COURSE CODE:	*VBB 202*
COURSE TITLE:	**Immunoglobulins: Types, Structures, Functions And Biomedical Importance**
NUMBER OF UNITS:	*3 Units*
COURSE DURATION:	*Three hours per week*

COURSE DETAILS:

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

COURSE CONTENT:

Relevance of biochemistry to veterinary medicine. Biochemistry of the living cell. PH and buffers. Enzymes: nature, properties and functions; enzyme kinetics; Allosteric effects; Co-enzymes; structure and role in cellular metabolism. Enzyme assay in clinical veterinary medicine. Chemistry and biochemistry of carbohydartes of importance in vet. medicine; Metabolism of carbohydrates: Glycolysis [Embden-Meyerhof pathway]. Metabolism of fructose and galactose. Kreb's(citric acid) cycle, Glycoxylate cycle. Alternate pathways of carbohydrate oxidation. The Uronic acid pathway. Metabolism of glycogen. Disorders of carbohydrate metabolism. The electron transport chain and oxidative phosphorylation. Lipids; classification, chemistry and functions; digestion, absorbtion and transportation. Lipoproteins etc. Biosynthesis of fatty acids; the triacylglycerols. Degradation of triacylglycerols, phospholipids and sphingolipids. β-oxidation of fatty acids. Ketone bodies and ketosis. Metabolism of cholesterol. Primary disorders of plasma lipoproteins (dyslipoproteinemias). Biochemistry of prostaglandins.

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 (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.

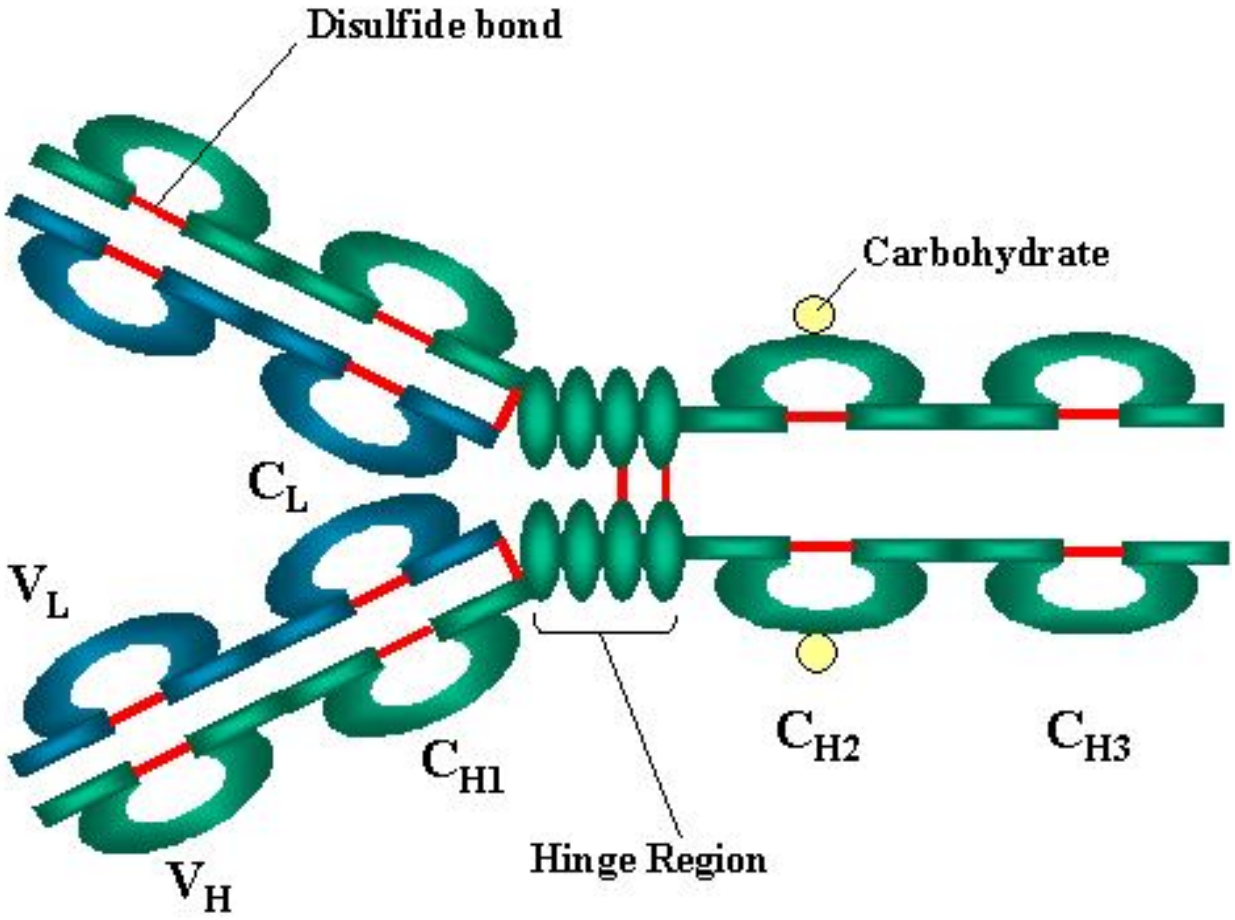
IgA Increases occur in:-Wiskott-Aldrich syndrome, cirrhosis of the liver, IgA myeloma, autoimmune disorders, rheumatoid arthritis, lupus erythromatosis etc

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

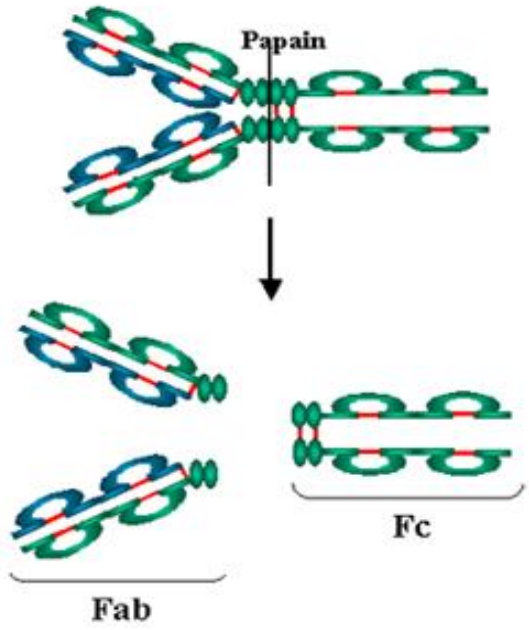
IgE Increases occur in:-atopic skin diseases e.g. eczema, hay fever, asthma, anaphylactic shock and IgE myelomas.

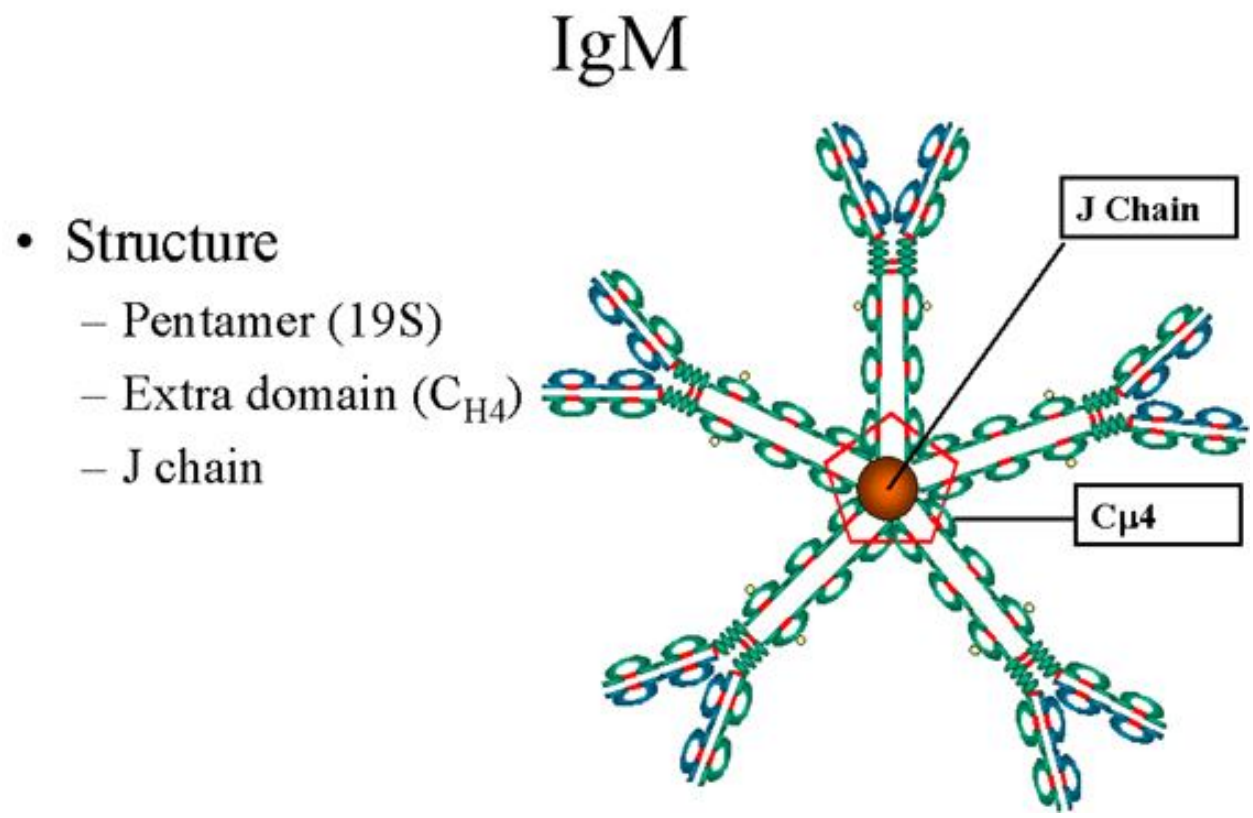
Decreases occur in:-congenital Aggammaglobulinemia, Hypogammaglobinemia etc


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

Immunoglobulin Fragments: Structure/Function Relationships

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





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 regionon 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.

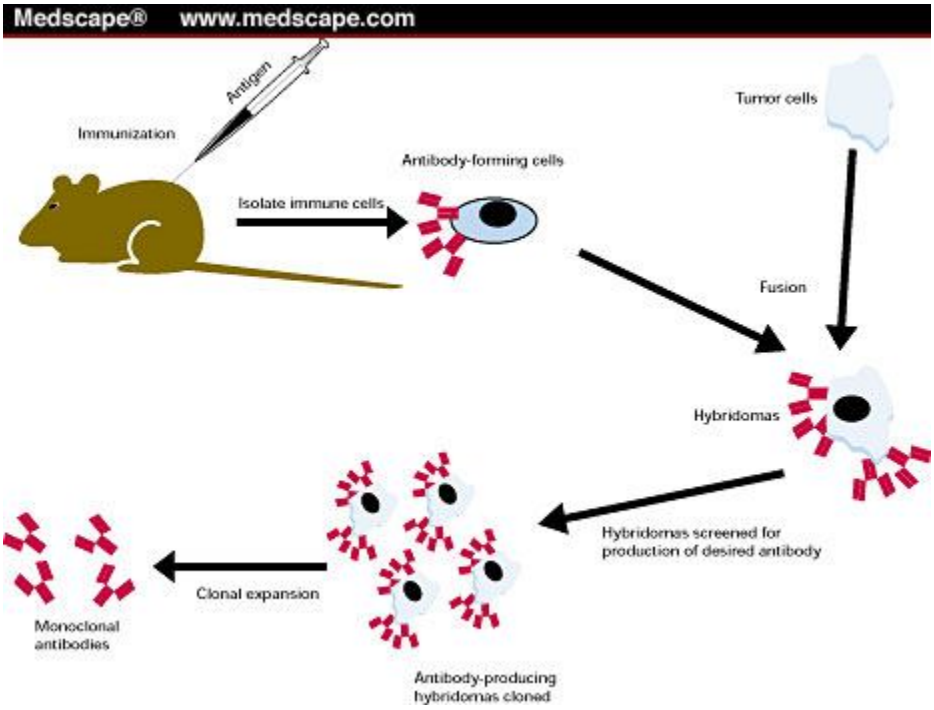
HYBRIDOMA TECHNOLOGY

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.

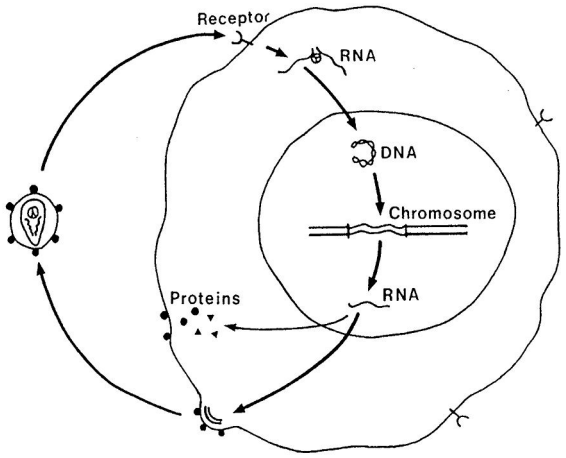
RETROVIRUSES, MOLECULAR BASES AND INVOLVEMENT IN CANCER, PROTONCOGENES AND ONCOGENES RETROVIRUSES.

- 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.

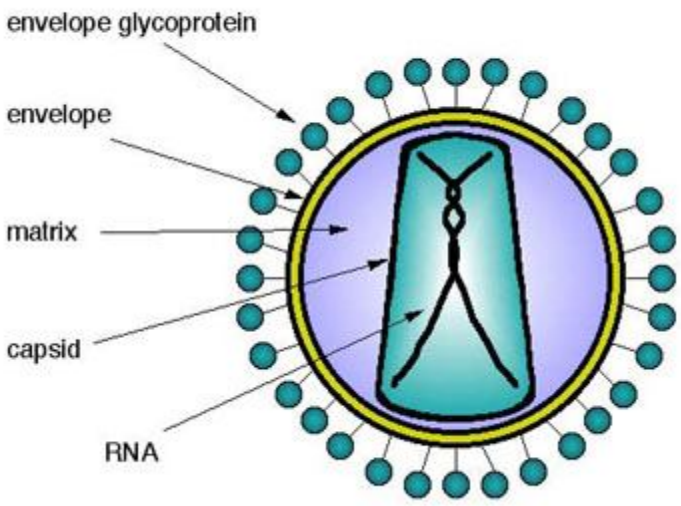
Retroviruses carry diploid, single-stranded RNA genomes in the virion and replicate by forming one double stranded DNA copy in the infected cell, by means of the viral enzyme, reverse transcriptase. This viral genome becomes integrated as a DNA called the ‘provirus’, into the chromosomal DNA of the host 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 celldifferentiation, 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 celldifferentiation, 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.

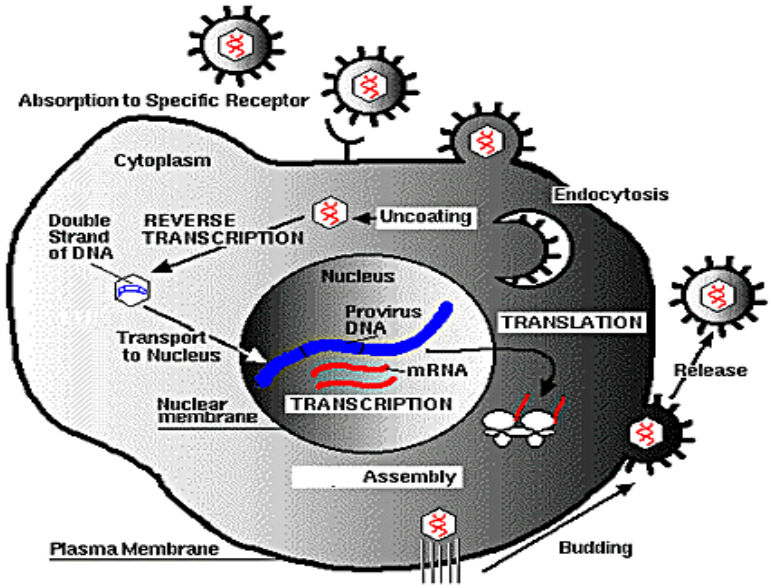
5) Double strand DNAis *circularized* and *integrated* into host cell DNA (see below) using a virally encoded integrase enzyme. This DNA is copied every time cellular DNA is copied. Thus, at this stage the provirus is just like a normal cellular gene.

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 andabnormal euploidy. Chromosomal defects usually have more profound effects on the phenotype than gene mutation and these changes occur during meiosis.

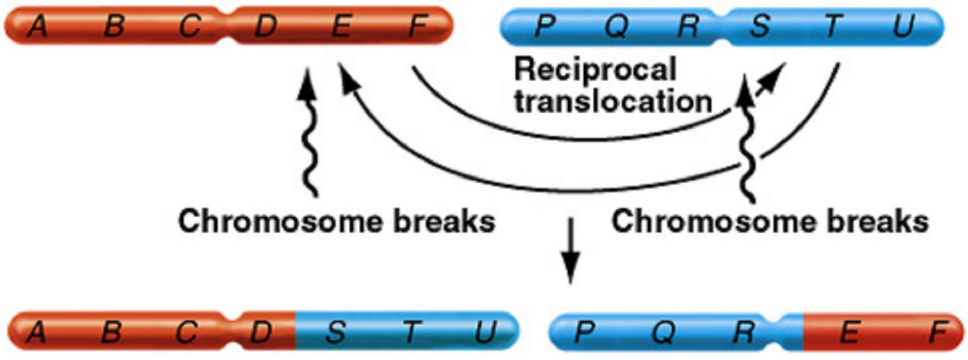
Mutations can be caused by copying errors in the genetic material during cell division, by exposure to ultraviolet or ionizing radiation, chemical mutagens, or viruses, or can be induced by the organism itself, by cellular processes such as hypermutation. In multicellular organisms with reproductive 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).

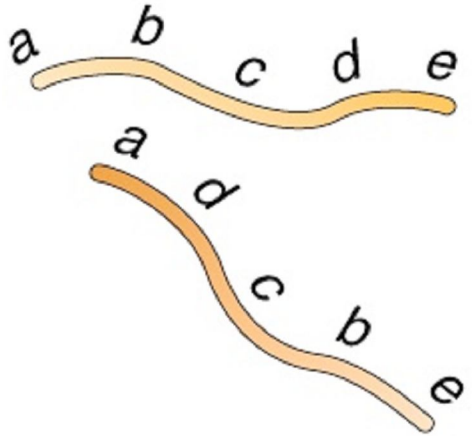
Translocations can often alter or abolish expression of the gene and gene products and maybe lethal. There are usually no consequences of translocation in homozygotes; genetic material is neither lost nor gained but in heterozygotes with non-reciprocal translocationgenetically imbalanced gametes result 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 itcan 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.

Hemolyticanemia 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 □2□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.

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).

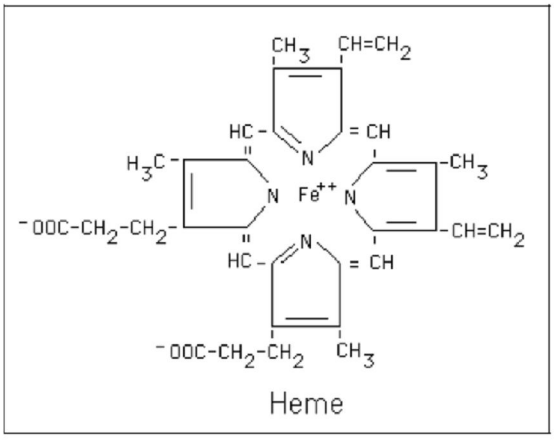
Porphyrins are generally known to form complexes with metal ions such as iron, magnesium, copper etc at the nitrogen atom. When an iron complex is formed the resulting compound is Heme, while chlorophyll is formed with a magnesium porphyrin complex. In nature the

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.

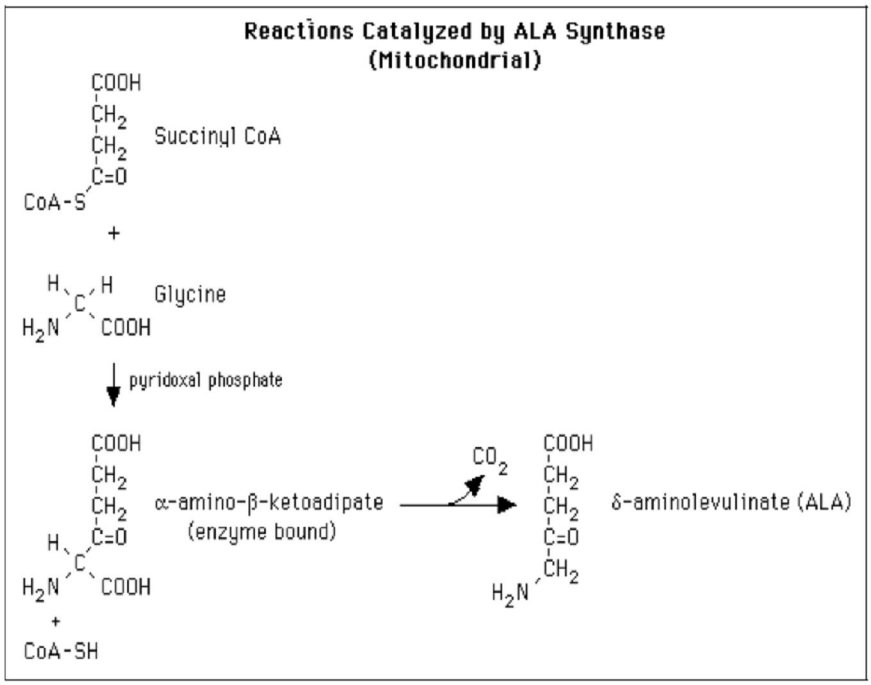


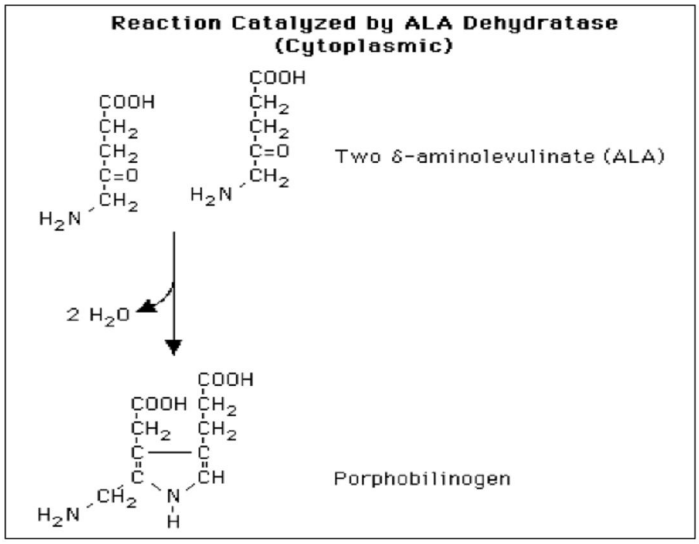
Heme

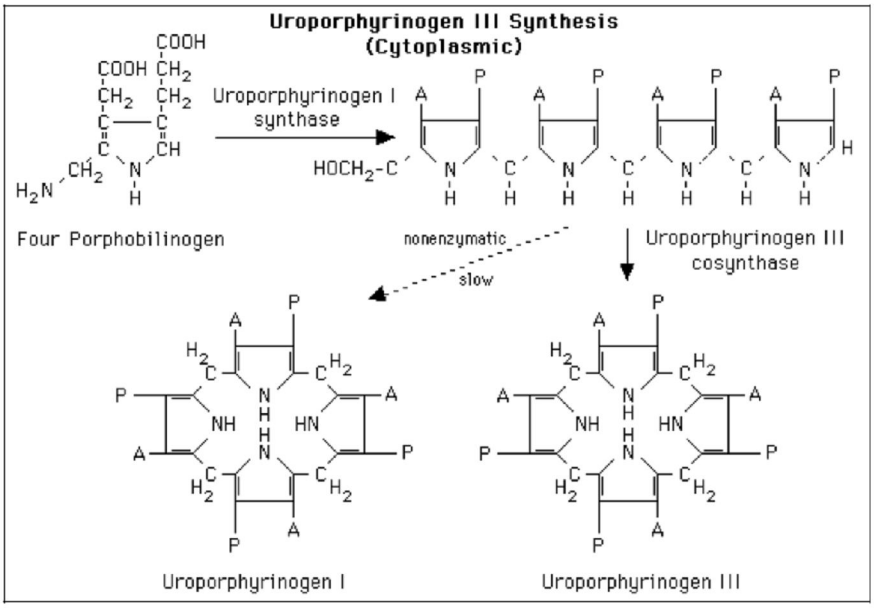
Porphyrinogens are a closely related compounds that have extra hydrogen atoms and also differ in pattern of double bonds available, hence are linked by methenyl bridges. They are colorless, do not fluoresce and are easily auto oxidized to porphyrins e.g. urobilinogen.

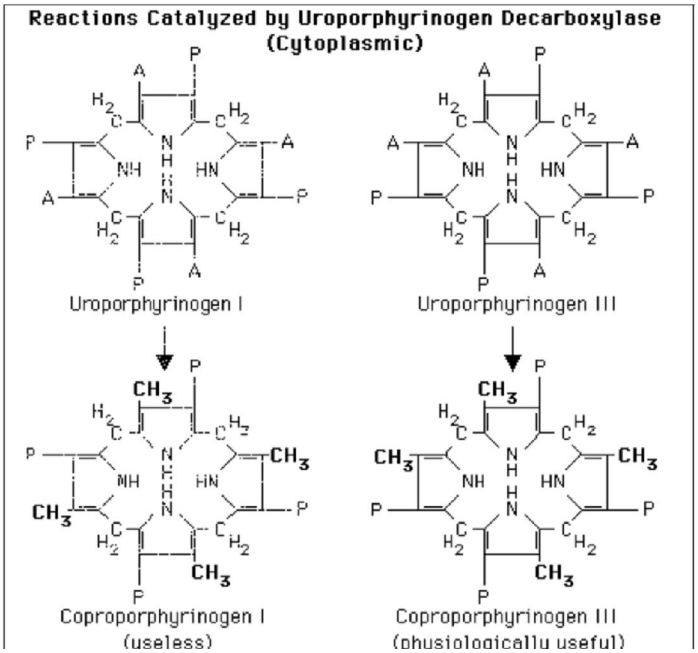
BIOSYNTHESIS OF HEME

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.

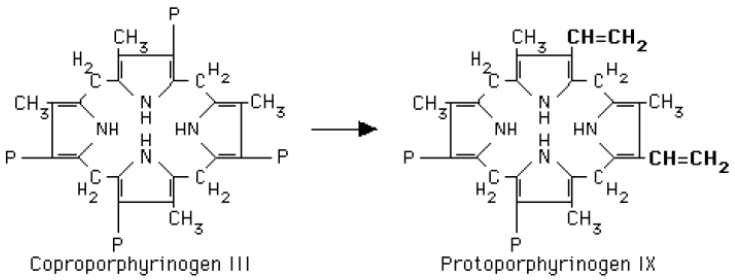



Reaction Catalyzed by ALA Dehydratase
(Cytoplasmic)
Two δ-aminolevulinate (ALA)
2 H2O
Porphobilinogen

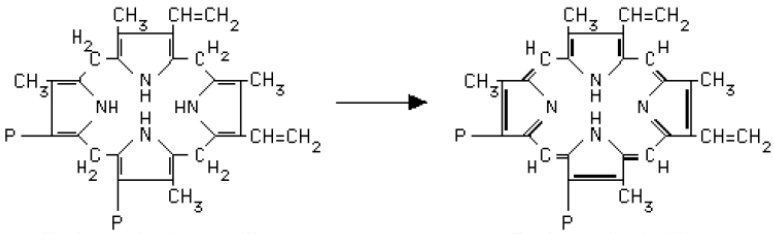

Uroporphyrinogen III Synthesis
(Cytoplasmic)
Uroporphyrinogen I
synthase
Four Porphobilinogen
nonenzymatic
slow
Uroporphyrinogen III
cosynthase
Uroporphyrinogen I
Uroporphyrinogen III

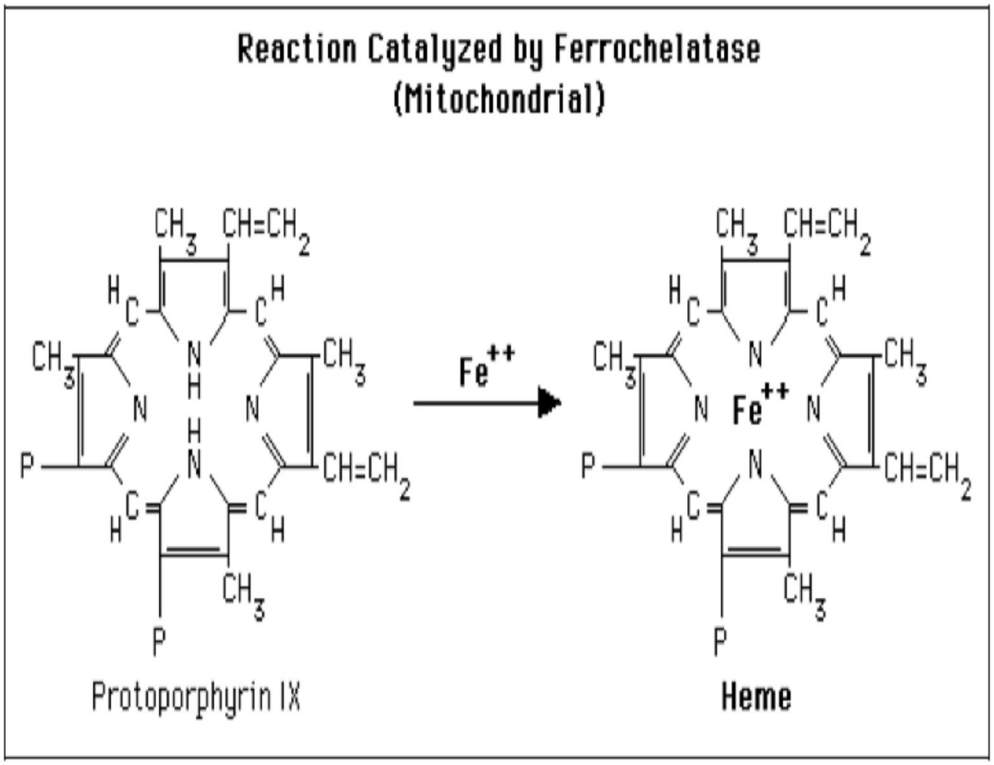

Reactions Catalyzed by Uroporphyrinogen Decarboxylase
(Cytoplasmic)
Uroporphyrinogen I
Uroporphyrinogen III
Coproporphyrinogen I
(useless)
Coproporphyrinogen III
(physiologically useful)

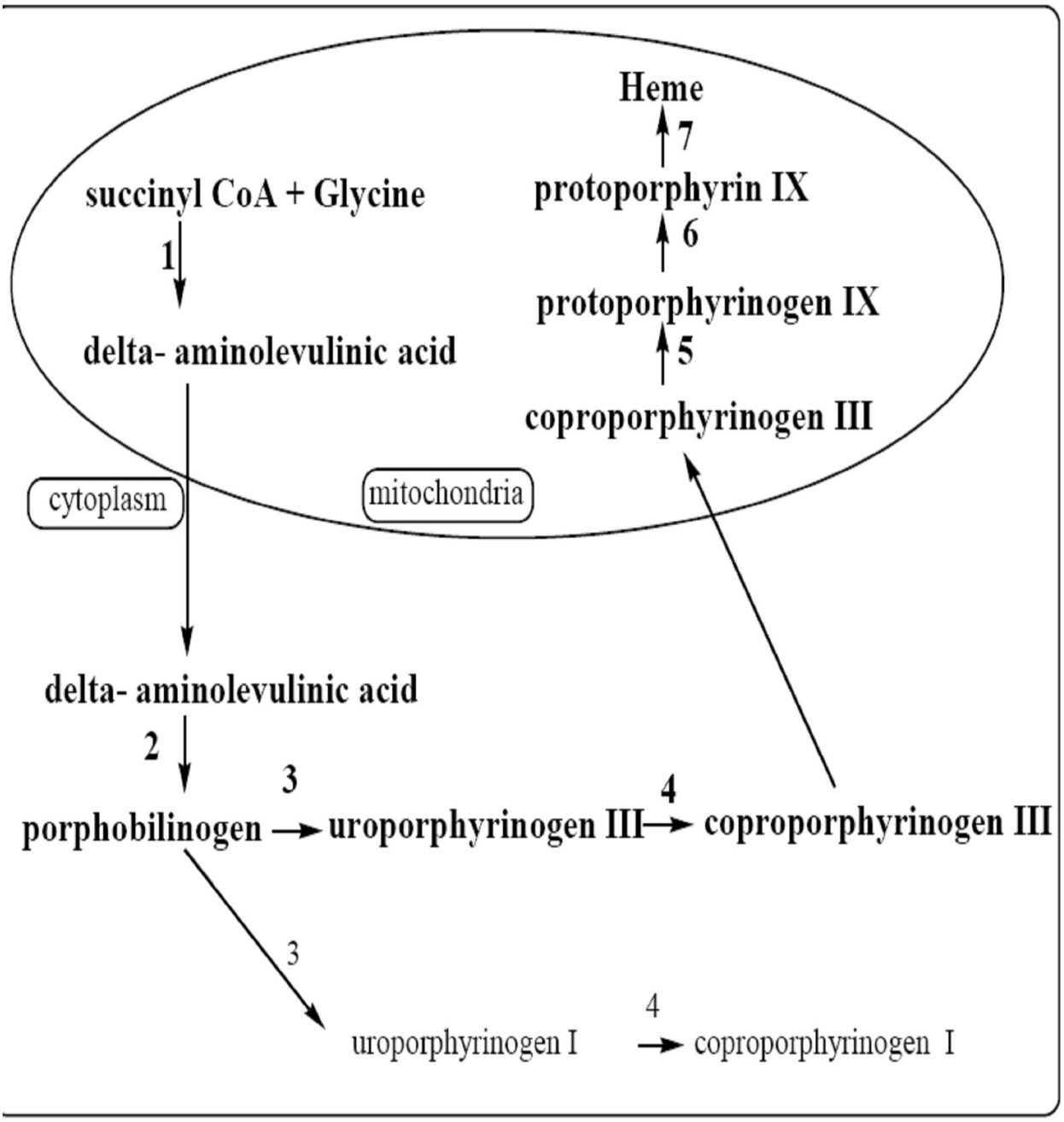
Reaction Catalyzed by Coproporphyrinogen III Oxidase (Mitochondrial)



Reaction Catalyzed by Protoporphyrinogen IX Oxidase (Mitochondrial)




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



Site and reactions of heme synthesis

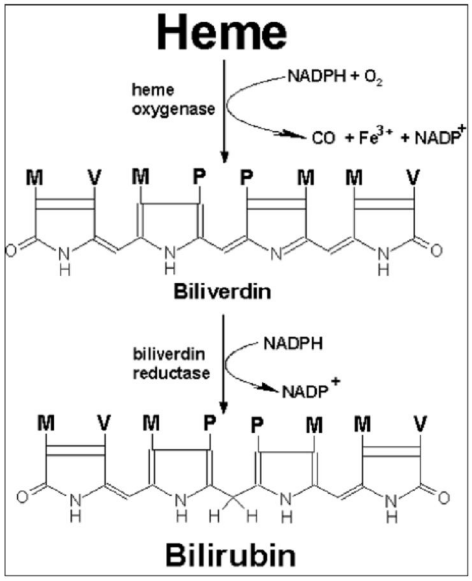
REGULATION OF HEME SYNTHESIS

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.

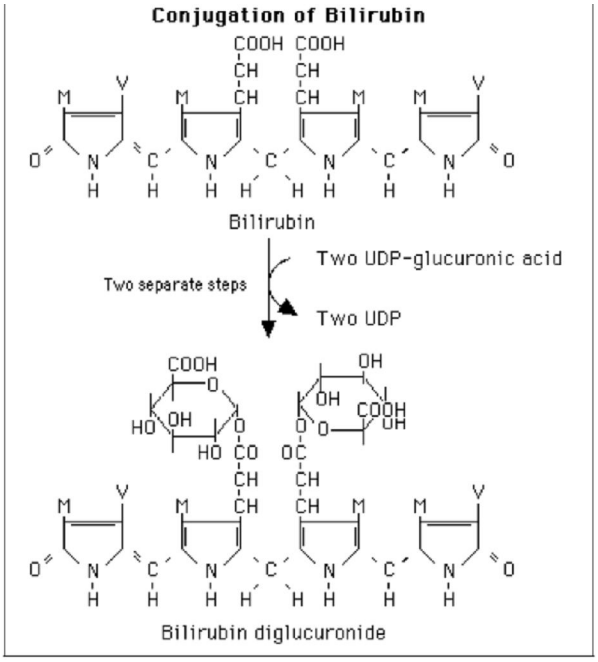
CATABOLISM OF HEME

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).

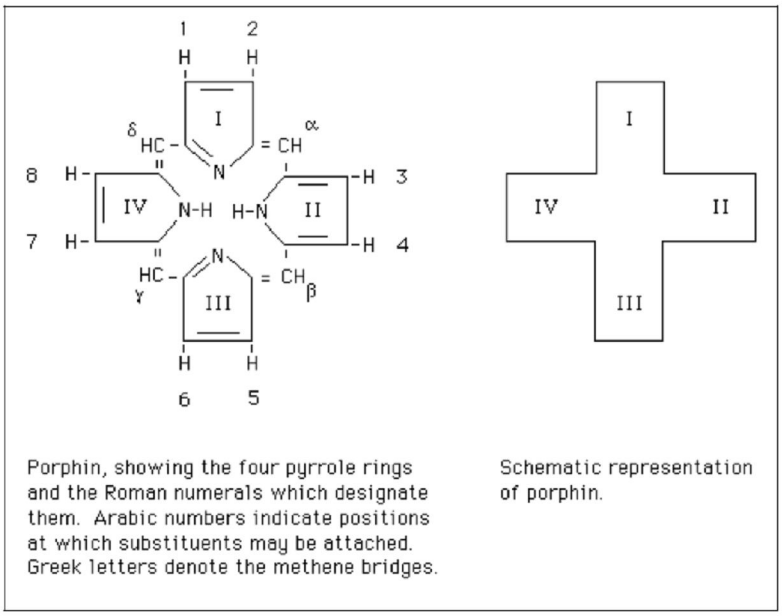
PORPHYRIAS

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.

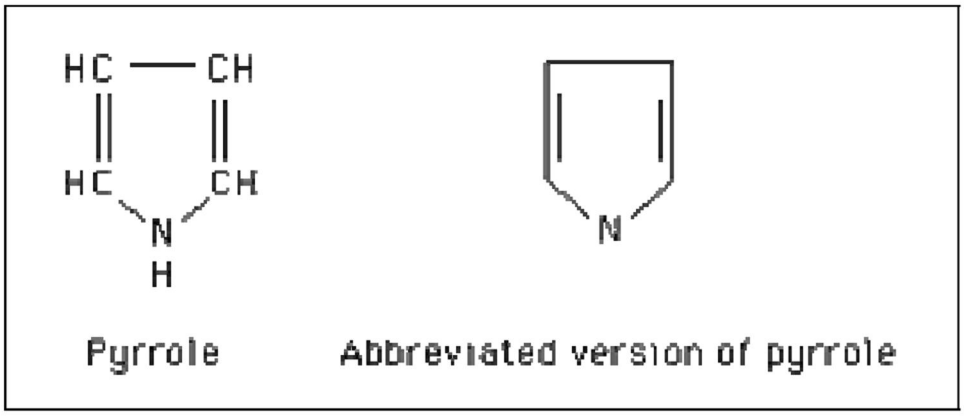
The most common porphyrias known is that caused by a defect in the enzyme porphobilinogen deaminase (PBG deaminase) called acute intermittent porphyrias.

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

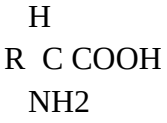
and plants.

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.

Amino Acids and Peptides 25

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 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	covalent

			albumin	
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 α1, α2, 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 Ca2+ 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.

α**1-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.

α**1-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.

α**1-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.

α**2-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.

α**2-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

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EXERCISES

ESSAY QUESTIONS

1. Draw DNA double helix. Describe its main features. Add a note on DNA functions.
2. Define RNA. Classify. Write structure and functions of each one.
3. Briefly describe nucleic acids.

SHORT QUESTIONS

1. Name different types of RNAs. Write main features and functions of mRNA.
2. Name differences between DNA and RNA.
3. Draw clover leaf structure of tRNA. Label its different parts. Mention functions of tRNA.
4. How eukaryotic DNA is organized?
5. Explain the following
(*a*) DNA as gene
(*b*) Denaturation of DNA
6. Write about functions of nucleic acids.
7. Write a note on DNA polymorphism.
8. How bacterial DNA is organized.
9. Write differences between prokaryotic and eukaryotic DNA.
10. Define plasmid. Give example. Write its importance.
11. Write a note on nucleosome.
12. Explain Ribosomal RNA. How it differs from other RNAs?
13. Write a note on unusual bases of RNAs.

MULTIPLE CHOICE QUESTIONS

1. Each polynucleotide chain
(*a*) Has direction. (*b*) Has 5′ and 3′ end.
(*c*) Has direction and two ends. (*d*) Has phosphodiester linkages.
2. ATTATA is sequence of a DNA segment. Each letter stands for
(*a*) Bases. (*b*) Nucleosides.
(*c*) Nucleotides. (*d*) Purine and pyrimidine bases.
3. Shine-Dalgarno sequence is present in
(*a*) Eukaryotic mRNA. (*b*) Prokaryotic mRNA.
(*c*) At 5′ end of prokaryotic mRNA. (*d*) At 3′ end of eukaryotic mRNA.
4. Ribosomes are
(*a*) Nucleic acids. (*b*) Proteins.
(*c*) Ribonucleo proteins. (*d*) Nucleosomes.
5. Loops in RNA molecules are
(*a*) Due to intra strand base pairing.
(*b*) Due to inter strand base pairing.
(*c*) Due to intra strand base pairing between complementary bases.
(*d*) Involved in transfer of genetic information.

FILL IN THE BLANKS

1. In polynucleotides phosphodiester linkage joins 3′-OH and 5′-OH belonging to ..... sugars.
2. ACGCATA is sequence of one DNA strand. Then ..... is sequence of opposite strand.
3. DNAs from different tissues of same species have ..... base composition.
4. When DNA is dehydrated it acquires ..... form.
5. An extra arm in tRNA is found between ..... and ..... arm.

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EXERCISES

ESSAY QUESTIONS

1. Classify proteins based on composition. Give examples for each class.
2. Explain terms primary, secondary, tertiary and quaternary structure of proteins. Write various forces that stabilize protein structure.
3. Describe immunoglobulins with respect to structure, classification and functions.
4. Describe plasma proteins.
5. Write an essay on functions of proteins with examples.

SHORT QUESTIONS

1. Define denaturation. Name methods of protein denaturation and write importance of this process

in medicine.
2. Write salient features of α-helix.
3. Write methods used for determination of primary structure of protein.
4. Explain primary structure of insulin.
5. Name acute phase proteins. In what conditions, they are elevated in blood ?
6. What is normal plasma protein level? Draw electrophoretic pattern of plasma proteins.
7. Write a note on super secondary structure of proteins.
8. Define abzymes. How they are produced ? Write their clinical importance.
9. Write a note on diseases associated with immunoglobulins.
10. Write briefly on Bence-Jones proteins.
11. Mention five structural features of β-pleated sheet.
12. Define primary structure. Write its importance.
13. Write about forces that stabilizes quaternary structure of protein.
14. Write normal plasma albumin level. Mention its functions.
15. Briefly write on various components of α1-globulins.
16. Write a note on charge properties of protein.
17. Name various components of β-globulins. Mention their functions.
18. Write short note on immunoglobulin structure.
19. Define isoelectric point of protein. Give an example. Write about properties of protein at isoelectric
point.
20. Write briefly about structure of albumin and collagen.
21. Name Edman's reagent. Write its importance.
22. Write about changes that occurs in protein properties on denaturation.
23. Define renaturation. Give an example.
24. Define derived proteins. Give examples.
25. Write a note on conjugated protein

MULTIPLE CHOICE QUESTIONS

1. All the following statements are correct regarding protein except:
(*a*) Proteins are involved in transport of gases.
(*b*) Proteins are involved in defence.
(*c*) Proteins act as buffers.
(*d*) Proteins are not found in all cells.
2. In fibrous proteins, polypeptide chains are
(*a*) Extended (*b*) Folded
(*c*) Twisted (*d*) Coiled
3. Hair pin turn of polypeptide chain is called as
(*a*) β-Turn (*b*) α-Turn
(*c*) γ-Turn (*d*) β-pleated turn
4. In the body, one gram of albumin holds

(*a*) 10 ml of fluid (*b*) 18 ml of fluid
(*c*) 25 ml of fluid (*d*) 20 fatty acids
5. Tumour marker present in liver cancer patient blood is
(*a*) Haptoglobulin (*b*) Acid protein
(*c*) α-Feto protein (*d*) Thyroxine
6. The concentration of Ig E class of immunoglobulin increases in blood in
(*a*) Allergic reactions (*b*) Cancers
(*c*) Cold conditions (*d*) Neonatal life

FILL IN THE BLANKS

1. ----------and ---------- are connective tissues proteins.
2. The isoelectric point of casein is ----------.
3. Gliadin of wheat is an example for ----------.
4. β-pleated sheet is stabilized by ---------- hydrogen bonds.
5. Quaternary structure of hemoglobin consists of ----------.
6. Emphysema is due to deficiency of ----------.
7. Plasma and urine of multiple myeloma patients contains ----------.
8. Immunoglobulins bearing catalytic activity are called as ---------

ESSAY QUESTIONS

1. Draw an animal cell diagram and label different cell organelle. Write functions of mitochondria,
golgi apparatus and lysosomes.
2. Describe structure and function of each cell organelle.
3. Write about cell cycle and cell death. Mention clinical importance of each one.

SHORT QUESTIONS

1. Name organic substances present in cell.
2. Define cytoskeletons of a cell. Name them. Write their functions.
3. Define cell cycle. Name stages of cell cycle. Explain any one stage.
4. Explain apoptosis.
5. Write a note on structure and function of mitochondria.
6. Draw mitochondria. Label its various parts.
7. Name different types of cell death. Explain each one.
8. Write a note on cytomembranes.
9. Name different types of endoplasmic reticulum of cell. Write structure and function of any one.
10. Write a note on intracellular membranous network.
11. Mention functions of nucleus, nucleolus and cytosol.
12. Write a note on lysosomol role in diseases.

MULTIPLE CHOICE QUESTIONS

1. In the cell cycle check points exist
(*a*) at G1/S boundary (*b*) at G1/G2 boundary
(*c*) at S/G2 boundary (*d*) at G1/M boundary
2. Lysosomes contain mainly
(*a*) Hydrolases (*b*) Proteases
(*c*) Lipases (*d*) Cathepsins
3. Cell death due to lack of oxygen is called as
(*a*) Necrosis (*b*) Atrophy
(*c*) Hypertrophy (*d*) Apoptosis
4. Peroxisomes are involved in
(*a*) Protein synthesis (*b*) Cell death
(*c*) Phospholipid synthesis (*d*) Triglyceride synthesis

FILL IN THE BLANKS

1. A well defined ---------- is absent in prokaryotes.
2. ---------- separates cell from its surroundings.
3. An important inner mitochondrial membrane phospholipid is ----------.
4. ---------- are called as suicide bags of cells.
5. A cytoskeleton filament present in the axons of nerve and sperm cell ----------.

EXERCISES

ESSAY QUESTIONS

1. Classify enzymes. Give examples for each class and write reactions with cofactors they catalyze.
2. Define enzymes. Write the effect of substrate concentration, temperature and pH on enzyme activity.
3. Define active site of an enzyme. Write its characteristics and explain models of active site.
4. Define coenzyme. Name four coenzymes and write reactions with cofactors in which they act as
coenzyme.
5. Define inhibition. Explain competitive and feedback inhibition with examples.
6. Describe enzyme regulation.
7. Write an essay on enzymes of diagnostic (clinical) importance.
8. Define allosteric enzymes. Describe kinetics of an allosteric enzyme with an example and model.
9. Name factors affecting enzyme catalyzed reactions. Explain each one of them with suitable examples.
10. Define cofactors. Explain their importance with suitable examples.
11. Write an essay on enzyme inhibition.

SHORT QUESTIONS

1. Define Km. Write its significance.
2. Define proenzymes. How they are converted to enzymes ?
3. Define non-competitive inhibition. What happens to Km and Vmax in this type of inhibition. Give examples.
4. Explain enzyme regulation by covalent modification.
5. Competitive inhibitors are chemotherapeutic agents. Justify with examples.
6. Define isoenzymes. Write their importance in diagnosis with examples.
7. Explain clinical significance of following serum enzymes.
(*a*) Transaminases
(*b*) Alkaline phosphatase
8. Explain group specificity with examples.
9. Define enzyme induction and repression. Explain with examples.
10. Explain effect of substrate concentration on enzymatic reaction.
11. Explain phenomenon of cooperativity.
12. Write diagnostic importance of lactatedehydrogenase and creatine phosphokinase.
13. Define metalloenzyme. Give examples.
14. Write on coenzymes of oxidation-reduction reactions.
15. How enzymes are named? Write about E.C. number.
16. What are enzyme profiles? How they are useful in diagnosis? Explain with example.
17. Define allosteric inhibition. Explain with an example.
18. Write on enzymes of myocardial infarction.
19. Explain ELISA technique. Write its application.

MULTIPLE CHOICE QUESTIONS

1. All of the following statements are correct for enzymes. Except
(*a*) Enzymes are proteins
(*b*) Enzymes are catalysts
(*c*) Enzymes speed up chemical reactions by lowering energy of activation.
(*d*) Enzymes alters equilibrium constant of the reaction which they catalyze.
2. The pH optimum of pancreatic proteases is
(*a*) 7.6 (*b*) 8.0
(*c*) 6.0 (*d*) 2.5
3. A competitive inhibitor
(*a*) Binds at active site (*b*) Does not bind at active site
(*c*) Alters Vmax only (*d*) Binds at allosteric site
4. A competitive inhibitor used in hypertension is
(*a*) Malonate (*b*) Allopurinol
(*c*) Captopril (*d*) Oxaloacetate
5. A non-competitive inhibitor that is used as nerve gas in World War II is

(*a*) Iodo acetate (*b*) Cyanide
(*c*) Di-isopropyl fluorophosphate (DFP) (*d*) Arsenite
6. In metalloenzymes metals are
(*a*) Attached to enzyme through coordinate bonds.
(*b*) Covalently attached to enzymes.
(*c*) Non-covalently attached to enzymes.
(*d*) Loosely attached to enzymes.
7. An allosteric enzyme
(*a*) Is usually made-up of many subunits. (*b*) Obeys Michaelis Menten kinetics.
(*c*) Undergo covalent modification. (*d*) Exist in pro-enzyme form.
8. γ-glutamyl transpeptidase level in blood increases in
(*a*) Alcoholic cirrhosis (*b*) Cancer
(*c*) Myocardial infarction (*d*) Pancreatitis

FILL IN THE BLANKS

1. In detergent industry enzymes are used as ----------.
2. Enzymes are for more efficient than ---------- catalysts.
3. The ability of enzymes to recognize optical isomers of a substrate is known as ----------.
4. Km of enzymes is important when they are used as ----------.
5. Affinity of enzyme towards substrate ---------- in competitive inhibition.
6. Heavy metals are known as enzyme ----------.
7. Angiotensin converting enzyme is an example for ---------- enzyme and metal ---------- enzyme.
8. An allosteric enzyme exist in ---------- state ---------- state.

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.

EXERCISES

ESSAY QUESTIONS

1. Give an account of naturally occurring nucleotides.
2. Define nucleoside, nucleotide. Give purine and pyrimidine based example for each. Write functions of nucleotides and nucleosides.

SHORT QUESTIONS

1. Name purine and pyrimidine bases found in nucleic acids.
2. Write structures of purine and pyrimidine bases indicating numbers of carbon and nitrogen atoms.
3. Write composition of nucleoside, nucleotide, dinucleotide and oligonucleotide.

4. Write functions of adenine nucleotides.
5. Write briefly about unusual nucleosides.
6. Name pyrimidine nucleoside analogs. Write their clinical importance.
7. Write cAMP and PAPS structures label components.
8. Write on nucleoside and nucleotide triphosphates.

VBB 204

WATER AND THE MAJOR IONS

Water

The living cell is made up of approximately 70% of water. It is an irregular tetrahedron molecule with oxygen at its center. It is essential for life and solubilizes and modifies the properties of biomolecules. H_2O is its chemical formula.

PROPERTIES

- It is dipolar(It has unequally distributed electrical charge)
- The elements of this compound are joined by weak hydrogen bonds which accounts for its liquidity at room temperature
- It is highly viscous
- It has a high surface tension.
- good solvent: water dissolves more compounds than any other liquid
- high heat capacity
- high melting and boiling point

Sources; water as such by drinking, water in the feed supply and metabolic water obtained from the oxidation of carbohydrates, fat and protein in the body.

FUNCTIONS

1. It's a vital constituent of cells and provides medium for chemical reactions
2. It provides fluidity to blood and other body fluids e.g. saliva, cerebrospinal fluid, gastric juices etc hence serves as a lubricant in the transport of feed to tissues

3. It acts as a medium of heat dissipation in the body.
4. An aid in excretion
5. A buffering agent to regulate pH (acidity or alkalinity) of body fluids 70% of the body is composed of water which is distributed in two major compartments in the body.
6. Water can act as both hydrogen donor and hydrogen acceptor i.e. acid and base, the basis of which it acts as a solvent to compounds which can also accept or donate protons themselves for H-binding with Water.

A number of factors affect the amount of water consumed by an animal including physiologic states, environmental temperature, and type of diet and so on. Dissolved within the body water are solutes composed chiefly of three categories of substances:

1. Organic compounds of large molecular e.g. proteins and these aid in distribution of water between the compartments of the by its effect on osmotic pressure.
2. Organic compounds of small molecular size e.g. glucose, urea etc exert little or no osmotic pressure but when in large quantities aid in retention of water.
3. Inorganic electrolytes or ions, these are found in large quantities within the fluids hence play a vital role in retention and distribution of body water.

MAJOR IONS: H^+, NA^+, K^+, CL^- AND HCO_3^-

Electrolytes are elements or compounds that dissociate in solution for example NaCl , KCl to give the constituent ions Na^+, K^+ and Cl^-, ions that are completely surrounded by water molecules. Positive ions are called cations while negative ions are called anions. These elements are involved in the maintenance of homeostasis (water, osmotic and PH status).the law of electrical neutrality states that the total number of +ve ions always equals the total number of –ve ions.

Hydrogen ion (proton) - H^+

H^+ ions are present in all body compartments and the maintenance of appropriate concentrations is essential for normal cellular functions. It has the largest concentration amongst cations in the plasma. It has negligible osmotic activity

The gradient of H^+ concentration between inner and outer mitochondrial membranes acts as a driving force for oxidative phosphorylation. In addition H^+ concentration in a fluid medium determines the ionization of weak acids and hence their functions within the body. Also H^+

Levels affect the surface charge and physical properties of proteins that make up the body. PH level is a measure of the H^+ concentration, hence H^+ concentration determines

the PH of blood and determines optimum environment for body chemical reactions.
H^+ is excreted in urine as $H_2PO_4^{2-}$.

Sodium ion (Na^+)

It is the major cation of the ECF and helps to regulate the volume of the ECF. Total body sodium is about 4000mEq, 50% is found in bones, 40% in ECF and 10% in soft tissues. Na^+ as well as other cations constitutes osmotically active particles. Sodium pump operates in all cells to keep the levels of Na^+ in the ECF always higher than that in the ICF, its activity is usually accompanied by opposite movement of K^+. Normal plasma concentration of Na^+ is about 136-145mEq/l while in the ICF its 12mEq/l.
NaCl (common salt) is the major source of Na^+ to the body, although it is also widely distributed in food materials mainly of animal sources.
Na^+ is readily absorbed from the intestines by sodium pump located in the Basal and lateral plasma membrane enterocytes and renal cells and Na-pump actively transports Na into the ECF.
FUNCTIONS

1. It maintains the crystalloid osmotic pressure of ECF, helping to retain water in the ECF.
2. It's involved in neuromuscular excitability/irritability.
3. It maintains viscosity by the sodium salt and along with K^+ helps to maintain the degree of hydration of plasma proteins.
4. It plays an active role in resting membrane potential, by keeping the Na conc. Far in the ECF than in the ICF (known as the resting membrane potential) causing a polarization creating a potential difference of up -70 to -90mV across the membranes.
5. In the same vein, the sudden increased permeability of the membrane to Na causing a rapid influx of Na into the cell occurs in the generation of action potential.

99% of Na^+ is filtered along in the glomerular filtrate and reabsorbed majorly in the proximal convoluted tubules. At the distal tubules , rennin (secreted from the juxtaglomerular cells) is produced due to decreased arterial pressure (decreased Na^+) and this stimulates the secretion of Aldosterone that causes reabsorbtion of Na^+, K^+ and to a lesser extent H^+ is lost in its place. Water subsequently moves in the direction of Na^+.

Potassium ion (K^+)

It is the major cation of the ICF and helps to maintain the intracellular osmotic pressure. Total body K^+ is about 3500mEq/l.
This ion is easily obtained from many foods such as fruits and vegetables etc.

K^+ is easily absorbed into the blood.
FUNCTIONS

1. EC K^+ is an important factor in skeletal and cardiac muscle contraction.
2. It is also involved in acid –base balance in the body
3. It is also actively involved in nerve impulse transmission and neuromuscular irritability.
4. Certain enzymes such as pyruvate kinase require K^+ as cofactor.

Plasma levels are about 3.5-5mEq/l while IC levels reach up to 150mEq/l.
It is obligatorily lost during Na^+ reabsorbtion from the tubules. It is also excreted in gastrointestinal tract, saliva, gastric juices, bile, pancreatic and intestinal juices.

Chloride ion (Cl^-)
It is the major anion of the ECF and forms inorganic anion of greatest quantity in the body .it also forms part of the osmotically active particles in plasma.
Cl^- is obtained from NaCl, and many other food substances. It is readily absorbed.
FUNCTIONS

1. It is involved in water distribution
2. Osmotic pressure maintenance and
3. Anion-Cation balance in ECF
4. It is important in the formation of gastric juices and hydrochloric acid.

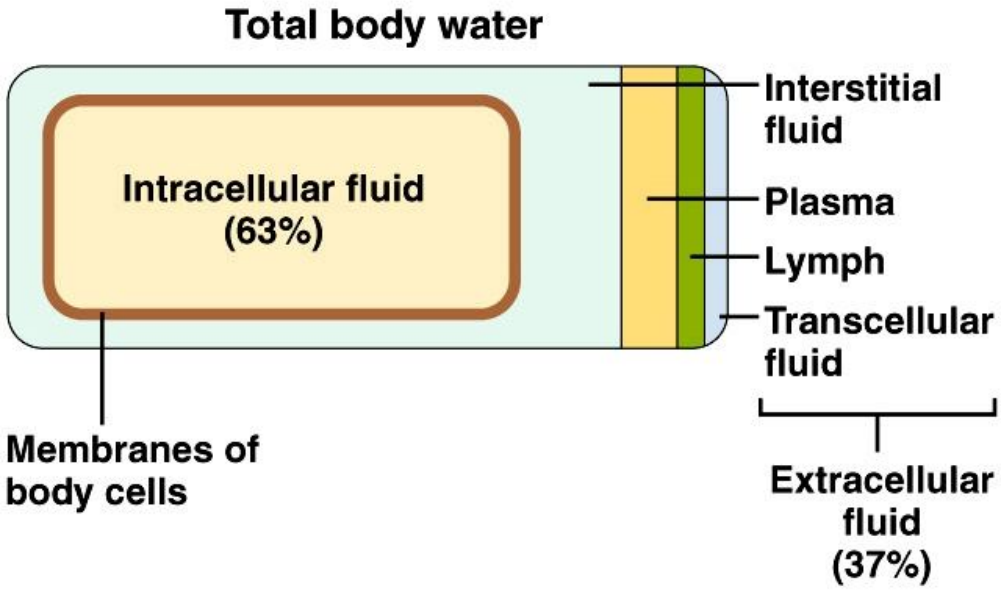
Intake, output and metabolism of Na^+ and Cl^- run in parallel; it is filtered in renal tubules and passively reabsorbed in the proximal tubules. Cl^- is also excreted in sweat.

FLUID AND ELECTROLYTE BALANCE

TOTAL BODY WATER

Total body water (TBW) is divided into two major compartments- the intracellular fluid (ICF) and the extra cellular fluid (ECF).the ICF accounts for approximately half to two third, the volume of body water while the ECF accounts for the rest. The two body fluid compartments differ markedly in solute and electrolyte compositions but are in osmotic equilibrium and water is freely diffusible between them. Movement of fluids is due to hydrostatic pressure and osmotic pressure.

The ECF Na^+ concentration largely determines the volume of the ECF, while that of the ICF is determined by K^+ concentration. Since water is freely permeable through cell membrane is no major osmotic gradient between ECF and ICF.



ECF

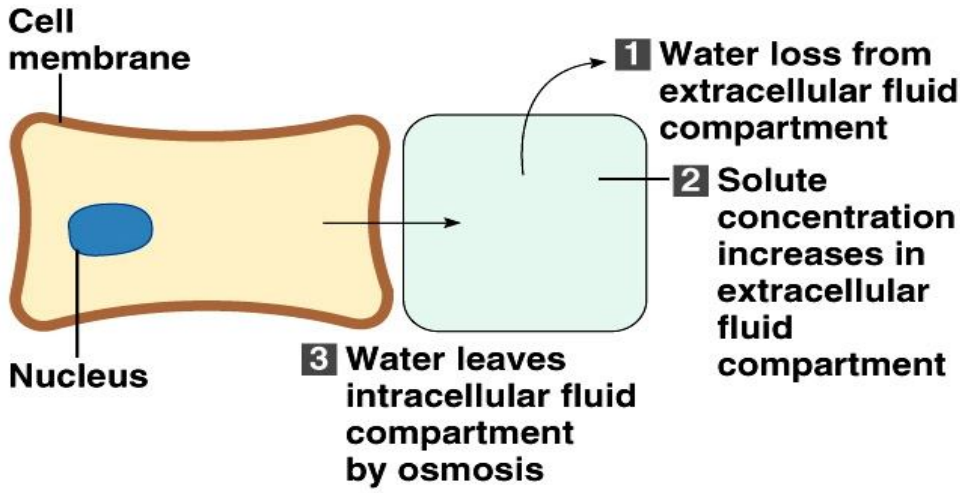
This consists of all the fluids located outside the cell including fluid in plasma (5-8%of TBW), interstitial fluid (25%), lymph, transcellular fluid (fluid content of gastrointestinal, respiratory tracts, intraocular fluids etc; 1-2%). All these fluid content Na^+ as the predominant cation at values ranging between 130-150mEq/l, this determines the ECF volume.

Deficient in Na^+ results in decrease in ECF volume whereas excess results in water retention in the compartment and can results to a condition called Edema (accumulation of fluids within the interstitial spaces). Cl^- and HCO_3^- are the major anions found in this compartment. Fluid generally moves from plasma to interstitial space via hydrostatic pressure and from IF to plasma through the force of colloid osmotic pressure and from the IF to lymph to venous plasma.

Homeostasis is maintained in response to changes that occur in the ECF.

ICF

This is all the fluid contained within the cells in the body. K^+ provides the osmotic skeleton for the ICF just as Na^+ does for the ECF. Changes in tonicity of the ECF are rapidly reflected in the ICF as also changes in tonicity as a result of movement of water out of the cells, thereby changing the ICF volume.

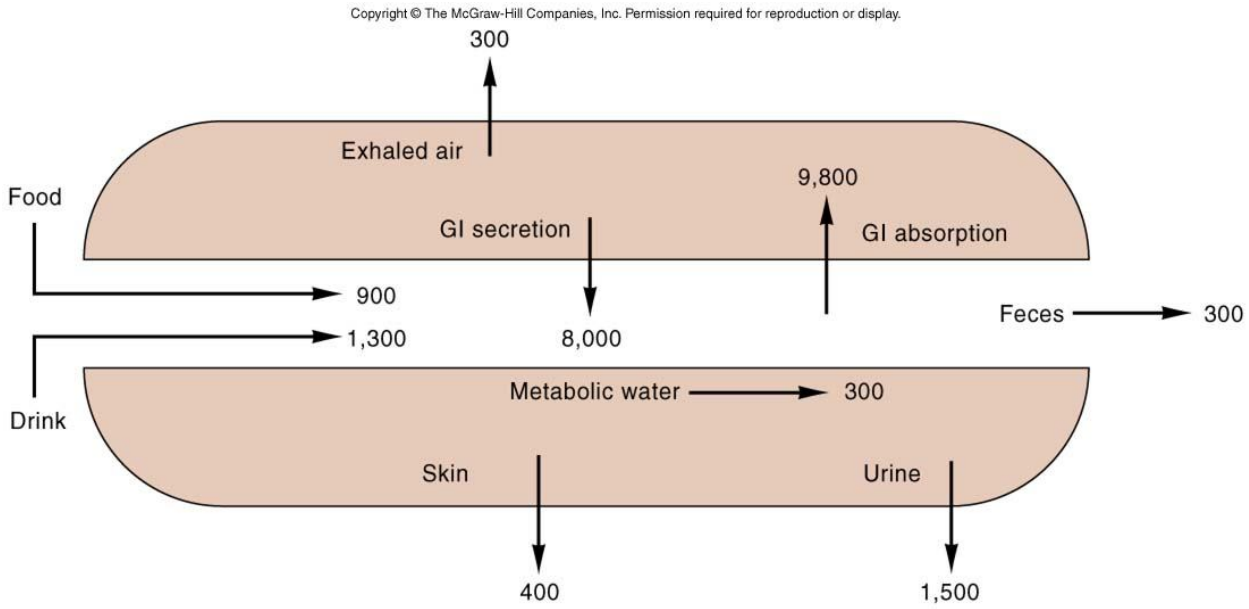


Hence when there is water retention in the ECF, Na^{+} concentration decreases, whereas the ICF volume increases. Other major ions found in this compartment are; Mg^{+2}, $HPO_4^{-2}$and proteins^{-}. Rapid fluid movement occurs between the ECF and ICF in response to changes in concentration of the ECF, and to ensure a balance, fluid or electrolyte gains must be equal to loss.

Fluid intake and output

Intake; fluid as water enters into the body by ingestion through drinking of water, as such, or from water in food. A small amount of water is also gotten from the metabolic breakdown of food products to carbon dioxide and water, via the oxidative phosphorylative pathway.

Output (or loss); is via kidneys (urine), sweat glands (insensible perspiration and sensible loss), & feces. Little amount of water is also lost through exhaled air.



REGULATION OF TOTAL BODY WATER AND ELECTROLYTES.

Generally homeostasis of water and electrolytes is maintained by ion transport, water movement and kidney functions.

1. Thirst can be stimulated to increase water intake.

2. An hormone ADH (anti diuretic hormone) can be secreted by the which causes the reabsorbtion of water at the kidney tubules; depending on the state of the body water content there may be either an increased secretion (to facilitate retention of more water e.g. as in time of dehydration) or decreased secretion (to facilitate loss of more water from the body as in cases of overhydration). The effects of either an increase or decrease in ADH secretion are an increase or decrease in urine output.

3. Renin is also secreted and this causes increased reabsorbtion of Na^+ and concurrently water by converting angiotensinogen to angiotensin I, which is subsequently converted to biologically active angiotensin II which exerts the effects. Renin is released in response to reduced renal perfusion produced by hypotension volume depletion or sympathetic activity.

4. Aldosterone produced in the adrenal cortex in response to changes in effective circulating fluid volume. It causes renal resorption of Na^+ in exchange for K^+ and H^+ in response to Na depletion.

5. Atrial Natriuretic Factor (ANF); this hormone results in natriuresis and duiresis by the kidneys.

Functions of electrolytes

Electrolytes are substances that exist as positive or negative charged particles in solution. To maintain electrical neutrality in biological fluids, there must be equal number of equivalents or milliequivalents of anions and cations in solution, therefore electrolytes in solution combine equivalents for equivalents i.e. positive and negative charges.

The osmotic properties of a solute in solution are related to the number of particles in solution and not on it weight or charge.

Concentrations of solutes in biological fluids are expressed as millimole (mmol/l), milliequivalents (mEq/l) or milliosmoles (mOsm/kg).

Electrolytes function to maintain the osmotic pressure and water balance between the compartment while individual electrolytes provide suitable environment for biological reactions and cell functions.

- Na^+ - major ion in ECF
    - Gain-loss imbalance is most common electrolyte problem
        1. Intake across digestive epithelium based on food content
        2. Loss in urine excretion & skin perspiration
    - Change in Na^+ level causes water movement, maintaining ECF Na^+ conc.

  Ex. Salty meal increases Na^+ level in digestive ECF, causing water input from digestive tract, increasing blood volume & pressure

    - Homeostatic mech: ADH, Aldosterone, Natriuretic peptides

- K^+ - major ion in ICF
    - Imbalance less common but more dangerous
    - Essential for nerve transmission
- Ca^{+2} - in ECF & ICF
    - Absorbed by active transport, increased by PTH & calcitriol
    - Essential in muscle contraction, neurotransmitter release, clotting, bone formation
- Mg^{+2} - mainly in ICF
    - Essential as enzyme cofactor, ATP use in contracting muscle, bone component
- PO_4^{-3} - most important function in ICF
    - Essential for bone mineralization, ATP, phosphorylation
- Cl^- - mainly in ECF, associated with Na

Dehydration and its correction

Dehydration occurs when there is more loss than gain of water or fluids and results in an increase in the osmotic concentration of the ECF relative to the ICF causing water to shift from ICF to the ECF with a resultant more concentration and lower volumes of both the ECF and ICF. It is usually caused by pathological conditions such as vomiting and diarrhea, or low water intake or exercise in hot weather.

The goal in correcting dehydration is not just to replace lost fluid but also lost electrolytes as well in order to increase the volume of both the ECF and ICF.

Overhydration

This occurs when there is more gain than loss of water from the body and results in the decrease in the osmotic concentration of the ECF and the shifting of water from the ECF into the ICF with resultant lower concentration in both compartments but higher volumes. It occurs in such conditions as excess intake of fluids, hypotonic solution infused, unable to eliminate urine, endocrine disorders etc

CALCIUM, PHOSPHORUS AND MAGNESIUM

CALCIUM

A total of approximately 1-1.5kg of body weight is made up of calcium where 99% is found in the bones and teeth and remaining 1% is in the ECF and other compartments. Ca^{2+} exists as carbonates or phosphates of calcium within the body, while in the plasma they exist in either the ionized form(which is the physiologically active form), bound to plasma proteins(mainly albumin) or complexes with organic acids, all these forms being in equilibrium with each other.

SOURCES; milk and other dairy products (cheese etc), egg yolk, bone meal, cabbage, nuts, figs etc

ABSORBTION; dietary Ca^{2+} is absorbed mainly from the duodenum and first half of the jejunum under the influence of a carrier protein CALBINDIN – a calcium dependent ATPase against an electrical and concentration gradient. Absorption is affected by certain factors including;

1. Vitamin D- presence of vitamin D in the gut promotes calcium absorption.

2. PH-acidity increases the absorption of calcium salts which are more soluble in these condition, while in alkaline medium decreases absorption by causes the formation of insoluble salts of calcium.

3. Composition of diet-a high protein diet (amino acids particularly Lysine and Arginnine) and organic acids e.g. citric acid increases the solubility of Ca^{2+} salts, fatty acids on the other hand cause formation of insoluble Ca^{2+} salts thereby decreasing Ca^{2+} absorption.

4. Parathyroid hormone through its stimulation of 1, α-hydroxylase which increases the production of Calcitriol (1, 25-$(OH)_2$-D_3), the active form of vitamin D, increases calcium absorption.

5. Calcitonin decreases the absorption of Ca^{2+}

6. Glucocorticoids diminish intestinal transport of Ca^{2+} hence its absorption.

7. Phytic and oxalic acids- presence of phytates and oxalates especially in cereals (phytates) and vegetables (oxalates) cause the formation of insoluble calcium salts which are excreted in feces and decrease Ca^{2+} absorption.

8. Presence of other minerals such as phosphates, phosphorus, iron and magnesium decrease Ca^{2+} absorption.

FUNCTIONS

1. Ca^{2+} is involved in calcification or mineralization of bones and teeth.

2. Also involved in coagulation of blood as factor IV causing the chelating of prothrombin to form thrombin in the clotting cascade.

3. Plays a role in neuromuscular transmission of impulses particularly at the pre and post synaptic junctions.

4. It is actively involved in muscle contraction and relaxation that produces body movement.

5. It in addition regulates microfilament mediated processes such as degranulation,cell motility etc

6. It activates regulatory kinases with or sometimes without binding to the regulatory protein Calmodulin.

7. It is needed for the excitability of nerves.

8. It plays a role in permeability of gap junctions

9. It acts as a secondary and tertiary messenger in signal transduction and hormone action
10. It mediates secretion of hormones
11. It's involved in systolic myocardial contraction and general excitability of heart.
12. Affects (decreases) vascular permeability hence reduces allergic exudates.

REGULATION AND EXCRETION

Levels of calcium in the body and particularly in the blood are strictly regulated by vitamin D- Calcitriol,and hormones such as Parathyroid hormone and Calcitonin. Excess Ca^{2+} is normally excreted in urine with little amounts also excreted in stool.

Calcitriol increases blood levels of calcium by increasing its absorption from the intestine while parathyroid hormone which is secreted by the parathyroid gland acts at three principal sites- the bones, kidneys and intestines also to increase blood levels of Ca^{2+} the hormone causes demineralization of bone leading to the release Ca^{2+} into blood, increased absorption of Ca^{2+} from the intestines and reabsorptionof the filtered ions from the glomerular filtrate at the kidneys.

Calcitonin is a peptide hormone secreted by the thyroid gland; it decreases blood Ca^{2+} concentration by inhibiting the resorbtion of bone having an opposite effect to parathyroid hormone together with which it causes remodeling of bone to achieve proper bone growth and development. Secretion and activities of these two hormones that regulate calcium blood conc. is under feedback regulation depending on the levels of blood Ca^{2+} and this influences quantity excreted.

Other factors that may influence blood Ca^{2+} conc. includes levels of Phosphorus ion which decreases Ca^{2+}(Ca and P ions have almost completely reciprocal relationships with respect to regulation and excretion from the body),pregnancy which places greater demands on blood Ca hence reduce total amount in blood, presence and absence of serum proteins, Ph of the blood with alkalosis decreasing Ca blood level through its facilitation of complexing of Ca with organic compounds in the blood.

PHOPHORUS

P levels in the body represent about 1kg of total body eight with 80% of this quantity found in the bones and teeth and about 10% in the muscles is found mainly intracellular, it occurs in either the organic (nucleic acids, phospholipids etc) or inorganic form.

SOURCES;milk,cereals, nuts, meat etc

Absorption; asorption is mainly from the jejunum and is influenced by Calcitriol which increases it. P in blood is mainly protein bound the skeleton is the major reservoir of P.

FUNCTIONS.

1. It is involved in the formation of bones and teeth.
2. It is an energy source as high energy phosphate bonds in ATP and other high energy compounds (CTP,GTP and CP) that maintain muscle contractility, neurological functions, electrolyte transport etc
3. It is a constituent of cyclic adenine and guanine nucleotides,cGMP,cAMP .
4. Composition of nucleoside coenzymes e.g. NAD,NADP
5. Involved in DNA and RNA synthesis
6. Forms physiologically important phosphate esters such as Phospholipids, Phosphoproteins, Glucose-6-phophate,Nucleic acids etc.
7. It also helps to maintain the critical intracellular concentration and provides substrate for bone mineralization.
8. It is also the source of the phosphate buffer system of the blood.
9. It helps in the activation of some enzymes by phosphorylation and is involved in the activities of several enzyme systems e.g. adenylate cyclase and 1, α-25-hydroxy vitamin D-hydroxylase.

REGULATION

Serum levels of P depends on levels from diet and on its excretion and reaborbtion from the kidney tubules which is under the influence of parathyroid hormone and calcitonin.

MAGNESIUM

This is the fourth most abundant cation in the body of animals and is second to potassium inside the cell. 60% of the body Mg is located in bones, 20% in skeletal muscles, 19% in other cells and 1% in ECF. It is an alkaline earth metal distinct from other transition elements in that it interacts with other chemical species with a stronger electrostatic bonding component and prefers oxygen to N atoms.

SOURCES; vegetables, cereals, nuts, beans, Bone Meal, dairy products etc.

20-30% of ingested Mg is absorbed from the small intestine, and this is influenced by malabsorbtion syndromes and other factors that affect passage of food. Other minerals such as Ca and Phosphates also decrease Mg absorption, while presence of proteins, lactose and vitamin D increases Mg absorption.

FUNCTIONS

1. It chelates important intracellular anionic ligands especially ATP. (Convert adenosine triphosphate (ATP) to adenosine pyrophosphoric acid (ADP), with the subsequent release of energy.)

2. It catalyses and activates more than 300 enzymes-being an essential cofactor for enzymes concerned with respiration,glycolysis and transmembrane transport of other cations e.g. Na and Ca. Mg affects enzyme activity by binding to the active sites of enzymes, ligand binding or induction of conformational changes during catalytic process as well as promotion of aggregation of multiple enzyme complexes

3. It helps to maintain low resting concentration of intracellular calcium by competing with Ca for binding sites on proteins (troponin molecule found at regular intervals along actin filaments) and membranes hence sequestering Ca into the sarcoplasmic reticulum. Magnesium acts to relax muscles after calcium stimulates contraction

4. It helps maintain normal muscle and nerve function.

5. Mg is known to play a crucial role in the maintenance of cell integrity such that deficiencies of Mg lead to development of cancer. Glutathione requires magnesium for its synthesis. Low magnesium is associated with dramatic increases in free radical generation without the cleaning and chelating work of glutathione (magnesium), cells begin to decay as cellular filth and heavy metals accumulate.

6. Magnesium has an effect on a variety of cell membranes through a process involving calcium channels and ion transport mechanisms. Magnesium is responsible for the maintenance of the trans-membrane gradients of sodium and potassium.

   The major excretory pathway for Mg is through the kidneys, but60-80% orally taken Mg is lost through feces while up to 0.75mEq/l is lost through sweat.

   Deficiency of Mg manifests as impairment of neuromuscular functions such as hyperirritability, tetany, convulsions and electrocardiographic changes. In cattle an endemic disease called grass staggers or grass tetany characterized by restlessness and convulsions followed by death frequently occurs.\

TRACE ELEMENTS

Trace elements occur in the human and animal body in milligrams per kilograms amount or less as against major elements which occur in gram per kg. Essential elements are elements required for life, whose deficient intake results in impairment of vital functions and only intake of physiologic amounts of the element can alleviate or prevent such a disturbance in function. Certain T.Es exists in the body whose exact role is not known such includes Arsenic, Mercury, and Cyanide etc

GENERAL CHARACTERISTICS OF TRACE ELEMENTS

1. Amplification; a very small amount of the element is necessary for optimal performance in the whole organism, hence a lack of such elements even in small quantities can result in disturbances. Trace elements are constituents of or interact with enzymes or hormones and regulate the metabolism of large biochemical substrates.
2. Specificity; they are specific in their functions and are most times not replaceable by even similar compounds.
3. Homeostasis; There exists mechanisms that regulate to achieve optimal body distribution of these T.Es including their absorbtion,storage and excretion e.g. the rate of absorption of T.Es generally decreases with its increasing concentration in the intestinal lumen or associated tissues. Active transport mechanisms have been suggested for Fe, Zn, and Cu. Excretions of T.Es is mainly through feces.
4. There are interactions between two or more T.Es, such as an overabundance of one element interfering with the metabolic activities of another one present in normal or marginal concentrations.

SULPHUR

Sulfur represents about 0.25 percent of our total body weight, similar to potassium. The body contains approximately 140 grams of sulfur-mainly in the proteins, although it is distributed in small amounts in all cells and tissues. Approximately half of the total of body sulphur is found in the Muscles, skin and bones, as well as concentrated amounts in hair and nails. A multivalent non-metal, it is an essential element for life as it is a building block of proteins, enzymes and vitamins. Sulfur is present in four amino acids: methionine (an essential amino acid) and in the non-essential cystine and cysteine, which can be made from methionine and taurine (used in production of bile acid for digestion).

SOURCES; “organic” sulphur is found in foods such as meat, fish, poultry, grains, legumes and vegetables such as Brussels sprouts, broccoli, onions and garlic.

FUNCTIONS

1. It plays an important function in the formation of amino acids. It is a component of keratin, the main protein of hair and nails. Sulfur is also present in the fur and feathers of animals. It also makes up collagen and elastin, the main proteins found in skin and connective tissue. And is involved in repairing damaged skin and maintaining healthy detoxification of the skin.
1. Organic sulphur can add flexibility to cell walls and allows easier passage of fluids. This may aid in eliminating pain, softening tissues and help movement.
2. Sulphur is also a component of the B vitamin Biotin and therefore contributes to fat metabolism. Sulphur is essential for insulin and thiamine production and therefore plays a role in carbohydrate metabolism.
3. Sulphur is a major component of joint tissue where it functions in the formation of cartilage, tendons and ligaments
4. Plays a role in detoxification of heavy metals in conjunction with the transport of oxygen across the cell membrane.
5. It is important in cellular respiration, as it is needed in the oxidation-reduction reactions that help the cells utilize oxygen, which aids brain function and all cellular activities.

ABSORPTION

It is generally believed that the sulphate ion is poorly absorbed. Sulfur is absorbed from the small intestine primarily as the four sulfur-containing amino acids or from sulfates in water or fruits and vegetables. Sulfur is stored in all body cells, especially the skin, hair, and nails. Excess amounts are eliminated through the urine or in the feces.

IODINE

The body contains about 25 mg of iodine. A small percentage of this is in the muscles, 20% is in the thyroid, and the rest is in the skin and bones.

Iodine is well absorbed from the stomach into the blood. About 30 percent goes to the thyroid gland, depending on the need. Iodine is eliminated rapidly. Most of the remaining 70 percent is filtered by the kidneys into the urine.

Iodine's main role in animal body is as constituents of the thyroidhormones, *thyroxine* (T4) and *triiodothyronine* (T3). These are made from addition condensation products of the amino acid tyrosine, and are stored prior to release in an iodine-containing protein called thyroglobulin. T4 and T3 contain four and three atoms of iodine per molecule, respectively. The thyroid gland actively absorbs iodide from the blood to make and release these hormones into the blood,

actions which are regulated by a second hormone TSH from the pituitary. Thyroid hormones are phylogenetically very old molecules which are synthesized by most multicellularorganisms, and which even have some effect on unicellular organisms. The thyroid hormones, thyroxine and triiodothyronine, are also needed for normal growth and development, protein synthesis, and energy metabolism

Sources; Iodized salt -- table salt with iodine added -- is the main food source of iodine. Seafood is naturally rich in iodine. Cod, sea bass, haddock, and perch are good sources. Kelp is the most common vegetable seafood that is a rich source of iodine. Dairy products also contain iodine. Other good sources are plants grown in iodine-rich soil.

MANGENESE

Mn is usually bound to proteins in the body either in the =2 or +3 valency states, it is associated with formation of connective tissue and bony tissue, growth reproduction as well as lipid and carbohydrate metabolism.

Sources; include cereals, vegetables, fruits nuts, liver, kidney etc

FUNCTIONS

1. It functions as a constituent of metalloenzyme and as an enzyme activator
2. It binds directly to substrates e.g. ATP or protein causing conformational changes that lead to enzyme activation. Some of the enzymes activated are non specific hence this can mask Mn defieciency, however Mn activation of glycosyltransferase, phosphoenol pyruvate carboxykinase and glutamine synthetase are specific.
3. Arginase, pyruvate carboxylase and Mn superoxide dismutase are examples of enzymes composed of Mn, while hydrolases, kinases and decarboxylases are examples of enzymes that can also be activated by Mn.

Mn within the cells are concentrated in the mitochondria and the body stores are located within the skeleton, absorption of Mn is poor especially in increased intake of the same. Ca and P also decrease its absorption. Absorbed Mn is bound to plasma proteins in blood and then transported to the liver from where its excess are removed via the kidney, as well as in bile and pancreatic secretions.

COPPER

Copper is distributed widely in the body and occurs in liver, muscle and bone. Cu is an important trace element associated with some metalloproteins. It is present both in the +1 and +2 valency states within the body and is involved mainly in

oxidation-reduction reactions. It is an important constituent of many compounds and enzymes including Ceruloplasmin, Cytochrome C Oxidase, Super oxide Dismutase, Tyrosinase (necessary for pigmentation of skin via the production of melanin) etc. most of these copper containing enzymes bind to and react directly with molecular oxygen.
It in addition plays a significant role in iron metabolism- Cu deficiency impairs Fe absorption and is accompanied by anemia.
Sources; meat, lequmes, nuts and cereals etc.
FUNCTIONS

1. In addition to its enzymatic roles, copper is used for biological electron transport. Blue copper proteins participate in electron transport and include Azurin and Plastocyanin.

Duodenum is the site of maximum absorption of Cu; it may also be absorbed in the stomach. Within the intestinal mucosa Cu reacts with metallothionein (a sulphurhydyl group rich protein that binds Cu) which can be competed with by other metal ions. The amount of ingested Cu that is eventually absorbed depends on sex (females have been shown to absorb more), chemical form of the ingested compound, other dietary constituents like trace elements, and the amount ingested.

Cu is stored in the liver after being transported as Cu- albumin or Cu –histidine complexes from blood as metallothionein like Cuproproteins, ceruloplasmin, from where it is transported to other cells to be used in Cu containing enzymes. It is excreted in feces after being secreted from bile into the intestine.

Deficiency of Cu results in weight loss, bone disorders, anemia and myocardial atrophy.

ZINC

Zn is the second most abundant trace element in the body with about 1.4-2.3g of it occurring in a matured animal body.

Prostrate, semen, liver, retina, bone and muscle tissues are rich in Zn. It is found in the +2 valency state and is an essential component of many metalloenzyme involved in all aspects of metabolism.

Sources; meat, fish, dairy products are good sources of available Zn.

FUNCTIONS

1.) It is an integral part of nearly 300 enzymes, contributing to their structural stability, in different species of life e.g. superoxide dismutase, carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerases, thymidine kinase, caboxypeptidase, alcohol dehydrogenase etc
2.) It also plays a role in protein synthesis and is involved in gene expression e.g. Zn finger proteins.
3.) It stabilizes the structures of proteins and nucleic acids
4.) Zn has been shown to be an important element in wound healing as it is necessary factor in the biosynthesis and integrity of connective tissue
5.) It is involved in normal fetal development and influences pregnancy outcome
6.) It is also involved in insulin secretion
7.) Biosynthesis of mononucleotides
8.) Vitamin A metabolism (stimulate the release of vitamin A into the blood).

20-30% of ingested Zn is absorbed mostly in the duodenum and early p

Art of jejunum by active energy dependent transport. This absorption is varied and depends on certain factors such as presence of dietary Ca, P, Fe, and Cu (which decrease Zn absorption); however diets rich in protein have the opposite effect.

Zn is transported in the blood bound to albumin (mainly) α-macroglobulin, transferin and free amino acids. It is mainly excreted in feces smaller amounts are excreted in urine and sweat.

MOLYBDENUM

It is a component of about 3 metalloenzymes in animals and man including Xanthine oxidase, Aldehyde oxidase and Sulfite oxidase.

Sources; cereals and dry legumes

FUNCTIONS

1.) Xanthine oxidase is involved in degradation of purines to uric acid.
2.) Aldehyde oxidase is involved in oxidation of aldehydes
3.) Sulfite oxidase is involved in final oxidation of sulphur containing amino acids.

Presence of Mo helps in the utilization of Cu while excessive amounts may result in Cu deficiency.

It is absorbed mainly in the stomach and small intestine, and stored in the liver small amounts are retained in the kidney and a skeleton.

Excess are excreted from kidneys and some from bile.

CHROMIUM

Cr is a transitional element that occurs mainly in the +3 and +6 valency state in biological systems. It is widely distributed throughout the body.

Sources; meat, whole grain products and yeast.

FUNCTIONS

It helps in the control of glucose, protein and lipid metabolism i.e. it is a potentiator of insulin action.

It is absorbed poorly from the upper small intestine and is bound to β-globulin fraction of serum proteins (transferin). It is excreted via the kidneys and it is found mainly in the liver-mitochondria, microsomes and cytosol.

Deficiency results in impaired glucose tolerance secondary to parenteral nutrition.

SELENIUM

It is a constituent of glutathione peroxidase and iodothyronine deiodinases and thioredoxin reductase (selenoproteins). It is present in tissues as selenocysteine and selenomethionine which serves as a store for Se which is released in cases of dietary insufficiencies.

The biologically active form of Se is selenocysteine and it is present in the selenoproteins.

Sources; plant (grown on Se rich soils) and animal tissues.

FUNCTIONS

It helps to defend the body against oxidative stress and is also involved in the synthesis and metabolism of thyroid hormones.

GSH (reduced glutathione) catalyses the breakdown of H_2O_2 , phospholipid hydroperoxides etc, it is present in RBC. Thioredoxin reductase is thought to have an immunological function and plays a role in reproduction.

Se is well absorbed in the GIT and may not be regulated homeostasis of Se is achieved by regulation of its excretion via the urine, however high intake of Se leads go exhalation of volatile forms.

COBALT

Co is an integral part of the B vitamin B12- cyanocobolamine which is required for the maturation of RBCs.

Sources; animal tissues.

FUNCTIONS

As a cofactor for some enzymes e.g. glcyl-glycine dipeptidase, invoved in bone marrow development for RBC maturation and in the formation of cobamide enzyme (adenosyl co-enzyme).

FLOURINE

It is obtained mainly from drinking water other sources include tea, salmon, sardine and mackerel. It is present in calcified tissues like bones and teeth.

It is absorbed from intestine and excreted mainly in the urine.

FUNCTIONS

It helps in tooth development-i.e. normal maintenance and hardening of enamel and prevention of dental caries. It also helps in the normal bone development as catalytic mounts of F is required for the conversion of phosphates and Ca hence helps prevent osteoporosis (softening of the bones).

Toxic levels of F in the body result in fluorosis characterized by mitochondrial damage, certain enzyme inhibition and negative effects on protein, steroid and collagen synthesis.

NICKEL

This occurs in trace amounts in humans and animal tissues. Dietary intake is poorly absorbed and it is excreted mainly in feces.

Ni plays an important role in some enzyme activities e.g. Arginase, carboxylase, trypsin and acetyl CoA synthetase.

It is also required for growth and reproduction.

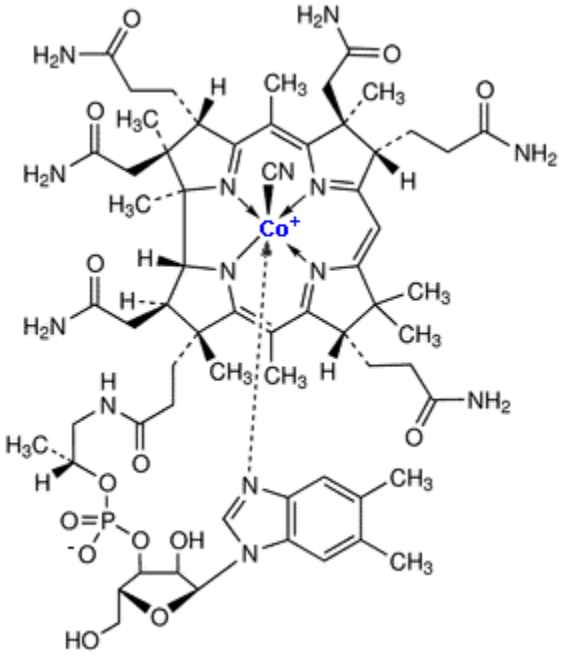
VITAMINS

WATER SOLUBLE VITAMINS: B AND C, BIOMEDICAL IMPORTANCE IN MAN AND ANIMALS.

VITAMIN B12;- CYANOCOBALAMINE

It is a hemopoeitic vitamin required for maturation o RBCs, and also involved in nucleic acid metabolism, methyl transfer, and myelin synthesis and repair. It serves as a carrier of one carbon group in metabolic reactions. The compound is composed of physiologically active substances classified as cobalamines or corronoids which is made up of tetrapyrole rings surrounding a central cobalt atom and nucleotide side chains. It has a molecular weight of about 1355.

Sources include clams, oysters, turkey, chicken, beef, and pork. Absorption is under the influence of an intrinsic factor, which takes place in the terminal ileum, it is then released from the factor and transported into blood. it is stored in liver and released to meet plasma needs. Excess vit. B12 is excreted by the kidneys.



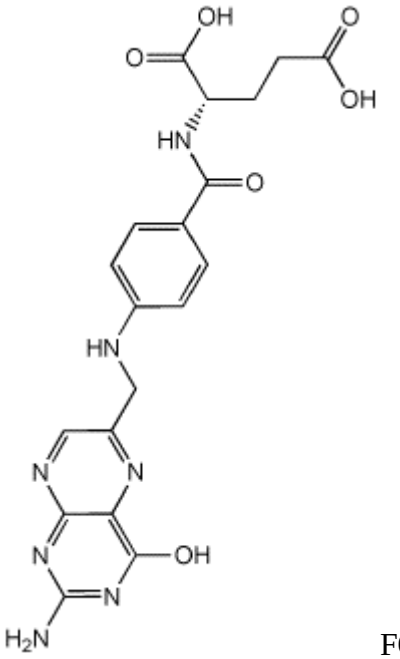
CYANOCOBALAMINE (VITAMIN B12)

FOLIC ACID

This consists of one molecule of P-aminobenzoic acid and glutamate to which a base, pteridine, has been attached sometimes (rarely) referred to as vitamin B-9, it is also is involved in maturation of red blood cells and the synthesis of purines and pyrimidines which are required for development of the fetal nervous system.

Sources include dried peas, dried beans, yeast, and leafy green vegetables such as spinach, endive, lettuce, and mustard greens.

It is absorbed in the duodenum and upper jejunum.



FOLIC ACID

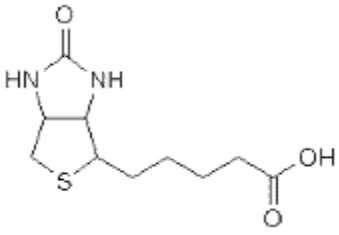
BIOTIN

This is an imidazole derivative, chemical name – Cis-tetrahydro-2-oxothienol [3,4-d]-imidazole-4- valeic acid.

Liver, egg yolks, green vegetables, and whole grains are rich sources of biotin. It acts as a coenzyme for carboxylation reactions essential to fat and carbohydrate metabolism e.g. pyruvate carboxylase, acetyl co A carboxylase, serving as a carrier on the enzyme.

Avidin found in egg white binds biotin and makes it unavailable for absorbtion into the body. Biocytin a form of biotin is readily absorbed and in the plasma is hydrolyzed to biotin and taken

up by tissues for use attached to an apoenzyme. By products of biotin in form of biotin sulfoxides and bisnorbiotin (trace amounts) is excreted along with free vitamins.



BIOTIN

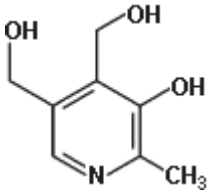
VITAMIN B6 (PYRIDOXINE)

Derivatives of pyridines and their phosphates including pyridoxine, pyridoxal and pyridoxamine.

Sources include brewer's yeast, liver, mackerel, avocado, bananas, meat, vegetables and eggs. Vitamin B6 is readily absorbed by intestinal mucosa cells that contain cytoplasmic pyridoxal kinase that catalyse the phosphorylation of the vitamin form, which is then absorbed to other cells by diffusion (the active form of the vit is the phosphate form especially pyridoxamine-5-phosphate).

B6 serves as a coenzyme in the catalysis of transamination, decarboxylation and threonine aldolase reactions. Vitamin B6 is important in the biosynthesis of heme and nucleic acid, as well as in lipid, carbohydrate, and amino acid metabolism. As a coenzyme in the breakdown of glycogen-phophorylase and in condensation of L-serine with palmitoyl Co A to form sphingomyelins.

The main by product of vit B6 metabolism is excreted in urine as 4-pyrodoxic acid formed by oxidation of the aldehyde and aldehyde dehydrogenation.

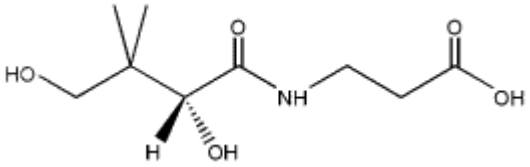


PYRIDOXINE(VITAMIN B6)

PANTOTHENIC ACID

This is a combination of pantoic acid and β-alanine. Also called vit B5. It is richly distributed in foods- whole grain cereals, legumes, eggs, and meat. It is critical in the metabolism and synthesis of carbohydrates, proteins, and fats.

It is readily absorbed in the intestine and goes into the cell for the formation of coenzymes. It is absorbed as pantetheine and pantothenate into circulation and it is within the cells that coenzyme forms are synthesized. The β-mecarptoethylamine derivative is excreted in urine and smaller fractions excreted in milk and colostrums.



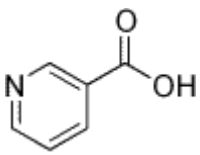
PANTOTHENIC ACID

NIACIN

This comprises nicotinic acid and nicotinamide. It can be synthesized from tryptophan in man but cats lack the metabolizing enzymes. Derivatives include nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are coenzymes in oxidation-reduction reactions vital in cell metabolism.

Sources include most plants and animal products, mushrooms and fish are good sources of niacin.

Both the acid and amide forms are readily absorbed in the GIT into circulation from where it diffuses into the cerebrospinal fluid. They are converted to the coenzyme forms in the liver, kidney blood and brain cells. Metabolites of this vitamin are excreted in urine.



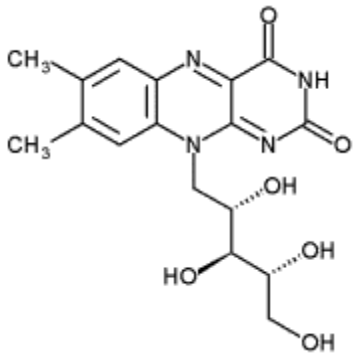
NIACIN

RIBOFLAVIN

Riboflavin – 7,8 dimethyl[1'-D-ribityl] isoalaxazine; a heterocyclic isoalloxazine ring attached to a sugar alcohol, ribitol. The coenzyme forms are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). It is involved in carbohydrate metabolism as an essential coenzyme in many oxidation-reduction reactions.

Sources are yeast, liver, kidney, heart and vegetables. It is synthesized in plants and microorganisms. Coenzyme forms of the vitamin release riboflavin during digestion in the intestine upon acidification in the stomach, it is absorbed in the proximal small intestine aided by bile salts and hen absorbed into cells the flavins are converted to the coenzymes.

Excess riboflavin is excreted in urine and to a lesser extent in feces.



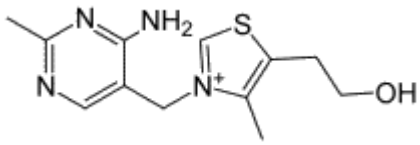
RIBOFLAVIN

THIAMIN(B1)

Thiamin is 3-[4-amino-2-methyl-pyrimidyl-5- methyl]-4-methyl-5-[β-hydroxyethyl] thiazole. The active coenzyme form is thiamin pyrophosphate (TPP) or the diphosphate. It is widely available in the diet. Small amounts are present in animal and plant tissues but are more abundant in unrefined cereals grains, liver ,heart, kidney and pork.

Thiamin is involved in carbohydrate, fat, amino acid, glucose, and alcohol metabolism, coenzyme in transketolase reactions etc.

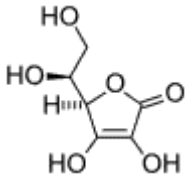
It is readily absorbed by the small intestine by active transport process and phosphorylated to TPP in the jejuna mucosa, then to portal blood. The diphosphate or triphosphate forms maybe stored in minute quantities in skeletal muscles, liver, heart and nervous tissue. Excess and metabolites of thiamin are excreted in urine.



THIAMIN (VIT.B1)

VITAMIN C

Vitamin C (ascorbic acid) plays a role in collagen, carnation, hormone, and amino acid formation. It is essential for wound healing and facilitates recovery from burns. Vitamin C is also an antioxidant, supports immune function, and facilitates the absorption of iron. Higher amounts can cause stomach upset and diarrhea. Vitamin C is found in fresh fruits and vegetables. Citrus fruits like oranges and lemons are good sources of vitamin C.



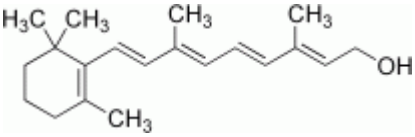
VITAMIN C

FAT SOLUBLE VITAMINS A,D,E,K THEIR BIOCHEMICAL FUNCTIONS IN MAN AND ANIMALS

VITAMIN A

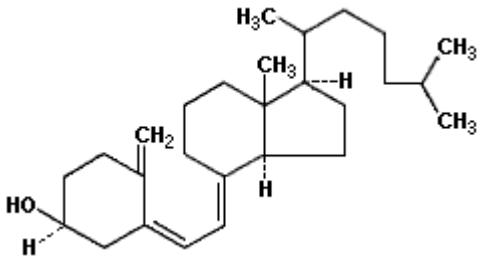
Vitamin A (retinol) is required for the formation of rhodopsin, a photoreceptor pigment in the retina. Vitamin A helps maintain epithelial tissues. Normally, the liver stores 90% of the body's Vitamin A. To use Vitamin A, the body releases it into the circulation bound to prealbumin (transthyretin) and retinol-binding protein. β-carotene and other provitamin carotenoids, contained in green leafy and yellow vegetables and deep- or bright-colored fruits, are converted

to Vitamin A. Carotenoids are absorbed better from vegetables when they are cooked or homogenized and served with some fats or oils. Deficiency impairs immunity and causes skin rashes and typical ocular effects such as dry eyes and night blindness.



VITAMIN D

Vitamin D has two main forms: D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D3 is synthesized in skin by exposure to sunlight (ultraviolet radiation) and obtained in the diet chiefly in fish liver oils and egg yolks. Vitamin D is a prohormone with several active metabolites that act as hormones. Vitamin D3 is metabolized by the liver to 25(OH)D, which is then converted by the kidneys to 1,25(OH)2D (1,25-dihydroxycholecalciferol, calcitriol, or active vitamin D hormone). 25(OH)D, the major circulating form, has some metabolic activity, but 1,25(OH)2D is the most metabolically active. Inadequate exposure to sunlight may cause vitamin D deficiency. Deficiency impairs bone mineralization and may contribute to osteoporosis.

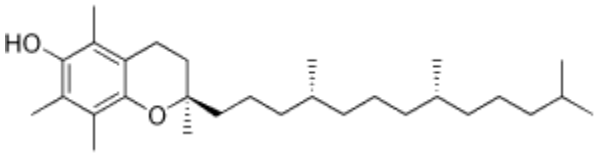


CHOLECALCIFEROL
(VITAMIN D_3)

VITAMIN E

Vitamin E is a group of compounds (including tocopherols and tocotrienols) that have similar biologic activities. The most biologically active is α-tocopherol, but β-, γ-, and δ-tocopherols also have important biologic activity. These compounds act as antioxidants, which prevent lipid peroxidation of polyunsaturated fatty acids in cellular membranes. Plasma tocopherol levels vary with the total plasma lipid levels. Vitamin E deficiency causes degeneration of the axons of neurons (nerve cells) resulting in neurologic deficits, and fragility of red blood cells which is generally diagnosed as hemolytic anemia. Vitamin E is found in spinach, watercress, mustard

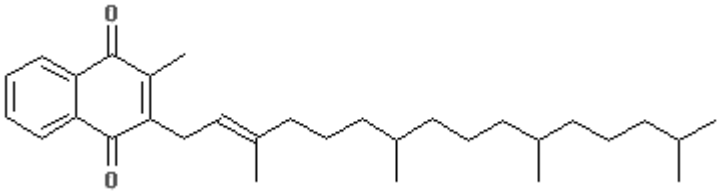
greens, and many green leafy vegetables. Good sources of Vitamin E are oily plant seeds such as peanuts and sunflower kernels.



ALPHA-TOCOPHEROL (VITAMIN E)

VITAMIN K

Vitamin K1 (phylloquinone) is dietary vitamin K. Dietary fat enhances its absorption. Vitamin K2 refers to a group of compounds (menaquinones) synthesized by bacteria in the intestinal tract; the amount synthesized does not satisfy the vitamin K requirement. Vitamin K controls the formation of coagulation factors II (prothrombin), VII, IX, and X in the liver. Vitamin K is widely distributed in green vegetables such as kale, spinach, and mustard greens. The bacteria of the normal gut also synthesize menaquinones.



VITAMIN K_1 (PHYLLOQUINONE)

DETOXIFICATION OF XENOBIOTICS

A variety of toxic substances or potentially toxic substances may enter human body. They are food additives, poisons, toxins, certain drugs, chemicals, environmental pollutants, pesticides and other foreign substances. They are called as Xenobiotics (Xenos (Greek) - Strange). When they are ingested either accidently or some other way they may be absorbed from the gastrointestinal tract and gain access to the organs and tissues of the body. In the body

xenobiotics undergo changes. These changes reduce the toxicity of xenobiotics. The conversion of highly toxic xenobiotics to less toxic substances is called detoxification or detoxication or biotransformation.

MEDICAL IMPORTANCE

1. Detoxification protects body and its organs from deleterious effects of toxins.
2. Detoxification removes most of drugs consumed from the body. Because of this drugs must be taken frequently during recovery from illness or disease.
3. Occasionally detoxification may generate toxic substance from relatively non-toxic substance.
4. Many anticancer agents work by inducing enzymes of detoxification.
5. Polymorphisms of enzymes of detoxification is associated susceptibility to diseases like myocardial infarction, cancer, inflammatory disease, alcoholic cirrhosis etc.

Generally detoxification converts less soluble toxic substance to more polar water soluble and hence the compound is easily excreted in urine. Some detoxified compounds may be excreted in feces through the bile. Liver is the organ involved in detoxification reactions. Detoxification of xenobiotics occur mainly in two stages (phases). In the first phase (stage) xenobiotics undergo three types of chemical reactions. They are oxidation, reduction (hydroxylation) and hydrolysis. The second phase involves conjugation of xenobiotics with variety of substances. Occasionally the detoxified products are sometimes more toxic than the original substance. Biotoxification is the word used to indicate such process.

I(*a*) Oxidation. Indole and Skatole are produced from tryptophan by the action of microbes. They are responsible for the disagreeble odour of the feces. They undergo oxidation.

Skatole → Skatoxyl, indole → Indoxyl

Benzene → Phenol, Benzaldehyde → Benzoic acid

Chloral → Trichloro acetic acid, Toluene → Benzoic acid

Ethylalcohol may be oxidized completely to CO2 and water. Similarly methanol may be oxidized to formaldehyde and formate.

Methanol → Formaldehyde → Formate

(*b*) **Reduction.** It is less common and less important than oxidation.

Picric acid → Picramic acid

Chloral hydrate (Sedative) → Trichloro ethyl alcohol

(*c*) **Hydroxylation.** Detoxification of number of drugs and steroids occur by hydroxylation. These reactions are catalyzed by cytochrome P450 dependent monooxygenases.

Phenobarbitol → Hydroxy phenobarbitol

Meprobamate (Tranquilizer) → Hydroxy meprobamate

Felbamate is structurally related to meprobamate. It is used in the treatment of epilepsy. It is eliminated by hydroxylation.

Felbamate → Hydroxyfelbamate

Cytochrome P450 (CYP) Enzymes

They are most important phase-I enzymes. They are involved in the detoxification and bio activation of xenobiotics present in food, organic solvents, tobacco smoke, drugs, pesticides,

environmental pollutants and alcoholic drinks. They are products of CYP super family of genes. Over 100 mammalian CYP genes and their products are studied extensively. Some members of CYP super family with their function are given below :

CYP Form Function

CYP1A1 Inducible member of CYP super family helps in detoxification of carcinogens, toxins.

CYP1A2 Catalyzes activation of carcinogenic aryl amines and aflatoxin B.

CYP3A4 Involved in biotransformation of many drugs.

CYP2E1 Involved in oxidation of volatile environmental chemicals and anesthetics.

Medical Importance

1. CYP enzymes are involved in biotransformation of several endogenous compounds and activation of certain carcinogens. Certain compounds of dietary origin inhibit activities of these enzymes thus acting as selective inhibitors of carcinogens or toxicity of chemicals.
2. Polymorphisms in the genes coding for CYP enzymes is associated with susceptibility to different diseases including alcohol related diseases like alcoholic cirrhosis and alcoholic pancreatitis.

(*d*) **Hydrolysis.** Many drugs are detoxified by hydrolysis.

Aspirin (Acetyl salicylic acid) → Salicylic acid + Acetic acid

Atropine (Psychoactive) → Tropic acid + Tropine

II. Conjugation. Conjugation means the chemical combination of one compound with another compound. Many toxic substances are detoxified after combining with compounds like glucuronic acid, glutathione, sulfate, cysteine, acetate, glycine and glutamine.

(*a*) **Conjugation reaction using glucuronic acid.** Glucuronic acid participates in detoxification reactions as its UDP derivative.

Phenol is detoxified by conjugation with glucuronic acid as UDP-Glucuronic acid. The enzyme is UDP Glucuronyl transferase

Phenol→ Phenyl glucuronide

Paraacetamol→ Conjugated product. The conjugating agent is UDP-Glucuronic acid

Benzoic acid→ Glucuronide monobenzoate

Antibiotic chloramphenicol undergo conjugation with glucuronate.

Chloramphenicol→Complex with glucuronate

Lamotrigine an antiepileptic drug is conjugated with glucuronic acid and excreted in urine.

Lamotrigine→ Conjugated product

Diclofenac sodium an analgesic and antipyretic is eliminated from the body by conjugation with glucuronic acid.

Diclofenac sodium→Conjugated product

Morphine, menthol, camphor, chloralhydrate, salicylic acid, PABA are excreted in conjugation with glucuronic acid.

(*b*) **Conjugation with glutathione.** Aliphatic or aromatic halogen substituted hydrocarbons are conjugated with glutathione. The conjugation is catalyzed by an inducible enzyme glutathione-S-transferase.
Dichloronitrobenzene is a halogen substituted aromatic hydrocarbon undergo conjugation with glutathione. The conjugated product is further acted upon by other enzymes to produce mercapturic acids which are excreted in urine.
Dichloronitrobenzene→Conjugated product→Mercapturic acid→Urine

Glutathione transferases (GST)

Glutathione-S-transferases are major enzymes of detoxification. They are involved in bioactivation
and detoxification of xenobiotics present in food, tobacco smoke, alcoholic drinks, pesticides, drugs, environmental pollutants, antitumor agents etc. They catalyze binding of large variety of electrophiles to sulfhydryl group of glutathione. Three types of mammalian glutathione-Stransferases
are identified. They are cytosolic, mitochondrial and microsomal GST.

Medical importance

1. Glutathione-S-transferases are involved in removal of chemical carcinogens. Since reactive ultimate carcinogenic form of chemical carcinogens are electrophiles GST is considered as important detoxification mechanism of carcinogen.
2. GST are involved in activation of unsaturated aldehydes, quinones, epoxides and hydroperoxides formed during oxidative stress.
3. Mammalian cytosolic GST exhibits polymorphism which increases susceptibility to carcinogenisis and inflammatory diseases.
4. Polymorphism of human microsomal GST is associated with increased risk of myocardial infarction and stroke.

(*c*) **Conjugation reactions using sulfate.** Paraacetamol, phenol, cresol, indoxyl and skatoxyl are compounds conjugated with sulfate. PAPS or active sulfate donates sulfate group.
Paraacetamol→Ethereal sulphate. The enzyme for the reaction is PAPS Transferase
PAPS is 3'-phosphoadenosine-5'-phosphosulphate
Skatoxyl/ Indoxyl→Ethereal sulphate. The enzyme is PAPS Transferase
Pain killer diclofenac sodium is conjugated with sulfate and excreted as ethereal sulphate.
(*d*) **Conjugation reactions using cyteine.** Naphthalene, anthracene, bromobenzene, chlorobenzene, iodobenzene and benzyl chloride are converted to mercapturic acids by conjugation with cysteine and acetylation.
(*e*) **Conjugation reactions using acetate.** Sulfa drugs are detoxified by acetylation.
Zonisamide an epilepsy drug is acetylated and excreted in urine.
Isonicotinic acid hydrazide used in treatment of tuberculosis undergo acetylation.
(*f*) **Conjugation reactions using glycine.** An example of conjugation with glycine is the

detoxification of benzoic acid.
(*g*) **Conjugation with glutamine.** Phenyl acetate is conjugated with glutamine.
Detoxification of cyanide : Cyanide is converted to thiocyanate. The reaction is catalyzed by Rhodanase.
Methylation. Some compounds are detoxified by methylation. S-adenosyl methionine serve as methyl donor.
BAL (British anti Lewisite) is methylated and excreted. BAL removes toxic metals such as arsenic, mercury and cadmium from body.
BAL is used as antidote for arsenic poisoning.

Biomethylation
Arsenic ingested is detoxified by methylation and excreted in urine. Biomethylation reduces toxicity of arsenic and facilitates its elimination from the body. Initially inorganic arsenic is methylated to monomethylarsenic acid and finally to dimenthyl arsenic acid.
Anti carcinogens and enzymes of detoxification
1. Several anticarcinogens exert their effect by inducing phase-I and phase-II enzymes. Most important phase-I enzymes are CYP enzymes.
2. Phase-II enzyme induction is common feature of many chemoprotectants of cancer. Induction of phase-II enzymes before or during exposure to carcinogen decreases or inhibits carcinogensis.
3. Glucuronyl transferases and GST of phase-II enzymes are induced by some anti-carcinogens.
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BIOCHEMISTRY OF AGING AND DIEASE.

AGING

Aging is the accumulation of irreversible processes of deterioration which follows the development of an organism. It is generally characterized by declining ability to respond to external or environmental stresses as a result of impaired adaptive and homoeostatic mechanisms. Aging is also known as **senescence.**

Physical/physiological changes in aging

- Vision and hearing decline
- Reduction in muscle strength and size
- Decreased flexibility of soft tissue, blood vessels, skin, joint cartilages that can result into arthritis etc
- Overall decline in body tone including intestinal motility, movement and decreased effectiveness of body organ functions
- Diminished sensitivity to triggers/stimulations
- Loss of number of functional cells in tissues and organs e.g. the brains loses some amount of neurons with age.
- Lowered metabolic activity, immune functions, heart, kidney, lungs, liver functions etc
- Graying of hair (occurs in animals too!)
- Some animals develop dull hair coat, brittle nails and had foot/hand pads
- Dental/gum diseases leading to teeth loss
- Bone marrow progressively gets replaced by fat

THEORIES OF AGING

Many of these theories are interlinked, in the same complex way the biological processes of the body and the many factors affecting it are linked.

DNA and Genetic theory: closely related to this theory is also the programmed theory of aging this theory implies that aging is regulated by biological clocks operating throughout the life span; it focuses upon the encoded programming within the DNA. DNA is the blue-print of individual

life obtained from our parents. It depends on changes in genes relating to the body repair, defense and maintenance mechanisms

Evolution theory; here it is suggested that that longevity is a product of evolutionary forces e.g. body weight brain weight and flight etc different species of animals have different life span, this provides evidence that longevity is genetically influenced. All adaptations that afford protection from predators and other hazards e.g. spines in porcupines justify greater developmental resources to build more durable animal and a longer maximum lifespan.

Neuroendocrine or hormonal Theory ; this theory Suggests the role of specific hormones in the aging process, notably cortisol, a hormone known to increase as organisms age and plays crucial role in stress; estrogen and so on. Generally it is known that the hypothalamus loses it precision regulatory ability and the receptors which uptake individual hormones become less sensitive to them. Accordingly, with increasing age the secretion of many hormones declines and their effectiveness (compared unit to unit) is also reduced due to the receptors down-grading.

Telomere or Telomerase Theory of Aging. Telomeres (the sequences of *nucleic acids* extending from the ends of *chromosomes*), shorten every time a cell divides. This shortening of telomeres is believed to lead to cellular damage due to the inability of the cell to duplicate itself correctly. Each time a cell divides it duplicates itself a little worse than the time before, thus this eventually leads to cellular dysfunction, aging and indeed death. Telomerase is an enzyme that appears to repair and replace telomeres helping to re-regulate the clock that controls the life-span of dividing cells, it is found only in germ and cancer cells.

Mitochondria theory; this theory is closely related to the free radical theory but emphasis of damage by FRs is placed on the mitochondria which is the power house of the cell where most endogenous generation of FRs occur. Mitochondria are the only cellular organelles possessing their own DNA, these DNA unlike nuclear DNA do not have protective heat shock and histone proteins, also lack DNA repair mechanisms, hence are liable to quick damage by FRs. The mitochondrial theory of AGING postulates that damage to mitDNA occur at a rate 10-20 times more than damage to nuclear DNA due to deficiencies in the oxidative phosphorylative pathway leading to loss of mitochondria functions (mitDNA code for the protein complexes of the electron transport chain) and imminently cellular function (due to insufficient production of energy). About 1-2% of oxygen leak from the respiratory chain to for reactive oxygen species.

Free radical theory; this theory of aging was developed by Denham Harman, free radicals (FR) are molecules that have one or more free electrons (unpaired electron) and is capable of existing independently, and this property makes it react with healthy molecules in a destructive way. Reactive species is a term used to describe FRs and other molecules that are easily converted to FRs and are powerful oxidizing agents. These compounds are found both intra and extracellularly and maybe produced endo and exogenously.

It is known that diet, lifestyle, drugs (e.g. tobacco and alcohol) and radiation etc., are all accelerators of free radical production within the body. However, there is also natural production of free-radicals within the body. This is the result of the production of energy, particularly from the mitochondria as a byproduct of oxidative metabolism. Other endogenous sources include phagocytic processes, prostaglandins, detoxification processes etc

Free radicals are known to attack long lived biopolymers in the body such as structural proteins, DNA, lipids (membranes of cells), prostaglandins etc. for instance attack on lipids in cell membrane can damage the membrane by disrupting fluidity and permeability, while lipid peroxidation (oxidative change caused by free radical on lipids) of mitochondrial membranes reduces electrical potential and the mitochondria's ability to generate energy through the electron transport chain. Also FR damage cause fragmentation of DNA, loss of function and structural integrity of proteins, disrupt protein synthesis etc

Oxidative stress is caused by FRs.

REDUCTION OF OXYGEN TO REACTIVE SPECIES

$O_2 + e + H^+$---------HO_2^* (hydroperoxyl radical)

HO_2^*---------$H^+ + O_2^-$ (Superoxide radical)

$O_2^- + 2H^+ + e$----------H_2O_2 (hydrogen peroxide)

$H_2O_2 + e$----------$OH^- + {}^{\cdot}OH$ (hydroxyl radical)

${}^{\cdot}OH + e + H^+$----------H_2O

Lipid peroxidation.

${}^{\cdot}OH + LH$--------${}^{\cdot}L + H_2O$; hydroxyl radical reacts molecules (LH) in the membranes of cells to produce lipid molecule radical (alkyl= ${}^{\cdot}L$)

$^{\cdot}L + O_2$ ------$LOO^{\cdot}$; The lipid radical then reacts with oxygen to form lipid peroxides (lipid peroxyl radicals, lipid molecules containing paired oxygen groups)

$LOO^{\cdot}$ + LH----------LOOH + $^{\cdot}L$.

The lipid hydroperoxides can promote a Fenton reaction;

Fe^{++} + LOOH----------Fe^{+++} + $^{\cdot}OL$ + H_2O

The lipid alkoyl radical ($^{\cdot}OL$) is more reactive and damages more than the lipid peroxide radical ($LOO^{\cdot}$). However if two alkoyl, alky or peroxide radicals collide they nullify each other after creating a cross link between two lipids.

FACTORS INFLUENCING THE OCCURRENCE OF OXIDATIVE STRESS

Antioxidants; these group of compounds delay or inhibit the occurrence of oxidative damage to target molecules by acting as replacement to such target cells, keeping formation of reactive species to a minimum, replacing and repairing damaged molecules, scavenging FRs, and binding metal ions required for the formation of highly reactive species e.g. Fe^{2+} , Cu^{+} etc. Antioxidants could be enzymes, minerals or compounds.

- Antioxidant enzymes found endogenously which play a crucial role in scavenging FRs these include superoxide dismutase (SOD), glutathione peroxidase and catalase. These enzymes are found in all cells
- SOD – catalyzes the reaction betw 2 superoxide ions to prd H_2O_2 and triplet oxygen. Catalase catalyzes the formation of water and free oxygen from H_2O_2,it is present in membrane limited organelles called peroxisomes which contains other enzymes involved in degrading amino acids and fatty acids with the production of H_2O_2 as a by prdt. Glutathione peroxidase (GP) catalyses the reduction of H_2O_2 to water by using the antioxidant compound glutathione.
- Glutathione is a tripepetide and a major antioxidant in the non-lipid portion of cells. It exists as reduced glutathione GSH and oxidized GSSG. GP takes hydrogen molecules from glutathione and transfers to H_2O_2 to yield water.
- Vitamin E is the main Fr trap in the lipid bilayer of membranes.
- Vitamin C acts as an antioxidant in the non lipid portion of cells and blood stream. Melatonin is a hormone produced by the pineal gland in decreasing quantities with age and it has been shown to be effective in protecting molecules against $^{\cdot}OH$.
- Uric acid (produced from purine degradation) can also act as an antioxidant by binding to ion metal like Fe.

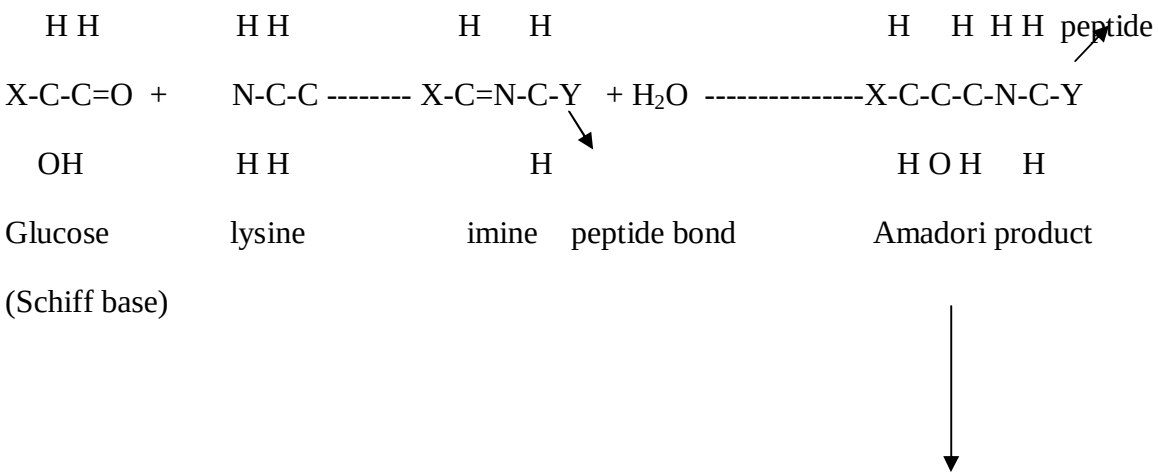
- A number of other compounds and chemicals notably found in plants e.g. lycopene, resveratrol, kolavirion etc has also been shown to have Fr radical scavenging capabilities.

Increase in FRs or reactive species; this can be influenced by

- excessive activation of phagocytes which produce FRs that may impose oxidative stress on tissues
- toxins form the environment e.g. cigarette smoke known to stimulate FRs production
- products of detoxification of toxins include FRs
- increased oxygen concentration or tension
- caloric restriction has been shown to increase life span of yeast cells , drosophila, worms and rodents, it is hypothesized that caloric restriction slows and reduces the overall metabolism (energy production, electron transport chain) hence also reduced production of reactive oxygen species.

Glycation theory; glycation is the formation of double bond between the glucose aldehyde and the lysine groups of amino acids with the elimination of water. An end product AGES- advanced glycation end products - is formed. AGES in tissues increases the rate of FR production to 50 times the rate of prdn in unglycated proteins. AGES attached to LDL-cholesterol accelerates oxidation and subsequent atherosclerosis. It can also aggravate protein cross linking; AGES may also be ingested in food. These compounds are known universal symptoms of aging and can adversely affect skin, lings, muscles, blood vessels and organ function in general.

The damage of proteins by FRs and glycation is also called Maillards reaction.



Oxidation catalysed by transition metals

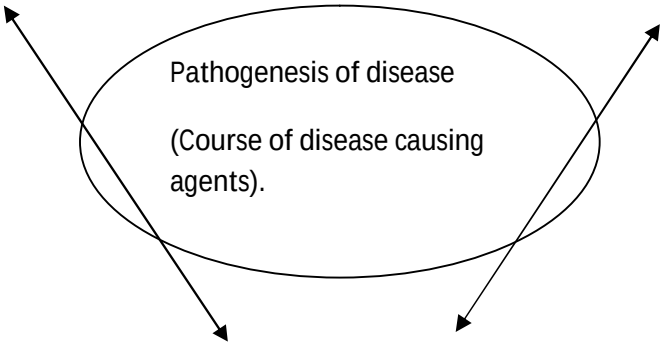
Advanced glycation end products (AGES)

BIOCHEMISTRY OF DISEASE

Biochemical changes occur as the basis for occurrence of disease. An understanding of the physiological biochemistry of the organism forms a baseline in understanding biochemistry of diseases.

Summarily;

CELL INJURY ⟷ FREE RADICALS



TISSUE DAMAGE

Changes therefore in the body of organisms with disease occurs as a result of the basic mechanism above and due to the body's effort to contain these changes for examples cell death to remove non-functional cells (apoptosis) such situation occurs to red cells as diseased ones are

rapidly removed from the circulation by spleen leading to anemia a common feature of many parasitic blood diseases e.g. trypanosomosis.

SOME CHANGES IN DISEASE AND BIOCHEMICAL BASES

1. Anemia- rapid breakdown of infected erythrocytes by the RES.
2. Hypoglycemia- excessive utilization of energy body cells to fight on going infections, and by the invading organisms to the detriment of host.
3. Hypergammaglobulinemia-increased synthesis of globulins to fight on-going invasion
4. Elevation of plasma enzymes- due to rapid tissue/cellular breakdown and release of contents into blood. The cell death is self induced as protective mechanism; apoptosis
5. Damage to more cells as a result of rapid release of FRs from invading organisms, phagocytes etc

Functions of the Liver

- Detoxification of endogenous and exogenous toxins
- Metabolism of CHOS, fats, and proteins
- Bile production
- Blood filtration
- Blood glucose regulation

Liver Function tests

The various liver function tests can be clarified broadly used on the major functions of the liver, including:-

1) Excretory functions

The liver is responsible for conjugating bilirubin, a production formed from the catabolism of hence to diglucuronide which is readily excreted in bile Bilirubin and other dyes like urobilinogens and sterobilinogen on be measured is blood (serum), and urine as important that for liver function. Bilirubin is estimated by Van Der Bergh reaction where diazotized suphanilic acid reacts with bilirubin to form a people colored complex-azobilirubin. For conjugated bilirubin the color change is produced immediately (direct), while for unconjugated color is produced only after addition of alcohol (indirect)

Only conjugated bilirubin is soluble in water and excretable wine, hence when there is obstructive jaundice, urine contains bilirubin as a means of excreting it from the body.

- Bromsulphthalein (BSP) test or sulphobromophthalein (organic anion)

  When this dye is injected the hepatic cells conjugation it with glutathione although a significant faction is excreted unconjugated, when a single bolus dose to 50g/l is given, the retention of the dye after 45minutes in normal individuals (is less than 5% impairment of the liver cell function causes an increase in BSP retention.

  Indocyacine green (ICG) is another dye also used.

2) Metabolic functions

The liver function tests are based on substances that are selectively metabolized by the liver e.g. galactose, ½ life of galactose in blood is about 10-15 minutes, but in defective liver is prolonged, antipyrine is rapidly and completely absorbed from the intestine and mostly metabolized by hepatic monoxygenase system, normal subject excrete 5-8% of this compound in their breathe on 2hrs while patients with cirrhosis excrete 2-3% and hepatitis 2-4%.

3) Synthetic functions liver

The liver functions in synthesis of almost all plasma proteins excepts Igs, so levels of plasma proteins may be arrested to determine the condition of the liver serum Albumin is appreciably reduced in all chronic liver disease but is not a good indicator of acute liver disease b/c of its long half life. Haptoglobulin and transferrin are better indicators of acute liver changes. Prolong prothrombin time is used an indicator of poor prognosis in chronic liver disease. Others are alpha feto-protein which is a tumor marker, whole level is markedly increased is blood during hepatocellular damage.

4) Serum Enzymes

Amino transferases levels in serum are used to indicate liver disease as they are elevated usually in almost all liver disease. Alkaline phosphatase (ALP) whose synthesis is induced by bile duct obstruction, have elevated levels in serum cholestasis and hepatic carcinomas as compared to parenchymal liver disease. Gamma glutamyl transferase levels are also used and are sensitive to biliary tract disease (usually obstructions).

Others include 5-nucleotidase and leucine amino peptidase and in special circumstances glutathione-S-transferase. Others are Arginase, Sorbitol dehydrogenase (esply used in large animals as against ALT in small animals). All in small animals) Glutamate dehydrogenase, gamma glutamyl transpeptidase etc.

Jaundice

Definition: Yellowish coloration of tissues as a result of higher than normal concentrations of bilirubin in plasma.

Types

In Hemolytic jaundice unconjugated bilirubin is increased hence the Van der Bergh test is indirect position, while in obstructive jaundice, conjugated bilirubin is elevated and the test direct. In hepatocellular jaundice and biphasic reaction is observed because both conjugated and unconjugated bilirubin is seen.

Claim of Jaundice	**Type of Bilirubin**	**Causes**
Prehepatic/hemolytic	Unconjugated	Abnormal red cells, Abs drugs and toxins, thalassemias, Hemoglobinopathies, Gilbert's syndrome etc
Hepatic/hepatocellular	Conjugated/unconjugated	Viiral hepatitis, toxic hepatitis, intrahepatic cholestasis
Post-hepatic/obstructive	Conjugated	Extra hepatic cholestasis, gall stories, tumors of bile duct, carcinoma of pancreas, Lymph Node enlargement etc

KIDNEY AND KIDNEY FUNCTION TESTS

The major function of the kidney is to excrete metabolic waste products and to maintain water, PH and electrolyte balance it also has endocrine functions of producing rennin, erythropoietin and calcitriol.

Kidney function tests can be broadly grouped into

1) Tests of glomerular filtration rate
2) Tests of tubular functions

Clearance Test

Measurement of GFR is a useful index for assessment of severity of renal damage. Clearance is defined as the quantity of blood or plasma completely cleared of a substance per unit time and is expressed as milliner per minute. It estimates the amount of plasma that must lower formed true glomeruli per minutes not complete remove of that subtract to acct for its appearing in urine.

$$\text{Clearance} = \frac{\text{mg of substance excreted per minute}}{\text{Mg of substance per ml of plasma/serum}}$$

$$C = \frac{u \times V}{P}$$

U = concentration of substance in urine P

P = concentration of substance plasma

V = ml of urine excreted per minute

Inulin – is neither absorbed nor secreted by the tubules: - its clearance is a good measure of GFR.

Diodrast – chi-iodo-pyridone acetic acid, it is used is urinary tract x-ray, because

It is filtered and excreted.

Para amino hippurate (PAH) is also used as above, hence a measure of renal plasma flow.

Creatinine is a waste prdt formed from creatine PO_4, it has a continuous production much hardly fluctuates, hence its excretion is a good measure of GFR

Urea is partially reabsorbed, so GFR is slighting more than urea-clearance.

Tubular function

- measurement specific gravity (SG) – indicating osmolality
- concentration test
- ADH test

Muscle Action

- mm comprise approx 50% of body man
- composed of long, multinucleated spindle shapes cells called myofibres
- These myofibres contain an array of specific contractile proteins and conductible membranes that give the muscle its excitable nature.
- Different types of mm exits- skeletal smooth cardiac muscles. These different mm tissues differ in myofibre constituents, vascular supply and nervous supply.
- Sarcolemma (plasmalemma of the skeletal myofibre) is the membrane of the muscle cells and is electrically excitable.
- Sarcolemma is also able to activate the contraction machinery located within the cells in response to signals it receives from the motor nerve, in contact with it.

- On this membrane surface (% mm cells0 are contained membrane spanning (transmembrane ion conducting pathways and channels gaits which regulate entry of Na+, K+, Ca2+ and + ions across the sarcolemma. There pathways and gaits open selectively in response to ligands, transmitters, or changes in voltage and they close by intrinsic regulatory processes.

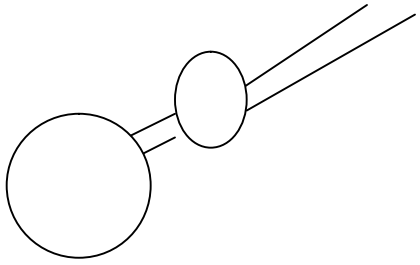
i) Voltage gated channels - have voltage sensing transmembrane domains and they are on essential for generation and modification of action potentials.
ii) Ligand gated ion channels – are essential for producing optimum myoplasmic calcium concentrations and establishing signal transduction pathway.

Motor End Plate

This is the neuromuscular junction, where there is a synapse, thus chemical transmission from the presynaptic axon terminal of a motor neuron (nerve) to the post gnaptic skeletal myofibre (muscle). The position of the NMJ on a mm fibre on vary among species, among different muscles an among fibres in a given muscle.

Axon Terminal

- The axon terminal rests on a 1o depression of sarcolemma called – primary cleft.
- The axon terminals contain nervous small vesicles that contain acetylcholine (ACH)
- ACH is a neurotransmitter, responsible for the excitation of skeletal myofibres.
- The space between the axon terminal the post synaptic sarcolemma comprises the synaptic cleft.
- the synaptic cleft is filled with basal lamina containing acetycholinesterase (AChE)
- When a nerve action potential arrives at the axon terminal there is activation (Opening) voltage gated calcium ion channels on the presyn mb, hence influx of Ca2= into the AT.



- This Ca2+ influx results in a Ca2+ dependent exocytose of Ach –containing vesicles from presyn. Mb. The Ach diffuses across the s/cleft to bind with Ach receptor on the muscle sarcolemma. AChR is an intergral transmembs protein having 5 subunits.
- Muscle excitation is initiated by the reversible binding of Ach to the AChR, though a local depolarization of the post syn Mb, leading to the increased conductance of Na+ and K+ though the AChR cation-channel
- Meanwhile voltage gated K+ channels in the presyn Mb close the voltage gated Ca2+ channel back, and restore the resting Mb potential in the axon.
- Also Ach binding to AChR is transient, and α is abolished by diffusion of Ach away from the receptors α hydrolysis by AChE.
- The large conductance of Na+ α K= lead to a wave of depolarization (Normal resting Po in MM fibre is about – 95mV) exceeding a threshold (-50mV) to cause a muscle action potential (MAP).
- This MAP is propagated over the surface of the myofibril α into its depth via transverse (T) - tubules).
- At the T-tubules depths in the myofibres junctional complexes adjacent terminal cisternae of the sarcoplasmic reticulum (SR) are formed called 'triads'. It is at this triad (which occurs twice in a sarcomere) that calcium ions are released and lead to mechanical shortening of the myofibres as a result of the transmission of the MAP.
- The SR function in the uptake storage and release of Ca2+ to regulate the conclusion of Ca2+ in the mm sarcoplasm which bathes the myofilaments and other organelles in the mm cell.
- The concentration of Ca2+ in the SR is aided by the presence of a protein calsequestrin found in the lumen of the SR cisternae.

Muscle contraction

- The contractile constituents of the sarcomere include the thick (made up of myosin) and thin (actin) filaments the thick myofilaments posses lateral projections that from reactive sites with action-(cyclically annotate and dissociate during muscle contraction and relaxation).

  Myosin – Asymmetric protein with 2 identical heavy chains and 2 pairs of light chains. The composition of heavy chains within sarcomere varies among spps, muscles and muscles cells.

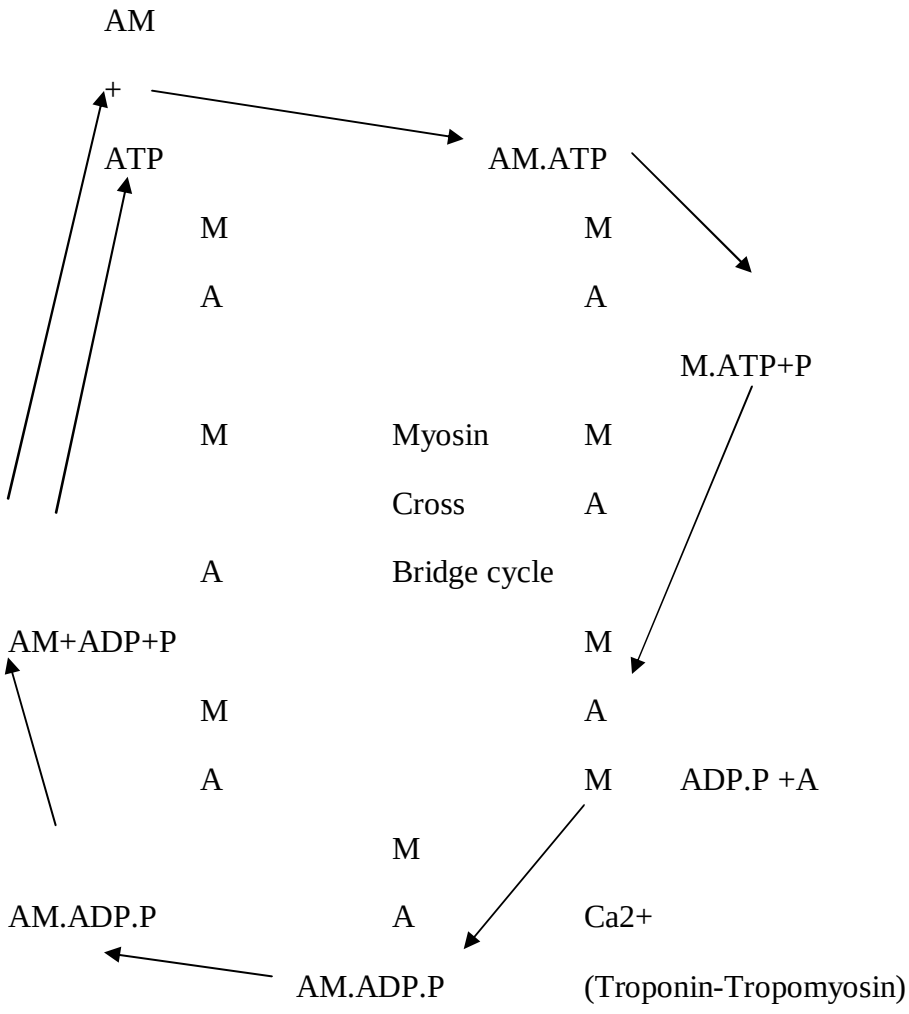
- Regulatory constituents of sarcomere include tropomyosin (a fibrous protein arranged along the length of the filaments) and Troponin (a globulin component TN-I (b)

Tropomyosin-binding TN-I component and (c) Calcium binding component. There two proteins work in concert with calcium to regulate muscle contraction.

- Within the sarcomeres, the myofilaments are supported by complex cytoskeletal network of intermediate filaments in addition to a number of accessory proteins which help to maintain the alignment of myofilaments and sarcomere, adjacent myofibrils attach sarcomere of peripheral myofibrils to sarcolemma etc. these from the structural constituents of the sarcomere.

Energetics of mm contraction

M.C results from transformation of chemical energy to mech. Energy.



Force generation Step

A = action, M= myosin

ATP is hydrolyzed to ADP and inorganic Phosphate under the catalysis of myosin ATPase on the myosin head –leading chemically cyclical association and dissociation of the contractile proteins (Action and myosin) while mechanically its sees as shortening so sarcomere (as a result of sliding of overlapping arrays).

1) A.M connected at cross bridges of myosin

2) A.M: ATP 2 molecules of ATP bind to M molecule

3) A, M + ATP +P, A and M dissociate

4) ATP hydrolyses, when A is not associated with M=ADP +P

5) GH of M moves to a new location on A = A.M This recombination of A and M is under control so Troponin and tropomyosin in response to calcium concentration

6) The GH of M den includes to a 45^{o} angle of attachment

7) ADP + P den detaches, x ATP is reformed through rephoshorylation

Each sarcomere shortens approximately 12mm

Rigor Mortis

Is the rigid and stiff condition of skeletal muscles that develops following death. After death all ATP stores one utilized, hence dissociation of action and myosin does not occur, and the cycle is terminated w large no of A-M complexes 4med w myosin head set at 45o.

NB: Cross bridges project from thick myofilaments and make contact with thin myofilaments

S2

S1 O Globular head

Myosin has both structural and enzymatic properties.

HORMONES

INTRODUCTION

Most glands of the body deliver their secretions by means of ducts. There are called exocrine glands.

There are few other glands that produce chemical substance that they directly secrete into the blood stream for transmission to various target tissues. These are ductless or endocrine glands. The secretions of endocrine glands are called as hormones.

DEFINITION OF HORMONES

It is a chemical substance which is produced in on part of the body, enters the circulation and is carried to distant target organs and tissues to modify their structures and functions.

SIMILARITIES OF HORMONES AND ENZYMES

- They act as body catalysts resembling enzymes in some aspect.
- They are required in small quantities.
- They are not used up during the reaction.

DISSIMILARITIES OF HORMONE AND ENZYME

- They are produced in an organ other than that in which they ultimately perform their action.
- They are secreted in blood prior to use.
- The circulating levels of hormones can give some indication of endocrine gland activity and target organ exposure. Because of the small amount of the hormones required, blood levels of the hormones are extremely low. In many cases it is ng/µg or MIU etc
- Structurally they are not always proteins. Few hormones are protein in nature, few are small peptides. Some hormones are derived from amino acids while some are steroid in nature.

The major hormone secreting glands are:

- Pituitary * Thyroid * Parathyroid * Adrenal * Pancreas
- Ovaries * Testes

Several other glandular tissues are considered to secrete hormones viz

- Juxtaglomeular cells of kidney: May produce the hormone erythropoietin which regulates erythrocytce maturation, erythropoiesis.
- Thymus: this produces a hormone that circulates from this organ to stem cells in lymphoid organ inducing them to become immunologically competent lymphocytes.
- Pineal gland: It produces a hormone that antagonizes the secretion or effects of ACTH. It also produces factors called glomerulotrophins that regulates the adrenal secretion of aldosterone
- Gastrointestinal tract: few hormones are also produced by certain specialized cells of GI tract and they are called GI hormones e.g. gastrin, secretin etc.

CLASSIFICATION OF HORMONES

According to Li the hormones can be classified chemically into three major groups.

- Steroid hormones: These are steroid in nature such as adrenocorticosteroid hormones, androgens, estrogens and progesterone
- Amino acid derivatives: These are derived from amino acid tyrosine e.g. epinephrine, norepinephrine and thyroid hormones.
- Peptides/protein hormones: These are either large proteins or small or medium size peptides e.g insulin, glucagon, parathormone, calcitonin, pituitary hormone etc.

Factors regulating Hormone Action

Action of a hormone at a target organ is regulated by four factors.

- Rate of synthesis and secretion: The hormone is stored in the endocrine gland.
- In some cases specific transport system in plasma
- Hormone-specific receptor in target cell membranes which differ from tissue to tissue
- Ultimate degradation of the hormone usually by the liver or kidneys

Mechanism of Action of Hormone

Although the physiological apparently secondary effects of most of the hormones have been rather completely known for a number of years, their primary biochemical mechanism of actions at a cellular/molecular level are also known in much details now. Many hormones serve as inducers or repressor in the genetically controlled synthesis of certain key cellular enzymes.

Although the exact site of action of any hormone is still not well understood, the following mechanisms of actions of hormone have been proposed.

1. **Interaction with nuclear chromatin (Nuclear Action):**

Steroid hormones act mostly by changing the transcription rate of specific genes in the nuclear DNA. The steroid hormone has a specific soluble, oligomeric receptor protein (mobile receptor) either in cytosol and or inside the nucleus. This brings about conformational changes and also changes in the surface of the receptor protein to favour its binding to the nuclear chromatin attached to nuclear matrix. The receptor-steroid complex is translocated to the nuclear chromatin and binds to a steroid-recognizing receptor site called the hormone-responsive element (HRE) of a DNA strand on the upstream side of the promoter site for a specific steroid responsive gene. The consequence change in the intracellular concentration of mRNA alters the rate of synthesis of a structural, enzymatic carrier or receptor protein coded by it. This results in ultimate cellular effects. The receptor-steroid complex subsequently leaves the acceptor site as the free receptor and the steroid. In addition to regulating the transcription, some steroid hormones may also act as regulatory agents for post transcriptional processing stability and transport of specific mRNAs.

2. Membrane Receptor

As per the suggestion of Heller, certain molecules cannot enter target cells through membrane lipid bilayer. This is achieved by the specific receptor molecules present on the surface of the plasma membrane. Many hormones seen specifically involved in the transport of a variety of substance across cell membrane. In general these hormones specifically bind to the receptor on cell membrane. They cause rapid secondary metabolic changes in the tissue but have little effect on metabolic activity of membrane free preparations. Most protein hormones and catecholamines activate transport of membrane enzyme systems by direct binding to specific receptors on the membrane.

3. Stimulation Of Enzyme Synthesis At The Ribosomal Level

Activity at the level of translation of information is carried by the mRNA on the ribosomes for the production of enzyme. Ribosomes taken from growth hormone treated animals have a modified capacity to synthesize protein in the presence of normal mRNA. Thus, in this case either increased production of new ribosomes or to create new population of more active or more selective ribosomes might be taking place.

4. cAMP And Hormone Action

3'-5'cAMP plays a unique role in the action of many protein hormones. Its level may be decreased or increased by hormonal action as the effect varies depending on the tissue. The hormones such as glucagons, catecholamines, PTH, etc. acts by influencing a change in intracellular cAMP concentration through the adenylate cyclase c-AMP system. The hormone binds to a specific membrane receptor. Different types of these receptors remain associated with wither G_s or G_i type of GTP-dependent trimeric nucleotide regulatory complexes of the membrane. Both G_s and G_i are made up of 3 subunits. G_s contain $\alpha_s\beta\gamma$ while G_i contains $\alpha_i\beta y$. Formation of the receptor hormone complex promotes the binding of GTP to the α subunit of either G_s or G_i. When α_s GTP is released it binds to adenylate cyclase located on the cytoplasmic surface of the membrane and changes its conformation to activate it. However in some cells calmodulin-$4Ca^{2+}$ is also required for activation. Adenylate cyclase catalyses the conversion of ATP to cAMP thus increased the intracellular concentration of the latter. On the other hand α_i-GTP inhibits adenylate cyclase by binding with it. This lowers the intracellular concentration of cAMP. The action of cAMP is mainly to activate some protein kinases allosterically.

Insulin can decrease hepatic cAMP in opposition to the increase caused by glucagon. Tissue levels of cyclic AMP can be influenced not only by hormone but also by nicotinic acid, imidazole, methylxanthine.

5. Role of Polyphosphoinositol and diacycglycerol in hormone action

Just like c-AMP other compounds such as 1,4,5- inositol triphosphate (ITP) and diacylglycerol (DAG) act as second messengers. This is especially found in case of vasopressin, TRH, GnRH, etc. These hormones activate the phospholipase c-polyphosphoinositol system to produce ITP and DAG by binding with the specific receptor protein on cell membrane, the hormone activates a trimeric nucleotide regulatory complex. The complex in turn activates phospholipase C on the inner surface of the membrane. ITP enhances the mobilization of ca^{2+} into the cytosol from intracellular ca^{2+} pool from mitochondria. Ca^{2+} then act as tertiary messenger. While DAG activates Ca^{2+} phosphatidyl-serine-dependent protein kinase c located on the inner surface of the membrane, by lowering its Km for Ca^{2+}. This enzyme then phosphorylates specific enzymes and other proteins in the cytosol to modulate their activities.

6. Role of Calcium in Hormone Action

The action of most protein hormones is inhibited in absence of calcium even though ability to increase or decrease cAMP is comparatively unimpaired. This calcium may be more terminal signal for hormone action then cAMP. It is suggested that ionized calcium of the cytosol is the important signal. The source of this calcium may be intracellular fluid or it may arise from mobilization of intracellular tissue bound calcium. As mentioned, membrane receptor binding may be responsible for this. The hormone receptor binding may directly inhibit the Ca^{2+}-ATPase. It may also directly open up voltage-independent Ca^{2+} channels in the membrane to increase the diffusion of Ca^{2+} into the cell down its inward concentration gradient resulting in increased cytosolic Ca^{2+} concentration which then acts as a second messenger to affect cellular activities. The receptor-hormone complex may produce ITP which in turn can increase cytosolic Ca^{2+} concentration by enhancing the mobilization of Ca^{2+} from mitochondrial and endoplasmic reticular pool. Calcium is involved in the regulations of several enzymes such as phospholipase A2, Ca^{2+}- phosophatidylserine dependent protein kinases, guanylate cyclase, adenylate cyclase, and glycogen synthetase. All these enzymes have special biochemical metabolic roles. Ca^{2+} also changes membrane permeability. Many of its effects are mediated through its binding to Ca^{2+}-dependent regulatory proteins like calmodulin and troponin.

7. Role of c-GMP in Hormone Action

Hormone such as insulin and growth hormone affect the guanylate cyclase c-GMP system. This will increase the intracellular concentration of c-GMP and activate c-GMP dependent protein kinase. The active c-GMP protein kinase would in turn bring about phosphorylation of specific cellular proteins to change their activities leading to relaxation of smooth muscle, vasodilation and other effect. It is likely that Ca^{2+} may act as a second messenger to activate guanylate cyclase and thereby increasing the concentration of c-GMP inside the cell.

8. Role of Phosphorylation of Tyrosine kinase

In fact a second messenger for insulin, growth hormone prolactin, oxytocin etc has not been identified so far. However, binding of them to their respective membrane receptors activates a specific protein kinase called tyrosine kinase which phosphorylates tyrosine residue of specific proteins. This may bring about some metabolic changes.

REGULATION OF HORMONE SECRETION

Hormones secretion is strictly under control of several mechanisms.

a. **Neuroendocrinal Control Mechanism**

Nerve impulses control some endocrine secretion. Cholinergic sympathetic fibers stimulate cathecholamine secretion from adrenal medulla. Centres in the midbrain, brainstem, hippocampus, etc can send nerve impulses which react with the hypothalamus through cholinergic and bioaminergic neurons. At the terminations of these neurons they release acetylcholine and biogenic amines to regulate the secretions of hypophysiotropic peptide hormones from hypothalamic peptidergic neurons. Some of the endocrine releases are controlled by either stimulatory or inhibitory hormones from a controlling gland, e.g corticosteroids are controlled by coticotropins and thyroid hormonrs are controlled by thyroptropin from anterior pituitary. The tropins are further regulation by hypothalamic releasing hormones.

b. **Feedback Control Mechanism**

It is due mainly to negative feedback that such control is brought about. When there is a high blood level of a target gland hormone. It may inhibit the secretion of the tropic hormone stimulating that gland. Adrenal cortex secretes a hormone called cortisol which brings about the inhibition of secretion of corticotrophin from anterior pituitary and corticotropin releasing hormones from the hypothalamus by a long-loop feedback. This leads to reduction in cortisol secretion.

c. **Endocrine Rhythms**

There are certain cyclic rhythmic associated with the secretion of hormone over a period of time. When there is a cyclic periodicity of 24 hrs, it is called as circadian rhythm. However, if it is more than 24hrs, it is named as infradian rhythm and when it is less than 24 hrs it is called as ultradian rhythm. Due to rhythm the highest and lowest concentration of corticotrophin is normally found in the morning and around midnight. Growth hormone and prolactin rise in the early hours of deep sleep. Cortisol peak is found between 4am and 8am. Endocrine rhythms results from cyclic activities of a biological clock in the limbic system supplemented by the diurnal light-dark and sleep activity cycles and mediated by the hypothalamus.

Pituitary Hormones

Control of secretion

Secretion of hormones from anterior pituitary are controlled by

- Nervous mechanism: by release of regulatory factors from hypothalamus
- Hormonal mechanism by feedback inhibition

Hypothaciamic Releasing Factors

Control of hormone secretion from pituitary is in part modulated by regulating factors or hormone from the hypothalamus. The median eminence of hypothalamus is connected directly to the pituitary stalk. Within this stalk is a portal system of blood vessels required to maintain normal secretary activity of the pituitary gland. The activities of the cells of the anterior lobe are controlled by the nerve cells of the hypothalamus which send axons to the capillary beds. The nerve endings liberate chemical substances, hypothalamic releasing factors or hormones. At present 10 discrete regulatory factors have been described that may affect the synthesis as well as secretion of specific pituitary hormone. They are:

Hypothalamic Hormone or factor	**Abbreviation**
* Corticotropin (ACTH) releasing hormone	CRH or CRF
* Thyrotropin (TSH) releasing hormone	TRH or TRE
* Follicle stimulating hormone (FSH)	FSH-RH or FSH-RF
* Luteinizing hormone (LH) releasing hormone	LH-RH or LH-RF
* Growth Hormone (GH)	GH-RH or GH-RF
releasing hormone	GH-RF
* Growth hormone release inhibiting	GH-RH or GIF
* Prolaction (PL) release	PL-RIH or PL-RIF
* Melanocyte stimulating hormones	mSH-RIH or
* (MSH) release inhibiting hormone	MSH-RIF
* Melanocyte stimulating hormone	MSH-RH or
* (MSH) releasing hormone	MSH- RF

Hormones of the Anterior Pituitary

The hormones secreted by the anterior lobe of the pituitary gland are:

- Growth hormone and
- Pituitary tropic hormones such as protactin, gonadotropins (FSH and LH), thyrotropic hormones (TSH) and adrenocorticotropic hormones (ACTH)

Growth Hormone.(GH)

Growth Hormone (GH) or somatotropin (STH) was first isolated in sufficient quantity from cattle, now it has been prepared in crystalline form several species including man.

Chemistry

Growth hormone from all mammalian species consists of a single polypeptide with a molecular weight of about 21500. It consists of 191 amino acids. There are two disulfide bridges between the adjacent cysteine residue (52 and 165 and 183 189). Although there is a high degree of similarity in the amino acid sequences of human, bovine and porcine GH, only human GH or that of other primates is active in man. GH can bring about some of the actions of prolactin and human placental lactogen (HPL) due to amino acid homology.

Metabolic Role

Growth hormone has a variety of effects on different tissues. The hormones act slowly requiring from 1-2 hours to several days before it biological effects are detectable. This slow action and its stimulatory effects on RNA synthesis suggest that it is involved in protein synthesis. The hormone acts by binding to specific membrane receptors on its target cells. But its exact mechanism of action and second messenger are not yet known.

1. **Protein Synthesis**

Growth hormone brings about positive nitrogen balance by retaining nitrogen. It stimulates overall protein synthesis with an associated retention of phosphorus probably by increasing tubular reabsorption. Blood amino acid and urea level are decreased. It facilitates the entry of amino acids into the cell. In addition, growth hormone facilitates protein synthesis in muscle tissue by a mechanism independent of its ability to provide amino acids. This protein synthesis carries on even if the amino acid transport is blocked.

- Growth hormones increases DNA and RNA synthesis
- It increases the synthesis of collagen

2. **Lipid Metabolism**

Growth hormone brings about lipolysis in a mild way by mobilizing fatty acids from adipose tissue by activating the hormone sensitive triacylglycerol lipase. Thus it increases circulating fatty acids.

3. **Carbohydrate Metabolism**

Growth hormone is a diabetogenic hormone, antagonizes the effect of insulin. Hypersecretion of GH can result in hyperglycemia, poor sugar tolerance and glycosuria. Growth hormone produces.

- Hyperglycaemia by increasing gluconeogenesis
- It reduces insulin sensitivity and thereby decreases the hypoglycaemic effect of insulin
- It brings about glycostatic effect, i.e increases liver glycogen it can also increase muscle and cardiac glycogen level probably by reducing glycolysis.

4. **Effect on Growth of Bones and Cartilages**

Growth hormone when secreted in abnormally high concentration prolongs the growth of epiphyseal cartilages to cause over growth of long bones. Acromegaly is found in adults. Hyposecretion causes stunted stature due to premature cessation of growth of the epiphyseal cartilages and consequently of long bones.

- The effect of growth hormone partly depends upon its calcium anabolic action. It promotes the retention of calcium and phosphate which helps in ossification and osteogenesis.
- It enhances the incorporation and hydroxylation of proline in the matrix collagen, incorporation of amines into glycosaminoglycans of cartilage, incorporation of sulphate into matric proteoglycans like chondroitin sulphates, the synthesis of DNA and RNA in chondrocytes.
- The growth effects are mediated by a peptide called insulin-like growth factor I (IGF-I) or somatomedin – C)

5. **Prolactin Action**

Growth hormone has a sequence homology with prolactin. Growth hormone binds to membrane receptors for prolactin and stimulates the growth and enlargement of mammary gland.

6. **Ion or Minerial metabolism**

It is observed that the intestinal absorption of calcium is increased by GH, since the bone growth and development is stimulated by growth hormone. Growth hormone retains Na, Ca, K, Mg, and PO_4^{3-}

PITUITARY TROPICHORMONES

In addition to GH, anterior pituitary gland secretes some tropic hormones usually called as pituitary tropins.

A tropin or tropic hormones is the one which influences the activities of other endocrine gland, principally those involved in stress and reproduction. These are carried by the blood to other target gland. The pituitary tropins are under the positive and negative control of peptide factors from hypothalamus. Further the tropic hormones are usually subject to feedback inhibition at the pituitary or hypothalamic level by hormone product of the final target gland. Prolactin (mammotropin), TSH (thyrotropin), FSH and LH (Gonadotropins), ACTH (Corticotropin) are the tropic hormones secreted by the pituitary gland.

A. Prolaction: PRL or Leuteotropic Hormone (LTH)

This is a monomeric simple protein (Mw23, 000). It contains 199 amino acids with three –s-s- linkages. It is secreted by lactotroph α-cells of anterior pituitary and has sequence homology with growth hormone.

Metabolic Role

- The main function of PRL is to stimulate mammary growth and the secretion of milk. By acting through specific glycoprotein receptors on plasma membrane of mammary gland cells, it stimulates mRNA synthesis. This ultimately leads to enlargement of breast (udder) during pregnancy. This is called mammotropic action.
- The synthesis of milk proteins such as lactalbumin, and casein takes place after parturition such an effect is called lactogenic action.
- Estrogen, thyroid hormones and glucocorticoids increases the number of prolactin receptors on the mammary cell membrane.
- Progesterone has the opposite effect.

B. **Thyrotrophic Hormone or Thyroid Stimulating Hormone (TSH)**

This is produced by basophil cells of anterior pituitary and is glycoprotein in nature. Its molecular weight is approximately 30,000. This consists of α and β submits.

- The α - subunit of TSH, LH and HCG and FSH are nearly identical
- The biological specificity of thyrotropin must therefore be in β -subunit. The α-subunit consist of 92 amino acids while β-subunit has 112 amino acids. Both α and β have several disulfide bridges. It carbohydrate content is 21% and it αand β chains bears two and one oligosaccharide chains linked by N-glycosidic linkages to specific asparagine residues. The chains are synthesized separately by separate structural genes and later undergo post-translation modification and glycosylation separately.

Metabolic Role

There are glycoprotein receptors on the thyroid cells membrane which binds to the receptor binding site on β-subunit of TSH. The complex then activates adenylate cyclase which catalyzes the formation of c-AMP which acts as the second messenger for most TSH actions an follows:

- TSH stimulates the synthesis of thyroid hormones at all stages such as Iodine uptake, organification and coupling.
- It enhances the release of stored thyroid hormones.
- It increases DNA content, RNA and translation of proteins, cell size.
- It stimulates glycolysis, TCA Cycle, HMP and phospholipids synthesis. Stimulation of last two does not involve c-AMP.
- It activates adipose tissue lipase to enhance the release of fatty acids (lipolysis)

C. ADRENOCORTICOTROPIC HORMONE (ACTH) OR CORTICOTROPIN: It is a single polypeptide containing 39 amino acids in its structure with a molecular weight of 4500 two forms have been isolated, α-corticotropin and β-corticotropin. Biological activity of ACTH resides in the first 23 amino acids from N-terminal end. The sequence of these 23 amino acids in the peptide chain is the same in all species tested. The remaining biologically inactive 16 amino acid residue varies accordingly to sources. ACTH is synthesized as a part of precursor peptide of mol.wt of 31500 with 260 amino acids. ACTH contains sequence of amino acids common for LPH, MSH and the endorphins. The precursor molecule is synthesized as a glycoprotein called pro-opiomelanocortin peptide (POMC). Various proteolytic enzymes hydrolyze POMC to give different peptides. Thus POMC is broken down into

- ACTH
- β-lipotropin (LPH). β-LPH is further cleaved into γ-LPH and endophins.

METABOLIC ROLE

The principal actions of corticotrophin are exerted on the adrenal cortex and extra adrenal tissue. ACTH increases the synthesis of corticosteroids by the adrenal cortex and also stimulates their release form the gland. Profound changes in the adrenal structure, chemical composition and enzymatic activity are observed as a response to ACTH. Total protein synthesis is found to be increased. Thus, ACTH produces both a tropic effect on steroid production and tropic effect an adrenal tissue. It is observed that ACTH has specific receptors on cells of fasciculata which increases c-AMP levels in the cell. This activation is calcium dependent. This result in DNA content aid RNA is transcribed. This leads to proliferation of fasciculata cells and growth of adrenal cortex.

- ACTH also stimulates the synthesis and secretion of glucocorticoids.
- ACTH is found to increase the transfer of cholesterol from plasma lipoproteins into the fasciculata cells.
- The ACTH induces rise in c-AMP, bring about phosphorylation and activation of cholesterol esterase. The enzyme action ultimately makes a large pool of free cholesterol.
- It activates the rate limiting enzyme for conversion of cholesterol to pregnenolone.
- It activates dehydrogenases of HMP to increase the concentration of NADPH required for hydroxylation.
- By activating adenylate cyclase of adipose tissue, it increases intracellular c-AMP which in turn activates hormone sensitive lipase. This enzyme is involved in lipolysis which increases the level of free fatty acids.
- It leads to increase ketogenesis.
- Direct effects on carbohydrate metabolism include :
- Lowering of blood glucose
- Increase in glucose tolerance
- Deposition of glycogen in adipose tissue is increased, regarded as due to stimulation of insulin secretion.
- It has MSH activity due to homology in amino acid sequence.

D. PITUITARY GONADOTROPINS

These tropic hormones influence the function and maturation of the testes and ovary, and are of two types.

- Follicle stimulating hormone (FSH)
- Luteinizing hormone (LH)

Both of them are glycoproteins with sialic acid, hexose and hexosamine as the carbohydrate moiety. Molecular weight of FSH is 25000 and that of LH is 40000. FSH, LH are dimers of α and β-chains linked non covalently. The α-chain is identical for TSH, FSH and LH of the same species. The β-chain of human FSH and LH has respectively 118 and 112 amino acid residues. Each chain has several disulfide bridges. A large precursor protein molecule for α and β chains is synthesized separately in gonadotroph β-cells.

METABOLIC ROLE OF FSH

It brings about it action by specific receptor binding and c-AMP

In females:

- It promotes follicular growth
- Prepares the Graafian follicle for the action of LH
- Enhances the release of estrogen induced by LH

In males:

- It stimulates seminal tubule and testicular growth
- Plays an important role in maturation of spermatozoa.

Role of FSH in Spermatogenosis

The conversion of primary spermatocytes into secondary spermatocytes in the seminiferous tubules is stimulated by FSH. In absence of FSH spermatogenesis cannot proceed. However, FSH by itself cannot cause complete formation of spermatozoa. For its completion testosterone is also required. Thus, FSH seems to initiate the proliferation process of spermatogenesis, and testosterone is apparently necessary for final maturation of spermatozoa. Since the testosterone is secreted under the influence of LH, both FSH and LH must be secreted for normal spermatogenesis.

Metabolic Role of LH

This hormone is also known as interstitial cells stimulating hormone (ICSH)

In females

- It causes the final maturation of Graafian follicle and stimulates ovulation
- Stimulates secretion of oestrogen by theca and granulosa cells.

- It helps in the formation and development of corpus luteum for luteinization of cells.
- In conjunction with luteotropic hormone (LTH) it is concerned with the production of estrogen and progesterone by the corpus luteum.
- In the ovary it can stimulate the non-germinal elements, which contain the interstitial cells to produce the androgens, androstenedione, dihydroepiandronstenedione (DHEA) and testosterone.

ACTION OF LH IN OVULATION (Ovulatory surge for LH): It is necessary for final follicular growth and ovulation. Without this hormone, even though large quantities of FSH are available the follicle will not progress to the stage of ovulation. LH acts synergistically with FSH to course rapid swelling of follicles shortly before ovulation. It is worth noting that especially large amount of LH called ovulatory surge is secreted by the pituitary during the day immediately preceding ovulation.

REGULATION OF TESTOSTERONE SECRETION BY LH: Testosterone is produced by the interstitial cells of leydig only when the testes are stimulated by LH from the pituitary gland, and the quantity of testosterone secreted varies approximately in proportion to the amount of LH available. Thus in males LH stimulate the development and functional activity of leydig cells (interstitial) and consequently testicular androgen.

ENDORPHINS AND ENCEPHALINS

Endorphins are a group of polypeptides which influence the transmission of nerve impulse. They are also known an opioides, because they bind to those receptors which bind opiates like morphine and play a role in pain perception. The opiodes first discovered were two penta-peptides in the brain and were named encephalin. They are of two types:

* Methionine - encephalin

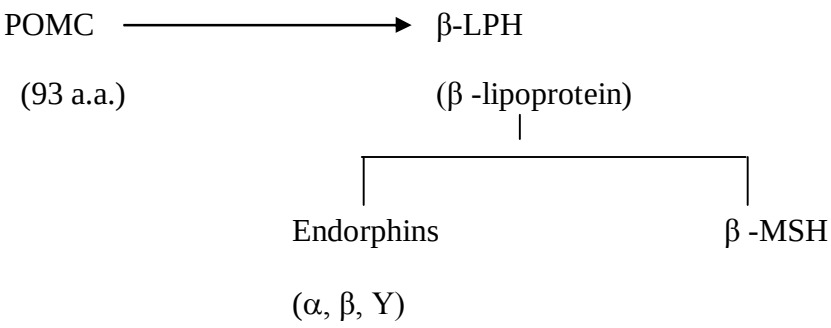
* Leucine - encephalin

FORMATION OF ENDORPHINS

β – lipoprotein (β-LPH) is the precursor for endorphin, all the three type α, β and γ and also for β-MSH.

β- lipotprotein (β-LPH) is derived from the precursor molecule "Pro-opiomelanocortin peptide (POMC). It is a single chain polypeptide containing 93 amino acids.

γ-LPH containing 60 amino acids is a part of β-LPH



TYPES OF ENDORPHINS

There are three types of endorphinsα, β.y

- The sequence of 31 amino acids at the C-terminal of β-LPH, (Obtained from POMC) i.e amino acid 104 to 134 gives β endorphin
- α- endorphin (104 to 117 amino acid) containing 17 amino acids less than the β from the C terminal end.
- γ-endorphin (104 to 118) containing 16 amino acid less than the β from the C-terminal end.

FUNCTION

Endorphins bind to the same CNS receptors like the morphine opiates and they play a role in the endogenous control of pain perception. They have highs analgesic potency than morphine.

HORMONE OF MIDDLE LOBE OF PITUITARY

MELANOCYTE STIMULATING HORMONES

The hormones secreted by intermediate lobe or middle lobe of pituitary gland are called melanocyte stimulating hormones or MSH. POMC is the precursor molecule which is cleaved by proteases to give ACTH and β-lipotropin. The ACHT is further cleaved to β-MSH which has 13 amino acids. There is also α-MSH which is present in larger quantities. Amino acids 11-17 of β-MSH are common to both α-MSH and ACTH. MSH darkens the skin and is involved in skin pigmentation by deposition of melanin by melanocytes.

HORMONE OF POSTERIOR PITUITARY LOBE

The hormones have been isolated and characterized from extracts of posterior pituitary gland. They are

- Vasopressin (pitressin) or Arginine Vasopressin (ADH)
- Oxytocin

Both are small peptides containing nine amino acids. Oxytocin differs from vasopressin with respect to 3rd and 8th amino acid residues. Their biological activities depend on C-terminal glycinamide, the side chain amide group of glutamine and asparagine, the hydroxyphenyl group of tyrosine and the intra-chain –s-s-linkage between cysteine of 1st and 6th amino acid. Posterior pituitary hormones are synthesized in neuro-secretory neuron. They are stored in the pituitary in association with two proteins neurophysin I and II with molecular weight, of 19000 and 21000 respectively. The release of these two hormones is independent of each other.

METABOLIC ROLES OF VASOPRESSIN

1. Antidiuretic action: Antidiuretic effect is it main function. It reabsorbs water from the kidneys by distal tubules and collecting ducts. It is found to be mediated through formation of c-AMP. It is released due to rise in plasma osmolarity. This leads to formation of hypertonic urine having low volume, high specific gravity and high concentration of Na^+, Cl^-, PO_4^{3-} and urea. Halothane, colchicine and vinblastine inhibit antidiuretic effect of vasopressin.

CLINICAL IMPORTANCE

- Condition of *Diabetes insipidus* is described due to failure in secretion or action of vasopressin. It is characterised by very high volumes of urine output up to 20-30 litre per day with a low specific gravity and excessive thirst.
- In primary, central or neurohypophyseal diabetes insipidus, vasopression secretion is poor.
- In nephprogenic diabetes insipidus, kidney cannot respond to vasopressin due to renal damage. The damage is common in psychiatric patients on lithium therapy.
- Inappropriate vasopressin secretion is characterized by persistently hypertonic urine, progressive renal loss of Na^+ with low plasma levels of Na^+, symptoms of

water intoxication like drowsiness, irritability, nausea, vomiting, convulsions, stupor and coma. It could be due to pulmonary infection and ectopic ADH secretion from lung tumor.

- Urea-retention effect: permeability of medullary collecting ducts to urea is increase by vasopressin. This leads to retention of urea and subsequently contributes to hypertonicity of the medullary interstitium. Urea retention effect can be reversed by phloretin.
- Pressor Effect: It stimulates the contraction of smooth muscles and this causes vasoconstriction by increasing cytosolic Ca^{2+} concentration.
- Glycogenolytic effect: By increasing intracellular calcium concentration.

METABOLIC ROLES OF OXYTOCIN

- Contraction of smooth muscle is the primary function of oxytocin. There are basically two effects, one on mammary glands called galactobolic effect and the other on uterus called as uterine effect.
- 1. **Galactobolic Effect**: This is released due to neuroendocrinal reflex such as sucking of nipples. By doing so it causes that contraction of myo-epithelial cells around mammary alveoli and ducts and the smooth muscle surrounding the mammary milk sinuses. Estrogen increases the number of oxytocin receptors during pregnancy while progesterone decreases the dame and also inhibits the secretion of oxytocin.

- 2. **Uterine effects**: It is found to be elevated at full term pregnancy. It causes contraction of uterine muscle for childbirth. Estrogen enhance while progesterone decreases oxytocin receptors as well as its secretion. Oxytocin is also secreted during coitus by the female uterus which promotes the aspiration of semen into the uterus. This is also augmented by rise in estrogen in the follicular phases of menstrual cycle.

THYROID GLAND AND ITS HORMONES

Hormones produced by Thyroid gland

- Follicular cells: produces T4, T3 and reverse T3
- Parafollicular C-cells: produces calcitonin (hence also called thyrocalcitonin)

- **THYROID HORMONES**
- The principal hormones secreted by the follicular cells of thyroid are
- Thyroxine (T4)

- Tri-iodothyronine (T3)
- Reverse T3

CHEMISTRY OF THYROID HORMONE

- The hormones T4, T3 and reverse T3 are iodinated amino acid tyrosine. The iodine in thyroxine accounts for 80% of the organically bound iodine in thyroid venous blood. Small amounts of "reverse' tri-iodothyronine, monoiodotyrosine (MIT) and other compound are also liberated.

BIOSYNTHESIS OF THYROID HORMONE

- Two raw materials (substrates) required by thyroid gland to synthesize the thyroid hormone are:
- Thyroglobulin
- Iodine
- Thyroglobulin: Thyroid hormone are synthesized by the iodination of tyrosine residue of a large protein called thyroglobulin

CHEMISTRY OF THYROGLOBULIN

- Thyroglobulin is a dimeric of glycoprotein, 19s in type (a macroglobulin) with a molecular weight of 660,000
- The receptor tyrosine molecules are present in this macroglobulin protein, each molecule containing 115 tyrosine residues.
- Carbohydrates accounts for 8 to 10% of the weight of thyroglobulin and iodide for about 0.2 to 1.0% depending on the iodine content of the diet. The carbohydrates are N-acetyl glucosamine, mannose, glucose, galactose, fucose and sialic acid.
- About 70% of the iodide in thyroglobulin exists as inactive precursors monoiodo-tyrosine (MIT) and di-iodotyrosine (DIT) while 30% is in the iodothyronyl residues T4 and T3.
- When iodine supplies is sufficient, T4; T3 ratio is about 7:1. In iodine deficiency, the ratio decreases, including MIT/DIT ratio. T3 and T4 after being synthesized remains in the bound form until it is secreted. When they are secreted the peptide bonds are hydrolyzed and free T3 and T4 enter the thyroid cells, cross them and are discharged into the capillaries

THYROID ACINAR CELLS HAVE THREE FUNCTIONS

- They synthesize thyroglobulin and store as colloid in follicles
- They collect and transport iodine for synthesis of the hormones in the colloid
- They remove T3 and T4 from thyroglobulin secreting the hormones into the circulation.

TRANSPORT

Within the plasma, T4 and T3 are mostly transported almost entirely in association with two proteins, the so called thyroxine binding proteins" which act as specific carrier agents for the hormones.

Two main carrier proteins are:

- Thyroxine-binding globuline (TBG)
- Thyroxine binding prealbumin (TBPA)
- When large amount of T4 and T3 are present and the binding capacities of the above two specific carrier proteins are saturated, the hormones can be bound to serum albumin. Approximately about 0.05% of the circulating thyroxine is in the free unbound form. Free T3 and T4 are the metabolically active hormones in the plasma

MECHANISM OF ACTION OF THYROID HORMONE

- Thyroid hormones are transported into their target cells by a "carrier mediated" active transport system of the cell membrane. Target organs include; liver, kidneys, adipose tissue, cardiac, neurons, lymphocytes, etc.
  1. Nuclear Action: T4 and T3 pass into the nucleus and bind directly to specific high affinity "nuclear receptors" which are histone chromatin proteins of specific genes. This receptor hormone binding increases the action of nuclear DNA dependent RNA polymerase increasing gene transcription, which in turn enhances m-RNA synthesis and induces synthesis of specific protein and enzyme.

1. Na^{+}-K^{+}- ATPase pump:

Thyroid hormone exerts most of metabolic effects by increasing 0_2-consumption. It has been suggested that much of the energy utilized by a cell is for driving the Na+-K+-ATPase pump. Thyroid hormones enhance the function of this pump by increasing the number of pump units, almost in all cells.

- **TRANSLATION OF PROTEINS**

Thyroid hormones may stimulate translation of proteins by directly increasing the binding of amino acid t-RNA complex to ribosome or by increasing the activity of peptidyl transferase or translocase enzymes.

METABOLIC ROLE OF THYROID HORMONES

1. ***Effect on protein metabolism***

* In hypothyroid children and in physiological doses, thyroid hormones when given in small doses, favour protein anabolism, leading to N-retention (positive N-balance) because they stimulate growth.

* Large, unphysiological doses of thyroxine, cause protein catabolism, leading to negative N-balance.

CLINICAL SIGNIFICANCE

- The catabolic response in skeletal muscle in cases of hyperthyroidism is sometimes so severe that muscle weakness is a prominent symptoms and creatinuria is marked, called thyrotoxic myopathy. The K^+ liberated during protein catabolism appears in urine and there is an increase in urinary hexosamine and uric acid excretion.
- Effect on bone proteins: Mobilization of bone proteins leads to hypercalcaemia and hypercalciuria with some degree of osteoporosis.
- Effect on skin: The skin normally contains a variety of proteins combined with polysaccharides hyaluronic acid and chondroitin sulphric acid.
- Clinical significance; in hypothyroidism, these complexes accumulate, promoting water retention, which produces characteristic puffiness of the skin, when thyroxine is administered, the proteins are mobilized and diuresis continues until the puffiness (myxoedema) is cleared.

- 2. **Effects on Carbohydrate metabolism**
- Net effect on carbohydrate metabolism:
- Increase in blood sugar (hyperglycaemia), and glycosuria
- Increase glucose utilization, and decreased glucose tolerance. Thyroid hormones are therefore, antagonistic to insulin

Thyroid hormone increase the rate of absorption of glucose from intestine

Decreased glucose tolerance may be contributed to also by acceleration of degradation of insulin.

Note: Diabetes mellitus is aggravated by coexisting thyrotoxicosis or by administration of thyroid hormone.

- Increased hepatic glycogenolysis, because they enhance the activity of Glucose-6-phosphatase
- In addition there is increased sensitivity to catecholamine; they potentiate the glycogenolytic effect of epinephrine by increasing the β-adrenergic receptors on hepatic cell membrane.
- Stimulate glycolysis as well as oxidative metabolism of glucose via TCA cycle and also increasing HMP shunt. Thyroxine increases the activity of G6PD enzyme in liver.
- Thyroid hormone causes a decrease of glycogen store in the liver and to a lesser extent, in the myocardium and skeletal muscle.
- At the same time, thyroid hormones increase hepatic gluconeogenesis by increasing the activities of pyruvate carboxylase and PEP carboxykinase.

3. Effect on Lipid Metabolism

- Increase lipolysis in adipose tissue thus increasing plasma FFA. This effect is rather indirect in the sense it increase sensitive, to catecholamine, by increasing the β-adrenergic receptor on adipose cell membrane.
- They may stimulate, at the same time lipogenesis by increasing the activities of malic enzymes, ATP citrate lyase and G6 PD.
- Cholesterol despite the fact that hepatic synthesis of cholesterol and phospholipids is depressed following thyroidectomy and is increased in thyrotoxicosis, the concentration of cholesterol and to lesser extent phospholipids in plasma is increased in hypothyroidism and decreased in hyperthyroidism.
  Decreased value in hyperthyroidism is explained as follows:

  Although thyroid hormones increase the rate of biosynthesis of cholesterol, they increase

- The rate of degradation
- Increase the formation of bile acids (cholic acid/ deoxycholic) acid and
- Increase biliary excretion, to a greater extent accounting for the lowered blood concentration.

Lipoproteins

The concentration of plasma lipoproteins of Sf 10-20 class (LDL) is frequently increased in hypothyroidism and decreased in thyrotoxicosis or following administration of thyroid hormones to normal subjects

2. Calorigenic Action

Thyroid hormones increase considerably O_2-consumption and oxygen coefficient of almost all metabolically active tissues.

Exceptions are Brain, testes, uterus lymphnodes, spleen and anterior pituitary. There is increase in heat production and BMR. These effects is due to**:**

- Induction of glycerol-3-P-dehydrogenase and other enzymes involved in mitochondrial oxidation.
- More important is increased activity and increased units of Na^+-K^+ ATPase pump.

It hydrolyzes ATP for transmembrane expression of Na^+, leading to enhanced heat production, O_2-consumption and oxidative phosphorylation.

5. **Vitamins**

- Administration of large amounts of thyroid hormones increases the requirement of certain members of vitamin B-complex (thiamine, pyridoxine, pantothenic acid) and for vitamin C. There are presumably related to the stimulation of oxidative and catabolic processes.
- Thyroxine is necessary for hepatic conversion of carotene to vitamin A and the accumulation of carotene in the blood stream in hypothyroidism is responsible for yellowish tint of the skin.

PARATHYROID GLANDS AND THEIR HORMONES

The parathyroid glands are intimately concerned with regulation of the concentration of

Ca and PO_4 ions in the blood plasma. This is accomplished by secretion of a hormone parathormone (PTH) by the chief cells, the net effect of which is:

- To increase the concentration of Ca and
- decrease the PO_4.

In addition to its effects on plasma ionized Ca via its action on bone, parathormone controls renal excretion of Ca and $P0_4$.

PARATHORMONE (PTH)

Chemistry: Parathormone is a linear polypeptide consisting of 84 amino acids. N-terminal amino acid is alanine and C-terminal is glutamine. Bovine PTH has Mwt of 9500. PTH from different spices differs only slightly in structure.

CORE OF ACTIVITY

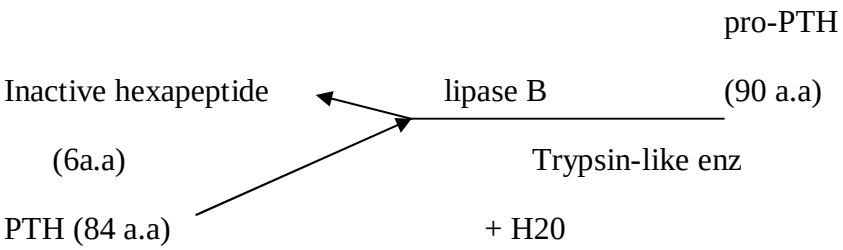
Studies on the synthetic PTH indicate that the amino acid sequence 1 to 29 or possibly 1 to 34 from N-terminal end is essential for the physiologic actions of this hormone on both skeletal and renal tissues.

Methionine is important amino acid and necessary for calcium mobilization effect. The N-terminal end up to 34 amino acids possesses the receptor binding ability.

Biosynthesis

PTH is initially synthesized in chief cells as a pro-hormone.

Pre-pro-PTH (115 a.a) —signal peptidase, +H2O→ inactive peptide (25 a.a)



- Pre-pro PTH: consisting of 115 amino acids is first formed in polysomes adhering on the rough ER membrane
- Pro-PTH: before the formation of Pre-pro PTH is completed its N-terminal end protrudes into the lumen of rough ER and a signal peptidase of rER membrane hydrolyzes the molecules to split off 25 a.a and thus pre-pro PTH is changed to pro-PTH having 90 amino acids.
- PTH: pro-PTH is transferred to rER lumen end moves to Golgi cisternae. A trypsin like enzymes called lipase B hydrolyses its N terminal amino acids and remove 6 amino acids rich in basic amino acids and thus converting pro-PTH to PTH.
  PTH thus formed is packaged and stored in secretory vesicles. Increased c-AMP concentration and a low Ca^{2+} level stimulates it release from secretory vesicles. On the other hand, a high concentration at Ca^{2+} stimulates the degradation of the stored PTH in secretory vesicles instead of it release.

MECHANISM OF ACTION

PTH increases serum Ca^{2+} level by acting on bones kidney and intestines.

a. Increasing cAMP level: PTH bind to specific receptor on the plasma membrane of bone cells, renal tubules cells, it activates the adenyl cyclase to form c-AMP in the cells. C-AMP acts as the "second messenger" which activate specific C-AMP dependent protein kinase which phosphorylate and thereby modulate the activities of specific proteins in the bone cell and kidney cells.
b. Role of Ca^{2+}: c-AMP also increases the Ca^{2+} concentration in these cells, which in turn may act as a messenger to modulate the activities of some intracellular proteins.
c. PH change in tissue: the hormone increase the amounts of both lactic and citric acid in the tissues and both of these acids may act to aid bone resorption.

METABOLIC ROLE OF PTH

The actions of PTH are reflected in the consequences of:

- its administration and
- removal of the parathyroid glands

A. The most conspicuous metabolic consequences of administration of PTH are:

- increase in serum Ca^{2+} concentration
- Decrease in serum inorganic PO_4 concentration.
- Increased urinary PO_4
- Removes Ca from bones particulars if dietary intake of Ca is inadequate.
- Increase in citrate content of blood plasma, kidney and bones
- Activates Vit. D in renal tissue by increasing the rate of conversion of 25-OH-Cholecalfiferol to 1, 25-di-OH-cholecalciferol, by stimulating α-1-hydroaylase enzymes
- Effect on Mg metabolism
  PTH has been reported to exert an influence on Mg metabolism. Primary hyperparathyroidism has been found to be associated with excessive urinary excretion of Mg and negative magnesium balance.

B. Actions on different Organs:

a. Action on kidneys: PTH acts through by increasing c-AMP. PTH binds to specific receptors on plasma membrane of renal cortical cells of both proximal and distal tubules and stimulates adenyl cyclase to produce c-AMP. c-AMP then is transported to apical/luminal part of the cell where it activated c-AMP dependent protein kinase, which phosphorylates specific proteins of the apical membrane to affect the several mineral transport across the membrane.

- PTH decreases the Trans-membrane transport and reabsorption of filtered Pi in both proximal and distal tubular cells and increases the urinary excretion of in organic phosphate (Posphaturia effect).
- Fall in serum PO_4 level leads to mobilization of PO_4 from bones, which also mobilizes Ca^{2+} along with it, resulting to hypercalcaemia.
- PTH stimulate α-1-hydroxylase enzyme located in mitochondria of proximal convoluted tubule cells, which converts 25-OH cholecalciferol to 1, 25-di-OH cholecalciferol which in turn increases the intestinal and renal absorption of Ca^{2+} resulting to hypercalcaemia..
- PTH inhibits the transmembrane transport of K^+ and HCO_3 to decrease their reabsorption by renal tubules.
- PTH increases the transmembrane transport and reabsoption of filtered Ca^{2+} in the distal tubules resulting initially to decrease urinary excretion of Ca^{2+}. But later on, PTH induced hypercalceamia enhances the amount of filtered Ca^{2+} which increases the renal excretion.

b. Action on Bones

PTH binds to specific receptors present on membrane of osteoclasts, osteoblast and osteocytes and increases c-AMP level in these cells which act through c-AMP dependent protein kinases.

Following actions are seen.

- Osteoclastic activity: it stimulates the different action and maturation of precursors cells of osteoclasts to mature osteoclasts.
- Osteoclastic osteolysis: PTH stimulates the osteoclasts through "second messenger" c-AMP to increase the resorption of bones which enhances mobilization of Ca and P from bones.
- Osteocytic osteolysis: PTH also stimulates osteocytes which increase bone resorption thus mobilizing Ca^{2+} and Pi; there occurs enlargement of bones lacunae.
- Action on alkaline posphatase:

Alkaline phosphatase activity varies as per PTH concentration. At low concentrations, PTH stimulates the sulfation of cartilages and increases the number of osteoblast and alkaline phosphates activities of bone osteoblasts. At higher levels of physiological concentrations, PTH inhibits alkaline phosphatase activity and collagen synthesis in osteoblast and decreases the Ca^{2+} retaining capacity of bones. PTH induced rise in intracellular c-AMP in osteoclast and osteocytes leads to secretion of lysosomal hydrolases and collagenases which increase breakdown of collagen and MPS in bones matrices.

C. Action an intestinal mucosa

PTH does not act directly on intestinal mucosal cells as the cells do not possess the specific receptors for PTH. But it increases the absorption of Ca^{2+} and PO_4 through production 1, 25-dioH cholecalciferol (Calcitriol).

CALCITONIN

Calcitonin is a calcium regulating hormone. It is proved that calcitonin originates from special cells, called c-cells, parafollicular cells. C-cells constitute an endocrine system which are derived from neural crest and are found in thyroid, parathyroids and in thymus.

CHEMISTRY

Calcitonin is a single chain lipophilic polypeptide having a most 3600. As many as four separate active fractions have been isolated and they have been designated as α, β, γ and δ-calcitonin. Amino acids sequences of calcitonin have now been established. It contains 32 amino acids; N-terminal amino acid is cysteine, and C- terminal prolinamide. An inter chain disulphide bridge joins two cysteine residue between position I and 7. Low number of ionizable groups are present, 5 of 6 possible – COOH groups been amidated. Isoleucine and lysine are absent conspicuously from the molecule. There is high content of Aspartic acid and threonine.

MECHANISM OF ACTION

1. Role of c-AMP: Calcitonin binds to specific calcitonin receptors on the plasma membrane of bone osteoclasts and renal tubular epithelial cells, activates adenyl cyclase which increase c-AMP level which mediates the cellular effect of the hormone. This is the principal mechanism by which calcitonin acts.
2. Celluar Shift: It has been suggested that calcitonin may directly affect the relative distribution of bone cells. The hormone both in vitro and in vivo produced a celluar shift, in which the number of osteoclasts decreased.
3. PH change: Calcitonin may regulate PH at cellular level producing more alkaline medium which diminishes resorption.

METABOLIC ROLE

Calcitonin acts both on (a) bone (b) Kidneys. Indirectly, the effects on these two organ systems account for.

Hypocalcaemia and hypophosphataemia

a. **Action on Bones**

- Calcitonin inhibits the resorption of bone by osteoclasts and thereby reduced mobilization of calcium and inorganic PO_4 from bones into the blood.
- It also stimulates influx of phosphates in bone
- There is decrease in activites of lysosonal bydrolases, pyrophosphatases and alkaline phosphates in bones.
- Decrease in collagen metabolism and decreased excretion of urinary OH-proline
- Whether or not calcitonin promotes bone formation is uncertain and controversial. But it has been established that the hormone in addition to causing a decrease in number of osteoclasts, it increase osteoblsat cells, which are thought to be involved in bone laying.

b. **Action on kidneys**

- The hormone acts on the distal tubule and ascending limb of loop of Henle and decrease tubular reabsorption of both calcium and inorganic phosphate thus producing calcinuria and phosphaturia.
- The hormone inhibits α-1-hydroxylase and inhibits synthesis of 1-25-d-OH-D3 thus decreasing calcicum absorption from intestine.
- Both the above effects account for hypocalcaemia.

INSULIN

Insulin is a protein hormone, secreted by β-cells of islets of Langerhars of pancreas. It plays an important role in metabolism causing increased carbohydrate metabolism glycogenolysis/ and glycogen storage, FA synthesis/TG storage and amino acid uptake/ protein synthesis. Thus insulin is an important anabolic hormone which act on variety of issues. Major target tissues of insulin are the muscles, liver, adipose tissues and heart.

Note: RBC, GI tract epithelial cells and renal tubular epithelial cells are rather generally unresponsive to insulin.

CHEMISTRY

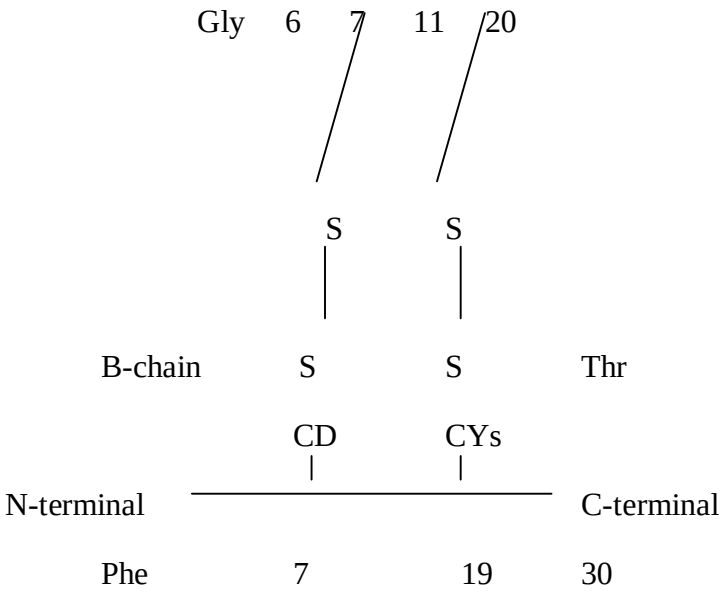
Insulin is a heterodimeric protein; it has been isolated from pancreas and prepared in crystalline form. For crystallization it requires zinc is also a constituent of stored insulin and normal pancreatic tissue is relatively rich in zinc. Insulin molecule is composed of two polypeptide chains, called A-chain and B-Chain, containing total of 51 amino acids. A-chains contains 21 amino acids and B-chain contains 30 amino acids. In A-chain, N-terminal amino acids is phenylalaine and C-terminal is threonine.

DISULFIDE BRIDGES

Both the chains are held together by two s-s-linkage. Cys 7 end Cys 20 of A chain are joined to Cys7 and Cys 19 of B chain respectively. In addition, the A-chain carries as intra-chain" s-s linkage between cyc 6 and cys 11.

A-chain S-S Cys Asn

N-terminal end ——————————— C-terminal end



INSULIN FROM OTHER SPECIES

Porcine insulin: Porcine insulin is similar to human insulin. It differs by only terminal amino acid No-30 of B-chain.

- In human: It is threonine
- In porcine: it is alanine in place of threonine. Removal of alanine (de-alaninated) retains the biological activity.

Note: De-alaninated insulin has been used in treatment of diabetes mellitus because of it low antigencity.

- Human insulin has been produced by recombinant DNA technology.

Important of S-S Bridges

Breaking of the disulfide bonds with alkali or reducing agents inactivate insulin. Digestion of insulin protein with proteolytic enzymes also inactivates the hormone; hence insulin cannot be given orally. Minimum calculated Mwt is 5734. Insulin can exist in different polymeric forms (dimmer, trimer etc) depending on pH, temperature and concentration.

BIOSYNTHESIS OF INSULIN

In biosynthesis of insulin, first “prepro-insulin” is formed which is converted to pro-insulin. The latter is finally converted to insulin.

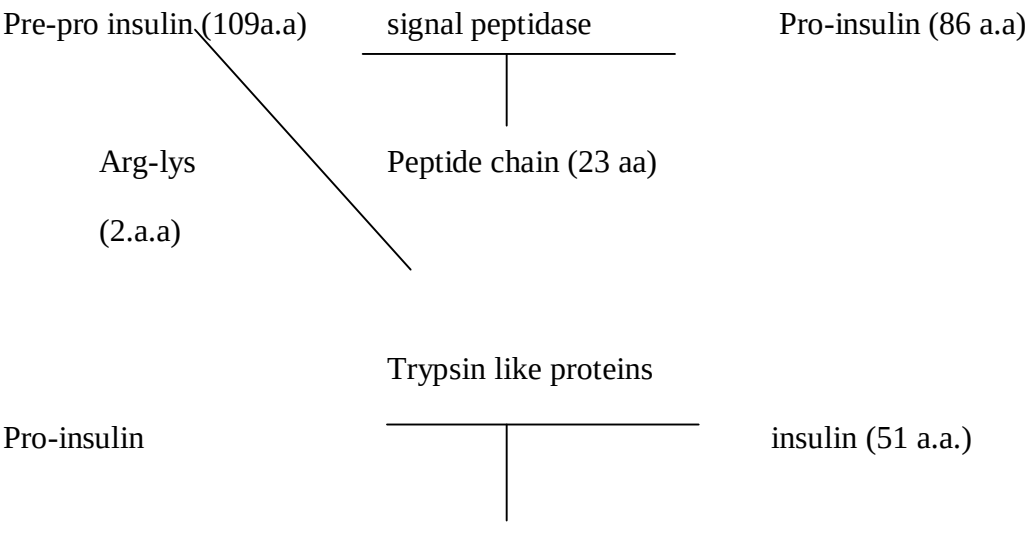
1. Synthesis of pre-pro insulin: Pre-pro insulin is syntheses in polysomes, attached to the membrane of rough endoplasmic reticulum in β-cell of islet of Langerhans. It is a polypeptide consisting of 109 amino acids, Mwt= 11,500
2. Conversion of pre-pro insulin to proinsulin

- Pre-proinsulin after synthesis is transferred to lumen of rER cisternae.
- A peptide chain consisting 23 amino acids in its N-terminal called leader sequence is split by an enzyme called signal peptidase present in the membrane of rER and pro-insulin is formed.
- Pro-insulin has 86 amino acids Mwt = 9000

3. Conversion of pro-insulin to insulin

Pro-insulin containing small vessicles are detached from ER and fuses with cisternae of Golgi apparatus.

- In the Golgi cisternae, Proinsulin is acted upon by a trypsin-like protease which hydrolyzes the peptide chain at two sites, so that an inactive connective C-peptide is liberated and two active peptide chain are left which forms the A and B chain.
- A carboxypeptidase B like enzymes splits the C-terminal peptide bonds in the two intermediates to release two C-terminal basic amino acids from each of them viz "Arg-63-lys 62" to form 'A' chains and "Arg 31-Arg 32" to form 'B' chain. C-peptide which is split off has 31 amino acids.
- Condensing vacuoles are pinched off from Golgi cisternae with equimolar amounts of insulin and C-peptide in their lumen. Insulin molecules form dimmers by hydrogen bonding between the peptide groups of phe 24 and tyr 26 residues of their B-chains. Gradually with increasing concentrations, condensing vacuoles change into secretory granules. In them insulin forms crystalloid forms of hexamers with two Zn^{2+} .C-peptides remain in the fluid surrounding the crystalloid granules.



Carboxy peptidase B

|

Arg-Arg (2 a.a)

Note: Pro-insulin is comparatively inactive biologically, but it can cross-react with antisera prepared against insulin.

- Plasma pro-insulin is not elevated in human diabetes or in normal after glucose stimulation, but it may be the predominant circulating form in some subjects with islet cell tumours.

CATABOLISM OF INSULIN

Insulin is very rapidly catabolised. Its plasma t½ is less than 3-5 minutes under normal conditions. Major organs where insulin is catabolised are liver, kidneys, and placenta.

About 50% of insulin is degraded in its single passage through the liver.

Mechanism

Two enzyme systems are involved for degradation of insulin

- Protease: an insulin-specific protease has been found in many tissues with highest concentration in liver and kidneys. The protease is-SH dependent and active at physiological PH.
- Second mechanism is more important. The enzyme is glutathione-insulin transhydrogenase (also called insulianse). This enzyme is found in higher concentration in liver and kidneys. Also present in skeletal muscles and placenta. This brings about reductive cleavage of the insulin molecule. Reduced glulathione (G-SH), acting as a co-enzyme for the transhydrogenase, donates the H-atoms for the reduction and is itself thus converted to oxidized glutathione.
- After insulin is reductively changes, the A-chains and B-chains are further hydrolyzed by proteolysis.

INSULIN RECEPTORS

Insulin acts on target tissue by binding to specific insulin receptors, which are glycoproteins. The human insulin is found on chromosome 19. The insulin receptors are being constantly

synthesized and degraded. Their t½ is 6-12 hrs only. It is synthesized as a single chain polypeptide pro-receptor" in the rER and is rapidly glycosylated in Golgi region. The "pro-receptor" has 1382 amino acids and most 190,000.

The pro-receptor is cleared to form mature "α" and "β" subunits ($\alpha_2\beta_2$) which is a heterodimer, linked by S-S bonds. Both subunits are extensively glycosylated and removal of sialic acid and galactose decreases insulin binding and insulin action. Insulin receptors are found in target cell membrane, up to 20,000 per cell.

Binding of insulin to the receptor, stimulates its, tyrosine kinase activity. Tyrosine kinase enzyme phosphorylates the phenolic –OH group of tyrosine residues in specific protein including that of a tyrosine in the β-chain of insulin receptor itself to modulate their activities, ATD + tryrosineprotein – ADP+ phosphor-tyrosine protein.

Regulation of insulin receptors

High blood insulin level decreases the number of insulin receptors on target cell membrane, probably through internalization of the insulin-receptor complex into the cell and thus decreases the insulin sensitivity of the target tissue.

MECHANISM OF ACTION OF INSULIN

When insulin binds to the specific receptor several events of actions take place.

- A conformational change of the receptor
- The receptor crosslink and form microaggregates
- The receptor complex is internalized and
- One or more signals is generated

But nature of the intracellular signal and intracellular second messenger" remains still uncertain and vague.

Various mechanisms have been proposed.

1. ***Role of c-AMP***: It is proposed that insulin promotes the phosphorylation of c-AMP phosphodiesterase. The active phosphodiesterase hydrolyses c-AMP and lowers the c-AMP level in the cells. The consequent fall in activities of c-AMP dependent protein kinase reduce phosphorylation of specific enzymes.

2. ***Role of c-GMP***: The insulin receptor binding may activate guanylate cyclase which forms c-GMP. Increased concentration of c-GMP act as second messenger" to activate c-GMP dependent protein kinase. These may phosphorylate some enzymes to modulate their activities

3. ***Role of protein phosphatase***: Insulin may act through the protein phosphates I which may dephosphorylate certain key enzymes thereby activating them. Best examples are the key enzyme glycogen synthase and pyruvate dehydrogenase complex. On the other hand inhibits phosphorylase enzyme and triacylglycerol lipase.

4. ***Action through tyrosine kinase***" Activity of β-subunit Receptor: The binding of insulin to its receptor enhances tyrosine kinase activity. Tyrosine kinase in turn phosphorylates pheolic-OH group of tyrosine residues of specific proteins leading to changes in enzyme activities.

5. **Role in mRNA translation**: Insulin is known to affect the activity or amount of at least more than 50 proteins in variety of tissue and many of these effects involves covalent modification. A role of insulin in the translation of mRNA has been proposed largely based on studies of ribosomal protein 6S, a component of the 40S ribosomal unit. Such a mechanism accounts for the general effect of insulin on protein synthesis in liver, heart muscle and skeletal muscles.

6. **Role on gene expression (Nuclear action):** insulin also affects the rate of transcription of specific genes, thereby regulates the synthesis of specific m-RNAs and thus changing the rate of synthesis of specific protein coded by them. e.g insulin decreases the transcription of gene involved in synthesis of the enzyme phosphoenol-pyrurate carboxy kinase (PEPCK), the key enzyme for gluconeogenesis. On the other hand insulin induce the synthesis of phosphofructokinase and pyruvate kinase required for glycolysis, by increasing the transcription of these genes.

METABOLIC ROLE OF INSULIN

A. Action on carbohydrate metabolism

Net effect is lowering of blood glucose level and

Increase glycogen store

The above is achieved by several mechanisms.

1. Increase glucose uptake:

- Insulin increases glucose uptake from extracellular fluid by the various tissues viz, muscles, adipose tissue, mammary glands, lens, etc.
- In adipose tissue and other extrahepatic tissues, insulin stimulates translocation of glucose transporters from their intracellular pool in Golgi cisternae to the plasma membrane where they participate as carrier in transportation of D-glucose and D-galactose across the membrane.
- Also in hepatocytes, insulin increases hepatic uptake of glucose (freely permeable to liver cells) it induces the synthesis of the enzyme glucokinase which simulataneously phosphorylates glucose, thereby lower intracellular concentration.

2. Increases glycolysis: increase utilization of glucose for providing energy which takes place in muscles, liver and many other tissues. Insulin enhances glycolysis because it induces the synthesis of key enzyme phosphofructokinase and also pyruvate kinase.
3. Increase conversion of pyruvate to acetyl -107 insulin increase aerobic oxidative decarboxylation of pyruvate to acetylcoa, because it causes dephosphorylation of pyruvate dehydrogenase complex which is thus converted to the form.
4. Stimulate glycogenesis: insulin stimulates glyogenesis in the liver and muscles by increasing dephosphorylation of the key and rate limiting enzyme, glycogen synthase, thus converting it to its active form. Insulin stimulates the protein phosphatase-1 directly, which brings about dephosphorylation.
5. Decrease Gluconeogenesis: Insulin reduces gluconeogenesis:

- By repressing the synthesis of the key rate limiting enzyme phosphoenol pyruvate carboxykinase (PEPCK) by decreasing the transcription rate of the gene.
- Also inhibits allosterically fructose-1 6-biphosphatase another key enzyme for gluconeogenesis.
- Insulin dephosphorylates fructose 2,6-biphosphatase so that it is converted to inactive form, which increases the concentration of fructose 2-6-biphosphate in the cell, which inturn allosterically inhibit fructose -1,6-biphosphatase.

6. Decrease glycogenolysis: insulin decreases glycogeneolysis.

- By dephosphorylating the key and rate limiting enzyme glycogen phosphorylase thus converting it to inactive form

- Also represses the enzyme glucose-6-phosphatase.

B. Action on lipid Metabolism

Net effects are lowering of free fatty acid level and increase in triglyceride store.

The above is achieved as follows:

1. Decrease lipohysis: Insulin decreases lipolysis in adipose tissue cells and consequently lower plasma FFA. Lipolysis is reduced due to

- Insulin activates phosphoprotein phosphatase which dephosphorylates the triacylgly cerolipase and thus converted to inactive form.
- At the same time, insulin activates phosphodiesterase which degrades c-AMP and prevent phosphorylation and reactivation of TG lipase

2. Increases fatty acid synthesis: Insulin increases the extramitochondrial denovo fatty acid synthesis by making available of more substrate acetyl CoA and also increasing the activity of acetylcoA carboxylase.

The above is done as follows:

- Insulin promotes dephosphorylation of pyruvate dehydrogenase complex and converts into active form so that more acetyl CoA is available from pyruvate
- Insulin induces the synthesis of ATP-citrate lyase to increase cleavage of citrate, so that more acetyl-CoA is available in cytosol.
- Insulin lowers the plasma FFA level, so prevent long chain acyl-CoA from inhibitory acetyl-CoA carboxylase.
- It induces the synthesis of acetyl-CoA carboxylase and fatty acid synthase, the cytosolic enzymes required for FA synthesis.
- Insulin activates acetyl-CoA carboxylase by dephosphorylation of the enzyme (Converting to active form).
- Provides more NADPH for the reductive steps in FA synthesis by stimulating HMP-shunt pathway.

3. Increase synthesis of TG: Insulin enhances TG synthesis in adipose tissues by:

- Proving more α-glycerol-P as glucose uptake and utilization is enhanced in adipocytes.
- Increased synthesis of FA provides the acyl CoA (FFA pool 1) required for TG synthesis.
- Insulin also induces the synthesis of lipoprotein lipase. This enzyme hydrolyzes TG of circulating chylomicrons and VLDL and releases FFA (FFA pool 2) which are taken up by the adipocytes and used for TG synthesis.

4 Decreases ketogenesis: As plasma FFA level is decreased less is oxidized by β-oxidation and less acetyl-CoA will be available for cholesterol synthesis and ketogenesis.

C. ACTION ON PROTEIN METABOLISM

Net effect is insulin promotes protein synthesis.

This is achieved as follows:

- Insulin increases amino acids uptake by the tissues by enhancing the rate of synthesis of membrane transporters for amino acids.
- Adequate supply of insulin is necessary for protein anabolic effect of growth hormone (permissive effect)
- Insulin increase protein synthesis by proving more amino acids in cells, by affecting gene transcription (nuclear level) by regulating specific m-RNA synthesis and affecting translation at ribosomal level.
- Regulation of ribosomal translation is done by two ways:
- Increase the synthesis of polyamines-required for ribosomal RNA synthesis, by increasing the synthesis of key and rate limiting enzyme ornithine decarboxylase.
- Secondly, insulin modulates ribosomal activity by causing phosphorylation of 6S ribosome (α component of 40S)

d. Action on mineral Metabolism

Decrease in concentration of K^+ and inorganic P in blood due to enhanced glycogenesis and phosphorylation of glucose.

e. Actions on growth and cell Duplication

Insulin stimulates growth in vivo and also cell proliferation in vitro. Cultural fibroblasts have been used most frequently in studies of cell proliferation. It has been found that insulin potentiates the ability of fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and epidermal growth and cell proliferation are seen in many tissues such as liver, mammary glands and adrenals and also in embryogenesis and tissue differentiation. There effects are largely due to stimulation of DNA replication, gene transcription, protein synthesis and modulation of various enzyme activities through phosphorylation dephosphorylation.

GLUCAGON HYPERGLYCAEMIC-GLYCOGENOLYTIC FACTOR

Glucagon is a hormone produced by α –cells of islet of Langerhan of pancreas and is an important hormone involved in

- Rapid mobilization of hepatic glycogen to give glucose by glycogenolysis and
- To a lesser extent FA from adipose tissue.

Thus it acts as a hormone required to mobilize metabolic substrates from storage depots.

CHEMISTRY

Glucagon has been purified and crystallized from pancreatic extracts and also the hormone has been synthesized. It is a polypeptide containing 29 amino acids. There are only 15 different amino acids in the molecule. Amino acid sequence has been determined, histidine is the N-terminal amino acid and threonine is the C-terminal. Mwt is approx 3485.

Unlike insulin

- It does not require zinc or other metals for it crystallization.
- Glucagons contains no cystine, proline, isoleucine but contains tyrosine, methionine and tryptophan

SYNTHESIS

It is synthesized first as a pro-hormone, proglucagon in α-cells. Lysosomal enzyme peptidase like carboxy-peptidase B and trypsin-like peptidase in α-cells hydrolyze pro-glucagon from both its N-terminal end and c-terminal end to yield glucagon and inactive peptides.

ENTERO-GLUCAGON OR GLUCAGON-LIKE IMMUNE REACTIVE FACTOR.

A glucagon-like immuno reactive factor (GLI) has been identified in gastric and duodenal mucosa. GLI is immunologically similar though not identical to the pancreatic hormone. Moreover, it is less active than pancreatic glucagons in stimulating adenyl cyclase and therefore cannot duplicate many of the function of pancreatic hormone. GLI is stimulated by absorbed glucose causing an apparent elevation of circulating pancreatic glucagons.

Recently, two different molecular fractions have been isolated:

- One having mol.wt =3500, has hyperglycaemic and glycogenolytic activity but far less potent than pancreatic glucagons.
- The other fraction, mol.wt=2000; devoid of the above activity.

Both have insulin releasing activity

MECHANISM OF ACTION

Glucagon binds to specific receptors on the plasma membranes of hepatocytes and adipocytes and activates adenyl cyclase to produce c-AMP in these cells, which is the principal "second messenger" and duplicates the functions of the hormone. C-AMP in turn activates c-AMP dependent protein kinases which further phosphorylates specific enzymes to increase/decrease their activities. C-AMP also induces synthesis of certain specific enzymes like glucose-6-phosphatases by increasing the transcription of their genes.

METABOLIC ROLE

1. Action on carbohydrate metabolism
   Net effect of the hormone is to increase the blood sugar level (hyperglycaemia). Hyperglycaemic effect is due to various causes.

- Glycogenolysis: glucagons increases glycogenolysis in liver. In muscles, it cannot bring about glycogenolysis as muscle cell membrane lacks the glucagons specific receptors glucagons also induces the synthesis of glucose-6-phosphatase enzyme.
- By increasing gluconeogenesis in liver: glucagon stimulates the conversion of lactic acid and glucogenic amino acids to form glucose.
- The increased hepatic c-AMP produced after glucagon action has been shown to increase protein kinases that catalyze nuclear histone phosphorylation in liver cell nucleus. This reaction inhibits the repressive effect normally exerted by histones on DNA and allows the initiation of a sequence of events leading to the synthesis off new enzyme proteins involved in gluconeogenesis. Thus, glucagons induces the synthesis of phosphoenol pyruvic carboxykinase, pyruvate carboxylase and fructose -1-6-biphosphatase enzyme, all key enzymes of gluconeogenesis.
- Also glucagon increases the pool of glucogenic amino acids in liver, so that they can be used for gluconeogenesis. This is achieved by increasing protein breakdown in liver and by reducing hepatic protein synthesis.

2. **On lipid metabolism**

- Lipolysis: In adipose tissue and also possibly in liver, glucagon increases the breakdown of TG to produce FFA and glycerol. FA undergo β-oxidation, increased breakdown may lead to ketone bodies formation and ketosis. Thyroid hormones help in the lipolytic action of glucagon, probably the hormones increases the number of glucagon specific receptors on adipocytes.
- Anti-lipogenic Action: Glucagon reduces F.A. synthesis. This is achieved in 2 ways:
- Increased lipolysis raise the concentration of FFA in blood. Long-chain acylCoA inhibits the rate limiting enzyme acetyl-CoA carboxylases.

- Increased c-AMP level in cells activates c-AMP dependent protein kinase which phosphorylates acetyl-CoA carboxylase. Phosphorylated form of the enzyme is inactive.

3. On protein metabolism

- Glucagon reduces protein synthesis by depressing incorporation of amino acids into peptide chains. This may be due to the inactivation of some ribosomal component by a protein kinase whose activity is enhanced by glucagon-induced rise in c-AMP.
- Glucagon also stimulates protein catabolism especially in liver thus increases the hepatic amino acid pool which is utilized for gluconeogenesis. Also increases urinary NPN and urea.

4. Action on Heart: Glucagon exerts a positive ionotropic effect on heart without producing increased myocardial irritability. Hence, use of glucagon in treatment of heart disease, viz in cardiac failure and cardiogenic shock.
   Advantage over non-epinephrine: glucagon increases the force of contraction, but does not produce any arrhythmias, tachycardia or increase in 0_2 consumption.

5. Calorigenic effect: glucagon increases heat production and rise in BMR. The calorigenic action is not due to hyperglycaemia perse but is probably due to increased hepatic deamination of amino acid, with thyroid hormones stimulating the utilization of deaminated residues. The calorigenic action requires the presence of thyroid and adrenocortical hormones and fails to occur in their absence.

6. **On mineral metabolism**:

   Potassium: glucagon increases K^+ release form the liver, an action which may be related to its glycogenolytic activity

   Calcium: Glucagon can increase the release of calcitonin from the thyroid.

SOMATOSTATIN

The peptide somatostatin (growth hormone release inhibiting factor) was first isolated from the hypothalamus and was implicated as a regulator of growth hormone secretion.

CHEMISTRY

It is a peptide consisting of 14 amino acids. There is an intrachain S-S linkage joining cysteine 3 and cysteine at position 14

Sources: there are three sources

- Hypothalamus
- Pancreas; somatostation is also secreted by δ-cells of islet of Langerhans of pancreas.
- GT tract: it is also produced by D-Cells of antral mucosa of stomach and also duodenal mucosa.

a. ***Hypothalamic somatostotin***

- Acts as a regulator of growth hormone secretion
- It inhibits growth hormone (GH) release
- It may also serve as a neurotransmitter

b. ***Pancreatic somatostatin***

- It inhibits both insulin and glucagon secretion and thus may serve as an intraislet regulator of secretion of these hormones. Thus act as intaorgan "synaptic transmitters" or neuromodulators.
- Somstostatin is secreted into the portal vein blood as a result of glucose or amino acid stimulus indicating extra-islet role
- Also directly inhibit secretion of both HCO_3^- and enzymes in pancreatic juice.

c. G.I. somatostatin

- Inhibit the secretions of gastrin, CCK and motilin.
- Also inhibits gastric acid secretion, secretion of Brunner's glands pancreatic HCO_3^- and enzymes secretions gastric emptying and gall bladder contraction.
  Since somatostatin can inhibit a variety of G.I functions (gastric emptying, G.T. motility), its major function may be to regulate nutritional influx at the level of GI tract.

ADRENAL STEROID HORMONES

Steroid Hormones Produced by Adrenal cortex about 50 steroids has been isolated from the adrenal cortex. But out of them only 7 (seven) are important and known to possess physiologic activity. They are all arrived from cholesterol which can be synthesized from active acetate and they contain the steroid nucleus, called cyclopentano perhydro phenanthrene nucleus. Seven important hormones are:

- 11-dehydro corticosterone (DOC)
- Cortisone
- Cortisol (17-OH cortisosterone)
- Aldosterone (mineralocorticoid)
- Androstenedione
- Dehydroepiandrosterone

Cortisol is the major free-circulating adrenocortical hormone (glucocorticoid) in human plasma.

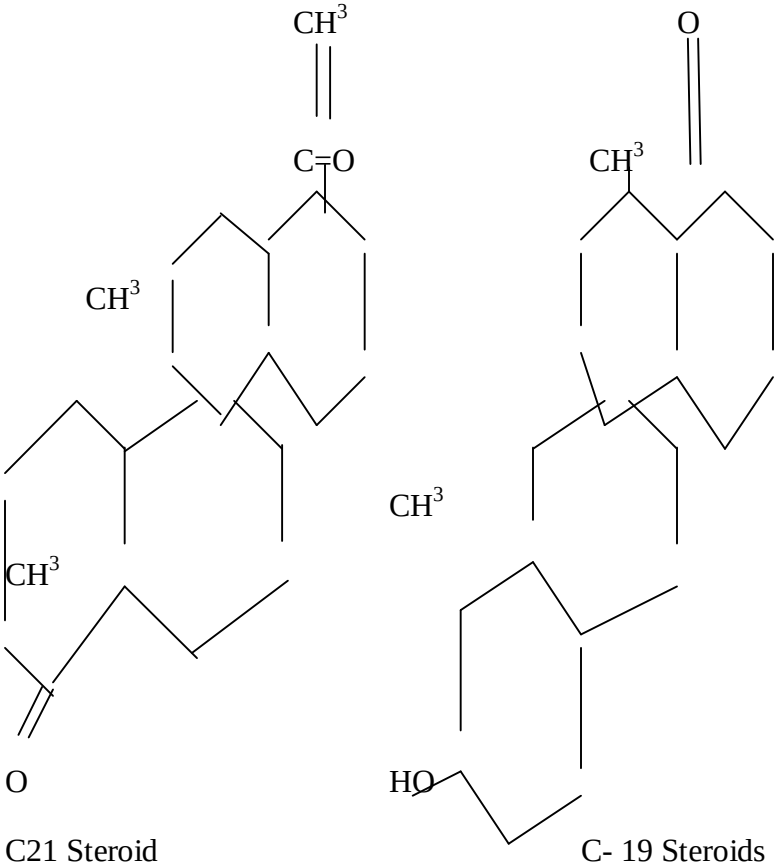
CLASSIFICATION

1. According to structure: Adrenocortical hormones are mainly of two structural types.

- C-21 steroids: those which have a two carbon side chain at position 17 of the D-ring and contain total 21 carbon atoms.
- C-19 steroids: Those which have on 0_2 atom or OH group at position 17 and contain 19 carbon atoms. Most of the C19 steroids have oxygen atom at position 17 and are therefore called as 17- oxosteroids (17-ketosteroids).
- Note: The C-21 steroids which have a –OH group at the position 17, in addition to the side chain are often called 17-OH corticoids or 17-OH corticosteroids

In general

- C- 19 steroids have androgenic activity and
- C-21 steroids have glucocorticoids and mineralocorticoids activity.



C21 Steroid C- 19 Steroids

According to function: Steriods are divided into three types according to function:

- Glucocorticoids: which primarily affect metabolism of carbohydrates, proteins and lipids and relatively minor effects on electrolytes and water metabolism e.g.cortisol, cortisone, corticosterone
- Mineralocorticoid are those which primarily affect the reabsorption of Na+ and excretion of K+ (mineral metabolism) and distribution of water in tissues e.g Aldosterone (chief mineralocorticoid). Others are cortisosterone, 11-deoxycortisol and 11-deoxycorticosterone
- Cortical sex hormones (Androgens and ertrogen) primarily affect secondary sex characters.

Relation of structure with functions:

1. Three structural features are essential for all known biological actions of the natural C21 adrenocortical hormones:

* a double bond of C4 and C5

* a ketonic group (C=O) at C3 and

* a ketonic group (C=O) at C20

2. Certain additional structural features have a profound effect upon the biological activity of these compounds:

* An-OH group at C21 enhance Na-retention and is required for activity in carbohydrate metabolism.

* The presence of 'O' either as –OH group or as O group, i.e hydroxyl or ketonic group of C11 is necessary for carbohydrate activity and decreases Na+ retention.

* An-OH group at C12 increases carbohydrate activity.

* A-CHO group at C18 necessary for mineral corticoid activity.

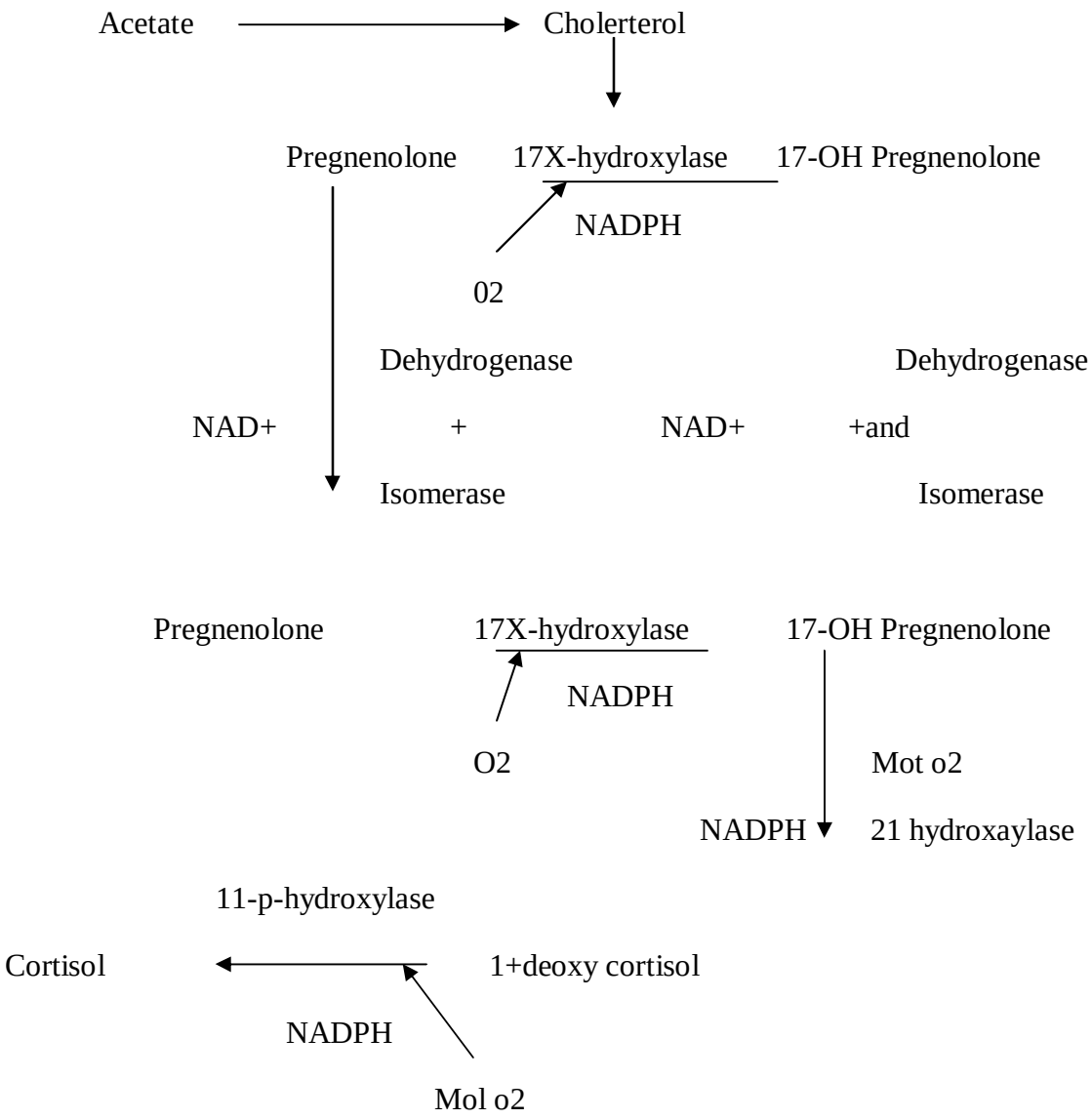
GLUCO CORTICOIDS

1. Biosynthesis of glucocorticoids:

   Common pathway for all cortico-steroids:

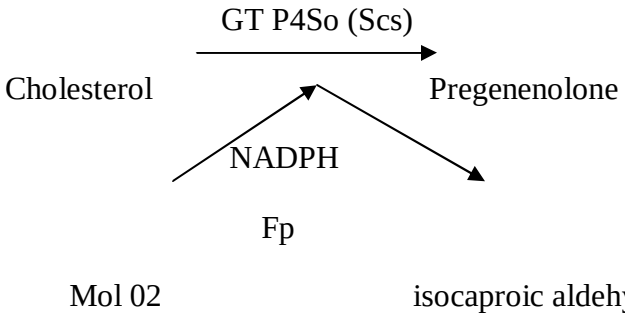
   Corticosteroids are synthesized by a common pathway from cholesterol in the adrenal cortex.

In all the three zones of adrenal cortex,



- cholesterol is first changned to form pregneolone (common pathway) from this, free cholesterol is released in the cytosol from cholesterol esters of cytoplasmic lipid droplets and transferred into mitochondria. An enzyme called cytochrone-P- 450-side chain cleavage enzyme (P450 sce) prevent in inner mitochondrial membrane hydroxylates cholesterol at (22 and C20 (also called 20, 22-desmolase) and then cleaves the side chain to form pregnenolone and isocaproic aldehyde. The enzyme

requires molecule O_2 and NADPH like all monoxygenases and also require FAD containing FP, and Fe_2S_2 protein (called adrenodoxin).



Glucocorticoid synthesis;

Glucocorticorids are synthesized in zona fasiculata cells.

ACTION OF ACTH ON CORTISOL FORMATION

ACTH stimulates the synthesis and secretion of glucocorticoids. It acts in several ways:

- Increase the availability of free cholesterol in fasciculata cells. This is achieved in two ways: through cyclic-AMP, activates the enzyme cholesteryl esterase which hydrolyzes cholesterol esters and increase free cholesterol in cells.
- Increase transfer of free cholesterol from plasma lipoproteins into fasciculata cells, probably by increasing lipoprotein receptors on plasma membrane of fasciculata cells.
- ACTH increases the conversion of cholesterol to pregnenolone, the rate limiting step.
- ACTH also stimulates the HMP_shunt pathway by increasing the activity of G-6-P D and phosphogluconate dehydrogenase. So that more NADPH is provided which is required for hydroxylation reactions.
- ACTH also increases the binding of cholesterol to mitochondrial cyt P450 necessary for hydroxylation reactions.

MECHANISM OF ACTION

All of the steroids act primarily at the level of cell nucleus (nuclear action) to increase m-RNA synthesis and increases protein synthesis.

- The first step occurs within minutes, which involves the binding of the steroids to a corresponding specific receptor protein present in cytosol.
- Glucocorticoids pass into target cells through plasma membrane and binds to specific glucocorticord receptor proteins present in cytosol.

The receptors occur in a wide variety of target tissues, viz liver, muscles, adipose tissue, lymphoid tissue, skin, bone, fibroblast etc.

Types of receptors: In humans, there are two types of receptor proteins.

α-form; containing approx 777 amino acids.

β- form having 742 amino acids.

Both differ in amino acid sequence in the c-terminal end. The receptor molecule has three distinct domains.

- A steroid binding domain near c-terminal
- A DNA binding domain near the middle of the molecule in c-terminal half ad
- A transcription activating domain near the N-terminal side.

A heat shock protein (hsp 90) binds to the receptor in the absence of hormone and prevents folding into the active conformation of the receptor protein.

Glucocorticoids bind to the specific receptor in cytosol to steroid-binding site. This binding causes dissociation of the hsp 90 stabilizer and permits conversion to the active configuration.

The steroid-receptor complex enters the nuclear and binds by DNA-binding site to the "hormone responsive elements (HRE) of specific nuclear gene. This modulates the transcription rate of these genes, leading to increase synthesis of many proteins and enzymes and also to decreased synthesis of some proteins like corticotrophin.

METABOLIC ROLE OF GLUCOCORTICOIDS

1. Metabolic Actions:

Points to note:

- In general, glucocortidoids have anti-insulin effects
- Glucocorticoids are catabolic to peripheral tissues and anabolic to liver.

a. Effects on carbohydrate metabolism: overall effect increases blood glucose level (hyperglycaemia)

Mechanism of hypergly aemia

1. Decreases glucose uptake; and utilization in muscles, in adipocytes and lymphoid cell by inhibiting the membrane transport of glucose into these cells.
2. Enhancing gluconeogenesis in liver: induces the synthesis of key gluconeogenic enzymes such as pyruvate carboxylase, PEP carboxykinase, fructose 1, 6-diphosphatase and also glucose-6-phosphatase.

- By making available more of substrate required for gluconeogenesis. This is achieved by
- Increasing protein catabolism in extrahepatic tissue
- Decreasing incorporation of amino acids in protein in peripheral tissues.
- Also increasing synthesis of some key enzymes required for amino acid catabolism like alanine transaminase, tyrosine transaminase, tryptophan pyrollase.

3. Decreases glycolysis in peripheral tissues

- In liver: glucocorticoids are anabolic. It increases the glycogen store in liver. This is due to:
- Increases in gluconeogenesis from amino acid and glycerol
- Activates protein-phosphatase-1 which dephosphorylates and activates glycogen synthesis:
- Stimulate the synthesis of glycogen synthase also

b. Effect on lipid metabolism: Net effect increase FFA in plasma and also glycerol. Glycerol is utilized for gluconeogeneris in liver.
In adipocytes.

- Glucocorticoids increase lipolysis and liberates FFA and glycerol by activating hormone sensitive TG lipase.
- As glucocorticoid decrease the uptake of glucose in adipose tissue, there will be reduction in α-glycerol phosphate as a result esterification suffers, hence net flow of FFA in plasma increase.

c. ***Effect on protein metabolism***

- In peripheral extrahepatic tissues, cortisol is catabolic and increase protein breakdown, leading to increase amino acids availability in plasma.
  Reasons of increased catabolism

- Enhances synthesis of key enzymes of amino acid catabolism like transaminase, tyrosine transaminase
  Tryptophen pyrrolase etc

- Also there is decreased incorporation of amino acids in protein molecule
- In liver: cortisol is anabolic, it increases protein synthesis it increases;
- Hepatic uptake of amino acids
- Incorporation of amino acids into ribosomal proteins.
- Increased m-RNA formation and synthesis of proteins including plasma protein
- In liver, cortisol also enhances urea synthesis from amino acids. There is increased synthesis of enzymes necessary for urea cycle, e.g arginino succinate synthetase, arginase etc.

MINERALO CORTICOIDS

Mineralo corticoids are C21 steroids, which influence the metabolism of Na+ and K+. The chief mineralocorticoid is aldosterone. It is produced by zona glomerulosa of the adrenal cortex. Structurally, it bears –OH group at C11 and aldehyde (CHO) group at C18.

Other corticosteroids which have mineralocorticoid activity are:

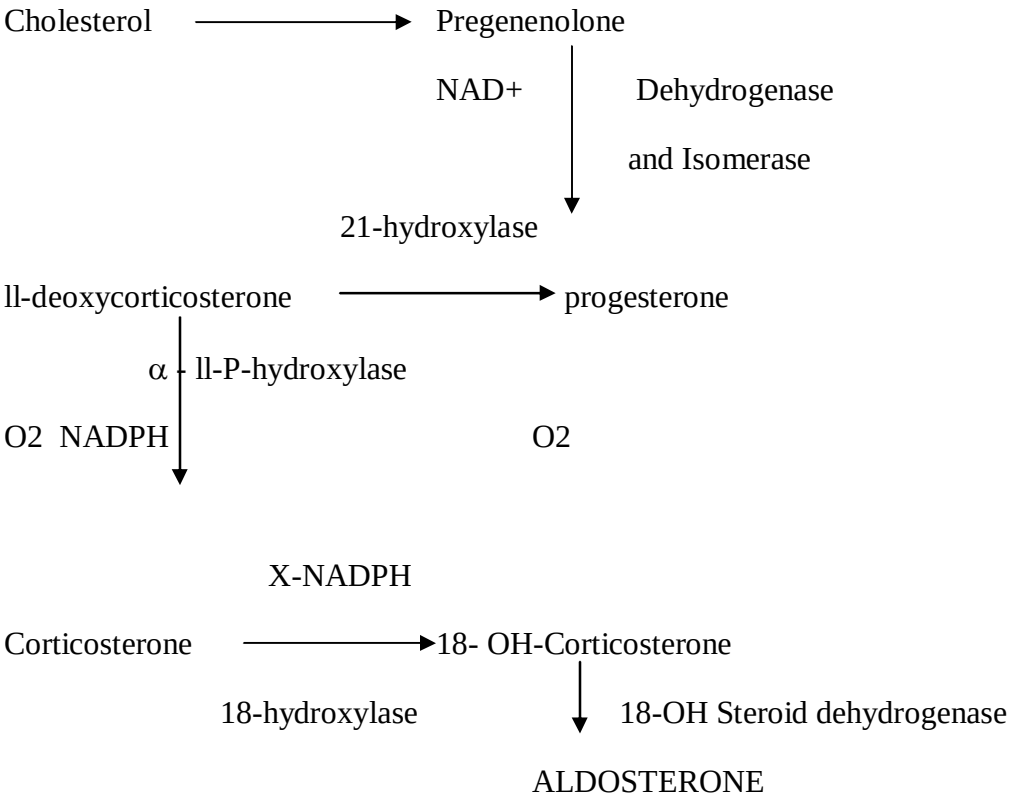
- Corticosterone. * II-deoxycortisol * II-deoxycorticosterone

Il-deoxycorticosterone is secreted in minute quantities and has almost the same effects as aldosterone, but a protency only $1/30^{th}$ that of aldosterone.

Biosynthesis

Mineralocorticoids are synthesized in zona glomerulosa cell only. They cannot be synthesized in other two layers of adrenal cortex. Only zona glomerulosa cells have the enzymes 18-hydroxylase and 18-hydroxysteroid dehydrogenase, which are lacking in other layers

Steps in synthesis:



MECHANISM OF ACTION

Mineralocorticoids enter the target cells through the plasma membranes and binds to a specific protein present in cytosol, and nucleosplasm, called mineralocorticoid receptors. They are present in epithelial cells of renal distal tubular cells and collecting ducts and also in gastro-intestinal mucosa, salivary gland duct and sweat ducts. The steroid receptor complex then enters the nucleus and binds to hormone responsive element of specific nuclear genes and increase the transcription rates of genes. Thus, aldosterone initiates an increase in mRNA synthesis, at the level of transcription of DNA. The induced mRNA stimulates protein synthesis at the ribosomal level.

METABOLIC ROLE OF ALDOSTERONE

a. Renal effects of Aldosterone

1. Effect on tubular reabsorption of sodium:

By far the most important effect of aldosterone and other mineralocorticoids is to increase the rate of tubular reabsorption of Na. Sodium is reabsorbed from the renal tubules

along their entire extent. Aldosterone has a specially potent effect in the distal tubule, collecting tubule and at least a part of loop of Henle.

Note: Total lack of aldosterone secretion can cause loss of as much as 12 gram of Na in the urine in a day, an amount equal to $1/7^{th}$ of all the sodium in the body.

2. Effect on tubular rebsorption of chlorides:

Aldosterone also increase the reabsorption of Cl ions from the tubules. This probably occurs secondarily to the increased Na reabsorption. Absorption of positively changed Na+ causes an electrical potential gradient to develop between the lumen and outside of the tubules with positivity on the outside.

This positivity in turn attracts negatively charged diffusible amino through the membrane since Cl- are by far the most prevalent anion in the tubular fluid, the absorption of Cl increase.

3. Increased renal secretion of K+: as aldosterone causes increased tubular reabsorption of Na+ at the same time it also increase loss of K+ in the urine by the renal distal tubules and collecting ducts. This may result from the elimination of K+ in exchange of the reabsorbed Na+.

Clinical significance:

Hypokalaemia and muscle paralysis: the loss of K+ in urine decrease K+ in ECF resulting to hypokalaemia. Thus at the same time that Na+ and Cl become increased in ECF, there will be group decrease in K+. The low K+ concentration sometimes leads to muscle paralysis, this is caused by hyperpolarization of the nerve and muscle fiber membrane which prevents transmission of action potentials.

4. Effect an acid-base balance (Alkalosis): A large proportion of Na+ reabsorption from the tubules results from an exchange reaction on which H+ are secreted into the tubules to take place of Na+ that is reabsorption is enhanced, in response to aldosterone, the H+ concentration in the body fluids is reduced. For each Na+ reabsorption by the H+ exchange, one HCO3 enters the ECF which shifts the reaction to alkaline side. Thus increased secretion of aldosterone promotes alkalosis, whereas decreased secretion produced acidosis.

b. ***Effect of aldosterone on fluid volume***:

1. **Effect on ECF volume**:

Mineralocorticoids greatly increase the quantities of Na+, Cl and HCO3 in the ECF increasing the electrolyte concentration in ECF. These in turn increase water reabsorption from the tubules by:

- Stimulating the hypothalamic OH system and
- Creating an osmotic gradient across the tubular membrane. When the electrolytes are absorbed, carries water through the membrane in the wake of electrolyte absorption
- Also increased electrolyte concentration of ECF causes thirst, thereby making the persons to drink excessive amount of water

Hence the final result is an increase in ECF volume, sometimes enough to course generalized oedema

2. **Effect on blood volume**:

The plasma volume increases almost proportionally during the early part of increase in ECF volume. Hence one of the effects of increased aldosterone secretion is a mild to moderate increase in blood volume.

c. Effect of aldosterone on sweat glands, salivary glands and gastric mucosa:

The mineralocorticoids have almost the same effect on the sweat glands, salivary glands intestinal glands as on the renal tubules, greatly reducing the lows of Na+ and Cl in the glandular secretions. The effect on the sweat glands is important to conserve body salt in hot environment whereas; the effect on intestinal gland is probably of importance to prevent salt loss in the gastrointestinal excretory products.

RENIN ANGIOTENSIN SYSTEM

Juxtaglomerular(JG) cells: Afferent arteriole of nephron show cytoplasmic granules which contain an enzyme called Renin. A fall in sodium concentration, hypovolemia, hypotension and a fall in intra cellular Ca2+ stimulate the release of rennin from JG cells to the blood. Brady Kinin and glucagon also stimulate release of renin.

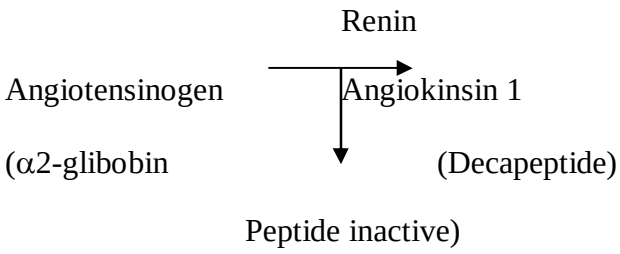
CHEMISTRY

Renin is a proteolytic enzyme mwt 35000 recently renin isoenzymes or renin like enzyme have been described in brain, placenta, and sub-maxillary duct and at the junction of uterine endometrium and myonetrium.

Action of Renin.

- Formation of Angiotensin I: Renin acts on a plasma substrate, andα 2-globulin, called angiotensinogen or Hypertensinogen, which is produced by the liver. The enzyme cleaves the leucyl-leucy bind between 10 and 11 positions from N-terminal end to

produce angiotensin 1, a decapetide and a polypeptide having 7400 amino acids inactive.



This is the rate limiting step. Cortisol and estradiol enhance the reaction, probably by increasing hepatic synthesis of angiotensinogen.

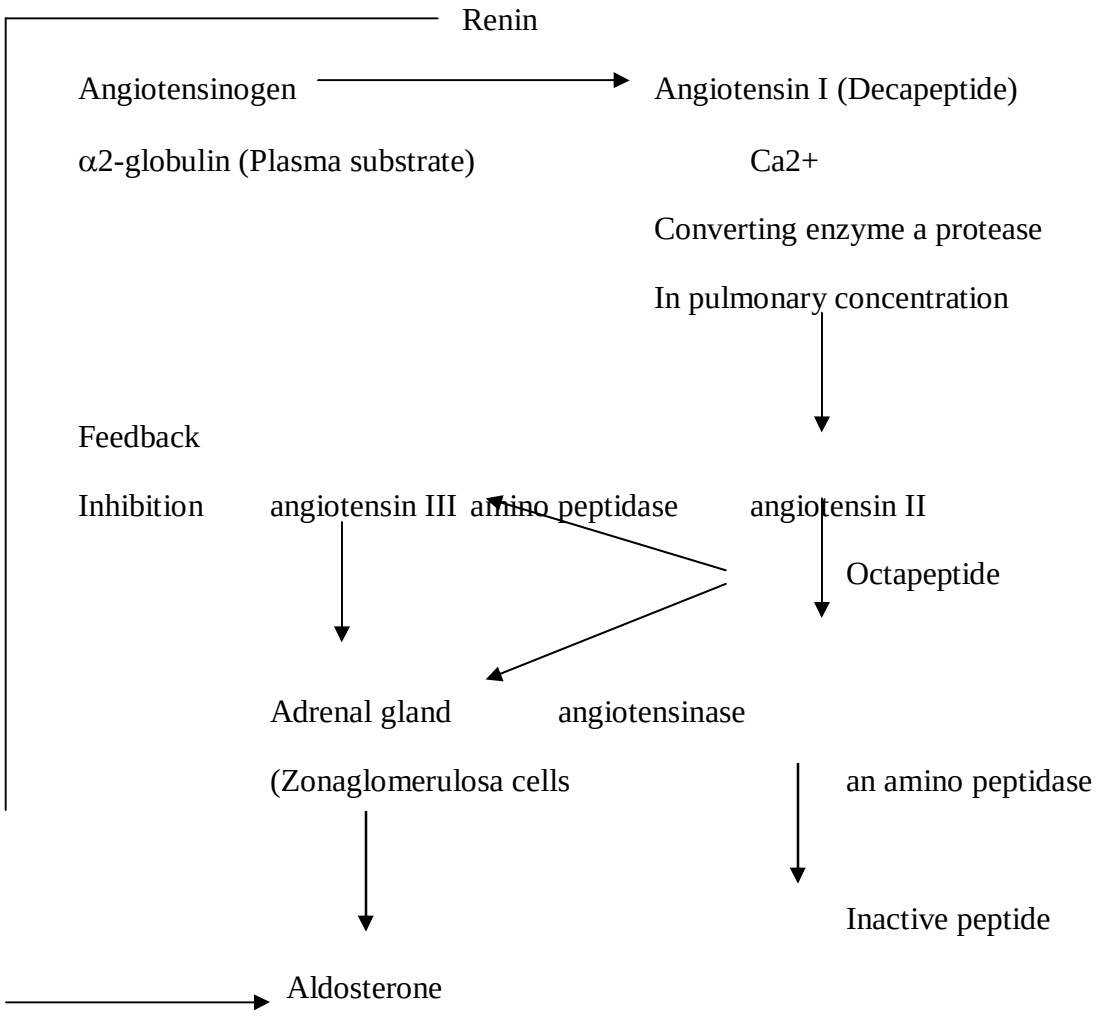
Formation of Angiotensin II

Angiotensin 1, a decapeptide, mot 1296, while circulatory, is acted upon by another enzyme, called converting enzyme (a protease) which occurs on the walls of small verses of living. The enzyme is Ca2+ dependent and it removes terminal histidyl-leucyl dipeptide in pulmonary circulation forming angiotensin II ahexapeptide mwt 1046 and an inactive dipeptide. Angiotensin II is the active component which acts a zona glomerulosa cells to increase synthesis of aldosterone and increases rate of release of the hormone.

In activation of Angiotensin II

An enzyme angiotensinase II by hydrolysis.

Angiotensin III: Recently in rat heptapeptide angiotensin IV has been isolated. It is claimed to be also present in humans. Both heptapeptide (angiotensin II) are claimed to be equipotent in stimulating aldosterone secretion aldosterone can inhibit the enzyme renin by feedback inhibition so that angiotensin II formation is decreased.



Inactivation of Renin: In addition to feedback inhibition by aldosterone.

- Renin is also destroyed by a cephalin derivatives in plasma and
- Also inhibited by a lysophospholipid, liberated by the action of phospholipase A2.

Actions of Angiotensin II:

Principal action is angiotensin II stimulates aldosterone synthesis in zona glomerulosa cells and increases rate of secretion of aldosterone.

Mechanism of action and effects:

Angiotensin II binds with specific reception an membrane of zona glomerulosa cells and

- Enhance cytosolic concentration of Ca2+ in cells and
- Formation of inositol-1, 4, 5- triphosphate.

The above act as a second messenger and in turn:

a. enhances conversion of cholesterol to pregnenolone
b. and corticosterone to aldosterone by increasing the activity of 18-hydroxylase

Aldosterone thus formed and secreted:

- Increases the active tubular reabsorption of Na+ and
- Consequently passive reabsorption of Cl and water. Renal retention of water restores the falling ECF volume and helps in long term increase of arterial blood pressure.

Others actions of Angiotensin II.

- Stimulates contraction of smooth muscles on the walls of alimentary canal uterus, arteries arterioles but unlike catecholamine it reacts with a specific receptor in the cell membrane of smooth muscles leading to a rise in intracellular Ca+ which then promotes contraction of smooth muscle fibers.
- May raise arterial B.P by causing arteriolar construction and is thought to be responsible for hypertension associated with ischaemic kidney.
- Also stimulates V.M centre in the hind brain leading to refer rise in cardiac output, reflex arteriolar constriction and a rise in B.P
- May stimulate vasopressin secretion, indirectly causing water retention
- May also stimulate synthesis and release of PG in renal medulla.

On the other hand PG particularly PGE, may act against angiotensin II and reduce the renal vasoconstrictor and antidiuretic effect of angiotensin II.

ADRENAL MEDULLARY HORMONES

Chemistry

1. Two biologically active compounds have been isolated from the adrenal medulla and synthesized. They are

- Epinephrine (Adrenaline or Adrenin)
- Norepinephrine (Noradrenaline or Arterenol)

2. The naturally occurring forms are laevorotatory, the synthetic are racemic, the form being almost twice as active as the latter.

3. The above two hormones are called catecholamines and are closely related to tyrosine and synthesized in body from tyrosine

Epinephrine is primarily synthesized and stored in adrenal medulla. Nor epinephrine is primarily synthesized in sympathetic nervous system and acts locally as neurotransmitter at the post synaptic cell. Norepinephrine is also synthesized and stored in adrenal medulla.

Biosynthesis

In adrenal pheochromocytes and renal cells, the synthesis of catecholamine is essentials same. Both are produced from the amino acid tyrosine.

STORAGE

- Epinephrine, norepinephrine and Dopamine are stored in the form of granules in the pheochromocytes of adrenal medulla.
- Norepinephrine only occurs in adrenergic nerve terminals as granules/ or vesicles 400 to 500 'A' and diameter. And some is probably free in cytoplasm. Both the hormones are stored in the granules in the adrenal medulla and in adrenergic neurons as a complex containing ATP in the ratio of about 4 molecules of hormone; one molecule of ATP and in combination with several incompletely characterized proteins like chromogenin A and Chromomembrane B.

Clinical importance:

As catecholamines cannot penetrate blood brain barriers the norepinephrinin the brain must be synthesized within that tissue. L-DOPA, the precursors of catecholamines does penetrate the barrier, it is hence, used to increase brain catecholamine synthesis in Parkinson's disease.

Mechanism of Action

1. **Role of c-AMP**

Catecholamines on binding to β-receptors (β1and β2) activate adenyl cyclase which increases c-AMP level in the cells. Increased c-AMP activates c-AMP dependent protein kinases which phosphorylates specific protein/or enzymes and activated / inactivate them. β-receptor action is mediated through increased intracellular c-AMP level.

- Catecholamines on binding to α-receptors inhibit adenyl cyclase, thus decreasing the intracellular c-AMP level. α–receptor action is mediated through decreasing intracellular c-AMP level.

2. Role of Ca2+ and phosphor-inositides:

Catecholamines on binding with α1 receptor effect the formation of inositol 1, 4 ,5 triphosphate and diacylglycerol, and or intracellular Ca^{2+} these may act as second messenger to produce tissue response during α-effects.

METABOLIC ROLE OF CATHECHOLAMINES

a. Glycogenolysis

1. Liver epinephrine stimulates rapid breakdown of glycogen of liver (glycogenolysis) producing hyperglycaemia.

Action mediated by two ways:

- It's binding to β2 receptors on hepatic cell membrane by increasing c-AMP level.
- Also exerts it effect by binding to α1 receptors on hepatic cell membrane, which increases intracellular Ca2+ level which act as second messenger.

The effect of c-AMP increase in hepatic cells is similar to glucagon. But measurement of c-AMP levels after epinephrine and/or glycogen indicate that glucagon is by for the more active hormone in liver tissue. Norepinephine has very little effect on blood glucose.

2. ***Muscle***: In muscle, epinephrine also causes breakdown of glycogen (glycogenolysis) by increasing c-AMP level (β-effect), but in this tissues it is more active than glucagon. Glucagon has very little effect or no effect due to lack of specific receptor. In exercising muscle, this can result in increased lactic acid formation, which passes to blood.

3. ***Heart muscle***: Increase c-AMP after epinephrine administration is seen in 2-4 seconds, the effect of epinephrine on cardiac output (lonotropic effect) is seen shortly after wards, whereas activation of phosphorylase is not detectable for 45 seconds.

4. ***Heart glycogen***: In vivo, actually epinephrine action can results in an increase in heat glycogen. This is probably secondary to the hormone action an adipose tissue causing adipose and increase FFA. Fatty acids as utilized as fuel. Increased glycogen is due to gluconeogenesis; the glucose is not utilized for energy and diverted to glycogen formation.

b. ***Lipolytic Action***: Both epinephrine and norepinephrine increase the breakdown of TG in adipose tissue by increasing c-AMP level (β effect). Net effect of lipolysis is rapid release of FFA and glycerol from adipose tissue to blood.

c. ***Glucogenic Action***: Epinephrine increase cyclic c-AMP which induces the synthesis of key enzymes pyruvate carboxylase, PEP carboxykinase and fructose -1,6-biphosphate. Increased FFA level in blood produced by lipolytic action can also activate hepatic gluconeogenesis.

d. ***Action on glucoses***: Epinephrine increase blood lactic acid level by promoting neither muscle glycolysis, nor epinephrine has very little effect on blood lactic acid.

e. ***Action on insulin Release***: Epinephrine has a direct inhibitory action on insulin release from β-cells of pancreas (α2-effect). Thus, in pancreases the α-adrenergic response to epinephrine predominates, c-AMP decrease and insulin release inhibited. However in the presence of α-blockers such as phentolamine (Regitine), the β-effect predominates and epinephrine cause increased c-AMP and increase insulin release.

f. ***Calorigenic Action***: Norepinephrine and epinephrine are almost equally potent in their calorigenic action. They produce a prompt rise in the metabolic rate which is independent of the liver,

* A smaller delayed rise which is abolished by hepatectomy and coincides with rise in blood lactic acid. The calorigenic action does not occur in the absence of the thyroid and adrenal cortex.

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