BIOMEDICAL IMPORTANCE OF IGS

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

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

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

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

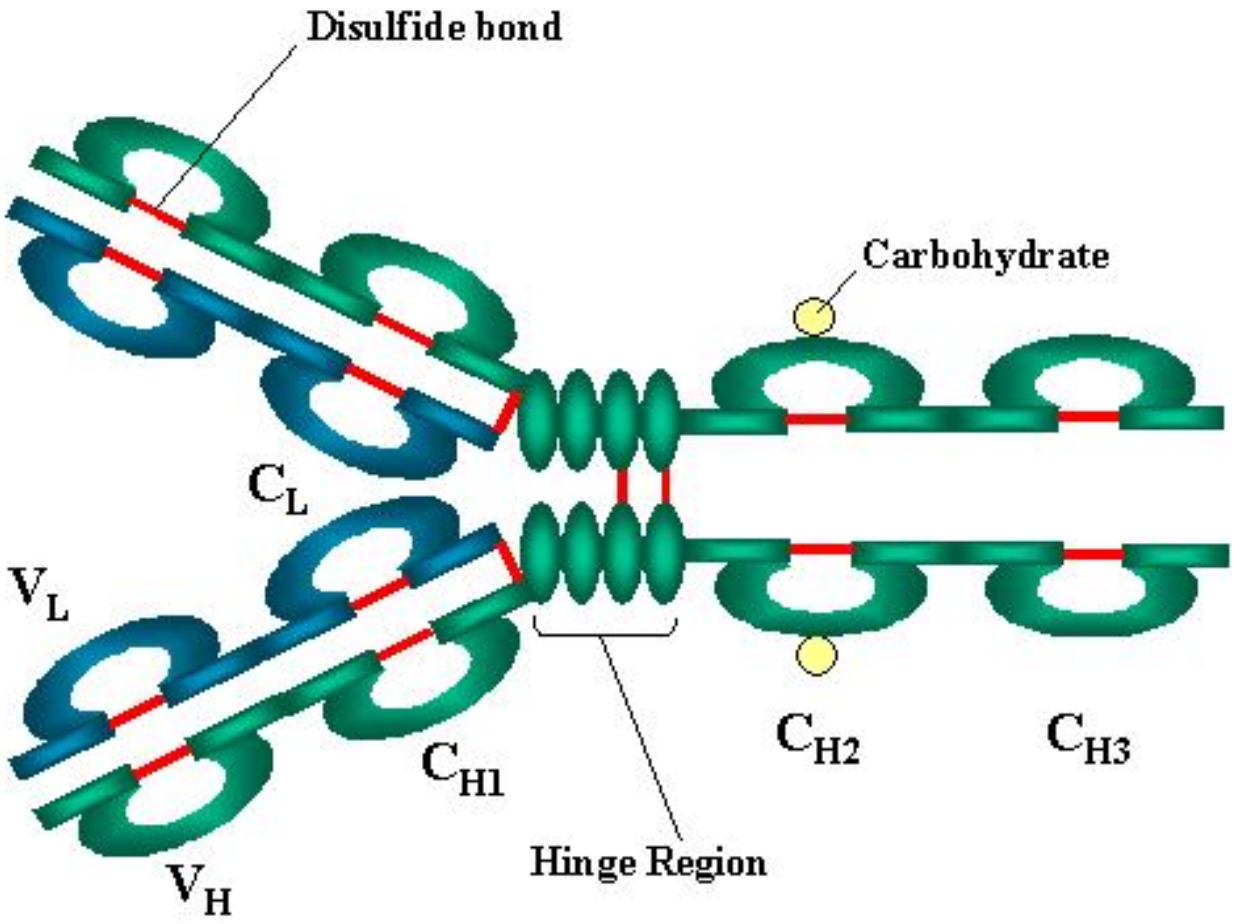
IgA Increases occur in:-Wiskott-Aldrich syndrome, cirrhosis of the liver, IgA myeloma, autoimmune disorders, rheumatoid arthritis, lupus erythromatosis etc

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

IgD Increases occur in: - chronic infections, IgD myelomas

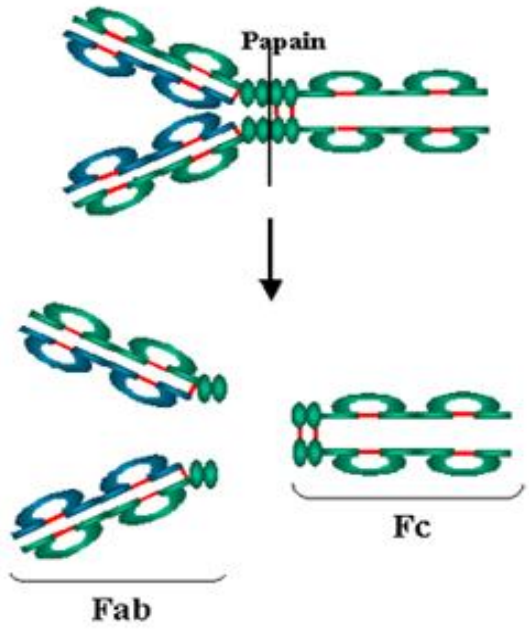
IgE Increases occur in:-atopic skin diseases e.g. eczema, hay fever, asthma, anaphylactic shock and IgE myelomas.

Decreases occur in:-congenital Aggammaglobulinemia, Hypogammaglobinemia etc


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

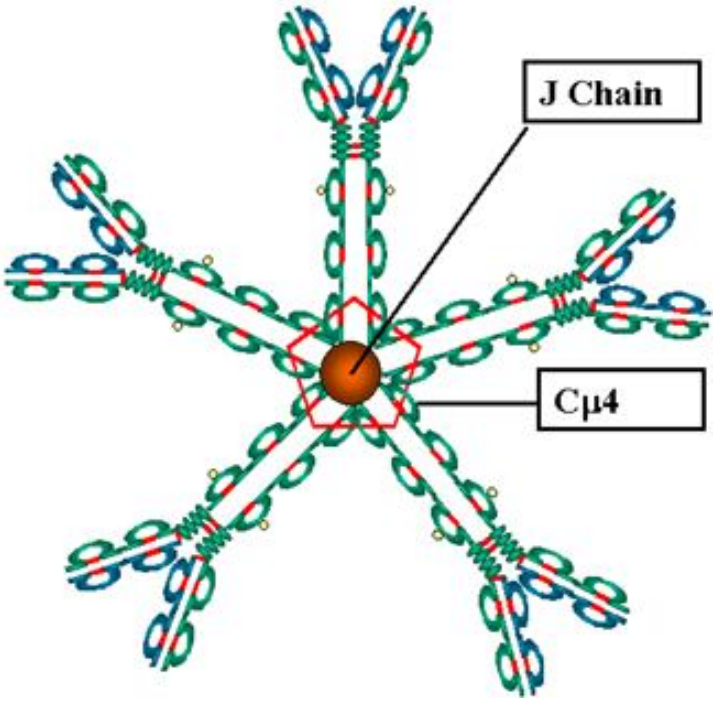
Immunoglobulin Fragments: Structure/Function Relationships

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



IgM

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



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

ANTIGENS AND ANTIGENIC DETERMINANTS

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

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

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

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

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

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

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

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

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

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

PROPERTIES OF ANTIGENS OR IMMUNOGENS

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

TYPES OF ANTIGENS

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

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

Nature of antigens

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

Vaccination

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

IMMUNOTHERAPY

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

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

There are two main types of immunotherapies;

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

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

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

MONOCLONAL ANTIBODIES (MAbs)

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

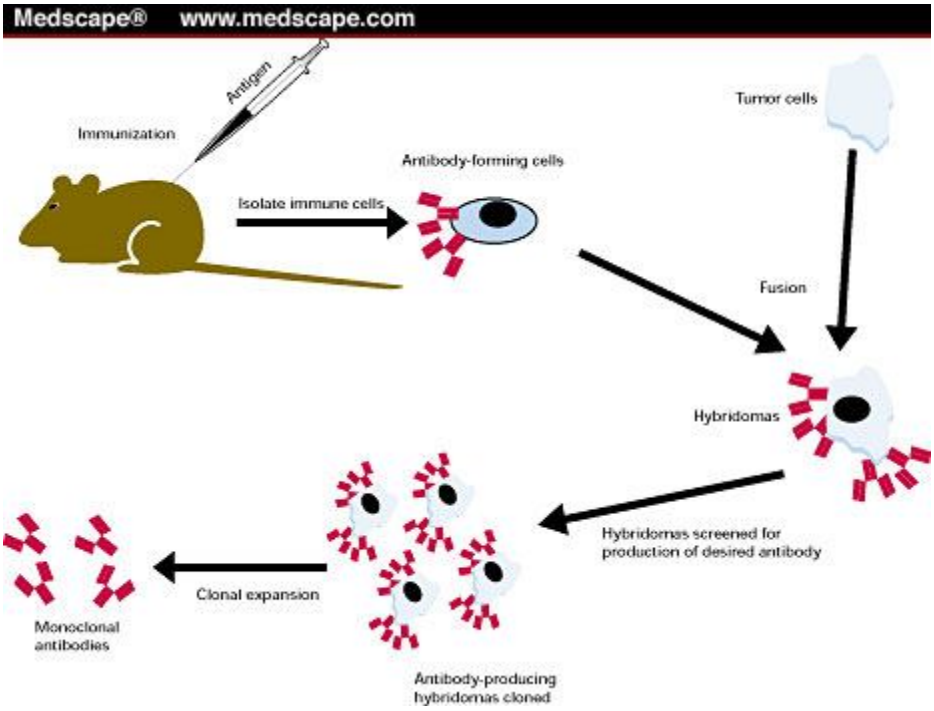
Monoclonal antibodies are the most widely used form of cancer immunotherapy at this time, they also widely used as reagents in immunoassay techniques (diagnosis of diseases).

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

HYBRIDOMA TECHNOLOGY

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

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



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

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

Autoimmune Disease

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

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

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

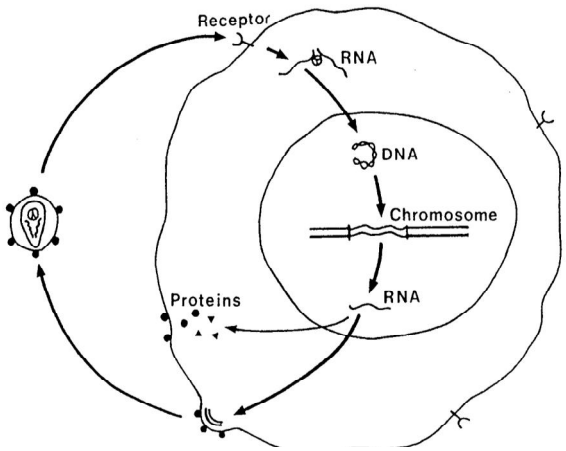
RETROVIRUSES.

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

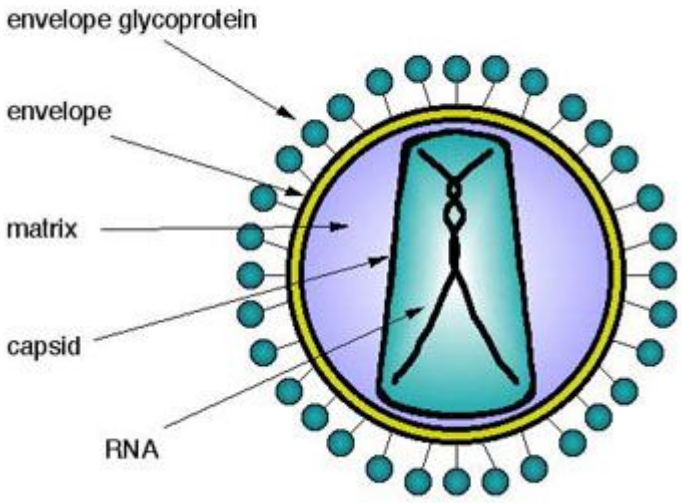
Retroviruses carry diploid, single-stranded RNA genomes in the virion and replicate by forming one double stranded DNA copy in the infected cell, by means of the viral enzyme, reverse transcriptase. This viral genome becomes integrated as a DNA called the ‘provirus’, into the

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

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



Basic structure of the retrovirus includes an outer envelope which comes from the host cell plasma membrane, coat proteins (surface antigens) ,inside the membrane is an icosahedral capsid containing proteins, that also coat the genomic RNA. There are two molecules of genomic RNA per virus particle with a 5' cap and a 3' poly A sequence. Thus, the virus is diploid. The RNA is plus sense (same sense as mRNA).About 10 copies of reverse transcriptase are present within the mature virus(a polymerase that copies RNA to DNA), Integrase (integrates the viral genome into the host genome), RNase H (cleaves the RNA as the DNA is transcribed so that reverse transcriptase can make the second complementary strand of DNA) and Protease (cleaves the polyproteins).



CANCER

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

PROTOONCOGENES AND ONCOGENES

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

However, if the activity remains high, or if proto-oncogenes are inappropriately reactivated later in life, cancer may occur.

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

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

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

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

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

MOLECULAR INVOLVEMENT OF RETROVIRUSES IN CANCER.

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

The following stages occur in the infection process:

1) Binding to a specific cell surface receptor

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

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

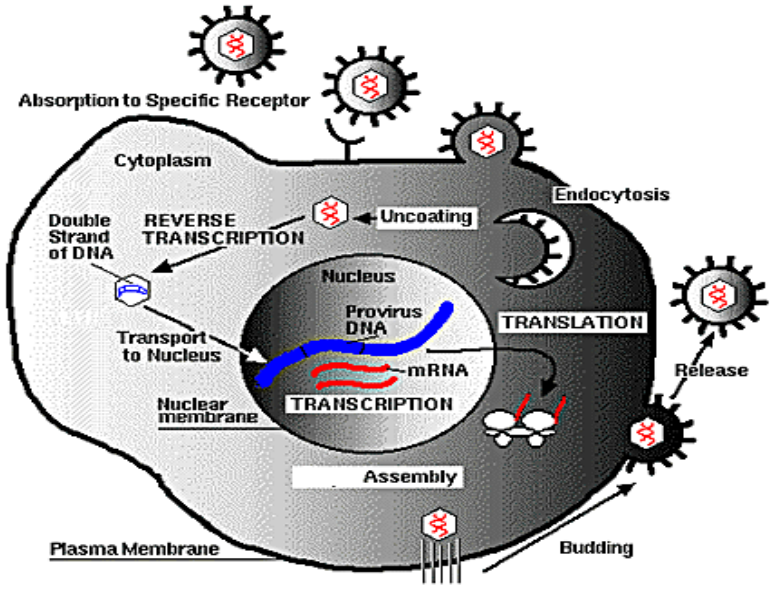
4) RNA is displaced and degraded by a virus-encoded RNase H activity. Reverse transcriptase now acts as a DNA-dependent DNA polymerase and copies the new DNA into a double strand DNA. This DNA form of the virus is known as a provirus.

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

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

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

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



Retrovirus replication

TRANSLOCATION AND GENE ARRANGEMENT IN DISEASE STATE OF ANIMALS.

GENETIC DEFECTS

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

Gene mutations refer to changes in gene structure as a result of change in the sequence of nucleotides of the DNA molecule in a particular region of the chromosome (alterations in DNA

sequences), which is transferred to the mRNA (during transcription) and results in amino acid or protein alteration (during translation) and is subsequently seen as spontaneous changes in the phenotype as against that which as originally genotypically typed. These changes include deletion, inversions, substitution and insertion.

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

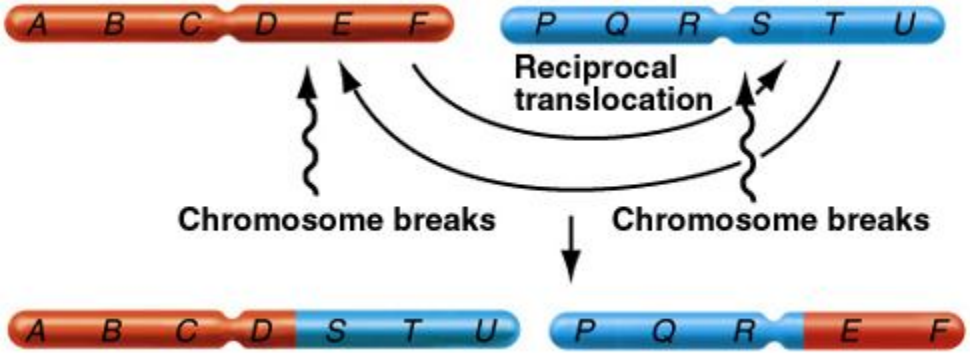
Mutations can be caused by copying errors in the genetic material during cell division, by exposure to ultraviolet or ionizing radiation, chemical mutagens, or viruses, or can be induced by the organism itself, by cellular processes such as hypermutation. In multicellular organisms with reproductive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

CHROMOSOMAL MUTATIONS

TRANSLOCATION

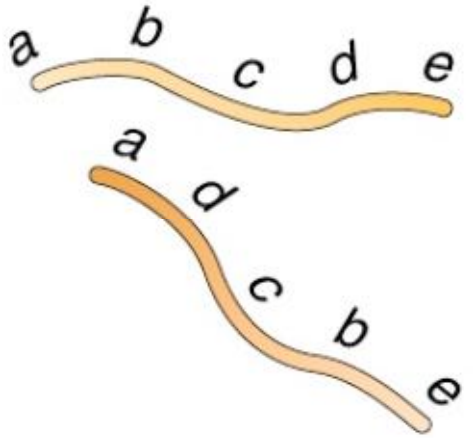
This occurs when a segment breaks off and rejoins of another end of the chromosome (reciprocal or balanced translocation) or another chromosome entirely (a non-homologous pairs; non-reciprocal or unbalanced translocation). Where there is translocation between non-homologous pairs, new pairs of homologous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

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



INVERSIONS

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



DELETIONS

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

Deletions maybe intragenic deletion; where small deletion within gene occurs and inactivates gene and has the same effect as a other null mutations of that gene, or multigene deletion in which case many genes are deleted, often with severe consequences such as gene imbalance. Pseudodominance is a phenomenon that can also result from deletion where it seems as if the recessive alleles are showing dominance because the dominants have been deleted and possible expression of deleterious recessive mutation.

TRANSPOSITION

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

DUPLICATION

This is the gain of a segment. It is usually a source of new genes and gene families.

It can result into tandem duplication where segment is attached adjacent to its duplicate (adjacent duplications) in same or reverse order or non-tandem/ insertional duplication, here duplicate

gene inserted elsewhere in the genome (same or reverse order). It may be a consequence of unequal crossing-over.
Most duplications have no phenotypic consequence but sometimes the effects can be seen due to increased gene dosage. Duplication plays a very important role in evolution through increase gene number and evolution of new genes (paralogs).
The mutant genes are displayed twice and the duplicate is usually harmless.

BLOOD: GENERAL PROPERTIES AND FUNCTIONS

BLOOD: GENERAL PROPERTIES AND FUNCTIONS

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

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

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

PROPERTIES OF BLOOD.

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

7. The proteinaceous component of blood (including clotting proteins) is produced predominantly by the liver, while hormones are produced by the endocrine glands and the watery fraction is regulated by the hypothalamus and maintained by the kidney.

   FUNCTIONS OF BLOOD

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

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

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

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

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

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

   Regulation of body pH and temperature.

THE RED CELL AND ITS METABOLISM

RBCs in mammals are non-nucleated biconcave shaped cells, highly flexible and lacking intracellular organelles. They are flattened and depressed in the center. Erythrocyte content consists mainly of hemoglobin. The precursors (Pronormoblast) of erythrocytes mature in the bone marrow, in a process called erythropoiesis, closely attached to a macrophage, these precursor cells manufacture hemoglobin until it accounts for some 90% of the dry weight of the cell, and as it matures the nucleus is squeezed out of the cell and is ingested by the macrophage. In addition the no-longer-needed proteins are expelled from the cell in vesicles called exosomes. RBCs are terminally differentiated, that is, they can never divide, and live for about 120 days after which they and engulfed and phagocytosed by cells of the RES predominantly in the spleen, bone marrow and liver. They are responsible for the transport of oxygen and carbon dioxide. In addition to their major function of O_2 and CO_2 transport RBCs also play some role in immune response by release of free radicals from damaged cells to destroy invading pathogens and also release S-nitrothiols that facilitate vasodilation when they (RBCs) are deoxygenated.

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

METABOLISM

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

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

BLOOD CLOTTING MECHANISMS

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

IRON AND ITS METABOLISM

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

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

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

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

SOURCES

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

ABSORBTION

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

Most intestinal iron absorption occurs in the duodenum and jejunum (the first two sections of the small intestine). Iron uptake is tightly controlled to prevent iron overload, so only 6-12% percent of dietary iron is absorbed by the intestines. Free iron in the intestines is reduced from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state on the luminal surface of intestinal enterocytes and transported into the cells through the action of the divalent metal transporter, DMT1, intestinal uptake of heme iron occurs through the interaction of dietary heme with the heme carrier protein (HCP1). The iron in the heme is then released within the enterocytes via the action the heme catabolizing enzyme heme oxygenase.

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

BODY DISTRIBUTION OF IRON

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

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

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

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

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

EXCRETION

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

ANEMIA AND HEAMACHROMATOSIS

ANEMIA

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

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

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

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

SIGNS

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

HEMACHROMATOSIS

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

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

The condition is primarily genetic due to inheritance of an autosomal recessive allele. HFE gene (a histocompatibility complex gene) regulates iron transfer into cells via its formation of complex with transferrin hence a mutation in this gene results in abnormal iron intake and storage. Secondary hemachromatosis which is not genetic can result from excess oral intake of iron or in patients receiving blood transfusion.

HAEMOGLOBIN: STRUCTURE, PROPERTIES AND BIOMEDICAL FUNCTIONS

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