tryptophan absorb ultra violet light at 280 nm. The ultra violet light absorption is also exhibited by proteins containing tryptophan. Hence, it is used for quantitative estimation of proteins and to study folding of protein molecules. Phenylalanine and tyrosine also absorb light in ultra violet region.

PEPTIDES

1. Peptides consist of 2 or more amino acid residues linked by peptide bond.
2. A peptide bond is formed when carboxyl group of an amino acid react with α-amino group of another amino acid. Peptide bond formation between two amino acids is always accompanied by loss of one water molecule. Further, peptide and proteins contain an amino (N–) terminus and carboxy (C–) terminus.
3. A peptide or protein is named starting with N-terminal amino acid and usually the N-terminal is located on the left hand side.
4. Animal, plant and bacterial cells contain wide variety of low molecular weight peptides (2-10 amino acids residues) having profound biological functions.

DIPEPTIDES

A dipeptide consist of two amino acid residues and one peptide bond.

Carnosine and Anserine

Are two peptides present in muscle and brain.

Carnosine consist of β-alanine and histidine (β-alanyl histidine). Anserine consist of β-alanine and N-methyl histidine (β-alanyl N-methyl histidine). Short hand formula for carnosine is β-ala-His.

Function
Remains unknown.

Aspartame
It consist of aspartate and phenylalanine (Aspartyl phenylalanine, Asp-Phe). It is present in African berry.
Function
It is a sweetening agent.
Tripeptides
A tripeptide consist of three amino acid residues and two peptide bonds.
Glutathione
Structure
It consists of glutamate. Cysteine and glycine. In glutathione, γ-carboxyl group of glutamate is involved in peptide linkage with cysteine hence it is named as γ-glutamyl cysteinyl glycine (Glu-Cys-Gly, G-SH,).
Functions
1. It acts as reducing agent in all cells. It assumes dimeric form on oxidation.
It is responsible for the maintenance of –SH groups of proteins in reduced form
2. It participates in the removal of H_2O_2 in erythrocytes.
3. It is required for removal of toxins from body.
4. It is involved in release of hormones.
5. It protects body proteins from radiation effects.
6. It is involved in cellular resistance to anticancer agents.
7. Glutathione regulates telomerase activity and of the cell cycle.
8. Glutathione is involved in modulation of apoptosis.

Thyrotropin Releasing Harmone (TRH)
Structure
It consists of glutamate, histidine and proline. It is an unusual tripeptide with blocked N and C terminals.
Function
It is a hormone secreted by hypothalamus.
Chemotactic Peptide
Structure
It consists of N-Formyl methionine, leucine and phenylalanine (f met-leu-phe). Its N-terminal contains formyl (–CHO) group.
Function
It is present in leukocytes. It plays an important role in chemotaxis.
Penta Peptides
They consist of five amino acids and four peptide bonds.

Enkaphalin

Structure

It consist of tyrosine, glycine, glycine, phenylalanine and methionine (Tyr-gly-gly-phe-met).

Function

It is present in brain. It binds to opiate receptors present in brain. So, it is body own opiate or analgesic. Enkaphalins containing six amino acid residues (hexa peptide), seven amino acid residues (hepta peptide) and eight amino acid residues (octa peptide) are also found in brain.

Other noteworthy peptides are

Angiotensin II. It is an octa peptide, found in lungs and other cells. It is a powerful vaso constrictor and raises blood pressure.

Bradykinin. It consist of nine amino acid residues (Nona peptide). It is a powerful vasodilator and anti inflammatory.

Oxytocin I. It is also a nona peptide. It stimulates uterus contraction.

Vasopressin. A nona peptide produced by pituitary gland. It has a disulfide bridge. It is also known as antidiuretic harmone (ADH).

Angiotensin I and Kallidin are examples for decapeptides containing ten amino acid residues.

CYCLIC PEPTIDES

1. They differ from normal peptides.
2. In these peptides N-terminus and C-teminus are linked by peptide bond resulting in cyclization of peptide.
3. An antibiotic gramicidin-S is a cyclic peptide. It consist of ten amino acids. So gramicidin-S is a cyclic decapeptide. Further it contains D-Phenyl alanine which is usually absent in life forms.
4. Tyrocidin is another cyclic decapeptide.

TOXIC PEPTIDES

1. Some peptides act as toxins.
2. α-amanitin is a bicyclic octapeptide present in a particular variety of mushrooms. It is extremely toxic to humans.
3. It is responsible for mushroom poisoning cases around the world.
4. When the mushrooms are consumed it causes pain in the gastrointestinal tract, vomitting, diarrhoea and nausae.
5. Death occurs within a week due of impairment of liver and kidney functions.

CYCLOTIDES (CYCLIC PEPTIDES)

In some peptides disulfide bonds are more. These disulfide bonds create knot with in the molecule. Two disulfide bonds and their connecting back bone segment form ring. They are known as cyclotides. These cyclic peptides show diverse actions. Some are anti HIV, anti bacterial and insecticidal agents. Some examples are given below:

1. ***Sunflower trypsin inhibitor (SFTI).*** It is smallest circular peptide with just 14 amino acids. It is an enzyme inhibitor.
2. ***RTD-1.*** It is a circular peptide present in leucocytes. It is a defensin. It consists of only 18 amino acids.
3. ***Microsin.*** It is 21 residue cyclic peptide. It is produced by E. coli. It is an antibiotic.

EXERCISES

ESSAY QUESTIONS

1. Classify amino acids. Give examples for each class.
2. Name five biologically important peptides. Write one function for each of them.
3. Write an essay on properties of amino acids.

SHORT QUESTIONS

1. Define amino acid and isoelectric pH. Write two properties of an amino acid at isoelectric pH.
2. Write composition of glutathione. How it differs from other peptides? Write two of its functions.
3. Explain acid-base properties of amino acids.
4. Define essential amino acids. Give examples.
5. Write structures of tyrosine, methionine and valine.
6. What are unusual amino acids? Give examples.
7. Define cyclic peptide. How it differs from other peptides? Write 2 examples with functions.
8. Write a note on semi essential amino acids.
9. Calculate isoelectric point of glutamic acid. How it differs from the isoelectric point of glycine?
10. What are the functions of amino acids?
11. Draw structure of peptide. Label its various parts.

MULTIPLE CHOICE QUESTIONS

1. Most of the amino acids found in human body are
(*a*) L-isomers (*b*) D-isomers
(*c*) D and L-isomers (*d*) Optical isomers
2. Which of the following amino acids has more pK values.
(*a*) Glycine (*b*) Alanine
(*c*) Glutamate (*d*) Glutamine
3. The isoelectric pH of lysine is equal to
(*a*) Arithamatic mean of amino groups pK values.
(*b*) Half of sum of amino group and carboxyl group pK values.
(*c*) Arithamatic mean of amino groups and carboxyl groups pK values.
(*d*) None of the above.
4. An example for unusual amino acid is
(*a*) Aspargine (*b*) Taurine

(*c*) Cystine (*d*) Anserine

5. All of the following statements are correct regarding peptide except

(*a*) It contains amino terminus (*b*) It contains carboxy terminus

(*c*) It contains peptide bonds (*d*) It contains only basic amino acids

FILL IN THE BLANKS

1. ----------absorbs light in ultraviolet region.
2. ----------is a dipeptide having sweet taste.
3. In a cyclic peptide N-terminus and C-terminus are linked by ---------- bond.
4. An unusual amino acid that function as neurotransmitter is ----------

PROTEIN

OCCURRENCE

Proteins are present in every cell of humans, animals, plant tissues, tissue fluids and in micro organisms. They account for about 50% of the dry weight of a cell. The term protein is derived from the Greek word *proteios* meaning holding first place or rank in living matter.

MEDICAL AND BIOLOGICAL IMPORTANCE

Proteins perform wide range of essential functions in mammalians.

1. Proteins are involved in the transport of substances in the body.

Example: Haemoglobin transports oxygen.

2. Enzymes which catalyze chemical reactions in the body are proteins.
3. Proteins are involved in defence function. They act against bacterial or viral infection.

Example: Immunoglobulins.

4. Hormones are proteins. They control many biochemical events.

Example: Insulin.

5. Some proteins have role in contraction of muscles.

Example: Muscle proteins.

6. Proteins are involved in the gene expres expression. They control gene expression and translation.

Example: Histones.

7. Proteins serve as nutrients Proteins are also involved in storage function.

Examples: Casein of milk, Ferritin that stores iron.

8. Proteins act as buffers.

Example: Plasma proteins.

9. Proteins function as anti-vitamins.

Example: Avidin of egg.

10. Proteins are infective agents.

Example: Prions which cause mad cow disease are proteins.

11. Some toxins are proteins.

Example: Enterotoxin of cholera microorganism.

12. Some proteins provide structural strength and elasticity to the organs and vascular system.

Example: Collagen and elastin of bone matrix and ligaments.

13. Some proteins are components of structures of tissues.

Example: α-keratin is present in hair and epidermis.

In order to understand how these substances though they are all proteins play such diverse functions their structures, and composition must be explored.

CHEMICAL NATURE OF PROTEINS

All proteins are polymers of aminoacids. The aminoacids in proteins are united through "Peptide" linkage. Sometimes proteins are also called as polypeptides because they contain many peptide bonds.

PROPERTIES OF PROTEINS

1. Proteins have high molecular weight, *e.g.,* the lactalbumin of milk molecular weight is 17000 and pyruvate dehydrogenase molecular weight is 7 × 106.
2. Proteins are colloidal in nature.
3. Proteins have large particle size.
4. Different kinds of proteins are soluble in different solvents.
5. Proteins differ in their shape.
6. Some proteins yield amino acids only on hydrolysis where as others produce amino acids plus other types of molecules.
7. **Charge properties:** Charge of a protein depends on the surroundings like amino acids. So, by changing the pH of surroundings the charge of protein can be altered. This property is used for separation of proteins.

Isoelectric point: Proteins have characteristic isoelectric points. At the isoelectric point its net charge is zero because the number of positive charges are equal to number of negative charges. So proteins are insoluble or have minimum solubility at isoelectric point. This property is used for the isolation of casein from milk. The isoelectric point for casein is 4.6. If the pH of the surrounding is raised above the isoelectric point, the protein is negatively charged *i.e.,* it exists as anion. Likewise, if the pH of the surrounding is lowered, the protein is positively charged *i.e.,* it exist as cation. Further, proteins do not move in an electrical field at isoelectric point like amino acids. However, if the pH of the medium is raised or lowered protein moves towards anode or cathode respectively. This property is exploited for the separation of proteins.

8. **Proteins act as buffers:** Since proteins are amphoteric substances, they act as buffers. Hemoglobin (Hb) of erythrocytes and plasma proteins are important buffers. Hb accounts for 60% of buffering action within erythrocytes and plasma proteins contributes to 20% of buffering action of blood

CLASSIFICATION OF PROTEINS

There is no single universally satisfactory system of protein classification so far.

1. One system classifies proteins according to their composition or structure.
2. One system classifies them according to solubility.

3. One system classifies them according to their shape.
4. Classification of proteins based on their function also found in literature.

Classification of proteins based on their composition

Proteins are divided into three major classes according to their structure.

1. **Simple proteins:** Simple proteins are made up of amino acids only. On hydrolysis, they yield only amino acids.

Examples: Human plasma albumin, Trypsin, Chymotrypsin, pepsin, insulin, soyabean trypsin inhibitor and ribonuclease.

2. **Conjugated proteins:** They are proteins containing non-protein part attached to the protein part. The non-protein part is linked to protein through covalent bond, non-covalent bond and hydrophobic interaction. The non-protein part is loosely called as prosthetic group. On hydrolysis, these proteins yield non-protein compounds and amino acids.

Conjugated protein → Protein + Prosthetic group

The conjugated proteins are further classified into subclasses based on prosthetic groups.

Different classes of conjugated proteins

Subclass Prosthetic group Examples Type of linkage

	SUBCLASS	**PR0STHETIC GROUP**	**EXAMPLES**	**TYPES OF LINKAGE**
1	**Lipoproteins**	**Lipids**	**Various classes of lipoproteins. Lipovitellin of eggs**	**Hydrophobic interaction**
2	**Glycoproteins**	**Carbohydrates**	**Immunoglobulin of blood, Egg albumin**	**covalent**
3	**Phosphoproteins**	**Phosphorus**	**Caesin of milk,Vitellin of egg yolk**	**Colvalent**
4	**Nucleoproteins**	**Nucleic acids**	**Chromatins, Ribosomes**	**Non covalent**
5	**Haemoproteins/Chromoproteins**	**Haem**	**Haemoglobin, myoglobin, chytochromes**	**Non covalent**
6	**Flavoproteins**	**Flavin nucleotides, FMN, FAD**	Succinate dehydrogenase	**Covalent**
7	**Metaloproteins**	**Iron**	**Ferritin ,chytochrome**	**Non covalent**
8	**Visualproteins**	**Retinal**	**Rhodopsin**	**Colvent**

3. **Derived proteins:** As the name implies this class of proteins are formed from simple and conjugated proteins. There are two classes of derived proteins.
(*i*) *Primary derived proteins:* They are formed from natural proteins by the action of heat or alcohol etc. The peptide bonds are not hydrolysed. They are synonymous with denatured proteins.
Example: Coagulated proteins like cooked-egg albumin.
(*ii*) *Secondary derived proteins:* They are formed from partial hydrolysis of proteins.
Examples: Proteoses, peptone, gelatin, and peptides.

Protein classification according to their solubility

1. **Albumins:** Soluble in water and salt solutions.
Examples: Albumin of plasma, egg albumin and lactalbumin of milk.
2. **Globulins:** Sparingly soluble in water but soluble in salt solutions.
Examples: Globulins of plasma, ovoglobulins of egg, lactoglobulin of milk.
3. **Glutelins:** Soluble in dilute acids and alkalies.
Examples: Glutenin of wheat, oryzenin of rice, zein of maize.
4. **Protamins:** Soluble in ammonia and water.
Examples: Salmine from salmon fish, sturine of sturgeon.
5. **Histones:** Soluble in water and dilute acids.
Example: Histones present in chromatin.
6. **Prolamines:** Soluble in dilute alcohol and insoluble in water and alcohol.
Examples: Gliadin of wheat, zein of corn.
7. **Sclero proteins:** Insoluble in water and dilute acids and alkalies.
Examples: Collagen, elastin and keratin.

Classification of proteins based on shape

Proteins are divided into two classes based on their shape.
1. **Globular proteins:** Polypeptide chain(s) of these proteins are folded into compact globular (Spherical) shape.
Examples: Haemoglobin, myoglobin, albumin, lysozyme, chymotrypsin.
2. **Fibrous proteins:** Poly peptide chains are extended along one axis.
Examples: α-keratin, β-keratin, collagen and elastin.

PROTEIN STRUCTURE

Since proteins are built from amino acids by linking them in linear fashion, it may be viewed as proteins having long chain like structures. However, such arrangement is unstable and polypeptide or protein folds to specific shape known as *conformation,* which is more stable. Various stages involved in the formation of final conformation from linear chain are divided into four levels or orders of protein structure. They are

1. Primary Structure

The linear sequence of amino acid residues in a polypeptide chain is called as primary structure. Generally disulfide bonds if any are also included in the primary structure.
Bonds responsible for the maintenance of primary structure are mainly peptide bonds and

disulfide bonds. Both of them are covalent bonds .

Primary Structure of Insulin

This protein consist of two polypeptide chains A and B. The two chains are covalently linked by disulfide bonds. The A chain has N-terminal glycine and C-terminal aspargine. The B chain has phenylalanine and alanine as N-and C-terminal residues, respectively. Insulin is a hormone and its molecular weight is 5,700.

2. Secondary Structure

Folding of polypeptide chain along its long axis is called as secondary structure of protein. Folding of polypeptide chain can be *ordered, disordered* or *random.* Secondary structure is often referred as *conformation.* So, proteins has *ordered secondary structure* or *conformation* and *random* or *disordered secondary structure* or *conformation.*

Ordered Conformation of Polypeptides

The polypeptide chain of some proteins may exist in highly ordered conformation. The conformation is maintained by *hydrogen bonds* formed between peptide residues.

Hydrogen bond

It is a weak ionic interaction between positively charged hydrogen atom and negatively charged atoms like oxygen, nitrogen, sulfur etc. It is indicated with broken lines (---).

There are two types of ordered secondary structure observed in proteins.

1. The polypeptide chain of α-keratin, which is present in hair, nails, epidermis of the skin is arranged as α-*Helix*. α-letter is given to this type of structure because it was first ordered structure noticed in proteins.
2. Polypeptide chain of β-keratin, which is present in silk fibroin and spider web is arranged in β-*pleated* sheet. The β-letter is given because it was observed later.

Main Features of α-Helix

1. In α-helix polypeptide, backbone is tightly wound round (coiled) long axis of the molecule.
2. The distance between two amino acid residues is 1.5 Å.
3. α-helix contain 3.6 amino acid residues per turn. The R-group of amino acids project outwards of the helix.
4. The pitch of the α-helix is 5.4 Å long and width is 5.0 Å .
5. The α-helix is stabilized by intra chain hydrogen bonds formed between –N–H groups and –C=O groups that are four residues back, *i.e.*, –N–H group of a 6th peptide bond is hydrogen bonded to –C=O group of 2nd peptide bond .
6. Each peptide bond participates in the hydrogen bonding. This gives maximum stability to α-helix.
7. α-helix present in most fibrous proteins is right handed. The right handed α-helix is more stable than the left handed helix.
8. α-helix is hydrophobic in nature because of intra chain hydrogen bonds.
9. An α-helix forms spontaneously since it is the most stable conformation of polypeptide chain.
10. Some amino acids act as terminators for α-helix.

Example: Proline.

11. Aromatic amino acids stabilizes α-helix.
12. Charged and hydrophobic amino acids destabilize α-helix.
13. Content of α-helix varies from protein to protein.

β-Pleated Sheet Features

1. In β-pleated sheet, the polypeptide chain is fully extended.
2. In β-pleated sheet, polypeptide chains line up side by side to form sheet . The side chains are above or below the plane of the sheet.
3. From 2 to 5, adjacent strands of polypeptides may combine and form these structure.
4. When the adjacent polypeptide chains run in same direction (N to C terminus) the structure is termed as parallel β-pleated sheet.
5. When the adjacent polypeptide chains run in opposite direction the structure is termed as anti-parallel β-pleated sheet.
6. The β-pleated sheet is stabilized by inter chain hydrogen bonds .
7. β-keratin contains anti parallel β-pleated sheet.
8. Both parallel and anti-parallel β-pleated sheet occur in other proteins. Amyloid protein present in Alzheimer's disease has anti parallel β-pleated sheet. It accumulates in the CNS.

Random Coil (Disordered) Conformation

Regions of proteins that are not organized as helices and pleated sheet are said to be present in random coil conformation. These are also equally important for biological function of proteins as those of helices and β-pleated sheet.

β-turn or β-bends (Reverse Turn)

Hair pin turn of a polypeptide chain is called as β-turn. The change in the direction of a polypeptide chain is achieved by β-turn. β-turn connects anti parallel β-sheets. Usually four aminoacids make up β-turn. Gly, Ser, Asp, proline are involved in β-turns.

Super Secondary Structure

In some globular proteins regions of α-helix and β-pleated sheet join to form super secondary structure or motifs. They are very important for biological function.

Super Helix

α-keratin consist of right handed α-helix as basic unit. Three such α-helices get cross linked by disulfide bonds and form super secondary structure.

Triple Helix

Collagen present in skin, cartilage, bone and tendons consists of left handed helix as basic unit. Three left handed helices are wrapped around each other to right handed super secondary structure triple helix.

3. Tertiary Structure

Three-dimensional folding of polypeptide chain is called as tertiary structure. It consists of regions of α-helices, β-pleated sheet, β-turns, motifs and random coil conformations. Interrelationships between these structures are also a part of tertiary structure.

Tertiary structure of a protein is mainly stabilized by non-covalent bonds. Non-covalent bonds present in tertiary structure

(*a*) Hydrophobic interaction (*b*) Electrostatic bonds

(*c*) Internal hydrogen bonds (*d*) vander waal's interactions

A. Hydrophobic interactions

The non-polar side chains of neutral amino acids tend to associate in proteins. These are called as hydrophobic interactions. They play significant role in maintaining tertiary structure.

B. Electrostatic bonds

These bonds are formed between oppositely charged groups of amino acid side chains. The ε-amino groups of lysine is positively charged and second (non-α-) carboxyl group of aspartic acid is negatively charged at physiological or body pH. These interact electrostatically to stabilize tertiary structure of protein. They are also called as salt bridges.

C. Internal hydrogen bonds

Amino acid side chains are involved in the hydrogen bond formation. Hydroxyl group of serine, threonine, the amino groups and carbonyl oxygen of glutamine and aspargine, the ring nitrogen of histidine participates in internal hydrogen bond formation.

D. Vander waals interactions

These are the weak interactions between uncharged groups of protein molecule. They also contribute to the stability of proteins.

4. Quaternary Structure

Proteins containing two or more polypeptide chains possess quaternary structure. These proteins are called as *oligomers*. The individual polypeptide chains are called as protomer, *monomers* or *subunits*. The protomers are united by forces other than covalent bonds. Occasionally, they may be joined by disulfide bonds.

The most common oligomeric proteins contain 2 or 4 protomers and are termed dimers and tetramers.

Forces that stabilize these aggregates (assembles of monomers) are:

1. Hydrogen bonding
2. Electrostatic interactions
3. Hydrophobic interactions
4. Vander waals interactions
5. Disulfide bridges (in some proteins)

Examples: 1. Haemoglobin consist of 4 polypeptide chains.

2. Hexokinase contains 2 subunits.
3. Pryuvate dehydrogenase contains 72 subunits.

Determination of Protein Structure

The primary structure of protein directs specific folding (secondary structure) and its tertiary structure. If there is a change in one of the amino acids of protein, then conformation of polypeptide chain alters, which results change in biological function. Further, the sequence of amino acids in proteins that gives them their striking specific biological actions. Therefore

knowledge of primary structure of a protein is required for the understanding of relationship of a protein's structure to its function at molecular level.

Determination of Primary Structure of Protein

1. Sanger's reagent

Sanger used FDNB (1-Fluoro-2, 4-Dinitrobenzene) to determine the amino acid sequence of a polypeptide chain from N-terminus. Sanger's reagent can be used to determine only one amino acid at a time because FDNB reacts with other amino acids. FDNB arylates free amino acid group and produces intense yellow 2, 4-dinitrophenyl residues of amino acids. These derivatives are separated by chromatography and identified

2. Edman's reagent

Edman used phenylisothiocyanate (Edman's reagent) for the determination of amino acid sequence of a protein from the N-terminus. Edmans reagent not only identifies N-terminus but also when used repeatedly provides complete sequence of the polypeptide chain. In Edman's reaction, the polypeptide chain is shortened by only one residue and rest of the polypeptide remains intact. The reaction is repeated and second residue is determined. By continued repetition, complete sequence of protein is determined starting from N-terminus

Edman's reaction for sequence determination of protein from N-terminus

Edman's reagent react with amino group and produces phenylthiocarbamyl derivatives on treatment with acid. Phenylthiocarbamyl derivative cyclizes to phenylthiohydantoins. They are estimated using chromatography.

Protein Folding

Let us examine how polypeptide chain attains native conformation as soon as it comes out of protein synthesizing machinery. Though exact mechanisms involved in protein folding are not known due to extensive investigations carried out some information on protein folding mechanisms is available.

Stages of Protein Folding

Protein folding occurs by stages:

(a) Domains formation

α-helical, β-pleated sheet, β-bend containing domains are formed in the initial step of folding of polypeptide chain. This self assembling process mostly depends on primary structure. It involves extensive interaction among amino acids residues side chains of polypeptide chain. It is governed by thermodynamic principles like free energy etc.

(b) Molten globule

In the next step domains from molten globule state in which secondary structure predominates and tertiary structure is highly disordered.

(c) Native conformation

Finally native conformation develops from molten globule state after several minor conformational changes and rearrangements.

(d) Oligomer formation

In the case of multimeric or oligomeric proteins after attaining specific conformation protomers or sub-units may assemble into native like structure initially. After some realignments it ultimately gives rise to final conformation of oligomer.

Additional Protein Folding Factors

Though self association of polypeptide chain into ordered conformation is largely determined by amino acid sequence (primary structure) recent research has shown that in some cases folding of protein requires additional factors. Some of them are enzymes and some are protein factors.

Protein Folding Enzymes

Two protein folding enzymes are known:

(a) Disulfide isomerase

In the newly formed protein molecules –SH groups of cysteine residues may form several intra or inter disulfide linkages. However, only few disulfide linkages may be essential for proper protein folding. The disulfide isomerase favours formation of such disulfide linkages by breaking unwanted linkages formed.

(b) Cis-trans prolyl isomerase

It aids folding process by catalyzing inter conversion of *cis-trans* peptide bonds of proline residues of folding protein.

Protein Factors

Chaperons (Chaperonins)

These proteins aid protein folding process by preventing formation of aggregates. Usually aggregate formation slows down protein folding process. Chaperons accelerate protein folding by blocking protein folding pathways of unproductive nature. They bind to hydrophobic parts of protein molecules and prevent formation of aggregates. They are also involved in protein refolding that occurs when proteins cross membrane structures.

Denaturation of Proteins

Denaturation is loss of native conformation. On denaturation, physical chemical and biological properties of a protein are altered.

Some of the changes in properties are:

1. Decreased solubility
2. Unfolding of polypeptide chain
3. Loss of helical structure
4. Decreased or loss of biological activity
5. More susceptible to action of enzymes
6. Increased chemical reactivity
7. Dissociation of subunits in case of oligomeric proteins.

Causes of Denaturation

1. High temperature
2. Extreme alkaline or acidic pH
3. Use of urea and guanidine at high concentration
4. UV radiation

5. Sonication
6. Vigorous shaking
7. Detergent like sodium dodecylsulfate also denatures protein
8. Treatment with organic solvents like ethanol, acetone etc.
9. Treatment with strong acids like trichloro acetic acid, picric acid and tungstic acid
10. Exposure to heavy metals like Pb^{2+}, Ag^{2+} and Cu^{2+}

Biomedical Importance

1. These properties are exploited for the separation of serum proteins from the other compounds of clinical importanc
2. Denaturation knowledge is required when activities of enzymes in biological fluids like blood are measured for diagnosis.
3. Purification of protein from mixture of proteins also needs denaturation properties.
4. Lead poisoning cases are treated with egg white to decrease toxicity of lead in the body.

Many cases of the process of denaturation is irreversible.

Examples of Denaturation

1. When egg white is exposed to high temperature coagulum is formed because heat denatures egg albumin. The solubility of denatured protein is decreased.
2. Formation of coagulum when albumin is exposed to high temperature.
3. Heat treatment of trypsin results in loss of biological activity.
4. Monellin is a dimeric protein has sweet taste. On denaturation the sweet taste is lost.

Renaturation

Though denaturation is irreversible in majority of the cases, in few cases, renaturation is observed.

Example: Ribonuclease denatures on exposure to heat but come back to its native conformation when temperature is lowered.

PLASMA PROTEINS

Plasma is non-cellular portion of blood. The total plasma protein level ranges from 6-7 gm/dl. Plasma contains many structurally and functionally different proteins. Plasma proteins are divided into two categories.

1. **Albumin:** Not precipitated by half-saturated ammonium sulfate.
2. **Globulin:** Precipitated by half-saturated ammonium sulfate.

The albumin constitutes over half of the total protein. Albumin level ranges from 3.5-5.5 gm/dl. Globulin ranges from 2-3 gm/dl. After the age of 40, albumin gradually declines with an increase in globulins. Albumin is found to be simple protein and a single entity. But globulin has been found to contain many components. Subglobulins are detected as bands on electrophoresis. They are $\alpha 1$, $\alpha 2$, β and γ-globulins.The different plasma protein bands are semi-quantitated using densitometer

Characteristics of Plasma Proteins

1. They are all glycoproteins except albumin. Sialic acid is the most important of all the sugars present in plasma proteins. Removal of sialic acid decreases the life span of

plasma proteins.
2. Each plasma protein has defined life span. The half life of albumin is 20 days and haptoglobin life span is 15 days.
3. Liver is the sole source of albumin, prothrombin and fibrinogen. Most of the α and β globulins are also of hepatic origin. γ-globulins are derived from lymphocytes.

Albumin

Liver produces about 12 gms of albumin per day.

Structure

It consists of single polypeptide chain of 584 amino acid residues with a molecular weight of 66,300. Charged amino acids (glutamate, aspartate and lysine) make up a quarter of the total amino acid residues. The acidic residues out number the basic amino acids hence molecule is highly negative charged which accounts for the high mobility of albumin towards anode. Secondary structure of the protein is over half is in the α-helical conformation. 15% as β-pleated structure and remaining in random coil conformation. The tertiary structure is that of globular protein. The overall shape resembles ovoid. The hydrophobic amino acid residues are present in the hydrophobic interior and polar amino acids are arranged to face the exterior of the albumin. This accounts for the high solubility of the albumin in water (aqueous solutions).

Functions

1. Albumin accounts for 75% of the osmotic pressure (25 mm Hg) in blood and responsible for maintenance of blood volume.
2. Albumin has major role in the regulation of fluid distribution.
3. One gram of albumin hold 18 ml of fluid in the blood stream. Decrease in albumin level leads to accumulation of fluid which results in edema.
4. It transports fatty acids from adipose tissue to liver. Albumin also binds many hydrophobic substances like bilirubin and several drugs. The binding of bilirubin is critical in neo-natal period.
5. Albumin act as a reservoir for Ca^{2+} in plasma. About 40% of plasma calcium is bound to albumin.
6. Albumin is also involved in the transport of thyroid hormones, glucocorticoids and sex steroids.
7. Albumin function as protein source for peripheral tissues. Each day liver replaces about 12 gm of albumin taken up by peripheral tissues. In certain conditions like stress and starvation the turn over rate of albumin is increased. Albumin is in dynamic equilibrium.
8. Albumin acts as a buffer.

α1-Globulin: Mainly α1-antitrypsin. It is a protease inhibitor. It is the major component of α1-fraction and accounts more than 90%. It inhibits trypsin, chymotrypsin, elastase and neutral protease. The major function of α1-antitrypsin is the protection of pulmonary tissue and other tissues from the destructive action of proteases.

α1-Acid glycoprotein (AAG): It is another major component of α1-globulins. It increases

in plasma in inflammatory conditions.
Other components of α1-globulins are
α-Lipoprotein: Functions in the transport of lipids (HDL). It transports cholesterol from extra hepatic tissue to liver.
Prothrombin: Blood clotting factor.
Retinolbinding protein: Transport of Vit A.
Thyroxine binding globulin: Transport of thyroxine.
α1-Fetoprotein: It is present only in fetal serum. Its presence in non-foetal serum indicates primary carcinoma of liver. It is referred as tumour marker.
α2-Globulins: The α2-fraction of globulins includes.
Haptoglobulin: It combines with haemoglobin in order to remove it from the circulation. Kidney cannot filter haemoglobin-haptoglobin complex because of its larger size.
α2-Macroglobulin: It functions as protease inhibitor. It combines with proteases and facilitates their removal from circulation. It also binds with cytokines and involved in zinc transport.
Ceruloplasmin: A copper binding plasma protein and function as ferrooxidase and converts $Fe^{2+} \rightarrow Fe^{3+}$
Erythropoietin: It is involved in erythropoiesis.
Pseudocholinesterase: It is only functional enzyme present in plasma. It hydrolyzes acetylcholine.
β-Globulins: They are
Transferrin: It accounts for about 60% of β-globulins. It is an iron transport protein.
β-*Lipoproteins:* Involved in the transport of cholesterol from liver to extrahepatic tissue (LDL).
Complement-3: It is one of the member of complement system present in plasma. It is involved in phagocytosis.
Other globulins present in plasma are:
Fibrinogen: It is similar to globulins because it is precipitated by half saturation with ammonium sulfate. It is a fibrous or filamentous protein. It is the precursor of fibrin, the blood clotting substances.
Prealbumin: It is a component of globulin fraction. Though it is a globulin by nature it is named as prealbumin because it migrates ahead of a albumin in electrophoresis. It is a carrier of thyroxine, Vitamin A and binds calcium.
Other blood clotting factors, plasminogen and several non-functional enzymes are also present in plasma.

Acute Phase Proteins or Reactants (APR)

1. The concentration of these proteins increases markedly during acute inflammation.
2. They are α1-antitrypsin, haptoglobin, ceruloplasmin, complement-3, fibrinogen and c-reactive protein. Their concentration increases in conditions like surgery, myocardial infraction, infections and tumours.

3. Acute phase reaction is general to any infection. They all play part in complex defensive process of inflammation.
4. The synthesis of these proteins by liver is triggered by interleukin at the site of injury.
5. The plasma levels of these APR raises at different rates. The levels of c-reactive protein raises first followed by α1-antitrypsin. The level of complement-3 raises at the end

γ-Globulins

The immunoglobulins and c-reactive protein (CRP) constitutes this fraction. C-reactive protein is so called because it forms precipitate with somatic C-polysaccharide of pneumococcus bacteria.

IMMUNOGLOBULINS

They are globulins produced as body's immune or defence against infection. Invasion of body by virus or microorganisms or foreign molecules is called *infection*. They are produced by B-lymphocytes, bone marrow and spleen in response to infection. Entry of foreign molecule into body triggers the synthesis of specific globulin, which selectively combines with foreign molecule and lead to its inactivation. The foreign molecule is called as *antigen* where as globulin produced against it is called as antibody. Even without infection the normal plasma contains hundreds of different antibody molecules.

Classification

The immunoglobulin (Ig) proteins of plasma are divided into three major classes Ig G, Ig A, Ig M and two minor classes Ig D, Ig E based on their composition.

Structure

The composition and shape of various classes of immunoglobulins have similar pattern and are represented by the structure of major G class of molecule *i.e.,* Ig G. Each Ig G molecule consist of 4 polypeptide chains and molecular weight is 150,000. The four polypeptide chains are of two types. They are two heavy chains or H chains or about 450 amino acids (molecular weight 50,000) and two light or L chains or about 220 amino acids (molecular weight 25,000). Over all shape of the molecule represents 'Y'. Two heavy chains intertwine to form the base of the Y, a disulfide bond links the L chain to H chain to form arm of the Y. The two heavy chains are held together by disulfide bonds formed between them at the hinge region of the Y

The H chain contains variable region of domain (VH) at the N-terminus and three constant domains (CH1, CH2, CH3) at the C-terminus. Likewise L chain consists of variable domain (VL) at the N-terminus and a constant domain (CL) at the C-terminus. The carbohydrate is attached to CH2 of the heavy chain. The amino acid sequence in the variable regions of H and L chains varies and are specific to the type of antibody. In contrast amino acid sequence in constant region of H and L chains are same in each class of immunoglobulins. The antigen binding site is called as *Fab site*. It consists of light chain and N-terminal half of the heavy chain. The remaining part of the immunoglobulin is called as Fc (fragment with constant domain).

The different classes of immunoglobulins vary in their size, distribution, function and composition. The main chemical differences are found in their H chains. They are named

according to the types of H chain present. There are five classes of H chains. They are γ, α, μ, δ, ε. However, there are only two classes of L chains κ or λ.

Different Classes of Immunoglobulins

1. Ig G class

It constitutes 70 to 80% serum immunoglobulins. Its composition is γ2L2 (γ2k2 or γ2λ2). It is the only class of antibody that is capable of crossing the placental barrier from the maternal to fetal circulation. It is the antibody of newborn until synthesis of immunoglobulins in the body *i.e.,* up to 2 years of age. Ig G antibodies bind to phagocytic cells thus making a link between antibody and phagocytes. Further, binding of Ig G to foreign cells increases their susceptibility to killer cell attack.

2. Ig A class

It accounts for 10-20% of immunoglobulins. Its basic composition is (α2L2), SCJ and it also exists as multimer of the basic unit (α2L2)*n* where *n* = 1, 2, 3 etc. It is the chief antibody present in mucous secretions of lungs and gastrointestinal tract. Mucosal cells add one more polypeptide chain known as *secretory component* (SC), joining H chains of Ig A dimers before passage into secretions. They form aggregates with antigen in the gut and lungs thus prevent the entry of such harmful substances into the body .

3. Ig M class

It accounts for about 5-10% of total immunoglobulins. Like Ig A class, it is also a multimer of basic tetramer. Its composition is (μ2L2)5 J *i.e.,* it is a pentamer of basic unit. The H chains are joined by JC chain. When these are present in secretions of mucous membranes they may contain SC component also. It is the largest of all the immunoglobulins.

IgM act as antigen receptor on B-lymphocytes. It is also involved in complement fixation.

IgM molecules are first to appear in infancy.

4. Ig D class

It accounts less than 0.5% of total immunoglobulins. Its composition is δ2L2. The biological activity of Ig D appears to be limited. It is not a secretory antibody. It is involved in the initiation of alternate pathway of complement fixation.

5. Ig E class

It is least concentrated and has shortest life span of all the immunoglobulins. Its composition is ε2L2. Ig E concentration increases in allergic reactions. It is a surface antibody of cells involved in anaphylactic response. The constant region of the antibody is bound to membrane receptor of leukocytes or mast cells and variable region is exposed to the outer surface. When the specific antigen reacts with antibody, it triggers the cells to release histamine and other vasoactive amines. The Ig E class also found in secretions of lungs and gut but the Ig Es lack the J chain and SC part found in Ig As and Ig M

Immunoglobulins Disorders

There are numerous disorders associated with different classes of immunoglobulins.

1. Multiplemyeloma

It is a malignant disease of single clone (cell type) of plasma cells of the bone marrow. These

plasma cells proliferate throughout bone marrow. Other bone marrow cells are reduced. Tumours of the plasma cells produce myeloma proteins.
The incidence is low in individuals younger than 60 years but raises with age. Symptoms include recurrent infections, weight loss, bone lesions, anaemia and haemorrhages.
Bence-Jones proteins
They are immunoglobulins light chains present in plasma and urine of multiple myeloma patients. The molecular weight is 2500. They are found with γ-globulin fraction on electrophoresis. The characteristic property of these proteins is their behaviour on heating. The normal plasma proteins precipitates between 60-70°C. The Bence-Jones proteins precipitate at 40-60 °C completely. Redissolving of the precipitate occurs as the temperature reaches boiling point. Subsequent cooling reprecipitates the protein and boiling redissolves it. They are identified in the urine of the suspected individuals based on this property.

2. Agammaglobulinemia
It is x-chromosome linked and affects only males. γ-globulins are absent in plasma of these patients. So they are prone to infections.
3. Hypogammaglobulinemia
Production of γ-globulins is decreased in these cases.
4. Autoimmune disorders
Sometimes body rejects its own proteins which becomes antigenic. This results in auto immune disorders due to production of antibodies against its own proteins. Rheumatoid arthritis is known auto immune disorder.

Catalytic Antibodies or Abzymes

1. Immunoglobulins bearing catalytic activity of an enzyme are produced using an enzyme active site as the antigen.
2. The first step consists of producing an antibody A1 against the active site of an enzyme.
3. Enzyme inhibition studies are used to confirm that A1 contains active site close to enzyme active site.
4. Then A1 is used to produce second generation A2 antibodies having specific catalytic activity.
5. They are used to remove toxins or viral coat proteins present in the body.

NUCLEIC ACIDS

OCCURRENCE

Two types of nucleic acids are present in all mammalian cells including humans. They are DNA-deoxy ribonucleic acid and RNA-ribonucleic acid. DNA is present in nucleus and mitochondria. RNA is present in nucleus and cytoplasm. Nucleic acids are also present in bacteria, viruses and plants.

MEDICAL AND BIOLOGICAL IMPORTANCE

1. Nucleic acids serve as genetic material of living organisms including humans.
2. Nucleic acids are involved in the storage, transfer and expression of genetic information.

3. Nucleic acids contain all the necessary information required for the formation of individual or organism.
4. Nucleic acids determine physical fitness of an individual to life.
5. Some nucleic acids act as enzymes and coenzymes. For example, RNA, act as catalyst and RNA is coenzyme for telomerase which seals ends of chromosomes.
6. DNA exhibits structural polymorphism. It assumes several forms depending on certain conditions. Several DNA variants are known.
7. Some RNAs without protein products are found recently in mammals, yeast and bacteria. They are involved in cellular functions.
8. Human Genome Project (HGP) is completed in 2000. It is considered as a major achievement of man after landing on moon. It is useful for finding causes of several diseases whose causes are unknown till. It may also lead to development of new therapeutics as well as diagnostics.

Chemical nature of nucleic acids

Nucleic acids are acidic substances containing nitrogenous bases, sugar and phosphorus. Both DNA and RNA are polynucleotides. They are polymers of nucleotides.

Phosphodiester linkage

In polynucleotides, nucleotides are joined together by phosphodiester linkage. Diester linkage of phosphate joins 3′ OH and 5′ OH belonging two separate sugars (Figure 16.1).

Nucleic acid structure

Primary structure of nucleic acids

Nucleotide sequence of a polynucleotide is known as primary structure of nucleic acid. The primary structure confers individuality to polynucleotide chain. Polynucleotide chain has direction. They are represented in 5′ → 3′ direction only. However, the phosphodiester linkage runs in 3′ → 5′ direction. Each poly nucleotide chain has two ends. The 5′ end carrying phosphate is shown on the left hand side and 3′ end carrying unreacted hydroxyl is shown on the right hand side . Primary structures of DNA and RNA exist in single stranded DNA and RNA organisms.

Since polynucleotide consists of various bases, sugars and phosphates writing a segment of polynucleotide showing structures of bases, sugars with attached phosphates is awkward or highly inconvenient. So, short hand or compact representation of polynucleotide has been proposed. In compact nomenclature or polynucleotide letters A, G, C and T represents nitrogenous bases adenine, guanine, cytosine and thymine, respectively. A vertical line represents sugar back bone. The branches of verticle lines with numerals 3′ and 5′ represents hydroxyl bearing carbon atoms of sugar. A branch at the middle of the verticle line represents hydroxyl bearing 3rd carbon atom of sugar. Another branch at the bottom of verticle line represents hydroxyl or phosphate bearing 5th carbon atom of sugar. The more compact representation of the same molecule is PAPCPGPTPA. Since primary structure is the sequence of nucleotides still more compact representation of the same

molecule is ACGTA. In this primary structure, letters A, G, C, T stands for nucleotides and sequence is written from left to right. Therefore, in DNA and RNA, letters A, G, C, T stands for nucleotides and sugar is deoxy ribose if the polynucleotide is a segment of DNA and sugar is ribose if it is a RNA segment. Remember that letters A, C, U, G, T stands for nucleosides in the case of nucleotides.

Structure of DNA

E. Chargoff and his colleagues extensively studied base composition of DNA. Their studies provided valuable information on the structure of DNA.

Characteristics of DNA base composition

1. In DNA, number of adenine residues is equal to the number of thymine residues *i.e.*, A = T. Further number of guanine residues is equal to number of cytosine residues *i.e.*, G = C. As corollary sum of purine residues is equal to sum of pyrimidine residues A + G = C + T.
2. DNAs from different tissues of same species have same base composition.
3. Base composition of DNA varies from one species to another species.
4. DNAs from closely related species have similar base composition.
5. DNAs of widely different species have different base composition.
6. DNA base composition of a species is not affected by age, nutritional state and environment.

In 1953, J.D. Watson and F.H.C. Crick proposed precise three dimentional model of DNA structure based on model building studies, base composition and X-ray diffraction studies. This model is popularly known as DNA double helix. Using this model, they also suggested a precise mechanism for the transfer of genetic information to daughter cells from parent cells.

Salient features of double helix

1. Two polynucleotide chains are coiled around a central axis in the form of right handed double helix. It represents secondary structure of DNA. It is present in double stranded DNA containing organisms.
2. Each polynucleotide chain is made up of 4 types of nucleotides. They are adenylate, guanylate, thymidylate and cytidylate.
3. Each polynucleotide chain has direction or polarity. Further each polynucleotide chain has 5′ phosphorylated and 3′ hydroxyl end.
4. The back bone of each strand consist of alternating sugar and phosphates. The bases projects inwards and they are perpendicular to the central axis.
5. The two strands run in opposite direction, i.e., they are anti-parallel.
6. The strands are complementary to each other. Base composition of one strand is complementary to the opposite strand. If adenine appears in one strand thymine is found in the opposite strand and vice versa. Where ever guanine is found in one strand cytosine is present in the opposite strand and vice versa.
7. **Base pairing** Bases of opposite strands are involved in pairing. Pairing occurs through

hydrogen bonding and it is specific. Adenine of one strand pairs with thymine of opposite strand through two hydrogen bonds. Guanine of one strand pairs with cytosine of opposite strand. Three hydrogen bonds between GC pair makes it more stronger than two hydrogen AT pair .

(*a*) DNA double helix

(*b*) Base pairing among complementary bases of opposite strands

(*c*) Alternating sugar and phosphate form back bone of strand. Bases project inwards and perpendicular to central axis

8. Complementarity of strands and base pairing are the outstanding features of Watson-Crick model. Specific base pairing immediately suggests a copying mechanism for DNA.

9. The large number of hydrogen bonds along entire length of DNA makes DNA molecule highly stable.

10. Major and minor grooves are present on double helix. They arise because glycosidic bonds of base pairs are not opposite to each other.

11. The base pairs are stacked and 3.4 Å apart. The pitch of the helix (One turn) is 34 Ao and accommodates ten base pairs.

12. Apart from hydrogen bonding, the double helix is stabilized by hydrophobic attraction between bases.

13. The width of double helix is 20 Å.

14. Watson-Crick model is known as B-DNA. Majority of the nuclear DNA is in B-form.

Functions of DNA

1. DNA is the genetic material of living systems. It is super chip ever made by man present in living systems.

2. DNA contains all the information required for the formation of an individual or organism.

3. The genetic information in DNA is converted to characteristic features of living organisms like colour of the skin and eye, height, intelligence, ability to metabolize particular substance, ability to with stand stress, susceptibility to disease and unable to produce or synthesize certain substances etc.

4. All the above phenotype characters of living organisms are intimately related to functions of proteins. Thus, DNA is the source of information for the synthesis of all cellular proteins. The segment of DNA that contains information for a protein is known as *gene*.

5. DNA is transmitted from parent to off spring and hence DNA flows from one generation to other in a given species. Further, DNA provides information inherited by daughter cells from parent cells.

6. The amount of DNA per cell is proportional to the complexity of the organism and hence to the amount of genetic information. The amount of DNA in mammalian cell is 1000 times more than bacteria. Likewise, bacteria contain more DNA than virus and plasmids.

7. The amount of DNA in any given species or cell is constant and is not affected by nutritional or metabolic states.

DNA as the gene

Studies on bacterial transformation carried out by Avery and his colleagues provided first experimental evidence to prove DNA is genetic material in living organisms. They used two types of pneumococci. They are virulent (pathogenic) and avirulent (non-pathogenic) types. DNA isolated from heat killed virulent organism when introduced into avirulent organism it transformed avirulent organism into virulent organism. Deoxy ribonuclease treatment of DNA isolated prior to introduction destroyed transforming capacity of DNA. These observations indicated that DNA is a genetic material.

Mitochondrial DNA

Eukaryotic mitochondria contains DNA. It is different from DNA present in nucleus. It account for 1% of cellular DNA. Base composition of mitochondrial DNA is different from nuclear DNA. Mitochondrial DNA is double stranded and circular.

Bacterial DNA

Bacteria like *E. Coli* contains single molecule of double stranded DNA. *E. Coli* DNA is 1.4 mm long which is 700 times bigger than the size of bacteria. Hence in bacteria also DNA is tightly packed or folded. In *E. Coli* the two ends of DNA are joined to form circular DNA. Histones are not used for packing of bacterial DNA because they are absent in bacteria. Super coiling of circular DNA allows its containment with in nuclear zone. Super-coiled DNA may be in association with some proteins, which stabilizes super coil.

Viral DNA

Viruses are extremely small particles. They are composed of a piece of DNA, which is surrounded by protein coat called *capsid*. Viral DNA may be single stranded or double stranded. Adeno virus (cold virus), Herpes virus and Pox virus are examples for double stranded viruses. Parvo virus is a example for single strand DNA virus.

Plasmids

They exist in bacteria as circular DNA molecules. Plasmid DNA is different from bacterial DNA. They are present in anti-biotic resistant bacteria. They contain genes for inactivation of anti-biotics. pBR 322 of *E. Coli* is an example for plasmid. Plasmids are used as vectors in genetic engineering.

Denaturation of DNA

When DNA molecule is heated it denatures and strands separate. Thermal denaturation of DNA is known as melting of DNA. Melting point of DNA is known as *Tm*. It is a characteristic of given DNA. If the heat denatured DNA is cooled base pairing occurs between strands and reformation of double, stranded molecule takes place. This process is known as *annealing*. It is very useful in genetic engineering particularly in DNA hybridization techniques

Ribonucleic acids (RNAs)

Ribonucleic acids are present in nucleus and cytoplasm of eukaryotic cells. They are also present in prokaryotes. They are involved in the transfer and expression of genetic information. They act as primers for DNA formation. Some RNA act as enzymes as well as coenzymes. RNA also function as genetic material for viruses.

Chemical nature of ribonucleic acids

Like DNAs, RNAs are also poly nucleotides. In RNA polymer, purine and pyrimidine nucleotides are linked together through phosphodiester linkage. The sugar present in a RNA is ribose.

There are mainly three types of RNAs in all prokaryotic and eukaryotic cells. The three types of RNA are 1. Messenger RNA or m-RNA, 2. Transfer RNA or t-RNA, 3. Ribosmal RNA or r-RNA. They differ from each other by size, function and stability.

Messenger RNA

It accounts for 1-5% of cellular RNA.

Structure

1. Majority of mRNA has primary structure. They are single-stranded linear molecules. They consist of 1000-10,000 nucleotides.
2. mRNA molecules have free or phosphorylated 3' and 5' end.
3. mRNA molecules have different life spans. Their life span ranges from few minutes to days.
4. Eukaryotic mRNA are more stable than prokaryotic mRNA.
5. The mRNA nucleotide sequence is complementary from which it is synthesized or copied.
6. Some eukaryotic mRNA molecules are capped at 5' end. The cap is methylated GTP (m7 GTP). Some mRNA contain internal methylated nucleotides. Capping protects mRNA from nuclease attack.
7. At 3′ end of most of eukaryotic mRNA, a polymer of adenylate (poly A) is found as tail. Poly A tail protects mRNA from nucleaes attack.
8. In prokaryotes 5′ end of mRNA contains a sequence rich in A and G. Such sequence is known as *Shine-Dalgarno sequence*. It helps attachment of mRNA with ribosome during protein synthesis.
9. Some prokaryotic mRNA has secondary structure. Intrastrand base paring among complementary
bases allows folding of liner molecule. As a result hairpin, or loop like
secondary structure is formed. (Figure 16.7b).

Functions

1. mRNA is direct carrier of genetic information from the nucleus to the cytoplasm.
2. Usually a molecule of mRNA contains information required for the formation of one protein molecule.
3. Genetic information is present in mRNA in the form of genetic code.
4. Sometimes single mRNA may contain information for the formation of more than one protein.

Transfer RNA

t-RNA accounts for 10-15% of total cell RNA.

Structure

They are the smallest of all the RNAs. Usually they consist of 50-100 nucleotides. They are single strand molecules. t-RNA molecules contain many unusual bases 7-15 per molecule. They are methylated adenine, guanine, cytosine and thymine, dihydrouracil, pseudo uridine, isopentenyl adenine etc. These unusual bases are important for binding of t-RNA to ribosomes and interaction of t-RNA with aminoacyl-t-RNA synthetases. About half of the nucleotides in t-RNA are involved in intrachain base pairing. As a result, double helical segments are formed in t-RNA. Further some bases are not involved in the base pairing resulting in loops and arms formation in t-RNA. Thus, folding in primary structure generate secondary structure. Though t-RNAs differ in chain lengths they have some common features with regard to secondary structure.

Secondary structure of t-RNA

Secondary structure of all the t-RNAs is in the form of clover leaf

1. An amino acid arm where amino acid is attached to 3′-OH of adenosine moiety of t-RNA. ACC is the common base sequence at this 3′-end.
2. Tϕc arm, which contains sequence of ribothymidine-pseudouridine-cytidine. Greek alphabet ϕ (Psi) stands for pseudo uridine. Thymine and pseudouracil are the two unusual bases found in this arm.
3. An anti-codon arm, which recognizes codon on mRNA.
4. DHU arm, which contains many dihydrouridine (UH2) residues.
5. The 5′ end of t-RNA is phosphorylated and residue is guanosine.
6. About 75% t-RNA molecules have extra arm. It consist of 3-5 base pairs. It is found between TϕC and anti-codon arm.

Tertiary structure of t-RNA

X-ray diffraction analysis indicated complex three-dimentional structure for t-RNA molecule. Three-dimentional structure of t-RNA looks like inverted or tilted L. The anti-codon arm is at the tip of the vertical arm of tilted L. The acceptor arm is at the tip of horizontal arm of tilted L. The D loop and TϕC loop are pushed into corner of tilted L.

Functions

1. It is the carrier of amino acids to the site of protein synthesis.
2. There is at least one t-RNA molecule to each of 20 amino acids required for protein synthesis.
3. Eukaryotic t-RNAs are less stable where as prokaryotic RNAs are more stable.

Ribosomal RNA

Ribosomal RNA or r-RNA accounts for 80% of total cellular RNA. It is present in ribosomes. In ribosomes, r-RNA is found in combination with protein. It is known as *ribonucleoprotein*. The length of r-RNA ranges form 100-600 nucleotides. Both prokaryotic and eukaryotic ribosomes contain r-RNA molecules. r-RNAs differ in sedimentation coefficients (S). There are four types of r-RNAs in eukaryotes. They are 5, 5.8, 18 and 28S r-RNA molecules. Prokaryotes contains 3 types of r-RNA molecules. They are 5, 16 and 23S r-RNA molecules.

Structure

r-RNA molecules have secondary structure. Intra strand base pairing between complementary

base generates double helical segments or loops. They are known as domains. 16S r-RNA with 1500 nucleotides has four major domains (Figure 16.8c). The three-dimentional tertiary structure of r-RNA is highly complex.

Functions

1. r-RNAs are required for the formation of ribosomes.
2. 16S RNA is involved in initiation of protein synthesis.

Differences between DNA and RNA

DNA	**RNA**
1. Sugar moiety is deoxy ribose	Sugar moiety is ribose
2. Uracil, a pyrimidine base is usually absent	Thymine, a pyrimidine base is absent
3. Double-stranded molecules	Single stranded molecules
4. Sum of purine bases is equal to sum of pyrimidine bases A + G = C + T A + G # C + T	Sum of purine bases is not equal to sum pyrimidine bases
5. Resistant to hydrolysis by alkali because of absence of hydroxyl group on 2 carbon atom of deoxyribose	Because of presence of hydroxyl group on 2 carbon atom of ribose RNA is easily hydrolyzed by alkali
6. Bases are not modified	Bases are modified
7. No catalytic activity	Some RNA are catalytically active
8. Only one form or type	More than three types
9. Usually not subjected to degradation in cell	Degraded in the cell by nucleases

NUCLEOTIDES

Occurrence

Nucleotides are present in all types of cells.

MEDICAL AND BIOLOGICAL IMPORTANCE

1. Nucleotides are high energy compounds.
2. Nucleotides are required for formation of co-enzymes of some members of vitamins B complex group.
3. Some nucleotides are called as ‘second messenger’ because many hormones exert their action through nucleotides.
4. Some nucleotides act as carrier or donor of activated sugars, sulphates and nitrogenous compounds.
5. Some nucleotides are involved in signal transduction.
6. Some nucleotides are involved in regulation of metabolic pathways.
7. Nucleotides act as alarmones. They regulate cell metabolism and alarms cell when all is not well in cell.

8. Synthetic analogs of nucleosides and nitrogenous bases are anticancer and antiviral agents.
9. Some nitrogenous bases are CNS stimulants.
10. Some bases act as anti-oxidants.
11. Some nucleotide analogs are mutagens.
12. Nucleosides also act as carriers of groups or compounds.
13. Nucleotides are building blocks of nucleic acids.
14. Purines play major role in cardiovascular biology in normal and pathological conditions. They are involved in cardiac aging, angiogenesis, hypertension etc. Purino receptors are identified in cardiovascular system.
15. Cyclic nucleotide cAMP is involved in regeneration of nervous tissues that are injured.
16. Some nucleotides are involved in regulation of ion channel activity. For example, ATP sensitive K+ channel couple cell metabolism to either cell excitability or potassium secretion.
17. Purine nucleotides support rotation of γ-subunit of ATP synthase of electron transport chain. Extra ring in purines is indispensable for the operation of molecular motor.

Chemical nature of nucleotides

Hydrolysis of nucleotides produce nitrogen bases, sugars and phosphate.
Nitrogenous bases. Nucleotides contain two types of nitrogenous bases. They are purine bases and pyrimidine bases.

Purine bases

They are derived from parent compound purine. Purine contains heterocyclic ring system. Fusion of pyrimidine ring with imidazole yields purine ring . The carbon (c) and nitrogen (N) atoms of purine ring are numberered in anti-clockwise direction .
The purines present in nucleotides are adenine and guanine.
Other purine bases are hypoxanthine and xanthine. They are intermediates in the formation of adenine and guanine nucleotides. Uric acid is another purine base. It is the end product of purine nucleotide catabolism.

Physicochemical properties of purine bases

1. Purine bases are sparingly soluble in water. Uric acid and xanthine tend to crystalize at physiological pH at high concentration.
2. Purine bases absorb light in UV region at 260 nm. This property is used for detection and quantitation of purine nucleotides.
3. Purine bases are capable of forming hydrogen bonds.
4. Purine bases like guanine exhibit keto-enol tautomerism at body pH. The ketoform predominates. However, small amount of enol form is present

5. Purine bases exhibit amino-imino tautomerism at body pH. However, amino form predominates

Pyrimidine bases

Pyrimidine bases are derived from parent compound pyrimidine. Pyrimidine is a heterocylic compound.

The pyrimidine bases present in nucleotides are cytosine, uracil and thymine.

Other pyrimidine bases are orotic acid and dihydroorotic acid. They are intermediates in the formation of pyrimidine nucleotides.

Physicochemical properties of pyrimidine bases

1. Pyrimidine bases are soluble in water at body pH.
2. Pyrimidine bases also absorb UV light at 260 nm. This property is used to detect and estimate pyrimidine nucleotides.
3. They are capable of forming hydrogen bonds.
4. They too exhibit keto-enol tautomerism as well as amino-imino tautomerism like purine bases.

Unusual or minor purine and pyrimidine bases

These bases are present in trace amounts in nucleotides compared to above mentioned bases. Hence, they are referred as minor bases or rare bases. They are dihydrouracil, thiouracil, isopentenyladenine, methyl adenine, dimethyl adenine, methylguanine, dimethylguanine, methyl cytosine and hydroxy methyl cytosine.

In plants some pharmacologically active purine bases are identified. They are caffeine of coffee, theophylline of tea, and the obromine of cocoa. Caffeine and theophylline act as CNS stimulants. Recently antioxidant function of caffeine has been discovered. Some inhalers contain theophylline which are used by asthmatics. Mostly it relieves nasal and bronchial congestion.

Sugars

Two types of pentose sugars are found in nucleotides. They are ribose and deoxy ribose. Nucleotides are named according to the type of sugar present. If the sugar is deoxyribose then nucleotide is named as deoxyribonucleotide. Similarly, if the sugar is ribose then nucleotide is named as ribonucleotide.

Some characteristic features of sugar present in nucleotides

1. Normally it is a 5-numbered furanose ring.
2. Only D-isomer is present.
3. Configuration around first carbon atom is ‘β’-form.
4. As mentioned earlier in deoxyribose, only hydrogen is present instead of OH group of 2 carbon atom of furanose ring.

Nucleosides

A nucleoside is composed of purine and pyrimidine base and sugar. In the case of purine nucleosides, the sugar is attached to N-9 of purine ring where as in pyrimidine nucleosides the sugar is attached to N-1 of pyrimidine ring . So, the type of linkage is Nglycosidic

and sugar can be ribose or deoxyribose.
corresponding nucleotides

NOMENCLATURE OF NUCLEOSIDES

Nucleosides are named as derivatives of bases. For example, adenine linked to ribose is called as adenosine. Capital letter A is used to indicate adenine containing nucleoside. If adenine is linked to deoxyribose then it is named as deoxy adenosine and it is abbreviated as dA.

Base Nucleosides Abbreviation

Adenine Adenosine A
Deoxyadenosine dA
Guanine Guanosine G
Deoxyguanosine dG
Hypoxanthine Inosine I
Xanthine Xanthosine
Cytosine Cytidine C
Deoxycytidine dC
Thymine Ribothymidine T
Deoxythymidine dT
Uracil Uridine U
Dehydrouracil Pseudouridine Ψ
Orotic acid Orotidine O

Nucleotides

They are phosphorylated nucleosides. Usually one or two of hydroxyl groups of ribose (deoxyribose) are phosphorylated. Thus, a nucleotide has three structural components. They are nitrogenous base, sugar and phosphate. Phosphate is attached to ribose through an ester linkage.

Nomenclature of nucleotides

Since nucleotides are phosphorylated nucleosides, the name of a nucleotide is composed of name of nucleoside and phosphate. The attachment position of phosphate to ribose is indicated with Arabic numeral. Further, a prime mark after numeral is used to differentiate numbered position of ribose from the numbered position of base. Usually nucleotides containing single phosphate are called as monophosphates. Thus a nucleotide of adenosine containing one phosphate on C-3 of ribose is named as adenosine monophosphate (AMP) and adenosine-3'-phosphate (A-3'-P) more precisely. If the sugar is deoxyribose then it is called as deoxy adenosine-3'-phosphate (dA-3'-P). If the phosphate is attached to C-5 of ribose then it is named as adenosine-5'-phosphate. Generally nucleotide mono phosphates in which phosphate is attached to C-5 of ribose are named without primed numeral. Hence, adenosine-5'-phosphate is called as *adenosine monophosphate* .

Because of phosphate nucleotides are acidic in nature. Hence they are named by adding word 'lic acid' to the name of the base or nucleoside. For example nucleotide of adenine is called as adenylic acid. Nucleotide of uracil is named as uridylic acid

Nucleoside di and triphosphates

They are nucleosides in which two or three phosphate groups are attached to C-5 or C- 3 of ribose. Since they are phosphorylated nucleosides they are nucleotides also. For example, adenosine with two phosphates attached to ribose is called as adenosine diphosphate (ADP). Likewise adenosine triphosphate (ATP). Phosphates are in acid anhydride forms.

Name of diphosphate Abbreviation	Name of triphosphate Abbreviation
Adenosine diphosphate ADP	Adenosine triphosphate ATP
Deoxy Adenosine dADP diphosphate	Deoxy Adenosine dATP triphosphate
Guanosine diphosphate GDP	Guanosine triphosphate GTP
Deoxy Guanosine dGDP Diphosphate	Deoxy Guanosine dGTP triphosphate
Cytidine diphosphate CDP	Cytidine triphosphate CTP
Deoxy Cytidine dCDP diphosphate	Deoxy Cytidine dCTP triphosphate
Thymidine diphosphate TDP	Thymidine triphosphate TTP
Deoxy Thymidine dTDP diphosphate	Deoxy Thymidine dTTP triphosphate
Uridine diphosphate UDP	Uridine triphosphate UTP

Dinucleotides

They consist of two nucleotides. They are joined together by phosphodiester linkage. 3'-OH of first nucleotide is linked to 5'-OH of second nucleotide through the phosphodiester linkage .

Two co-enzymes, which are dinucleotides are NAD+ (NADP+) and FAD. But in these dinucleotides, nucleotides are held together through anhydride linkage formed between phosphate of first nucleotide and phosphate of second nucleotide . Further in FAD the glycosidic linkage between sugar and base is absent.

Oligonucleotides

They consist of less than ten nucleotides but more than two nucleotides. Nucleotides are joined by phosphodiester linkage.

Example: oligo adenylate.

Naturally occurring nucleotides

Cells contain several free nucleotides. Several biological processes depends on free nucleotides.

Adenine nucleotides and their functions

1. ATP is energy currency of cell. In mammalian cells, its concentration is about 1 mM/L.
2. Oxidative phosphorylation of respiratory chain requires ADP. ADP is a high energy compound.

3. ATP, ADP and AMP are allosteric effectors of several enzymes.
4. Several hormones exerts their action through cyclic AMP or cAMP .
5. Phosphoadenosine phosphosulfate (PAPS) is the donor of sulfate groups in many biosynthetic reactions .
6. Adenine nucleotides are constituents of FAD and NAD+, NADP+ (Fig. 14.6), coenzyme A and vitamin B12 co-enzyme.
7. Diadenosine triphosphate and diadenosine poly phosphate are neurotransmitters and affect platelet aggregation and blood pressure.
8. Oligoadenylate is mediator for interferon action.
9. ATP is required for protein biosynthesi

Guanine nucleotides and their functions

1. GTP and GDP are high energy compounds. They participate in energy-dependent reactions.
2. GTP is required for protein biosynthesis.
3. Many hormones mediate their action though cyclic GMP or cGMP. cGMP is involved in vasodilation and smooth muscle relaxation.
4. G-proteins, which requires GTP and GDP are involved in signal transduction of several biological processes like vision, taste, metabolic regulation, olfaction, and cancer.
5. RNA is catalytically active in presence of GMP or Ribozyme action depends on GMP.
6. GDP is carrier of activated sugars in biosynthesis of mucopolysaccharides.

Hypoxanthine nucleotides

1. IDP and IMP are high energy compounds.
2. IMP is intermediate in purine ribonucleotide synthesis.

Uracil nucleotides

1. UTP and UDP are high energy compounds.
2. UDP is carrier of activated sugars and amino sugars needed for the synthesis of glycogen, glycoportein, gangliosides etc.
3. UDP-glucuronate serve as donor of glucuronide in conjugation reactions. For example, formation of bilirubin diglucuronide and detoxication reactions.

Cytosine nucleotides

1. CTP and CDP are high energy compounds.
2. CDP-choline serve as donor of choline in biosynthesis of phospholipid.
3. CMP-NANA is donor of NANA in biosynthesis of gangliosides.
4. Cyclic CMP also exist in cells.

Adenine nucleoside

S-adenosyl methionine is a adenine nucleoside. It is the donor of methyl groups in biosynthesis reactions.

Purine and pyrimidine analogs

Several synthetic analogs of purines and pyrimidines are used as anti-cancer agents. Their actions are detailed in next chapter.

Purine analogs

1. Mercaptopurine
2. Thioguanine
3. 2-Aminopurine
4. Allopurinol
5. Azathiopurine. A modified mercaptopurine. It is an immune suppressive agent.

Pyrimidine analogs

1. 5-Flurouracil

Nucleoside analogs

Nucleoside analogs containing modified bases or sugars are used as anti-cancer agents, anti-viral agents and mutagens.

1. **Deazauridine** It is nucleoside with unnatural base. It is anti-cancer drug.
2. **6-Azauridine** Another nucleoside with unnatural base. An anti-cancer agent.
3. **Adenine arabinoside (Ara-A)** It is a nucleoside with abnormal pentose. It acts as anti-cancer agent as well as anti-viral agent.
4. **Arabinosyl cytosine (Ara-C)** It is a cytosine arabinoside used in cancer treatment.
5. **AZT (3'-azido-3'-deoxy thymidine) or Azido thymidine** It is used in treatment of AIDS. It can prevent progression of the disease if given at an early stage.
6. **Dideoxy cytidine** It is used in viral infections.
7. **Bromodeoxy uridine** It is a mutagen.
8. **Iododeoxy uridine** It is an anti-viral agent.
9. **Fluorodeoxy uridine** It is anti-cancer agent

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