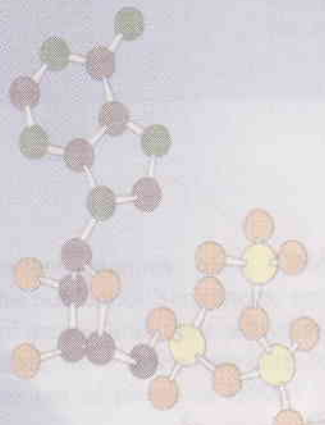
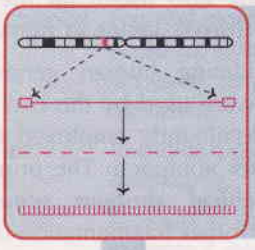
CURRENT TOPICS



Section VI

28 Human Genome Project



The Human Genome speaks :

"I am the outcome of international collaborative research;
Composed of 3.2 billion nucleotide base pairs;
With 30,000 to 40,000 protein coding genes;
That represents only 1.1 to 1.5% of the genome"

The most important features of a DNA molecule are the nucleotide sequences, and the identification of genes and their activities. Since 1920, scientists have been working to determine the sequences of pieces of DNA.

THE BIRTH AND ACTIVITY OF HUMAN GENOME PROJECT

The human genome project (***HGP***) was conceived in 1984, and officially begun in earnest in October 1990. The primary objective of HGP was to ***determine the nucleotide sequence of the entire human nuclear genome.*** In addition, HGP was also entrusted to elucidate the genomes of several other model organisms e.g. *Escherichia coli*, *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (roundworm), *Mus musculus* (mouse). James Watson (who elucidated DNA structure) was the first Director of HGP.

In 1997, United States established the ***National Human Genome Research Institute*** (***NHGRI***). The HGP was an international venture involving research groups from six countries — USA, UK, France, Germany, Japan and China, and several individual laboratories and a large number of scientists and technicians from various disciplines. This collaborative venture was named as ***International Human Genome Sequencing Consortium*** (***IHGSC***) and was headed by Francis Collins. A total expenditure of $3 billion, and a time period of 10–15 years for the completion of HGP was expected. A second human genome project was set up by a private company — ***Celera Genomics***, of Maryland USA in 1998. This team was led by Craig Venter.

Announcement of the draft sequence of human genome

The date ***26th June 2000*** will be remembered as one of the most important dates in the history of science or even mankind. It was on this day, Francis Collins and Craig Venter, the leaders of the two human genome projects, in the presence of the President of U.S., jointly announced the working drafts of human genome sequence. The detailed results of the teams were later published

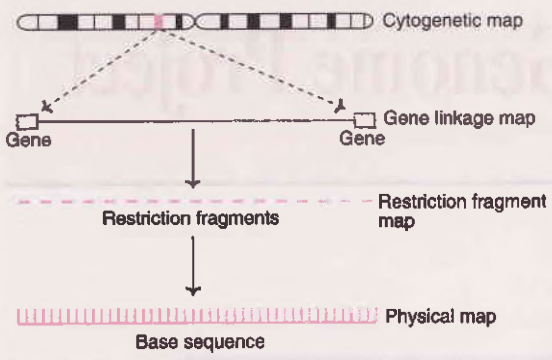


Fig. 28.1 : Different types of genome maps.

in February 2001 in scientific journals Nature (IHGSC) and Science (Celera Genomics).

The human genome project results attracted worldwide attention. This achievement was hailed with many descriptions in the media.

- The mystery of life unravelled.
- The library of life.
- The periodic table of life.
- The Holy grail of human genetics.

MAPPING OF THE HUMAN GENOME

The most important objective of human genome project was to construct a series of maps for each chromosome. In ***Fig.28.1***, an outline of the different types of maps is given.

1. **Cytogenetic map :** This is a map of the chromosome in which the active genes respond to a chemical dye and display themselves as bands on the chromosome.

2. **Gene linkage map :** A chromosome map in which the active genes are identified by locating closely associated marker genes. The most commonly used DNA markers are ***restriction fragment length polymorphism (RFLP), variable number tandems repeats (VNTRs) and short tandem repeats (STRs).*** VNTRs are also called as ***minisatellites*** while STRs are ***microsatellites.***

3. **Restriction fragment map :** This consists of the random DNA fragments that have been sequenced.

4. **Physical map :** This is the ultimate map of the chromosome with highest resolution base sequence. Physical map depicts the location of the active genes and the number of bases between the active genes.

APPROACHES FOR GENOME SEQUENCING

A list of different methods used for mapping of human genomes is given in ***Table 28.1***. These techniques are also useful for the detection of normal and disease genes in humans.

For elucidating human genome, different approaches were used by the two HGP groups. ***IHGSC*** predominantly employed map first and sequence later approach. The principal method was ***heirarchical shotgun sequencing.*** This technique involves fragmentation of the genome into small fragments (100–200 kb), inserting them into vectors (mostly bacterial artificial chromosomes, BACs) and cloning. The cloned fragments could be sequenced.

Celera Genomics used ***whole genome shotgun approach.*** This bypasses the mapping step and saves time. Further, Celera group was lucky to have ***high-throughput sequenators*** and ***powerful computer programmes*** that helped for the early completion of human genome sequence.

Whose genome was sequenced?

One of the intriguing questions of human genome project is whose genome is being sequenced and how will it relate to the 6 billion or so population with variations in world? There is no simple answer to this question. However, looking from the positive side, it does not matter whose genome is sequenced, since the phenotypic differences between individuals are due to variations in just 0.1% of the total genome sequences. Therefore many individual genomes can be used as source material for sequencing.

Much of the human genome work was performed on the material supplied by the Centre for Human Polymorphism in Paris, France. This institute had collected cell lines from sixty different French families, each spanning three generations. Thus, the material supplied from Paris was used for human genome sequencing.

Table 28.1 A list of principal methods used for mapping of genomes (and also normal and disease genes in humans)

Method	*Comments*
DNA sequencing	Physical map of DNA can be identified with highest resolution.
Use of probes	To identify RFLPs, STS and SNPs.
Radiation hybrid mapping	Fragment genome into large pieces and locate markers and genes. Requires somatic cell hybrids.
Fluorescence *in situ* hybridization (FISH)	To localize a gene on chromosome.
Sequence tagged site (STS) mapping	Applicable to any part of DNA sequence if some sequence information is available.
Expressed sequence tag (EST) mapping	A variant of STS mapping; expressed genes are actually mapped and located.
Pulsed-field gel electrophoresis (PFGE)	For the separation and isolation of large DNA fragments.
Cloning in vectors (plasmids, phages, cosmids, YACs, BACs)	To isolate DNA fragments of variable lengths.
Polymerase chain reaction (PCR)	To amplify gene fragments
Chromosome walking	Useful for cloning of overlapping DNA fragments (restricted to about 200 kb).
Chromosome jumping	DNA can be cut into large fragments and circularized for use in chromosome walking.
Detection of cytogenetic abnormalities	Certain genetic diseases can be identified by cloning the affected genes e.g. Duchenne muscular dystrophy.
Databases	Existing databases facilitate gene identification by comparison of DNA and protein sequences.

(RFLP–Restriction fragment length polymorphism; STS–Sequence tagged site; SNP–Single nucleotide polymorphism; YAC–Yeast artificial chromosome; BAC–Bacterial artificial chromosome)

HUMAN GENOME SEQUENCE—RESULTS SUMMARISED

The information on the human genome projects is too vast, and only some highlights can be given (***Table 28.2***). Some of them are briefly described.

Table 28.2 Major highlights of human genome

- The draft represents about 90% of the entire human genome. It is believed that most of the important parts have been identified.
- The remaining 10% of the genome sequences are at the very ends of chromosomes (i.e. telomeres) and around the centromeres.
- Human genome is composed of 3200 Mb (or 3.2 Gb) i.e. 3.2 billion base pairs (3,200,000,000).
- Approximately 1.1 to 1.5% of the genome codes for proteins.
- Approximately 24% of the total genome is composed of introns that split the coding regions (exons), and appear as repeating sequences with no specific functions.
- The number of protein coding genes is in the range of 30,000–40,000.
- An average gene consists of 3000 bases, the sizes however vary greatly. Dystrophin gene is the largest known human gene with 2.4 million bases.
- Chromosome 1 (the largest human chromosome) contains the highest number of genes (2968), while the Y chromosome has the lowest. Chromosomes also differ in their GC content and number of transposable elements.
- Genes and DNA sequences associated with many diseases such as breast cancer, muscle diseases, deafness and blindness have been identified.
- About 100 coding regions appear to have been copied and moved by RNA–based transposition (retro-transposons).
- Repeated sequences constitute about 50% of the human genome.
- A vast majority of the genome (~ 97%) has no known functions.
- Between the humans, the DNA differs only by 0.2% or one in 500 bases.
- More than 3 million single nucleotide polymorphisms (SNPs) have been identified.
- Human DNA is about 98% identical to that of chimpanzees.
- About 200 genes are close to that found in bacteria.

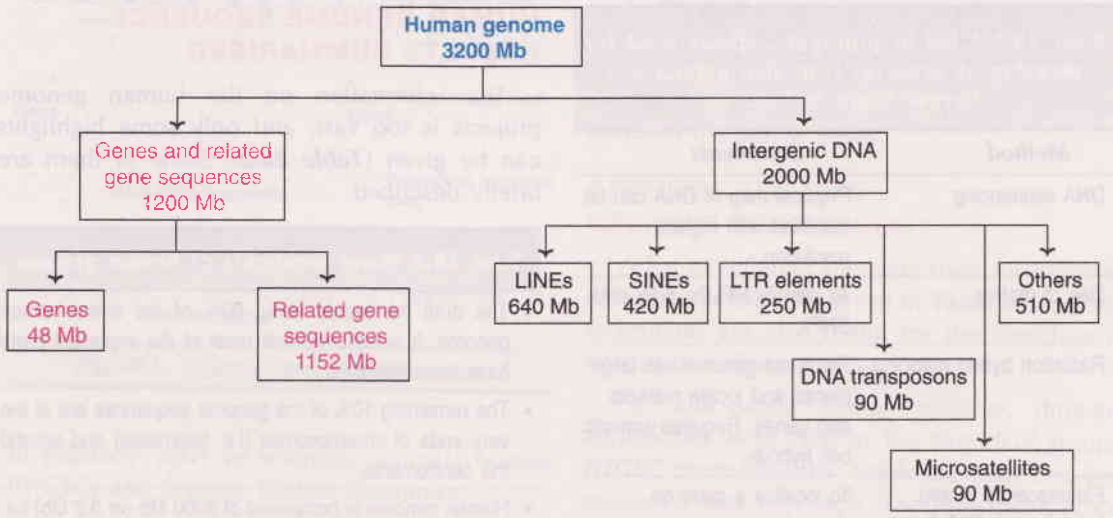


***Fig. 28.2** : An overview of the organization of human genome (LINEs-Long interspersed nuclear elements; SINEs–Short interspersed nuclear elements; LTR-Long terminal repeats).*

Most of the genome sequence is identified

About 90% of the human genome has been sequenced. It is composed of 3.2 billion base pairs (3200 Mb or 3.2 Gb). If written in the format of a telephone book, the base sequence of human genome would fill about 200 telephone books of 1000 pages each. Some other ***interesting analogs/sidelights of genome*** are given in ***Table 28.3***.

Individual differences in genomes : It has to be remembered that every individual, except identical twins, have their own versions of genome sequences. The differences between individuals are largely due to ***single nucleotide polymorphisms (SNPs)***. SNPs represent positions in the genome where some individuals have one nucleotide (i.e. an A), and others have a different nucleotide (i.e. a G). The frequency of occurrence of SNPs is estimated to be one per 1000 base pairs. About 3 million SNPs are believed to be present and at least half of them have been identified.

Organization of human genome

An outline of the organization of the human genome is given in ***Fig.28.2***. Of the 3200 Mb,

Table 28.3 Some interesting analogs/sidelights about human genome

- The base sequence in human genome would fill about 200 telephone books of 1000 pages each.
- If the genome is recited at the rate of one base per second for 24 hours a day, it would take a century to recite the book of life.
- If a typist types at the rate of 60 words per minute (i.e. 360 letters) for 8 hours a day, he/she would take around 50 years to type human genome.
- If the DNA sequence is typed in lines 10 cm containing 60 nucleotide bases and printed, the human genome sequence (from a single cell) would stretch a distance of 5000 km.
- If the DNA in the entire human body is put end to end, it would reach to the sun and back over 600 times (**Note :** The human body contains 100 trillion cells; the length of DNA in a cell is 6 feet; the distance between the sun and earth is 93 million miles).
- The total expenditure for human genome project was $3 billion. The magnitude of this huge amount has to be appreciated. If one starts counting at a non–stop rate of a dollar per second, it would take about 90 years to complete.

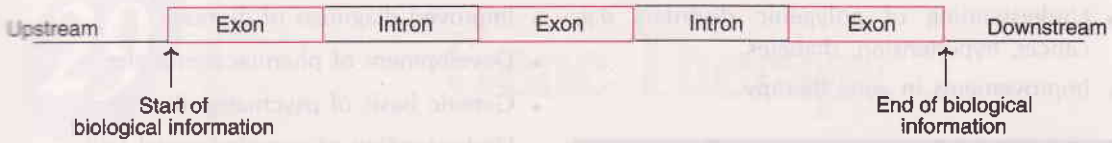


Fig. 28.3 : A diagrammatic representation of a typical structure of an average human gene.

only a small fraction (48 Mb) represents the actual genes, while the rest is due to gene-related sequences (introns, pseudogenes) and intergenic DNA (long interspersed nuclear elements, short interspread nuclear elements, microsatellites, DNA transposons etc.). ***Intergenic DNA*** represents the parts of the genome that lie between the genes which have no known function. This is appropriately regarded as ***junk DNA***.

Genes present in human genome

The two genome projects differ in their estimates of the total number of genes in humans. Their figures are in the range of 30,000–40,000 genes. The main reason for this variation is that it is rather difficult to specifically recognize the DNA sequences which are genes and which are not.

Before the results of the HGP were announced, the best guess of human genes was in the range of 80,000–100,000. This estimate was based on the fact that the number of proteins in human cells is 80,000–100,000, and thus so many genes expected. The fact that the number of genes is much lower than the proteins suggests that the ***RNA editing*** (RNA processing) is widespread, so that a single mRNA may code for more than one protein.

A diagrammatic representation of a typical structure of an average human gene is given in ***Fig.28.3***. It has exons and introns.

A broad categorization of human gene catalog in the form of a pie chart is depicted in ***Fig.28.4***. About 17.5% of the genes participate in the general biochemical functions of the cells, 23% in the maintenance of genome, 21% in signal transduction while the remaining 38% are involved in the production of structural proteins, transport proteins, immunoglobins etc.

Human genes encoding proteins

It is now clear that only 1.1-1.5% of the human genome codes for proteins. Thus, this figure 1.1-1.5% represents exons of genome.

As already described, a huge portion of the genome is composed of introns, and intergenic sequences (junk DNA).

The major categories of the proteins encoded by human genes are listed in ***Table 28.4***. The functions of at least 40% of these proteins are not known.

BENEFITS/APPLICATIONS OF HUMAN GENOME SEQUENCING

It is expected that the sequencing of human genome, and the genomes of other organisms will dramatically change our understanding and perceptions of biology and medicine. Some of the benefits of human genome project are given.

- Identification of human genes and their functions.

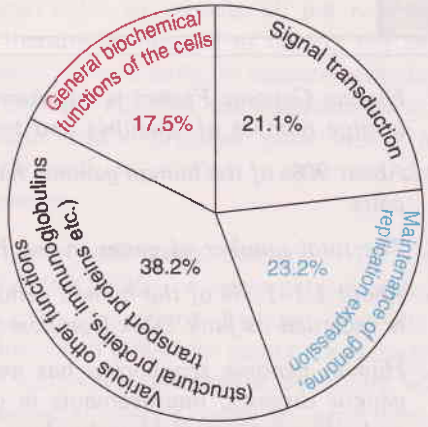


Fig. 28.4 : A pie chart showing a broad categorization of the human gene catalog (About 13000 genes whose functions are not known are not included).

- Understanding of polygenic disorders e.g. cancer, hypertension, diabetes.
- Improvements in gene therapy
- Improved diagnosis of diseases
- Development of pharmacogenomics
- Genetic basis of psychiatric disorders
- Understanding of complex social trait
- Improved knowledge on mutations
- Better understanding of developmental biology
- Comparative genomics
- Development of biotechnology

TABLE 28.4 Different categories of proteins encoded by human genes (*based on the Human Genome Project report, 2001*)

Category of proteins	*Percentage*	*Actual number of genes*
Unknown functions	41.0%	12,809
Nucleic acid enzymes	7.5%	2,308
Transcription factors	6.0%	1,850
Receptors	5.0%	1,543
Hydrolases	4.0%	1,227
Regulatory proteins (G-proteins, cell cycle regulators etc.)	3.2%	988
Protooncogenes	2.9%	902
Structural proteins of cytoskeleton	2.8%	876
Kinases	2.8%	868

*(**Note** : This table is based on the rough draft of human genome reported by Celera Genomics. The percentages are derived from a total of 26,383 genes)*

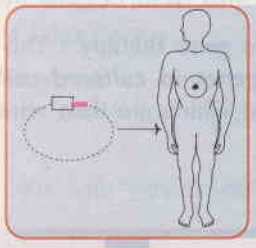
ETHICS AND HUMAN GENOME

The research on human genomes will make very sensitive data available that will affect the personal and private lives of individuals. For instance, once it is known that a person carries genes for an incurable disease, what would be the strategy of an insurance company? How will the society treat him/her? There is a possibility that ***individuals with substandard genome sequences may be discriminated***. Human genome results may also promote racial discrimination categorizing the people with ***good*** and ***bad genome sequences***. Considering the gravity of ethics related to a human genome, about 3% of the HGP budget was earmarked for ethical research.

SUMMARY

1. *Human Genome Project is an international venture involving several laboratories, and a large number of scientists and technicians from various disciplines.*
2. *About 90% of the human genome has been sequenced. It is composed of 3.2 billion base pairs.*
3. *The total number of genes in the humans is in the range of 30,000–40,000.*
4. *About 1.1–1.5% of the human genome codes for proteins while the remaining portion is regarded as junk DNA (composed of introns and intergenic sequences).*
5. *Human genome sequencing has wide range of applications–better understanding of genetic diseases, improvements in gene therapy, development of pharmacogenomics, and advancement of biotechnology.*

29 Gene Therapy



The gene therapy speaks :

"I represent the insertion of genes into cells;
The preferred being somatic cells to, treat diseases;
Although unsuccessful and unable to satisfy now;
I am highly optimistic about my future!"

Advances in biochemistry and molecular biology have helped to understand the genetic basis of inherited diseases. It was a dream of the researchers to replace the defective genes with good ones, and cure the genetic disorders.

Gene therapy is the process of inserting genes into cells to treat diseases. The newly introduced genes will encode proteins and correct the deficiencies that occur in genetic diseases. Thus, gene therapy primarily involves ***genetic manipulations*** in animals or humans ***to correct a disease,*** and keep the organism in good health. The initial experiments on gene therapy are carried out in animals, and then in humans. Obviously, the goal of the researchers is to benefit the mankind and improve their health.

An overview of gene therapy strategies is depicted in ***Fig.29.1***. In ***gene augmentation therapy***, a DNA is inserted into the genome to replace the missing gene product. In case of ***gene inhibition therapy***, the antisense gene inhibits the expression of the dominant gene.

APPROACHES FOR GENE THERAPY

There are two approaches to achieve gene therapy.

1. **Somatic cell gene therapy :** The ***non-reproductive (non-sex) cells*** of an organism are referred to as somatic cells. These are the cells of an organism other than sperm or egg cells, e.g., bone marrow cells, blood cells, skin cells, intestinal cells. At present, all the research on gene therapy is directed to correct the genetic defects in somatic cells. In essence, somatic cell gene therapy involves the ***insertion of a fully*** functional and ***expressible gene into a target somatic cell*** to correct a genetic disease permanently.

2. **Germ cell gene therapy :** The ***reproductive (sex) cells*** of an organism constitute germ cell line. Gene therapy involving the introduction of DNA into germ cells is passed on to the successive generations. For safety, ethical and technical reasons, germ cell gene therapy is ***not being attempted at present.***

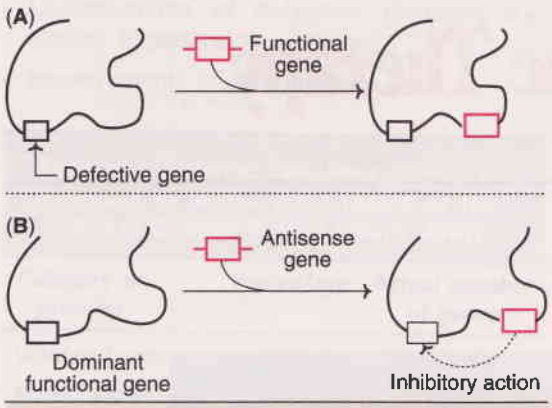


***Fig. 29.1** : Overview of two major gene therapy strategies (A) Gene augmentation therapy (B) Gene inhibition therapy.*

The genetic alterations in somatic cells are not carried to the next generations. Therefore, ***somatic cell gene therapy is preferred*** and extensively studied with an ultimate objective of correcting human diseases.

A large number of genetic disorders and other diseases are currently at various stages of gene therapy trials. A selected list of some important ones is given in ***Table 29.1***.

There are two types of gene therapies.

I. ***Ex vivo* gene therapy :** This involves the ***transfer of genes in cultured cells*** (e.g., bone marrow cells) which are then reintroduced into the patient.

TABLE 29.1 Human gene therapy trials

Disease	*Gene therapy*
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA).
Cystic fibrosis	Cystic fibrosis transmembrane regulator (CFTR).
Familial hypercholesterolemia	Low density lipoprotein (LDL) receptor.
Emphysema	α_1-Antitrypsin
Hemophilia B	Factor IX
Thalassemia	α- or β-Globin
Sickle-cell anemia	β-Globin
Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyltransferase (HGPRT).
Gaucher's disease	Glucocerebrosidase
Peripheral artery disease	Vascular endothelial growth factor (VEGF)
Fanconi anemia	Fanconi anemia C
Melanoma	Tumor necrosis factor (TNF)
Melanoma, renal cancer	Interleukin-2 (IL-2)
Glioblastoma (brain tumor), AIDS, ovarian cancer	Thymidine kinase (herpes simplex virus)
Head and neck cancer	p^{53}
Breast cancer	Multidrug resistance I
AIDS	*rev* and *env*
Colorectal cancer, melanoma, renal cancer	Histocompatability locus antigen-B_7 (HLA-B_7)
Duchenne muscular dystrophy	Dystrophin
Short stature*	Growth hormone
Diabetes*	Glucose transporter-2, (GLUT-2), glucokinase
Phenylketonuria*	Phenylalanine hydroxylase
Citrullinemia*	Arginosuccinate synthetase

** Mostly confined to animal experiments*

II. ***In vivo* gene therapy :** The ***direct delivery of genes into the cells*** of a particular tissue is referred to as *in vivo* gene therapy.

EX VIVO GENE THERAPY

The *ex vivo* gene therapy can be applied to only selected tissues (e.g., bone marrow) whose cells can be cultured in the laboratory.

The technique of *ex vivo* gene therapy involves the following steps (***Fig.29.2***).

1. Isolate cells with genetic defect from a patient.
2. Grow the cells in culture.
3. Introduce the therapeutic gene to correct gene defect.
4. Select the genetically corrected cells (stable transformants) and grow.
5. Transplant the modified cells to the patient.

The procedure basically involves the ***use of the patient's own cells for culture and genetic correction, and then their return back to the patient***. This technique is therefore, not associated with adverse immunological responses after transplanting the cells. *Ex vivo* gene therapy is efficient only if the therapeutic gene (remedial gene) is stably incorporated and continuously expressed. This can be achieved by use of vectors.

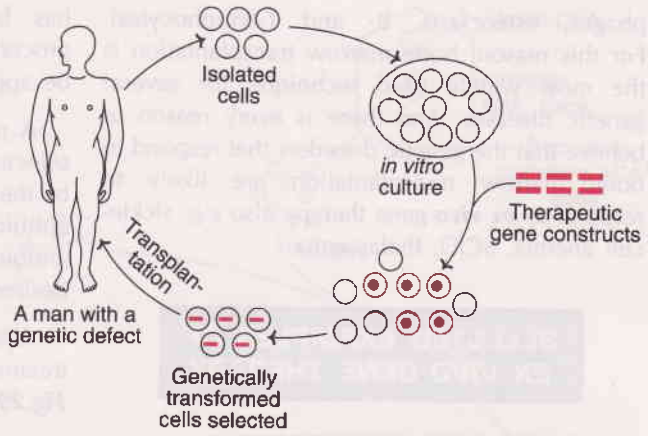


Fig. 29.2 : *The procedure for ex vivo gene therapy.*

VECTORS IN GENE THERAPY

The ***carrier particles or molecules used to deliver genes to somatic cells*** are referred to as vectors. The important vectors employed in *ex vivo* gene therapy are listed below and briefly described next.

- Viruses
- Human artificial chromosome
- Bone marrow cells.

VIRUSES

The vectors frequently used in gene therapy are viruses, particularly ***retroviruses***. RNA is the genetic material in retroviruses. As the retrovirus enters the host cell, it synthesizes DNA from RNA (by reverse transcription). The so formed viral DNA (referred to as provirus) gets incorporated into the DNA of the host cell. The proviruses are normally harmless. However, there is a tremendous risk, since some of the retroviruses can convert normal cells into cancerous ones. Therefore, it is absolutely essential to ensure that such a thing does not happen.

HUMAN ARTIFICIAL CHROMOSOME

The human artificial chromosome (HAC) is a synthetic chromosome that can replicate with other chromosomes, besides encoding a human protein. As already discussed above, use of retroviruses as vectors in gene therapy is associated with a heavy risk. This problem can be overcome if HAC is used. Some success has been achieved in this direction.

BONE MARROW CELLS

Bone marrow contains ***totipotent embryonic stem (ES) cells***. These cells are ***capable of dividing and differentiating into various cell types*** (e.g., red blood cells, platelets, macro-

phages, osteoclasts, B- and T-lymphocytes). For this reason, bone marrow transplantation is the most widely used technique for several genetic diseases. And there is every reason to believe that the genetic disorders that respond to bone marrow transplantation are likely to respond to ***ex vivo*** gene therapy also e.g. sickle-cell anemia, SCID, thalassemia.

SELECTED EXAMPLES OF *EX VIVO* GENE THERAPY

THERAPY FOR ADENOSINE DEAMINASE DEFICIENCY

The ***first*** and the most publicised human ***gene therapy*** was carried out to correct the deficiency of the enzyme adenosine deaminase (ADA). This was done on September 14, 1990 by a team of workers led by Blaese and Anderson at the National Institute of Health, USA (The girl's name is Ashanti, 4 years old then).

Severe combined immunodeficiency (SCID)

SCID is rare inherited immune disorder associated with T-lymphocytes, and (to a lesser extent) B-lymphocytes dysfunction. About 50% of SCID patients have a defect in the gene (located on chromosome 20, and has 32,000 base pairs and 12 exons) that encodes for adenosine deaminase. In the deficiency of ADA, deoxyadenosine and its metabolites (primarily deoxyadenosine 5'-triphosphate) accumulate and destroy T-lymphocytes. T-Lymphocytes are essential for body's immunity. Besides participating directly in body's defense, they promote the function of B-lymphocytes to produce antibodies. Thus, the ***patients of SCID*** (lacking ADA) ***suffer from infectious diseases and die at an young age***. Previously, the children suffering from SCID were treated with conjugated bovine ADA, or by bone marrow transplantation.

Technique of therapy for ADA deficiency

The general scheme of gene therapy adopted for introducing a defective gene in the patient has been depicted in ***Fig.29.2***. The same procedure with suitable modifications can also be applied for other gene therapies.

A plasmid vector bearing a proviral DNA is selected. A part of the proviral DNA is replaced by the ADA gene and a gene (G 418) coding for antibiotic resistance, and then cloned. The antibiotic resistance gene will help to select the desired clones with ADA gene.

A diagrammatic representation of the treatment of ADA deficient patient is depicted in ***Fig.29.3***.

Circulating lymphocytes are removed from a patient suffering from ADA deficiency. These cells are transfected with ADA gene by exposing to billions of retroviruses carrying the said gene. The genetically-modified lymphocytes are grown in cultures to confirm the expression of ADA gene and returned to the patient. These lymphocytes persist in the circulation and synthesize ADA. Consequently, the ability of the patient to produce antibodies is increased. However, there is a limitation. The lymphocytes have a short life span (just live for a few months), hence the transfusions have to be carried out frequently.

Transfer of ADA gene into stem cells

In 1995, ADA gene was transferred into the stem cells, obtained from the umbilical cord blood, at the time of baby's delivery. Four days after birth, the infant received the modified cells back. By this way, a permanent population of ADA gene producing cells was established.

IN VIVO GENE THERAPY

The ***direct delivery of the therapeutic gene*** (DNA) ***into the target cells*** of a particular tissue of a patient constitutes *in vivo* gene therapy (***Fig.29.4***). Many tissues are the potential candidates for this approach. These include liver, muscle, skin, spleen, lung, brain and blood cells. Gene delivery can be carried out by ***viral*** or ***non-viral vector systems***. The success of ***in vivo*** gene

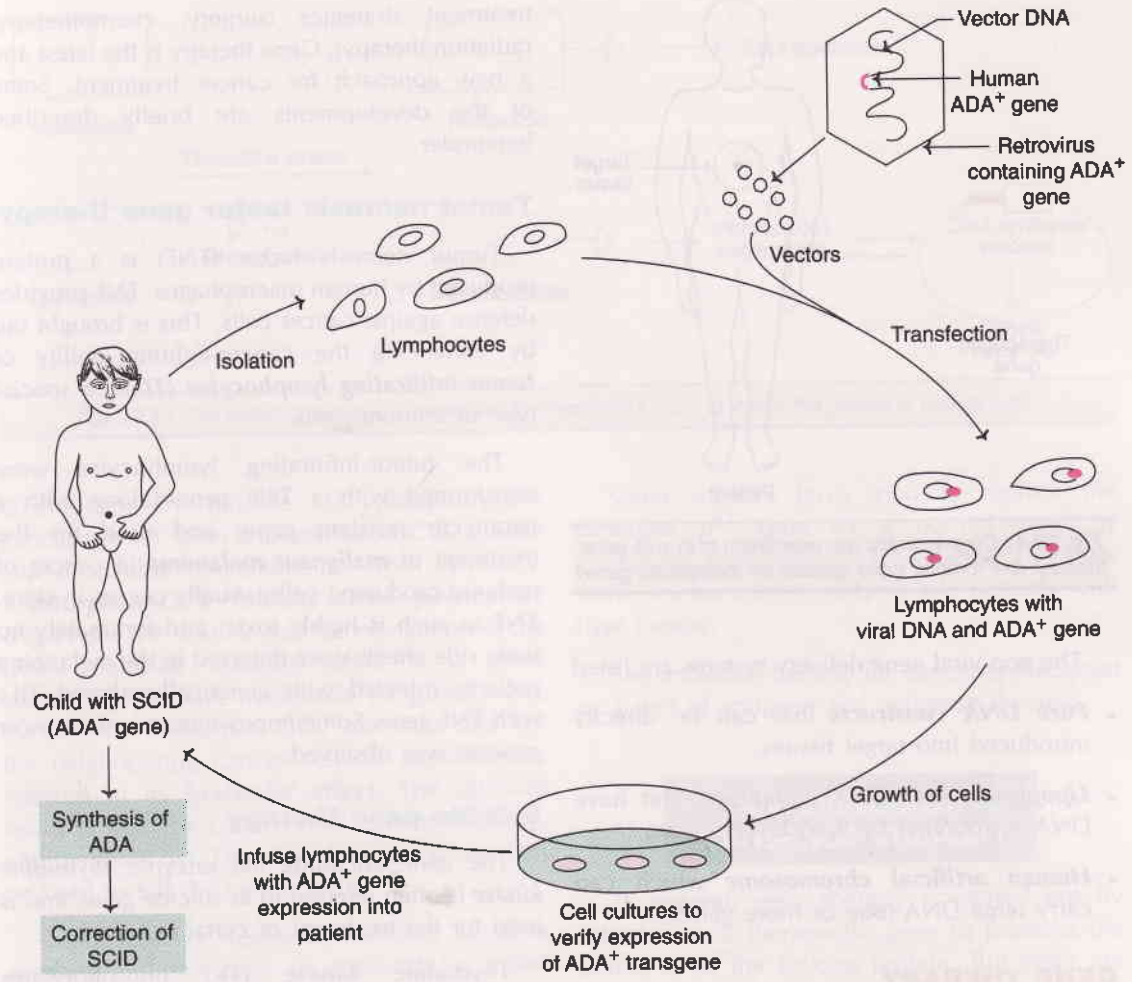


***Fig. 29.3** : Treatment of adenosine deaminase (ADA) deficient patient by somatic ex vivo gene therapy (SCID-Severe combined immunodeficiency).*

therapy mostly depends on the following parameters

- The efficiency of the uptake of the remedial (therapeutic) gene by the target cells.
- Intracellular degradation of the gene and its uptake by nucleus.
- The expression capability of the gene.

GENE DELIVERY BY VIRUSES

Many viral vector systems have been developed for gene delivery. These include retroviruses, adenoviruses, adeno-associated viruses and herpes simplex virus.

GENE DELIVERY BY NON-VIRAL SYSTEMS

There are certain limitations in using viral vectors in gene therapy. In addition to the prohibitive cost of maintaining the viruses, the viral proteins often induce inflammatory responses in the host. Therefore, there is a continuous search by researchers to find alternatives to viral vector systems.

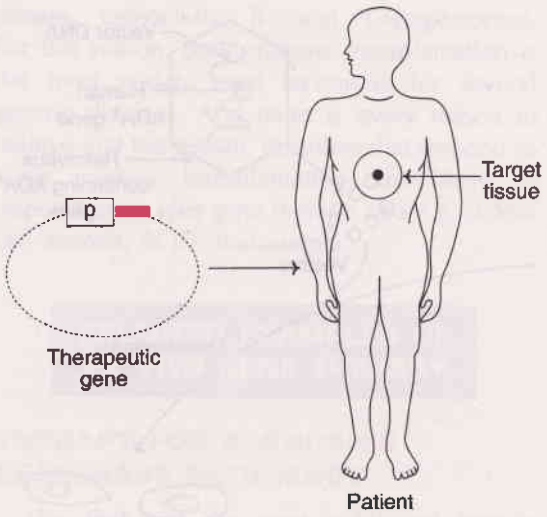


Fig. 29.4 : Diagrammatic representation of in vivo gene therapy. (p-Promoter gene specific for therapeutic gene)

The non-viral gene delivery systems are listed

- ***Pure DNA constructs*** that can be directly introduced into target tissues.
- ***Lipoplexes***, lipid-DNA complexes that have DNA surrounded by lipid layers.
- ***Human artificial chromosome*** which can carry large DNA (one or more genes).

GENE THERAPY STRATEGIES FOR CANCER

Cancer is the leading cause of death throughout the world, despite the intensive treatment strategies (surgery, chemotherapy, radiation therapy). Gene therapy is the latest and a new approach for cancer treatment. Some of the developments are briefly described hereunder.

Tumor necrosis factor gene therapy

Tumor necrosis factor (TNF) is a protein produced by human macrophages. TNF provides defense against cancer cells. This is brought out by enhancing the cancer-fighting ability of ***tumor-infiltrating lymphocytes (TILs)***, a special type of immune cells.

The tumor-infiltrating lymphocytes were transformed with a TNF gene (along with a neomycin resistant gene) and used for the treatment of ***malignant melanoma*** (a cancer of melanin producing cells, usually occurs in skin). TNF as such is highly toxic, and fortunately no toxic side effects were detected in the melanoma patients injected with genetically altered TILs with TNF gene. Some improvement in the cancer patients was observed.

Suicide gene therapy

The gene encoding the enzyme ***thymidine kinase*** is often referred to as suicide gene, and is used for the treatment of certain cancers.

Thymidine kinase (TK) phosphorylates nucleosides to form nucleotides which are used for the synthesis of DNA during cell division. The drug ***ganciclovir*** (***GCV***) bears a close structural resemblance to certain nucleosides

BIOMEDICAL / CLINICAL CONCEPTS

- *Theoretically, gene therapy is the permanent solution for genetic diseases.*
- *A large number of genetic disorders and other diseases are at various stages of gene therapy trials e.g. sickle-cell anemia, cystic fibrosis, AIDS, cancer.*
- *Ganciclovir (a drug with structural resemblance to thymidine) has been used (suicide gene therapy) for the treatment of brain tumors, although with limited success.*
- *Despite extensive research and trials, as of now, no disease has been permanently cured by gene therapy. However, a breakthrough may come at anytime.*

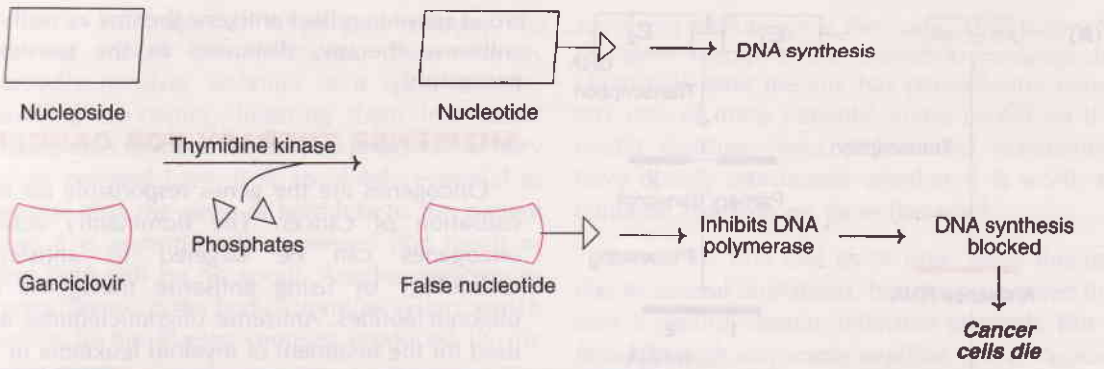


Fig. 29.5 : The action of ganciclovir mediated by thymidine kinase to inhibit the growth of cancer cells.

(thymidine). By mistake, TK phosphorylates ganciclovir to form triphosphate-GCV, a false and unsuitable nucleotide for DNA synthesis. ***Triphosphate-GCV inhibits DNA polymerase (Fig.29.5).*** The result is that the elongation of the DNA molecule abruptly stops at a point containing the false nucleotide (of ganciclovir). Further, the triphospate-GCV can enter and kill the neighbouring cancer cells, a phenomenon referred to as ***bystander effect***. The ultimate result is that the cancer cells cannot multiply, and therefore die. Thus, the drug ganciclovir can be used to kill the cancer cells.

Ganciclovir is frequently referred to as a ***prodrug*** and this type of approach is called ***prodrug activation gene therapy***. Ganciclovir has been used for treatment of brain tumors (e.g., glioblastoma, a cancer of glial cells in brain), although with a limited success.

Gene replacement therapy

A gene named ***p*53** codes for a ***p***rotein with a molecular weight of ***53*** kilodaltons (hence p^{53}). p^{53} is considered to be a ***tumor-suppressor gene***, since the protein it encodes binds with DNA and inhibits replication. The tumor cells of several tissues (breast, brain, lung, skin, bladder, colon, bone) were found to have altered genes of p^{53} (mutated p^{53}), synthesizing different proteins from the original. These altered proteins cannot inhibit DNA replication. It is believed that the damaged p^{53} gene may be a causative factor in tumor development.

Some workers have tried to replace the damaged p^{53} gene by a normal gene by employing adenovirus vector systems. There are some encouraging results in the patients with liver cancer.

The antisense therapy for cancer is discussed as a part of antigene and antisense therapy.

ANTIGENE AND ANTISENSE THERAPY

In general, gene therapy is carried out by introducing a therapeutic gene to produce the defective or the lacking protein. But there are certain disorders (cancer, viral and parasitic infections, inflammatory diseases) which result in an overproduction of certain normal proteins. It is possible to treat these diseases by ***blocking transcription*** using a single-stranded nucleotide sequence (antigene oligonucleotide) that hybridizes with the specific gene, and this is called ***antigene therapy***. ***Antisense therapy*** refers to the ***inhibition of translation*** by using a single-stranded nucleotide (antisense oligonucleotide). Further, it is also possible to inhibit both transcription and translation by blocking (with oligonucleotides) the transcription factor responsbile for the specific gene expression.

Nucleic acid therapy refers to the ***use of DNA or RNA molecules for therapeutic purposes***, as stated above. The naturally occurring sequences of DNA and RNA (with suitable modifications)

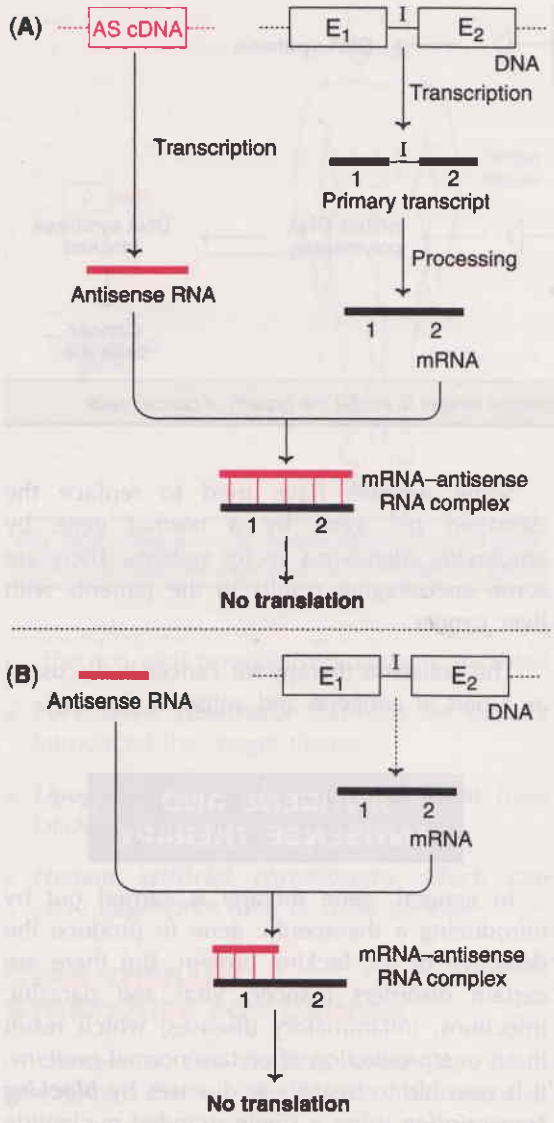


***Fig. 29.6** : Inhibition of translation by antisense RNA (**A**) The cloned AS cDNA introduced into cells to produce antisense RNA (**B**) Antisense RNA directly introduced into cells. (AS cDNA = Antisense complementary DNA; E_1, E_2-Exons in a gene; I-Intron)*

or the synthetic ones can be employed in nucleic acid therapy. Theoretically, there is a vast potential for use of nucleic acids as therapeutic agents. But most of the work that is being carried out relates to the use of RNA in antisense therapy. Some of these are described below (**Note** : Some authors use antisense therapy in a broad sense to reflect antigene therapy as well as antisense therapy, discussed in the previous paragraph).

ANTISENSE THERAPY FOR CANCER

Oncogenes are the genes responsible for the causation of cancer. The dominantly acting oncogenes can be targeted in antisense technology by using antisense transgenes or oligonucleotides. Antisense oligonucleotides are used for the treatment of myeloid leukemia in as early as 1991.

Antisense RNA molecules are more frequently used in cancer therapy. This approach is effective only if the antisense oligonucleotide (antisense mRNA) specifically binds to the target mRNA, and blocks protein biosynthesis (translation). This can be achieved in two ways, as illustrated in ***Fig.29.6***.

The antisense cDNA can be cloned and transfected into cells. Antisense mRNA is synthesized by transcription. This can readily bind with the specific mRNA and block translation (***Fig.29.6A***). The mRNA is actually formed by a gene containing exons and introns through transcription, followed by processing.

The other way to block translation is to directly introduce antisense RNA into the cells. This hybridizes with target mRNA and blocks translation (***Fig.29.6B***).

The antisense mRNA therapy was tried for the treatment of a brain tumor namely malignant glioma and the cancer of prostate gland. In case malignant glioma, the protein insulin-like growth factor I (IGF-I) is overproduced, while in prostate cancer, insulin-like growth factor I receptor (IGF-IR) protein is more synthesized. For both these cancers, the respective antisense cDNAs can be used to synthesize antisense mRNA molecules. These in turn, are used to block translation, as briefly described above, and illustrated in ***Fig.29.6***.

THE FUTURE OF GENE THERAPY

Theoretically, gene therapy is ***the permanent solution for genetic diseases***. But it is not as

simple as it appears since gene therapy has several inbuilt complexicities. Gene therapy broadly involves isolation of a specific gene, making its copies, inserting them into target tissue cells to make the desired protein. The story does not end here. It is absolutely essential to ensure that the gene is harmless to the patient and it is appropriately expressed (too much or too little will be no good). Another concern in gene therapy is the body's immune system which reacts to the foreign proteins produced by the new genes.

The public, in general, have ***exaggerated expectations*** on gene therapy. The researchers, at least for the present, are ***unable to satisfy them***. As per the records, by 1999 about 1000 Americans had undergone clinical trails involving various gene therapies. Unfortunately, the gene therapists are unable to categorically claim that gene therapy has permanently cured any one of these patients! Some people in the media (leading news papers and magazines) have openly questioned whether it is worth to continue research on gene therapy!!

It may be true that as of now, gene therapy due to several limitations, has not progressed the way it should, despite intensive research. But ***a breakthrough may come anytime***, and of course, this is only possible with persistent research. And a day may come (it might take some years) when almost every disease will have a gene therapy, as one of the treatment modalities. And gene therapy will revolutionize the practice of medicine!

SUMMARY

1. *Gene therapy is the process of inserting genes into cells to treat diseases. Somatic cell gene therapy, involving the insertion of an expressible gene into somatic cells, is the preferred approach.*
2. *Ex vivo gene therapy involves the transfer of genes in cultured cells which are then reintroduced into the patient. The direct delivery of genes into the cells of a particular tissue is regarded as in vivo gene therapy.*
3. *Gene therapy was successfully carried out in a patient of severe combined immunodeficiency (caused by the deficiency of the enzyme adenosine deaminase).*
4. *Antigene therapy involves blocking of transcription (by antigene oligonucleotide) while in antisense therapy, translation is inhibited (by antisense oligonucleotide). These approaches are in the experimental stages for the therapy of cancer and AIDS.*
5. *Although as of now, gene therapy has not offered any permanent cure to any human patients, a breakthrough may come anytime. And gene therapy may revolutionize the practice of medicine.*

30 Bioinformatics

The bioinformatics speaks :

"I am the product of biology and informational technology;
International computer network (internet) is my brain;
Biological databases represent my body;
I have revolutionized the advances in biology."

Bioinformatics is ***the combination (or marriage!) of biology and information technology***. Basically, bioinformatics is a recently developed science using information to understand biological phenomenon. It broadly involves the computational tools and methods used to manage, analyse and manipulate volumes and volumes of biological data.

Bioinformatics may also be regarded as a part of the ***computational biology***. The latter is concerned with the application of quantitative analytical techniques in modeling and solving problems in the biological systems. Bioinformatics is an interdisciplinary approach requiring advanced knowledge of computer science, mathematics and statistical methods for the understanding of biological phenomena at the molecular level.

History and relevance of bioinformatics

The term bioinformatics was first ***introduced in 1990s***. Originally, it dealt with the management and analysis of the data pertaining to ***DNA, RNA and protein sequences***. As the biological data is being produced at an unprecedented rate, their management and interpretation invariably requires bioinformatics. Thus, bioinformatics now includes many other types of biological data. Some of the most important ones are listed below

- Gene expression profiles
- Protein structure
- Protein interactions
- Microarrays (DNA chips)
- Functional analysis of biomolecules
- Drug designing.

Bioinformatics is largely (not exclusively) a computer-based discipline. Computers are in fact very essential to handle large volumes of biological data, their storage and retrieval.

We have to accept the fact that there is no computer on earth (however advanced) which

can store information, and perform the functions like a living cell. Thus a highly complex information technology lies right within the cells of an organism. This primarily includes the organism's genes and their dictates for the organisms biological processes and behaviour.

BROAD COVERAGE OF BIOINFORMATICS

Bioinformatics covers many specialized and advanced areas of biology.

Functional genomics : *Identification of genes and their respective functions.*

Structural genomics : Predictions related to functions of proteins.

Comparative genomics : For understanding the genomes of different species of organisms.

DNA microarrays : These are designed to measure the levels of gene expression in different tissues, various stages of development and in different diseases.

Medical informatics : This involves the management of biomedical data with special reference to biomolecules, *in vitro* assays and clinical trials.

COMPONENTS OF BIOINFORMATICS

Bioinformatics comprises three components

1. **Creation of databases :** This involves the organizing, storage and management of the biological data sets. The databases are accessible to researchers to know the existing information and submit new entries. e.g. protein sequence data bank for molecular structure. Databases will be of no use until analysed.

2. **Development of algorithms and statistics :** This involves the development of tools and resources to determine the relationship among the members of large data sets e.g. comparison of protein sequence data with the already existing protein sequences.

3. **Analysis of data and interpretation :** The appropriate use of components 1 and 2 (given above) to analyse the data and interpret the results in a biologically meaningful manner. This includes DNA, RNA and protein sequences, protein structure, gene expression profiles, and biochemical pathways.

BIOINFORMATICS AND THE INTERNET

The internet is an ***inter***national computer ***net***work. A computer network involves a group of computers that can communicate (usually over a telephone system) and exchange data between users.

It is the ***internet protocol*** (IP) that determines how the packets of information are addressed

BIOMEDICAL / CLINICAL CONCEPTS

- *Bioinformatics has largely benefited biological and medical sciences, particularly related to molecular biology and biotechnology. Some applications are listed :*
  - *Sequencing of macromolecules (proteins, DNA, RNA)*
  - *Human genome sequencing*
  - *Molecular modelling of biomolecules*
  - *Handling of vast biological data*
  - *Designing of drugs for the treatment of diseases*
  - *Development of models for the functioning of cells, tissues and organs*
- *As such, there is no field of biological science that is not benefited by bioinformatics.*

and routed over the network. To access the internet, a computer must have the correct hardware (modem/network card), appropriate software and permission for access to network. For this purpose, one has to subscribe to an ***internet service provider*** (ISP).

World wide web (www) : www involves the exchange of information over the internet using a programme called ***browser***. The most widely used browsers are Internet explorer and Netscape navigator.

www works on the basis of ***Uniform resource locator*** (URL) which is a document with a unique address. URLs takes the format ***http.//*** (***h***yper***t***ext ***t***ransfer ***p***rotocol) that can identify the protocol for communication over www.

BIOLOGICAL DATABASES

The collection of the ***biological data*** on a computer which can be manipulated to appear ***in varying arrangements and subsets*** is regarded as a database. The biological information can be stored in different databases. Each database has its own website with unique navigation tools.

The biological databases are, in general, publicly accessible. Selected examples of biological databases are briefly described (***Table 30.1***).

Table 30.1 Selected examples of biological databases in bioinformatics

Database(s)	*Salient features*
Primary nucleotide sequence databases	
GenBank (www.ncbi.nih.gov/GeneBank/)	Provides nucleotide sequence databases maintained by the National Center for Biotechnology Information (NCBI), USA.
Other nucleotide sequence databases	
UniGene (www.ncbi.nih.gov/UniGene/)	The nucleotide sequences of GenBank in the form of clusters, representing genes are available.
Genome Biology (www.ncbi.nlm.nih.gov/Genomes/)	The information about the completed genomes is available.
Protein sequence database	
SWISS-PROT (www.expasy.ch/sport)	Provides the description of the structure of a protein, its domains structure, post-translational modifications, variants etc. It has high level of integration with other databases and minimal level of redundancy.
Protein sequence motif databases	
PROSITE (www.expasy.ch/prosite/)	Provides information on protein families and domains. It also has patterns and profiles for sequences and biological functions.
Macromolecular databases	
PDB (www.rcsb.org/pdb)	This is the primary database for 3-dimensional (3-D) structures of biological macromolecules (determined by X-ray and NMR studies).
Other databases	
KEGG (www.genome.ad.jp/kegg/)	The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database with latest computerised information on biomolecules and cell biology. KEGG provides details on information pathways, interacting molecules and the connecting links with genes.

Nucleotide sequence databases

The nucleotide sequence data submitted by the scientists and genome sequencing groups is at the databases namely GenBank, EMBL (European Molecular Biology Laboratory) and DDBJ (DNA Data Bank of Japan). There is a good coordination between these three databases as they are synchronized on daily basis.

Besides the primary nucleotide databases (referred above), there are some other databases also to provide information on genes, genomes and ongoing research projects.

Protein sequence databases

Protein sequence databases are usually prepared from the existing literature and/or in consultation with the experts. In fact, these databases represent the translated DNA databases.

Molecular structure of databases

The three dimensional (3-D) structures of macromolecules are determined by X-ray crystallography and nuclear magnetic resonance (NMR). PDB and SCOP are the primary databases of 3-D structures of biological molecules.

Other databases

KEGG database is an important one that provides information on the current knowledge of molecular biology and cell biology with special reference to information on metabolic pathways, interacting molecules and genes.

APPLICATIONS OF BIOINFORMATICS

The advent of bioinformatics has revolutionized the advancements in biological science. And biotechnology is largely benefited by bioinformatics. The best example is the sequencing of human genome in a record time which would not have been possible without bioinformatics. A selected list of applications of bioinformatics is given below.

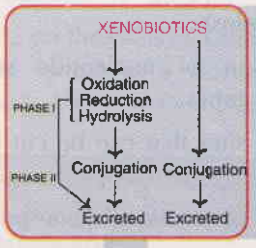
- Sequence mapping of biomolecules (DNA, RNA, proteins).
- Identification of nucleotide sequences of functional genes.
- Finding of sites that can be cut by restriction enzymes.
- Prediction of functional gene products.
- To trace the evolutionary trees of genes.
- For the prediction of 3-dimensional structure of proteins.
- Molecular modelling of biomolecules.
- Designing of drugs for medical treatment.
- Handling of vast biological data which otherwise is not possible.
- Development of models for the functioning various cells, tissues and organs.

The above list of applications however, may be treated as incomplete, since at present there is no field in biological sciences that does not involve bioinformatics.

SUMMARY

1. *Bioinformatics (a computer-based discipline) represents an alliance between biology and information technology.*
2. *The storage, management and interpretation of vast biological data invariably requires bioinformatics.*
3. *Bioinformatics comprises three components-creation of data base, development of algorithms and statistics, and analysis of data and interpretation.*
4. *Biological databases, containing the biological information, are publicly accessible e.g. GenBank (www.ncbi.nih.gov/GeneBank).*
5. *Bioinformatics has revolutionized the advancements of biological and medical sciences e.g. sequencing of human genome.*

31 Metabolism of Xenobiotics (Detoxification)



The detoxification speaks :

"I deal with the metabolism of foreign compounds;
Through oxidation, reduction, hydrolysis and conjugation,
To convert xenobiotics into soluble forms;
For their effective elimination from the body."

Man is continuously exposed to several foreign compounds such as drugs, pollutants, food additives, cosmetics, pesticides etc. Certain unwanted compounds are produced in the large intestine by the bacteria which enter the circulation. These include indole from ***tryptophan***, ***cadaverine*** from lysine, tyramine from tyrosine, phenol from phenylalanine etc. In the normal metabolism of the body, certain waste compounds (e.g. bilirubin) are formed. A vast majority of the foreign compounds or the unwanted substances, produced in the body, are toxic and, therefore, they should be quickly eliminated from the body.

The term ***detoxication or detoxification*** refers to ***the series of biochemical reactions occurring in the body to convert the foreign (often toxic) compounds to non-toxic or less toxic, and more easily excretable forms.***

Detoxification—a misnomer?

Detoxification is rather misleading, since sometimes a detoxified product is more toxic than the original substance (e.g. procarcinogens to carcinogens). It appears that the body tries to get rid of a foreign substance by converting it into a more soluble (often polar), and easily excretable compound, and this may be sometimes associated with increased toxicity (e.g. conversion of methanol to formaldehyde).

In recent years, the term detoxification is replaced by ***biotransformation*** or metabolism of xenobiotics (*Greek* : xenos—strange, foreign) or simply ***metabolism of foreign compounds.***

Site of detoxification

The detoxification reactions are carried out ***mainly in the liver*** which is equipped with the enzyme machinery. Kidney and other organs may sometimes be involved. The products formed by detoxification are mostly excreted by the kidneys, less frequently excreted via feces or expired air.

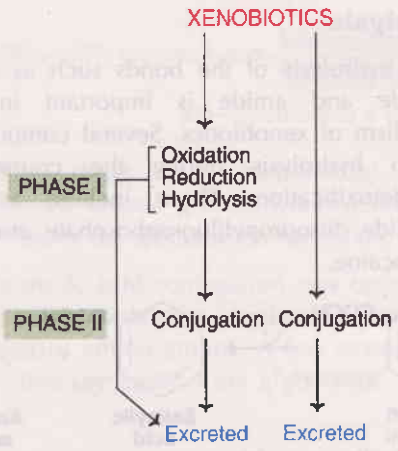


Fig. 31.1 : Phase I and phase II reactions in the metabolism of xenobiotics.

MECHANISM OF DETOXIFICATION

The metabolism of xenobiotics may be divided into two phases which may occur together or separately (***Fig.31.1***).

Phase I : The reactions of phase I are ***oxidation***, ***reduction*** and ***hydrolysis.***

Phase II : These are the conjugation reactions, involving compounds such as glucuronic acid, amino acids (glycine), glutathione, sulfate, acetate and methyl group.

Generally, detoxification of a compound involves phase I as well as phase II reactions. For instance, oxidation followed by conjugation is the most frequent process in the metabolism of xenobiotics.

Oxidation

A large number of foreign substances are detoxified by oxidation. These include alcohols, aldehydes, amines, aromatic hydrocarbons and sulfur compounds. In general, aliphatic compounds are more easily oxidized than aromatic ones.

Alcohols : Aliphatic and aromatic alcohols undergo oxidation to form the corresponding acids.

$CH_3OH \longrightarrow HCOOH$
Methanol — Formic acid

$C_2H_5OH \longrightarrow CH_3COOH$
Ethanol — Acetic acid

$C_6H_5CH_2OH \longrightarrow C_6H_5COOH$
Benzyl alcohol — Benzoic acid

Aldehydes : Aldehydes are oxidized to produce the corresponding acids.

$C_6H_5CHO \longrightarrow C_6H_5COOH$
Benzaldehyde — Benzoic acid

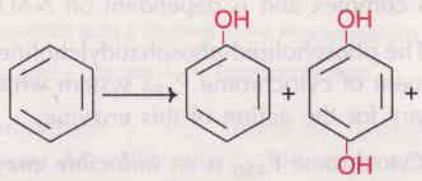
$C.Cl_3CHO \longrightarrow CCl_3COOH$
Chloral — Trichloroacetic acid

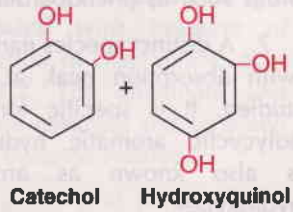
Amines and their derivatives : Alipahtic amines are converted to the corresponding acids, liberating urea while aromatic amino acids are oxidized to phenols.

$RCH_2NH_2 \longrightarrow R\text{–}COOH + NH_2\text{–}CO\text{–}NH_2$
Aliphatic amine — Aliphatic acid — Urea

$C_6H_5NH_2 \longrightarrow HO\text{–}C_6H_4\text{–}NH_2$
Aniline — p-Amino phenol

Aromatic hydrocarbons : Benzene may be oxidized to mono, di- and trihydroxy phenols as shown below





Sulfur compounds : Organic sulfur is oxidized to sulfuric acid.

Drugs : Meprobamate is a tranquilizer. It is oxidized to hydroxymeprobamate and excreted in urine.

Role of cytochrome P_{450}

Most of the oxidation reactions of detoxification are catalysed by monooxygenase or cytochrome P_{450}. This enzyme, also called

mixed function oxidase, is associated with the microsomes. The usage P_{450} refers to the absorption peak (at 450 nm), exhibited by the enzyme when exposed to carbon monoxide.

Most of the reactions of cytochrome P_{450} involve the addition of a hydroxyl group to aliphatic or aromatic compounds which may be represented as

$$RH + O_2 + NADPH \longrightarrow ROH + H_2O + NADP^+$$

Salient features of cytochrome P_{450}

1. Multiple forms of cytochrome P_{450} are believed to exist, ranging from 20 to 200. At least 6 species have been isolated and worked in detail.

2. They are all ***hemoproteins***, containing heme as the prosthetic group.

3. Cytochrome P_{450} species are found in the highest concentration in the microsomes of liver. In the adrenal gland, they occur in mitochondria.

4. The mechanism of action of cytochrome P_{450} is complex and is dependent on NADPH.

5. The phospholipid-phosphatidylcholine is a constituent of cytochrome P_{450} system which is necessary for the action of this enzyme.

6. Cytochrome P_{450} is an ***inducible enzyme***. Its synthesis is increased by the administration of drugs such as phenobarbitol.

7. A distinct species namely cytochrome P_{448} (with absorption peak at 448 nm) has been studied. It is specific for the metabolism of polycyclic aromatic hydrocarbons, hence it is also known as aromatic hydrocarbon hydroxylase.

Reduction

A few examples of detoxification by reduction are given.

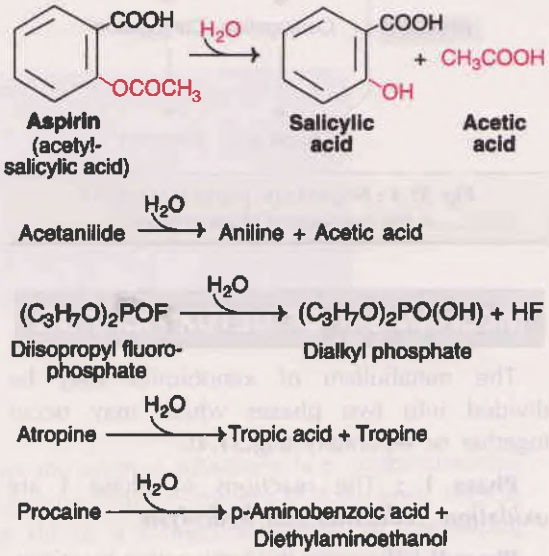
$C_6H_2OH(NO_2)_3 \longrightarrow C_6H_2OH(NO_2)_2NH_2$
Picric acid — Picramic acid

$CCl_3.CH(OH)_2 \longrightarrow CCl_3CH_2OH$
Chloral — Trichloroethanol

$C_6H_5NO_2 \longrightarrow C_6H_5NH_2$
Nitrobenzene — Aminobenzene

Hydrolysis

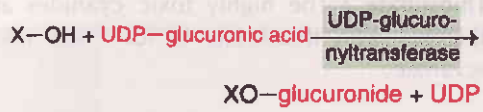
The hydrolysis of the bonds such as ester, glycoside and amide is important in the metabolism of xenobiotics. Several compounds undergo hydrolysis during the course of their detoxification. These include aspirin, acetanilide, diisopropylfluorophosphate, atropine and procaine.



Conjugation

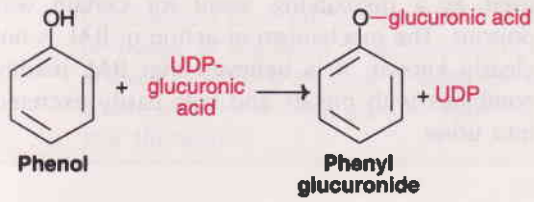
Several xenobiotics undergo detoxification by conjugation to produce less toxic and/or more easily excretable compounds. Conjugation is the process in which a ***foreign compound combines with a substance produced in the body.*** The process of conjugation may occur either directly or after the phase I reactions. At least 8 different conjugating agents have been identified in the body. These are glucuronic acid, glycine, cysteine (of glutathione), glutamine, methyl group, sulfate, acetic acid and thiosulfate.

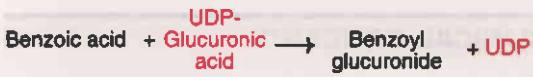
Glucuronic acid : Conjugation with glucuronic acid is the most common. The active form of glucuronic acid is UDP-glucuronic acid produced in the uronic acid pathway (***Chapter 13***). The microsomal enzymes UDP-glucuronyl transferases participate in glucuronide formation. A general reaction of glucuronide conjugation is shown below (X-OH represents xenobiotic).

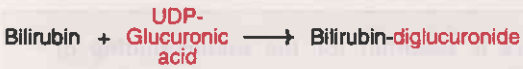


Certain drugs (e.g. barbiturates) when administered induce glucuronyltransferase and this increases the glucuronide formation.

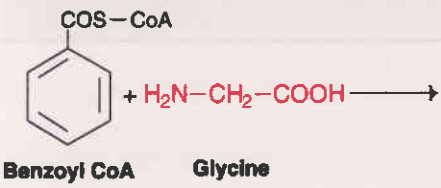
Glucuronic acid conjugation may occur with compounds containing hydroxyl, carbonyl, sulfhydryl or amino groups. A few examples of glucuronide conjugation are given here.

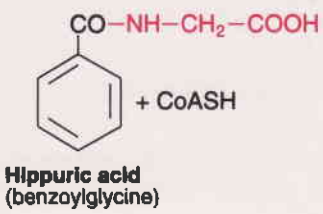






Glycine : Many aromatic carboxylic acids (e.g. benzoic acid, phenylacetic acid) are conjugated with glycine. Hippuric acid is formed when glycine is conjugated with benzyl CoA. The excretion of hippuric acid (*Greek* : hippos–horse) was first reported in 1829 in the urine of cows and horses.





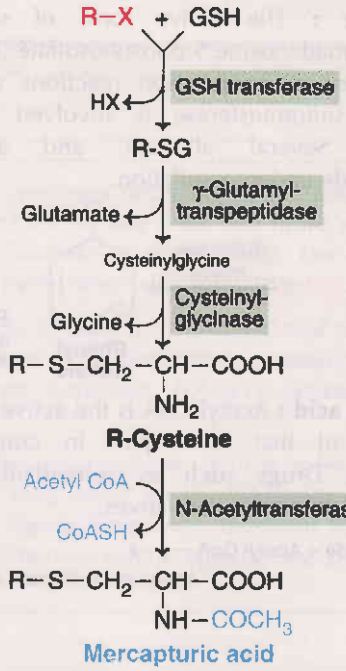


Fig. 31.2 : Role of glutathione in conjugation to form mercapturic acid (R-X–A xenobiotic; GSH–Glutathione).

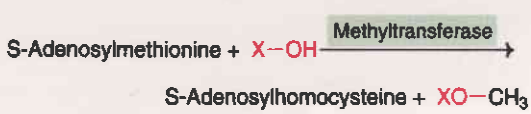
Phenylacetic acid + Glycine ⟶ Phenylaceturic acid

Cholic acid + Glycine ⟶ Glycocholic acid

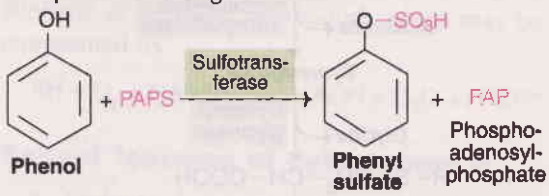
Glutathione : The tripeptide glutathione (Glu-Cys-Gly), is the active conjugating agent. A wide range of organic compounds such as alkyl or aryl halides, alkenes, nitro compounds and epoxides get ***conjugated with cysteine of glutathione***. The formation of mercapturic acid is depicted in ***Fig.31.2***. The glutamate and glycine of glutathione are removed and an acetyl group is added to the cysteine residue.

Glutamine : Phenylacetic acid is conjugated with glutamine to form phenylacetyl glutamine. Conjugation with glutamine is, however, relatively less important.

Methyl group : The methyl group ($-CH_3$) of ***S-adenosylmethionine*** is frequently used to methylate certain xenobiotics. This is catalysed by the enzyme methyltransferase.



Sulfate : The active form of sulfate—3'-phosphoadenosine 5-phosphosulfate (***PAPS***)—participates in conjugation reactions and the enzyme sulfotransferase is involved in this process. Several aliphatic and aromatic compounds undergo sulfation.



Acetic acid : Acetyl CoA is the active form of acetic acid that takes part in conjugating reactions. Drugs such as sulfanilamide are converted to acetyl derivatives.

Sulfanilamide + Acetyl CoA ⟶ Acetyl sulfanilamide + CoASH

Thiosulfate : The highly toxic cyanides are conjugated with thiosulfate to form less toxic thiocyanate.

Cyanide + Sodium thiosulfate ⟶ Thiocyanate + Sodium sulfate

Detoxification by drugs : It may be surprising to know that some drugs are administered to detoxify foreign substances. The toxic effects of certain metals such as arsenic, mercury and cadminum could be overcome by administering BAL (British antilewisite). This compound was developed during the World War II and was used as a detoxifying agent for certain war poisons. The mechanism of action of BAL is not clearly known. It is believed that BAL readily combines with metals and gets easily excreted into urine.

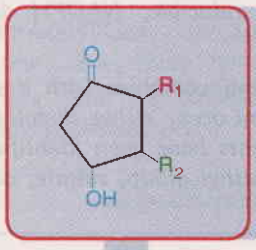
BIOMEDICAL / CLINICAL CONCEPTS

- *Knowledge of the metabolism of xenobiotics is essential for the understanding of toxicology, pharmacology and drug addiction.*
- *The body possesses the capability to get rid of the foreign substances by converting them into more easily excretable forms.*
- *Detoxification is not necessarily associated with the conversion of toxic into non-toxic compounds. For instance, methanol is metabolized to a more toxic formaldehyde.*
- *Detoxification primarily occurs in the liver through one or more of the reactions, namely oxidation, reduction, hydrolysis and conjugation.*
- *British antilewisite (BAL), a compound developed during Second World War, was used to detoxify certain war poisons.*

SUMMARY

1. *Detoxification deals with the series of biochemical reactions occurring in the body to convert the foreign (often toxic) compounds to non-toxic or less toxic and more easily excretable forms. Liver is the major site of detoxification. In recent years, the term detoxification is replaced by biotransformation or metabolism of xenobiotics.*
2. *Detoxification may be divided into phase I (oxidation, reduction, hydrolysis) and phase II reactions (conjugation). Oxidation is a major process of detoxification, involving the microsomal enzyme cytochrome P_{450} which is an inducible, NADPH dependent hemoprotein.*
3. *Conjugation is a process in which a foreign compound combines with a substance produced in the body. The process of conjugation may occur either directly or after phase I reactions. At least 8 different conjugating agents have been identified in the body—glucuronic acid, glycine, cysteine, glutamine, methyl group, sulfate, acetic acid and thiosulfate.*

32 Prostaglandins and Related Compounds



The prostaglandins speak :

"Twenty carbon compounds are we!
Synthesized from arachidonic acid;
Act as local hormones in function;
Widely used as therapeutic agents."

Prostaglandins and their related compounds—prostacyclins (PGI), thromboxanes (TXA), leukotrienes (LT) and lipoxins are collectively known as ***eicosaniods***, since they all contain 20 carbons (*Greek* : eikosi-twenty). Eicosanoids are considered as locally acting hormones with a wide range of biochemical functions.

History : Prostaglandins (PGs) were first discovered in human semen by Ulf von Euler (of Sweden) in 1930. These compounds were found to stimulate uterine contraction and reduce blood pressure. von Euler presumed that they were synthesized by prostate gland and hence named them as prostaglandins. It was later realized that PGs and other eicosanoids are synthesized in almost all the tissues (exception–erythrocytes). By then, however, the name prostaglandins was accepted worldwide, and hence continued.

The prostaglandins E and F were first isolated from the biological fluids. They were so named due to their solubility in ether (PGE) and phosphate buffer (PGF, F for fosfat, in Swedish). All other prostaglandins discovered later were denoted by a letter—PGA, PGH etc.

Structure of prostaglandins

Prostaglandins are derivatives of a hypothetical 20-carbon fatty acid namely ***prostanoic acid***, hence known as ***prostanoids***. This has a cyclopentane ring (formed by carbon atoms 8 to 12) and two side chains, with carboxyl group on one side. Prostaglandins differ in their structure due to substituent group and double bond on cyclopentane ring. The different prostaglandins are given in ***Fig.32.1***.

The structures of the most important prostaglandins (PGF_2 and $PGF_2\alpha$), prostacyclins (PGI_2), thromboxanes (TXA_2) and leukotrienes (LTA_4) along with arachidonic acid are depicted in ***Fig.32.2***. A subscript numeral indicates the number of double bonds in the two side chains. A subscript α-denotes that the hydroxyl group at C_9 of the ring and the carboxyl group are on the same side of the ring.

Prostanoic acid

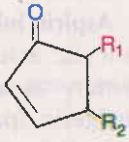
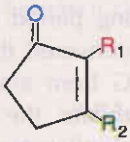
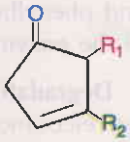
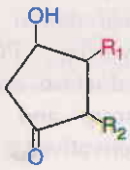
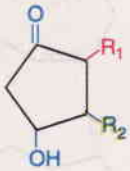
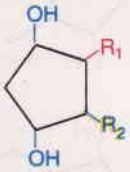
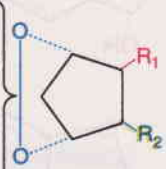
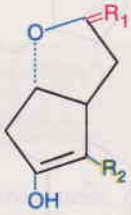
Prostaglandin	Substituent group	Structure
PGA	Double bond $C_{10} - C_{11}$ Keto group C_9	
PGB	Double bond $C_8 - C_{12}$ Keto group C_9	
PGC	Double bond $C_{11} - C_{12}$ Keto group C_9	
PGD	—OH group at C_9 Keto group at C_{11}	
PGE	Keto group at C_9 —OH group at C_{11}	
PGF	—OH group at C_9 and at C_{11}	
PGG	Two oxygen atoms present at C_9 and C_{11} are bonded; hydroperoxide group at C_{15}	
PGH	Ring the same as in PGG; —OH group at C_{15}	
PGI	Attachment of oxygen between C_6 and C_9 forming another 5-carbon ring	

Fig. 32.1 contd. next column

Fig. 32.1 : Prostaglandins with substituent groups and structures
(Note : Prostanoic acid is the parent nucleus for all the PGs; R_1 represents C_1 to C_7 of prostanoic acid, except in PGI where R_1 is C_1 to C_5; R_2 represents C_{13} to C_{20} of prostanoic acid).

Synthesis of prostaglandins

Arachidonic acid (5,8,11,14-eicosatetraenoic acid) is the precursor for most of the prostaglandins in humans. The biosynthesis of PGs was described by scene Bergstrom and Bengt Samuelsson (1960). It occurs in the endoplasmic reticulum in the following stages, as depicted in ***Fig.32.3.***

1. Release of arachidonic acid from membrane bound phospholipids by phospholipase A_2—this reaction occurs due to a specific stimuli by hormones such as epinephrine or bradykinin.

2. Oxidation and cyclization of arachidonic acid to PGG_2 which is then converted to PGH_2 by a reduced glutathione dependent peroxidase.

3. PGH_2 serves as the immediate precursor for the synthesis of a number of prostaglandins, including prostacyclins and thromboxanes.

The above pathway is known as ***cyclic pathway of arachidonic acid.*** In the linear pathway of arachidonic acid, leukotrienes and lipoxins are synthesized (details given later).

Cyclooxygenase–a suicide enzyme : It is interesting to note that prostaglandin synthesis can be partly controlled by suicidal activity of

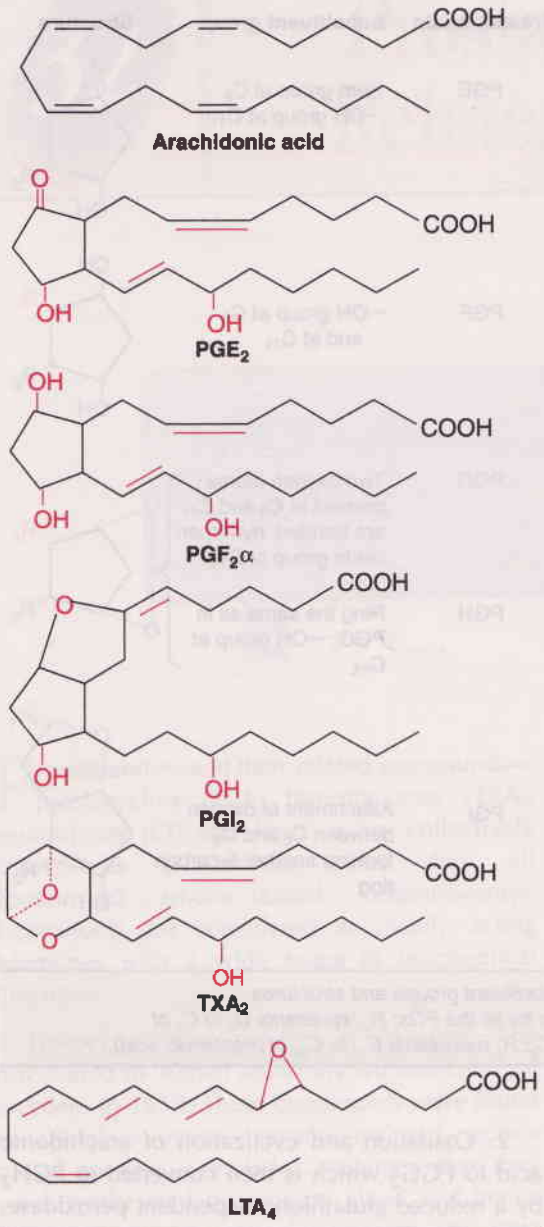


Fig. 32.2 : The structures of arachidonic acid, common prostaglandins (PGE_2 and $PGF_2\alpha$), prostacyclins (PGI_2), thromboxanes (TXA_2) and leukotrienes (LTA_4).

the enzyme cyclooxygenase. This enzyme is capable of undergoing self-catalysed destruction to switch off PG synthesis.

Inhibition of PG synthesis : A number of structurally unrelated compounds can inhibit prostaglandin synthesis. Corticosteroids (e.g. cortisol) prevent the formation of arachidonic acid by inhibiting the enzyme phospholipase A_2.

Many non-steroidal ***anti-inflammatory drugs*** inhibit the synthesis of prostaglandins, prostacyclins and thromboxanes. They do so by ***blocking*** the action of the enzyme ***cyclo-oxygenase***.

Aspirin inhibits PG synthesis : Aspirin (acetyl salicylic acid) has been used since nineteenth century as an antipyretic (fever-reducing) and analgesic (pain relieving). The mechanism of action of aspirin however, was not known for a long period. It was only in 1971, John Vane discovered that aspirin inhibits the synthesis of PG from arachidonic acid. Aspirin irreversibly ***inhibits*** the enzyme ***cyclooxygenase***. Other antiinflammatory drugs, such as indomethacin and phenylbutazone act as reversible inhibitors of the enzyme cyclooxygenase.

Degradation of prostaglandins : Almost all the eicosanoids are metabolized rapidly. The lung and liver are the major sites of PG degradation. Two enzymes, namely 15-α-hydroxy PG dehydrogenase and 13-PG reductase, convert hydroxyl group at C_{15} to keto group and then to C_{13} and C_{14} dihydro-derivative.

Biochemical actions of prostaglandins

Prostaglandins act as ***local hormones*** in their function. They, however, differ from the true hormones in many ways. Prostaglandins are produced in almost all the tissues in contrast to hormonal synthesis which occurs in specialized glands. PGs are not stored and they are degraded to inactive products at the site of their production. Further, PGs are produced in very small amounts and have low half-lives.

Prostaglandins are involved in a variety of biological functions. The actions of PGs differ in different tissues. Sometimes, PGs bring about opposing actions in the same tissue.

Overproduction of PGs results in many symptoms which include pain, fever, nausea, vomiting and inflammation.

Prostaglandins mediate the regulation of blood pressure, inflammatory response, blood clotting, reproductive functions, response to pain, fever etc.

1. **Regulation of blood pressure :** The prostaglandins (PGE, PGA and PGI_2) are vasodilator in function. This results in increased blood flow and decreased peripheral resistance to ***lower the blood pressure***. PGs serve as agents in the treatment of hypertension.

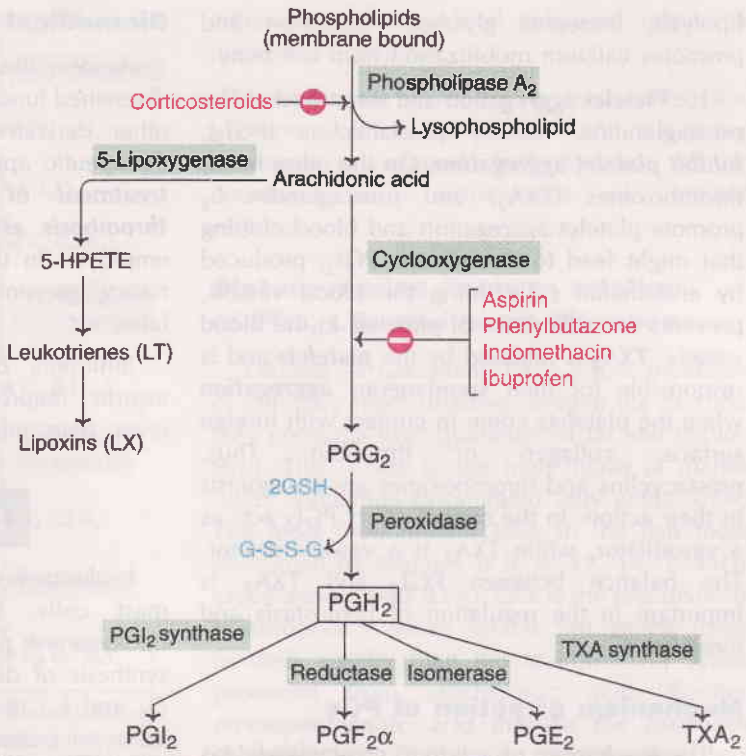


Fig. 32.3 : *Overview of biosynthesis of prostaglandins and related compounds (5-HPETE–5-Hydroxyperoxycicosatetraenoic acid; PG–Prostaglandins; PGI_2–Prostacyclin I_2; TXA_2–Thromboxane A_2).*

2. **Inflammation :** The prostaglandins PGE_1 and PGE_2 ***induce*** the symptoms of ***inflammation*** (redness, swelling, edema etc.) due to arteriolar vasodilation. This led to the belief that PGs are natural mediators of inflammatory reactions of rheumatoid arthritis (involving joints), psoriasis (skin), conjunctivitis (eyes) etc. Corticosteroids are frequently used to treat these inflammatory reactions, since they inhibit prostaglandin synthesis.

3. **Reproduction :** Prostaglandins have widespread applications in the field of reproduction. PGE_2 and PGF_2 are ***used for*** the medical ***termination of pregnancy*** and ***induction of labor***. Prostaglandins are administered to cattle to induce estrus and achieve better rate of fertilization.

4. **Pain and fever :** It is believed that pyrogens (fever producing agents) promote prostaglandin biosynthesis leading to the formation of PGE_2 in the hypothalamus, the site of regulation of body temperature. ***PGE_2*** along with histamine and bradykinin ***cause pain***. Migraine is also due to PGE_2. Aspirin and other non-steroidal drugs inhibit PG synthesis and thus control fever and relieve pain.

5. **Regulation of gastric secretion :** In general, prostaglandins (PGE) inhibit gastric secretion. ***PGs*** are used for the ***treatment of gastric ulcers***. However, PGs stimulate pancreatic secretion and increase the motility of intestine which often causes diarrhea.

6. **Influence on immune system :** Macrophages secrete PGE which decreases the immunological functions of B-and T-lymphocytes.

7. **Effects on respiratory function :** PGE is a bronchodilator whereas PGF acts as a constrictor of bronchial smooth muscles. Thus, PGE and PGF oppose the actions of each other in the lungs. PGE_1 and PGE_2 are used in the treatment of asthma.

8. **Influence on renal functions :** PGE increases glomerular filtration rate (GFR) and promotes urine output. Excretion of Na^+ and K^+ is also increased by PGE.

9. **Effects on metabolism :** Prostaglandins influence certain metabolic reactions, probably through the mediation of cAMP. PGE decreases

lipolysis, increases glycogen formation and promotes calcium mobilization from the bone.

10. **Platelet aggregation and thrombosis :** The prostaglandins, namely prostacyclins (***PGI_2***), ***inhibit platelet aggregation***. On the other hand, thromboxanes (TXA_2) and prostaglandin E_2 promote platelet aggregation and blood clotting that might lead to thrombosis. PGI_2, produced by endothelial cells lining the blood vessels, prevents the adherence of platelets to the blood vessels. ***TXA_2*** is released by the ***platelets*** and is responsible for their spontaneous ***aggregation*** when the platelets come in contact with foreign surface, collagen or thrombin. Thus, prostacyclins and thromboxanes are antagonists in their action. In the overall effect PGI_2 acts as a vasodilator, while TXA_2 is a vasoconstrictor. The balance between PGI_2 and TXA_2 is important in the regulation of hemostasis and thrombosis.

Mechanism of action of PGs

The mechanism of action of prostaglandins is not known for certain. They bind to the specific cellular receptors and bring about their action at the molecular level. It is believed that PGs may act through the mediation of cyclic nucleotides. PGE increases cAMP levels whereas PGF elevates cGMP.

Biomedical applications of PGs

As described above, prostaglandins perform diversified functions. And for this reason, PGs (or other derivatives) are the most exploited in therapeutic applications. They are used in the ***treatment of gastric ulcers, hypertension, thrombosis, asthma*** etc. Prostaglandins are also employed in the medical termination of pregnancy, prevention of conception, induction of labor etc.

Inhibitors of prostaglandin synthesis (e.g. aspirin, ibuprofen) are utilized in controlling fever, pain, migraine, inflammation etc.

LEUKOTRIENES

Leukotrienes are synthesized by leucocytes, mast cells, lung, heart, spleen etc., by lipoxygenase pathway of arachidonic acid. The synthesis of different leukotrienes (A_4, B_4, C_4, D_4 and E_4) through the intermediate, 5–hydroperoxyeicosatetraenoic acid (5–HPETE) is depicted in ***Fig.32.4***.

Anaphylaxis is a violent and fatal allergic reaction. It is now known that leukotrienes (C_4, D_4 and E_4) are the components of ***slow-reacting substances of anaphylaxis*** (SRS-A), released after immunological challenge. SRS–A is 100–1,000

BIOMEDICAL / CLINICAL CONCEPTS

- *Prostaglandins, synthesized in almost all the tissues (exception–erythrocytes) of the body, act as local hormones.*
- *PGs perform diversified biochemical functions. These include lowering of blood pressure, inhibition of gastric HCl secretion, decrease in immunological response and induction of labor.*
- *Overproduction of PGs causes symptoms such as pain, fever, vomiting, nausea, inflammation etc. Aspirin/ibuprofen/corticosteroid administration inhibits PG synthesis and relieves these symptoms.*
- *Platelet aggregation that may lead to thrombosis is promoted by thromboxanes and prostaglandins E_1 and inhibited by prostacyclins.*
- *Leukotrienes are implicated in hypersensitivity (allergy) and asthma.*
- *Consumption of fish foods containing the unsaturated fatty acid namely eicosapentaenoic acid is advocated to prevent heart attacks.*

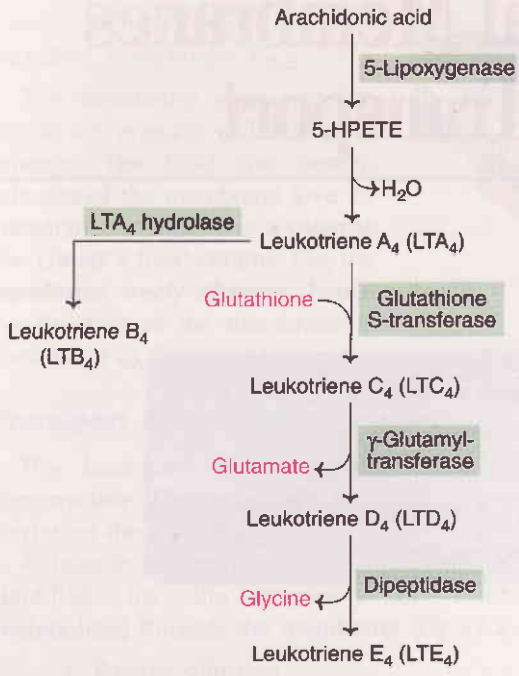


Fig. 32.4 : Synthesis of leukotrienes from arachidonic acid (5-HPETE–5-Hydroperoxycicosatetraenoic acid).

times more potent than histamine or prostaglandins in its action as a stimulant of allergic reactions. Leukotrienes are implicated in asthma, inflammatory reactions, hypersensitivity (allergy) and heart attacks.

Leukotrienes cause contraction of smooth muscles, bronchoconstriction, vasoconstriction, adhesion of white blood cells and release of lysosomal enzymes.

Lipoxins are involved in vasoactive, and immunoregulatory functions. There is a strong evidence to support that lipoxins act as counterregulatory compounds of immune response.

Dietary marine lipids in relation of PGs, LTs and heart diseases

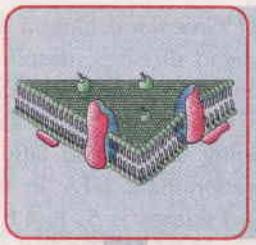
Eskimos of Greenland have a low incidence of coronary heart diseases, despite the fact that they consume high quantities of fat and cholesterol. This is due to the high intake of marine lipids containing unsaturated fatty acids (UFA). The most predominant UFA in the fish foods consumed by Eskimos is 5, 8, 11, 14, 17-eicosapentaenoic acid (EPA). EPA is the precursor for leukotrienes-5 series which are much lower in their activity than the leukotriene-4 series, produced from arachidonic acid. Further, ***eicosapentaenoic acid inhibits the formation thromboxanes (TXA_2)***. As already described, TXA_2 promotes platelet aggregation and thrombosis.

The diet rich in marine lipids (with EPA) decreases plasma cholesterol and triacylglycerols. These factors, along with reduced synthesis of TXA_2 are believed to be responsible for the low incidence of heart attacks in Eskimos.

SUMMARY

1. *Prostaglandins (PGs) and related compounds prostacyclins (PGI), thromboxanes (TXA) and leukotrienes (LT) are collectively known as eicosanoids. They are the derivatives of a hypothetical 20 carbon fatty acid, namely prostanoic acid. Prostaglandins are synthesized from arachidonic acid, released from the membrane bound phospholipids. Corticosteroids and aspirin inhibit PG synthesis.*
2. *Prostaglandins act as local hormones and are involved in a wide range of biochemical functions. In general, PGs are involved in the lowering of blood pressure, induction of inflammation, medical termination of pregnancy, induction of labor, inhibition of gastric HCl secretion, decrease in immunological response and increase in glomerular filtration rate. Thromboxanes (TXA_2) and prostaglandin E_1 promote while prostacyclins (PGI_2) inhibit platelet aggregation.*

33 Biological Membranes and Transport



The plasma membrane speaks :

"I earmark the cell territory;
For protection from hostile environment;
Regulate solute import and export
By passive or active transport."

The plasma membrane is an envelope surrounding the cell (***Refer Fig.1.1***). It separates and protects the cell from the external hostile environment. Besides being a protective barrier, plasma membrane provides a connecting system between the cell and its environment. The subcellular organelles such as nucleus, mitochondria, lysosomes are also surrounded by membranes.

Chemical composition

The membranes are composed of lipids, proteins and carbohydrates. The actual composition differs from tissue to tissue. Among the lipids, ***amphipathic lipids*** (containing hydrophobic and hydrophilic groups) namely ***phospholipids***, ***glycolipids*** and ***cholesterol***, are found in the animal membranes.

Many animal cell membranes have thick coating of complex polysaccharides referred to as ***glycocalyx***. The oligosaccharides of glycocalyx interact with collagen of intercellular matrix in the tissues.

Structure of membranes

A ***lipid bilayer model*** originally proposed for membrane structure in 1935 by Davson and Danielle has been modified.

Fluid mosaic model, proposed by Singer and Nicolson, is a more recent and acceptable model for membrane structure. The biological membranes usually have a thickness of 5-8 nm. A membrane is essentially composed of a lipid bilayer. The hydrophobic (nonpolar) regions of the lipids face each other at the core of the bilayer while the hydrophilic (polar) regions face outward. Globular proteins are irregularly embedded in the lipid bilayer (***Fig.33.1***). Membrane proteins are categorized into two groups.

1. **Extrinsic (peripheral) membrane proteins** are loosely held to the surface of the membrane and they can be easily separated e.g. cytochrome c of mitochondria.

2. **Intrinsic (integral) membrane proteins** are tightly bound to the lipid bilayer and they can be separated only by the use of detergents or

organic solvents e.g. hormone receptors, cytochrome P_{450}.

The membrane is asymmetric due to the irregular distribution of proteins. The lipid and protein subunits of the membrane give an appearance of mosaic or a ceramic tile. Unlike a fixed ceramic tile, the ***membrane freely changes***, hence the structure of the membrane is considered as fluid mosaic.

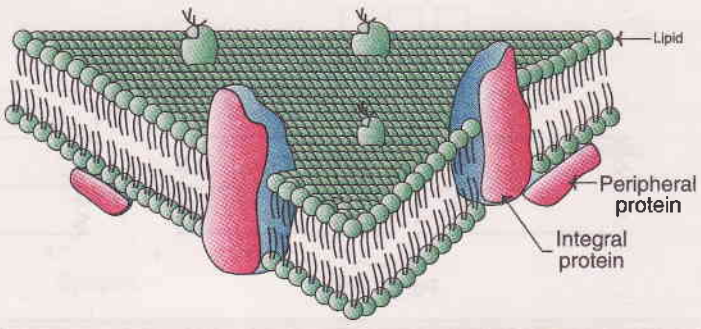


Fig. 33.1 : *The fluid mosaic model of membrane structure.*

Transport across membranes

The biological membranes are relatively impermeable. The membrane, therefore, forms a barrier for the free passage of compounds across it. At least three distinct mechanisms have been identified for the transport of solutes (metabolites) through the membrane (***Fig.33.2***).

1. Passive diffusion
2. Facilitated diffusion
3. Active transport.

1. **Passive diffusion :** This is a simple process which depends on the concentration gradient of a particular substance across the membrane. Passage of water and gases through membrane occurs by passive diffusion. This process does not require energy.

2. **Facilitated diffusion :** This is somewhat comparable with diffusion since the solute moves along the concentration gradient (from higher to lower concentration) and no energy is needed. But the most important distinguishing feature is that facilitated diffusion occurs through the mediation of carrier or transport proteins. Specific carrier proteins for the transport of glucose, galactose, leucine, phenylalanine etc. have been isolated and characterized.

Mechanism of facilitated diffusion : A ping-pong model is put forth to explain the occurrence of facilitated diffusion (***Fig.33.3***). According to this mechanism, a transport (carrier) protein exists in two conformations. In the pong conformation, it is exposed to the side with high solute

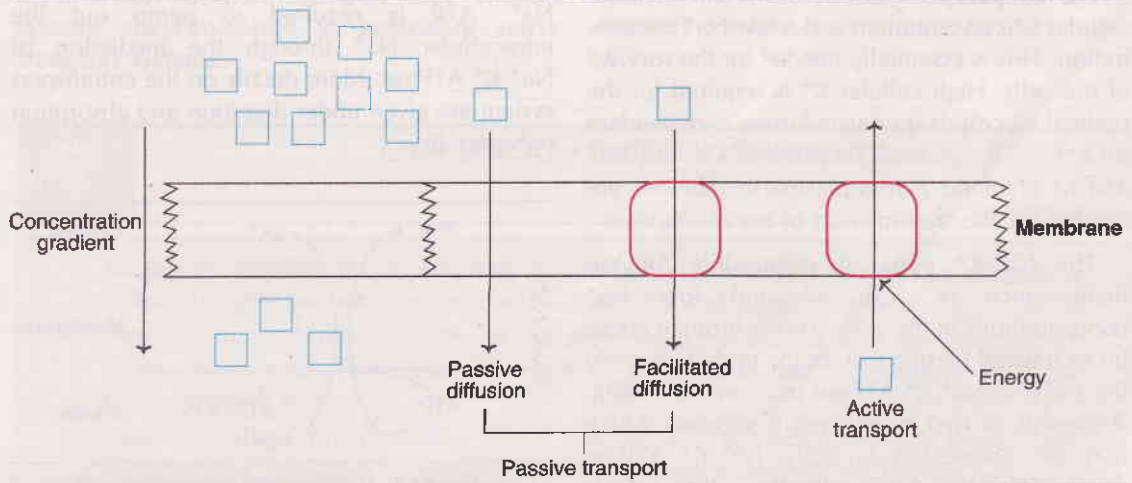


Fig. 33.2 : *Mechanism of transport across biological membrane*
(Note : *Transport molecule are represented in blue; the carrier proteins in red).*

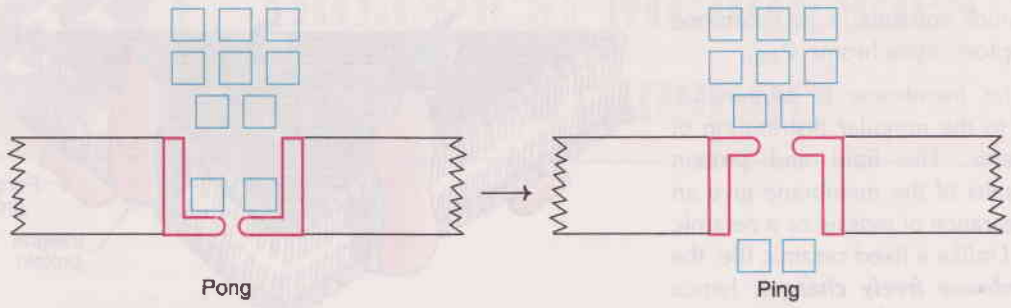


Fig. 33.3 : A diagrammatic representation of 'ping-pong' model for facilitated diffusion.

concentration. This allows the binding of solute to specific sites on the carrier protein. The protein then undergoes a conformational change (ping state) to expose to the side with low solute concentration where the solute molecule is released. Hormones regulate facilitated diffusion. For instance, insulin increases glucose transport in muscle and adipose tissue; amino acid transport in liver and other tissues.

3. **Active transport :** Active transport occurs ***against a concentration gradient*** and this is dependent on the ***supply of metabolic energy (ATP).*** Active transport is also a carrier mediated process like facilitated diffusion. The most important ***primary active transport systems*** are ion-pumps (through the involvement of pump ATPases or ion transporting ATPases).

Na^+-K^+ pump : The cells have a high intracellular K^+ concentration and a low Na^+ concentration. This is essentially needed for the survival of the cells. High cellular K^+ is required for the optimal glycolysis (pyruvate kinase is dependent on K^+) and for protein biosynthesis. Further, Na^+ and K^+ gradients across plasma membranes are needed for the transmission of nerve impulse.

The Na^+-K^+ pump is responsible for the maintenance of high K^+ and low Na^+ concentrations in the cells. This is brought about by an integral plasma membrane protein, namely the enzyme Na^+-K^+ ATPase (mol. wt. 250,000). It consists of two α and two β subunits which may be represented as $(\alpha\beta)_2$. Na^+-K^+ ATPase pumps $3Na^+$ ions from inside the cell to outside and brings $2K^+$ ions from the outside to the inside with a concomitant hydrolysis of intracellular ATP. The Na^+-K^+ pump, depicted in ***Fig.33.4,*** is summarized.

$$3\ Na^+\ (in) + 2K^+\ (out) + ATP \longrightarrow 3Na^+\ (out) + 2K^+\ (in) + ADP + Pi$$

A major portion of the cellular ATP (up to 70% in nerve cells) is in fact utilized by Na^+-K^+ pump to maintain the requisite cytosolic Na^+ and K^+ levels. Ouabain (pronounced as Wah-báin) inhibits Na^+-K^+ ATPase. ***Ouabain*** is a steroid derivative extracted from the seeds of an African shrub. It is a poison used to tip the hunting arrows by the tribals in Africa.

Na^+-cotransport system : The amino acids and sugars are transported into the cells by a Na^+-cotransport system. This process essentially consists of the passage of glucose (or amino acid) into the cell with a simultaneous movement of Na^+. ATP is required to pump out the intracellular Na^+ through the mediation of Na^+-K^+ ATPase. More details on the cotransport system are given under digestion and absorption (***Chapter 8***).

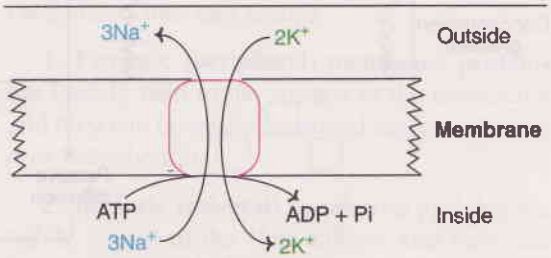


*Fig. 33.4 : Diagrammatic representation of Na^+—K^+ pump (**Note** : Red colour block represents the enzyme Na^+—K^+ ATPase).*

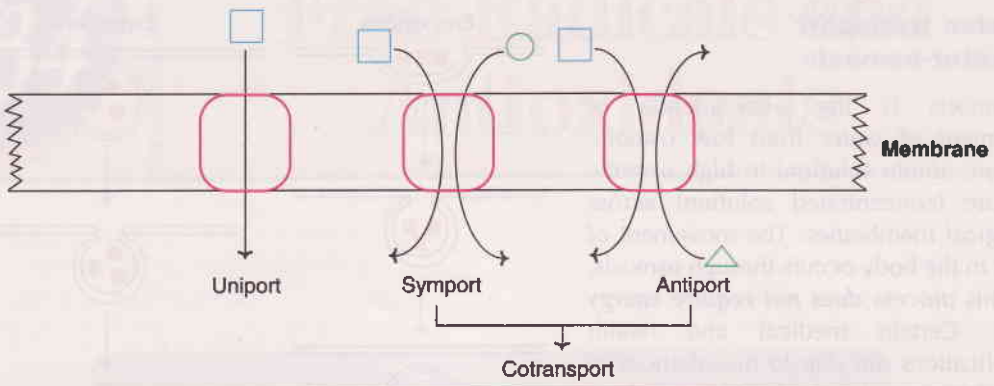


Fig. 33.5 : Diagrammatic representation of transport systems.

Transport systems

The transport systems may be divided into 3 categories (***Fig.33.5***).

1. **Uniport system :** This involves the movement of a single molecule through the membrane e.g. transport of glucose to the erythrocytes.

2. **Symport system :** The simultaneous transport of two different molecules in the same direction e.g. transport of Na^+ and glucose to the intestinal mucosal cells from the gut.

3. **Antiport system :** The simultaneous transport of two different molecules in the opposite direction e.g exchange of Cl^- and HCO_3^- in the erythrocytes. Uniport, symport and antiport systems are considered as ***secondary active transport systems.***

Cotransport system : In cotransport, the transport of a substance through the membrane is coupled to the spontaneous movement of another substance. The symport and antiport systems referred to above are good examples of cotransport system.

Proton pump in the stomach : This is an antiport transport system of gastric parietal cells. It is brought out by the enzyme ***H^+–K^+ ATPase*** to maintain highly acidic (pH≈1) conditions in the lumen of the stomach. Proton pump antiports two cytoplasmic protons ($2H^+$) and two extracellular potassium ($2K^+$) ions for a molecule of ATP hydrolysed. The chloride ions secreted by Cl^- channels combine with protons to form gastric HCl.

BIOMEDICAL / CLINICAL CONCEPTS

- *Biological membranes are relatively impermeable protective barriers that provide a connecting link between the cell (or its organelle) and its environment.*
- *The cells must contain high K^+ and low Na^+ concentrations for their survival. Na^+-K^+ pump, which consumes a major portion of the cellular metabolic energy (ATP), is responsible for this.*
- *Ouabain inhibits Na^+-K^+ ATPase (Na^+-K^+ pump). It is extracted from the seeds of an African shrub and used as poison to tip the hunting arrows by the tribals.*
- *Disturbances is osmosis are associated with diarrhea, edema, inflammation of tissues etc.*

Passive transport of water-osmosis

Osmosis is the phenomenon of movement of water from low osmotic pressure (dilute solution) to high osmotic pressure (concentrated solution) across biological membranes. The movement of water in the body occurs through osmosis, and this process ***does not require energy*** (ATP). Certain medical and health complications are due to disturbances in osmosis. e.g. edema, diarrhea, cholera, inflammation of tissues. The reader may refer ***Chapter 40*** for more information on osmosis, water and electrolyte imbalance in cholera/diarrhea.

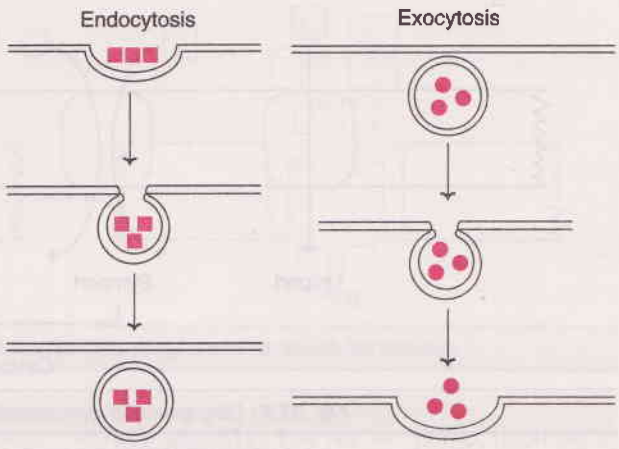


Fig. 33.6 : *Diagrammatic representation of endocytosis and exocytosis*
(Note : *The red coloured particles indicate the transport material).*

Transport of macromolecules

The transport of macromolecules such as proteins, polysaccharides and polynucleotides across the membranes is equally important. This is brought about by two independent mechanisms namely ***endocytosis***—intake of macromolecules by the cells and ***exocytosis***—release of macromolecules from the cells to the outside.

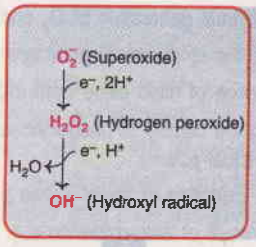
Endocytosis : It is estimated that approximately 2% of the exterior surface of plasma membrane possesses characteristic ***coated pits***. These pits can be internalized to form coated vesicles which contain an unusual protein called ***clathrin***. The process of endocytosis is depicted in ***Fig.33.6***. The uptake of low density lipoprotein (LDL) molecules by the cells is a good example of endocytosis.

Exocytosis : The release of macromolecules to the outside of the cells mostly occurs via the participation of Golgi apparatus. The macromolecules are transported to the plasma membrane in vesicles and let out (***Fig.33.6***). The secretion of hormones (e.g. insulin, parathyroid hormone) usually occurs by exocytosis.

SUMMARY

1. *The biological membranes are the barriers that protect the cell and the subcellular organelles from the hostile environment. The membranes are primarily composed of a lipid bilayer onto which the globular proteins are irregularly embedded to form a fluid mosaic model.*
2. *Transport of molecules through membranes occurs either by passive diffusion, facilitated diffusion or active transport. Active transport occurs against a concentration gradient which is dependent on the supply of metabolic energy (ATP). Na^+-K^+ pump is responsible for the maintenance of high K^+ and low Na^+ concentrations inside the cells, an essential requisite for the survival of cells.*
3. *The transport systems are divided into 3 categories—uniport, symport and antiport.*
4. *The transport of macromolecules takes place by endocytosis (ingestion by the cells) and exocytosis (release from the cells).*

34 Free Radicals and Antioxidants



The free radicals speaks :

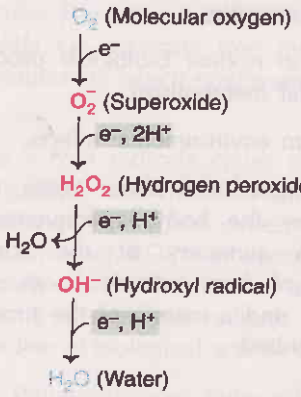
"We exist as independent molecular species;
Generated by cellular metabolism and environmental effects;
Implicated in the causation of several diseases;
Destroyed by antioxidants to protect cells/tissues/body."

The supply of oxygen is absolutely essential for the existence of higher organisms. As the saying goes too much of even the best is bad. Very high concentrations of O_2 are found to be toxic, and can damage tissues. The present day concept of oxygen toxicity is due to the involvement of ***oxygen free radicals*** or ***reactive oxygen species (ROS)***. In fact, the generation of reactive metabolites of O_2 is an integral part of our daily life.

A ***free radical*** is defined as a molecule or a molecular species that ***contains one or more unpaired electrons***, and is ***capable of independent existence.***

Types of free radicals

Oxygen is required in many metabolic reactions, particularly for the release of energy. During these processes, molecular O_2 is completely reduced, and converted to water. However, if the reduction of O_2 is incomplete, a series of reactive radicals are formed, as shown in the next column.



Besides the above (O_2^-, H_2O_2, OH^-), the other free radicals and reactive oxygen species of biological importance include singlet oxygen ($^1O_2$), hydroperoxy radical (HOO^-), lipid peroxide radical (ROO^-), nitric oxide (NO^-) and peroxynitrite ($ONOO^-$).

The common characteristic features of free radicals are listed

- Highly reactive
- Very short half-life

- Can generate new radicals by chain reaction
- Cause damage to biomolecules, cells and tissues

Free radicals and reactive oxygen species (ROS)—not synonymous : By definition, a free radical contains one or more unpaired electrons. e.g. O_2^-, OH^-, ROO^-. There are certain non-radical derivatives of O_2 which do not contain unpaired electrons e.g. H_2O_2, $^1O_2$. The term reactive oxygen species is used in a broad sense to collectively represent free radicals, and non-free radicals (which are extremely reactive) of the biological systems. However, most authors do not make a clear distinction between free radicals and ROS, and use them interchangebly.

SOURCES AND GENERATION OF FREE RADICALS

The major sources responsible for the generation of free radicals may be considered under two categories

I. Due to normal biological processes (or cellular metabolism).

II. Due to environmental effects.

It is estimated that about 1-4% of the O_2 taken up by the body is converted to free radicals. A summary of the sources for generation of free radicals is given in the ***Table 34.1***, and a couple of the processes are briefly described.

Table 34.1 Sources along with some examples for generation of free radicals

I Cellular metabolism

- Leakage of electrons from the respiratory chain (ETC).
- Production of H_2O_2 or O_2^- by oxidase enzymes (e.g. xanthine oxidase, NADPH oxidase).
- Due to chain reactions of membrane lipid peroxidation.
- Peroxisomal generation of O_2 and H_2O_2.
- During the synthesis of prostaglandins.
- Production of nitric oxide from arginine.
- During the course of phagocytosis (as a part of bactericidal action).
- In the oxidation of heme to bile pigments.
- As a result of auto-oxidation e.g. metal ions [Fe^{2+}, Cu^{2+}]; ascorbic acid, glutathione, flavin coenzymes.

II Environmental effects

- As a result of drug metabolism e.g. paracetamol, halothane, cytochrome P_{450} related reactions.
- Due to damages caused by ionizing radiations (e.g. X-rays) on tissues.
- Photolysis of O_2 by light.
- Photoexcitation of organic molecules
- Cigarette smoke contains free radicals, and trace metals that generate OH^-.
- Alcohol, promoting lipid peroxidation.

Lipid peroxidation

Free radical-induced peroxidation of membrane lipids occurs in three stages-initiation, propagation and termination

Initiation phase : This step involves the removal of hydrogen atom (H) from polyunsaturated fatty acids (LH), caused by hydroxyl radical

$$LH + OH^- \longrightarrow L^- + H_2O$$

Propagation phase : Under aerobic conditions, the fatty acid radical (L^-) takes up oxygen to form peroxy radical (LOO^-). The latter, in turn, can remove H-atom from another PUFA (LH) to form lipid hydroperoxide (LOOH).

$$L^- + O_2 \longrightarrow LOO^-$$

$$LOO^- + LH \longrightarrow LOOH + L^-$$

The hydroperoxides are capable of further stimulating lipid peroxidation as they can form alkoxy (LO^-) and peroxyl (LOO^-) radicals.

$$2LOOH \xrightarrow{Fe,\ Cu} LO^- + LO_2^- + H_2O$$

$$LOOH \longrightarrow LO^- + LOO^- + \text{aldehydes}$$

Termination phase : Lipid peroxidation proceeds as a chain reaction until the available PUFA gets oxidized.

Malondialdehyde (MDA) as a marker for lipid peroxidation

Most of the products of lipid peroxidation are unstable e.g. carbonyls, esters, alkanes, alkenes, 2-alkenal, 2,4-alkadienal, MDA. Of these, malondialdehyde (−CHO−CH_2−CHO−) is the most extensively studied, and is used as a biochemical marker for the assessment of lipid peroxidation. MDA and other aldehydes react with thiobarbituric acid and produce red-coloured products namely ***thiobarbituric acid reactive substances (TBARS)*** which can be measured colorimetrically.

The estimation of serum MDA is often used to assess oxidative stress, and free radical damage to the body.

Damages caused by lipid peroxidation

The products of lipid peroxidation are highly destructive. They damage the membranes, cells and even tissues. Lipid peroxidation has been implicated in many diseases (See harmful effects of free radicals).

Generation of ROS by macrophages

During the course of phagocytosis, inflammatory cells, particularly the macrophages produce superoxide (O_2^-), by a reaction catalysed by NADPH oxidase (***Fig.34.1***). This O_2^- radical gets converted to H_2O_2, and then to hypochlorous acid (HClO). The superoxide radical along with hypochlorous ions brings about ***bactericidal action***. This truly represents the ***beneficial affects of the free radicals*** generated by the body.

A large amount of O_2 is consumed by macrophages during their bactericidal function, a phenomenon referred to as ***respiratory burst***. It is estimated that about 10% of the O_2 taken up by macrophages is utilized for the generation of free radicals.

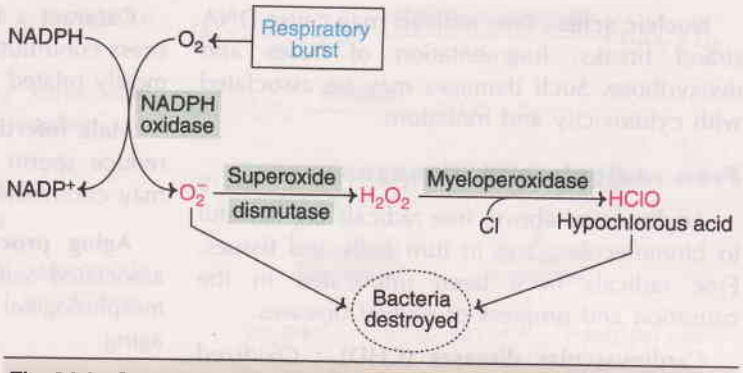


Fig. 34.1 : Generation of free radicals by macrophages and respiratory burst.

HARMFUL EFFECTS OF FREE RADICALS

Free radicals and biomolecules

Free radicals are highly reactive, and are capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrates, nucleic acids). The fact is that ***free radicals beget free radicals*** i.e. generate free radicals from normal compounds which continues as a chain reaction.

Proteins : Free radicals cause oxidation of sulfhydryl groups, and modification of certain amino acids (e.g. methionine, cysteine, histidine, tryptophan, tyrosine). ROS may damage proteins by fragmentation, cross-linking and aggregation. The net result is that free radicals may often result in the loss of biological activity of proteins.

Lipids : Polyunsaturated fatty acids (PUFA) are highly susceptible to damage by free radicals. Details have been given under ***lipid peroxidation***.

Carbohydrates : At physiological pH, oxidation of monosaccharides (e.g. glucose) can produce H_2O_2 and oxoaldehydes. It appears that the linkage of carbohydrates to proteins (glycation) increases the susceptibility of proteins to the attack by free radicals. This character assumes significance in diabetes mellitus where protein glycation is associated with many health complications e.g. diabetic microangiopathy, diabetic nephropathy.

Nucleic acids : Free radicals may cause DNA strand breaks, fragmentation of bases and deoxyribose. Such damages may be associated with cytotoxicity and mutations.

Free radicals and diseases

As discussed above, free radicals are harmful to biomolecules, and in turn cells and tissues. Free radicals have been implicated in the causation and progress of several diseases.

Cardiovascular diseases (CHD) : Oxidized low density lipoproteins (LDL), formed by the action of free radicals, ***promote atherosclerosis*** and CHD.

Cancer : Free radicals ***can damage DNA***, and cause mutagenicity and cytotoxicity, and thus play a key role in carcinogenesis. It is believed that ROS can induce mutations, and inhibit DNA repair process, that results in the inactivation of certain tumor suppressor genes leading to cancer. Further, free radicals promote biochemical and molecular changes for rapid growth of tumor cells.

Inflammatory diseases : Rheumatoid arthritis is a chronic inflammatory disease. The free radicals produced by neutrophils are the predominant causative agents. The occurrence of other inflammatory disorders—chronic glomerulonephritis and ulcerative colitis is also due to the damages caused by ROS on the extracellular components (e.g. collagen, hyaluronic acid).

Respiratory diseases : Direct exposure of lungs to 100% oxygen for a long period (more than 24 hrs) is known to destroy endothelium and cause lung edema. This is mediated by free radicals. ROS are also responsible for adult respiratory distress syndrome (ARDS), a disorder characterized by pulmonary edema.

Cigarette smoke, as such, contains free radicals, and further it promotes the generation of more free radicals. The damages caused to lungs in the smokers are due to ROS.

Diabetes : Destruction of islets of pancreas due to the accumulation of free radicals is one of the causes for the pathogenesis of insulin-dependent diabetes mellitus.

Cataract : Increased exposure to oxidative stress contributes to cataract formation, which is mostly related to aging.

Male infertility : Free radicals are known to reduce sperm motility and viability, and thus may contribute to male infertility.

Aging process : Free radicals are closely associated with the various biochemical and morphological changes that occur during normal aging.

Other diseases : Free radicals play a key role in Parkinson's disease, Alzheimer's disease, multiple sclerosis, liver cirrhosis, muscular dystrophy, toxemia of pregnancy etc.

ANTIOXIDANTS IN BIOLOGICAL SYSTEM

To mitigate the harmful/damaging effects of free radicals, the aerobic cells have developed antioxidant defense mechanisms. A biological antioxidant may be defined as a ***substance*** (present in low concentrations compared to an oxidizable substrate) that ***significantly delays*** or ***inhibits oxidation*** of a ***substrate***. Antioxidants may be considered as the ***scavengers of free radicals***.

The production of free radicals and their neutralization by antioxidants is a normal bodily process. There are different ways of classifying antioxidants.

I. Antioxidants in relation to lipid peroxidation

1. ***Preventive antioxidants*** that will block the initial production of free radicals e.g. catalase, glutathione peroxidase.

2. ***Chain breaking antioxidants*** that inhibit the propagative phase of lipid peroxidation e.g. superoxide dismutase, vitamin E, uric acid.

II. Antioxidants according to their location

1. ***Plasma antioxidants*** e.g. β-carotene, ascorbic acid, bilirubin, uric acid, ceruloplasmin, transferrin.

2. ***Cell membrane antioxidants*** e.g. α-tocopherol.

3. ***Intracellular antioxidants*** e.g. superoxide dismutase, catalase, glutathione peroxidase.

III. Antioxidants according to their nature and action

1. ***Enzymatic antioxidants*** e.g. superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase.

2. ***Non-enzymatic antioxidants***

(a) ***Nutrient antioxidants*** e.g. carotenoids (*β-carotene*), *α-tocopherol, ascorbic acid,* selenium.

(b) ***Metabolic antioxidants*** e.g. glutathione, ceruloplasmin, albumin, bilirubin, transferrin, ferritin, uric acid

The antioxidant enzyme system

The antioxidant enzymes are truly the scavangers of free radicals. The major reactions of these enzymes are depicted in ***Fig.34.2***, some highlights are given below.

Superoxide dismutase : It converts superoxide (O_2^-) to hydrogen peroxide and O_2 (***Fig.34.2A***). This is the first line of defense to protect cells from the injurious effects of superoxide.

Catalase : Hydrogen peroxide, produced by superoxide dismutase, is metabolised by catalase (***Fig.34.2B***).

Glutathione peroxidase : It detoxifies H_2O_2 to H_2O, while reduced glutathione (G–SH) is converted to oxidized glutathione (GS–SG). The reduced glutathione can be regenerated by the enzyme ***glutathione reductase*** utilizing NADPH (***Fig.34.2C***). The hexose monophosphate shunt is the major source of NADPH.

Nutrient antioxidants

Tocopherols (vitamin E) : Vitamin E is fat soluble, and among the tocopherols, α-tocopherol is biologically the most active. It is an antioxidant present in all cellular membranes, and protects against lipid peroxidation.

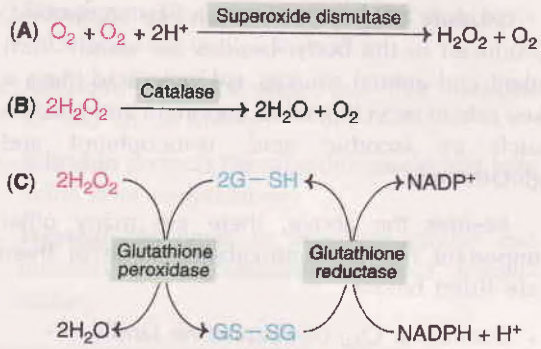


Fig. 34.2 : The antioxidant enzyme system (G–SH-reduced glutathione; GS–SG-oxidized glutathione).

α-Tocopherol can directly act on oxyradicals (e.g. O_2^-, OH^-, singlet oxygen), and thus serves as an important chain breaking antioxidant.

Ascorbic acid (vitamin C) : It is a vitamin that participates in many normal metabolic reactions of the body. Ascorbic acid is an important water-soluble antioxidant in biological fluids. Vitamin C efficiently scavanges free radicals, and inhibits lipid peroxidation. It also promotes the regeneration of α-tocopherol (from α-tocopheroxyl radical produced during scavenging of ROS).

Carotenoids : These are the natural compounds with lipophilic properties. About 500 different carotenoids have been identified, among them ***β-carotene is the most important.*** It can act as an antioxidant under low partial pressure of O_2. β-Carotene usually functions in association with vitamins C and E. ***Lycopene***, a fat soluble pigment is a carotenoid. It is responsible for colour of certain fruits and vegetables (e.g. tomato). Lycopene possesses antioxidant property. ***Lutein*** and ***zeaxanthin*** are also carotenoid pigments that impart yellow or green colour to fruits and vegetables. These pigments can also serve as antioxidants.

Selenium : It is an essential trace element, and is proved to be a significant antioxidant. Selenium works with vitamin E in fighting free radicals. It is also required for the function of an important antioxidant enzyme, namely ***glutathione peroxidase.***

α-Lipoic acid : It is vitamin-like compound, produced in the body, besides the supply from plant and animal sources. α-Lipoic acid plays a key role in recycling other important antioxidants such as ascorbic acid, α-tocopherol and glutathione.

Besides the above, there are many other important nutrient antioxidants, some of them are listed below

- Coenzyme Q_{10} of ubiquinone family
- Proanthocyanidins of grape seeds
- Catechins of green tea
- Curcuminoids of turmeric
- Quercetin of onions

In the ***Table 34.2***, some important nutrient antioxidants and their dietary sources are given. ***Consumption of a variety of nutrient antioxidants*** is important, since each antioxidant targets certain types of damaging free radicals.

Table 34.2 Nutrient antioxidants and their dietary sources

Antioxidant	*Dietary Source*
Vitamin E (tocopherols)	Unprocessed vegetable oils (cotton seed oil, peanut oil, sunflower oil) whole grains, leafy vegetables, legumes
Vitamin C (ascorbic acid)	Citrus fruits (oranges, grapes) gooseberry (amla), guava, green vegetables (cabbage, spinach), cauliflower, melons
β-Carotene (provitamin A)	Carrots, green fruits and vegetables, spinach, turnip, apricots.
Lycopene	Tomatoes, and their products (tomato sauce), papaya, pink guava, watermelon.
Leutein and zeaxanthin	Egg yolk, fruits, green leafy vegetables, corn, green peas.
Selenium	Sea foods, meats, organ meats, whole grains
α-Lipoic acid	Red meat, liver, yeast
Coenzyme Q_{10}	Organ meats (best heart), beef, chicken.
N-Acetylcysteine	Available as supplement or drug.
Proanthocyanidins	Grape seeds
Catechins	Green tea
Curcuminoids	Turmeric
Quercetin	Onions, red wine, green tea
Ellagic acid	Berries, walnuts, pomegranates
Hesperidin	Citrus fruits (oranges), lemon.

Metabolic antioxidants

Glutathione : Reduced glutathione (GSH) plays a key role in the biological antioxidant enzyme system (***See Fig.34.2C***). GSH and H_2O_2 are the twin substrates for glutathione peroxidase. The reduced glutathione (GSH) gets regenerated from the oxidized glutathione (GS−SG) through the participation of glutathione reductase and NADPH. It is sugested that the ability to synthesize GSH decreases as age

BIOMEDICAL / CLINICAL CONCEPTS

- *Free radicals have been implicated in the causation and progress of several diseases e.g. atherosclerosis and CHD, cancer, respiratory diseases, aging.*
- *The estimation of serum malondialdehyde is often used to assess oxidative stress and free radical damage to the body.*
- *The respiratory burst of macrophages, accompanied by the generation of ROS (H_2O_2 and HClO), brings about bactericidal action, and is beneficial to the body.*
- *Dietary consumption of a variety of nutrient antioxidants (vitamins C and E, β-carotene, lycopenes, Se, β-lipoic acid) is desirable since each antioxidant targets certain types of damaging free radicals.*

advances, and this has been implicated in certain diseases e.g. cataract.

There are many more metabolic antioxidants of biological importance. A selected few of them are listed below

- **Uric acid**, a powerful scavenger of singlet oxygen ($^1O_2$) and OH^- radicals.
- **Ceruloplasmin** inhibits iron and copper dependent lipid peroxidation.
- **Transferrin** binds to iron and prevents iron-catalysed free radical formation.
- **Albumin** can scavange the free radicals formed on its surface.
- **Bilirubin** protects the albumin bound free fatty acids from peroxidation.
- **Haptoglobin** binds to free hemoglobin and prevents the acceleration of lipid peroxidation.

SUMMARY

1. *Free radicals are the molecules or molecular species containing one or more unpaired electrons with independent existence. e.g. O_2^-, H_2O_2, OH^-, $^1O_2$.*
2. *ROS are constantly formed during the normal cellular metabolism, (e.g. lipid peroxidation) and due to various environmental influences (e.g. ionizing radiations).*
3. *Free radicals are highly reactive and are capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrates, nucleic acids), and have been implicated in the causation of many diseases e.g. cardiovascular diseases, cancer, inflammatory diseases.*
4. *To mitigate the harmful effects of free radicals, the aerobic cells have developed antioxidant defense mechanisms-enzymatic antioxidants (superoxide dismutase, catalase) and non-enzymatic antioxidants (glutathione, Se, α-tocopherol, β-carotene).*

35 Environmental Biochemistry

The environment speaks :

"Composed of living and non-living entities;
Co-existing and friendly with you all;
Continuously insulted by your activities of pollution;
That threatens only your healthy existence!"

Environment constitutes the non-living (air, water, land, energy etc.) as well as the living (biological and social) systems surrounding man. Environmental biochemistry primarily deals with the metabolic (biochemical) responses and adaptations in man (or other organisms) due to the environmental factors.

A healthy environment is required for a healthy life which is however, not really possible or practicable. This is mainly because of the atmospheric (climatic) changes and environmental pollution.

Environmental biochemistry is a very vast subject. The basic concepts regarding the atmospheric changes and environmental pollution on humans are dealt with here.

ATMOSPHERIC (CLIMATIC) CHANGES

The climatic changes include cold, heat etc. The body makes every effort to maintain its normal temperature (despite cold and heat surroundings) for optimal physiological and biochemical functions.

EXPOSURE TO COLD

Short-term exposure to cold causes shivering (mainly due to skeletal muscle) to produce extra heat. Heat is generated by the hydrolysis of ATP.

Non-shivering phase

Chronic exposure to cold results in non-shivering phase which is characterized by several metabolic adaptations.

- **Energy metabolism :** Heat generation by a process called ***chemical thermogenesis*** occurs in non-shivering phase. The foodstuffs undergo oxidation to generate heat at the expense of growth and other anabolic processes. Elevation in BMR, and increased intake of foods are observed.
- **Lipid metabolism :** Stored fat (triacylglycerol) in the adipose tissue is mobilized to supply

free fatty acids for oxidation and production of energy. Brown edipose tissue, particularly in neonatal life, significantly contributes to thermogenesis.

- **Hormonal changes :** Thyroxine, a hormone closely associated with energy metabolism, is elevated. Further, corticosteroids are increased on exposure to cold.

EXPOSURE TO HEAT

There is a continuous generation of heat by the body due to the ongoing biochemical processes, referred to as ***metabolic heat***. This heat has to be exchanged with the environment to maintain a constant body temperature. On exposure to heat in surroundings, as happens in summer, the body is subjected to an uncomfortable situation (since temperature of the surroundings is much higher than that of the body). However, heat is still lost from the body through sweating and evaporation. Normally, the body (thermoregulation) gets acclimatized to higher temperature within 3-5 days.

Heat stroke : It is characterized by the failure of the heat regulatory system (thermoregulation) of the body. The manifestations of heat stroke include high body temperature, convulsions, partial (some times total) loss of consciousness. In extreme cases, heat stroke may cause irreversible damage to brain. The treatment for the heat stroke involves rapid cooling of the body.

The milder form of heat stroke is referred to as ***heat syncope***. Although the body temperature is not raised much in this condition, the blood pressure falls and the person may collapse suddenly. Heat syncope is easily reversible.

ENVIRONMENTAL POLLUTION

Environmental pollution may be regarded as the addition of extraneous (foreign) materials to air, water or land which adversely affects the quality of life. Pollution may be caused by physical, chemical or biological processes.

The term ***pollutant*** refers to a substance which increases in quantity due to human activity and adversely affects the environment (e.g. carbon monoxide, sulfur dioxide, lead). A substance which is not present in nature but released during human activity is the ***contaminant*** (e.g. methyl isocyanate, DDT, malathion). A contaminant however, is regarded as a pollutant when it exerts detrimental effects. Environmental pollution may be considered in different ways—industrial pollution; agricultural pollution; pollution due to gaseous wastes, liquid wastes and solid wastes. Environmental pollution with reference to air, water and foodstuffs is briefly discussed.

AIR POLLUTION

The major components of air include nitrogen (78.1%), oxygen (20.93%) and carbon dioxide (0.03%), along with water vapour and suspended particles. The rapid growth of industries coupled with changing lifestyles of man (urbanization, smoking, use of motor vehicles etc.) largely contribute to air pollution. The major chemical constituents of air pollution are sulfur dioxide, oxides of carbon (CO_2 and CO), oxides of nitrogen, hydrocarbons and particulates. The biochemical affects of air pollution are described.

Sulfur dioxide

Sulfur dioxide (SO_2) is the most dangerous pollutant gas to man. Industrial activities such as burning of coal and oil emit large quantities of SO_2.

Sulfur dioxide pollution primarily affects respiratory system in man. Irritation of the respiratory tract and increasing airway resistance (breathing difficulty) are observed. Lung tissue may get damaged due to acidic pH. Further, dipalmityl lecithin, the phospholipid acting as the lung surfactant, gets affected. Continuous exposure to SO_2 (> 1 ppm) for several days causes bronchitis and in some individuals lung cancer. Atmospheric SO_2 when dissolved in rain water becomes very acidic (acid rain) damaging soil, plants and vegetables. Exposure of plants to SO_2 destroys leaves.

Carbon monoxide

Carbon monoxide (CO) is mostly produced by incomplete combustion of fuel or carbon-containing compounds. Automobiles, aircrafts, rail engines and burning of coal in factories contribute to CO pollution.

Carbon monoxide combines with hemoglobin to form carboxyhemoglobin (***Refer Chapter 10***). This causes a drastic reduction in the supply of O_2 to tissues. At a CO concentration around 1 ppm, impairment in mental performance and visual perception take place. With a further increase in CO level, headache, dizziness and loss of consciousness occur. Death may be inevitable in persons exposed to above 750 ppm of CO.

Carbon dioxide

Carbon dioxide (CO_2), constituting only a fraction (0.03%) of the atmospheric gases, plays a significant role in controlling the climate. This is done by trapping the heat radiation from the earth's surface. Without the presence of CO_2, the earth would be as cold as moon!

Carbon dioxide is often referred to as greenhouse gas. The term ***greenhouse effect*** refers to an ***elevation in CO_2*** near earth's surface that traps sunlight and ***increases atmospheric temperature***. Deforestation, burning of coal, oils etc., elevate atmospheric CO_2 resulting in greenhouse effect. Hence the global propaganda for increased plantation of trees!

Fortunately, marginal variations in atmospheric CO_2 are tolerated by the cells. The body gets adapted to prolonged exposure to higher concentrations of CO_2 (even upto 1%) with minor alterations in electrolyte balance.

Nitrogen dioxide

Nitrogen dioxide (NO_2) like carbon monoxide (CO), combines with hemoglobin and reduces the supply of O_2 to the tissues. NO_2 is more harmful to human health than CO. It is fortunate that the atmospheric concentration of NO_2 is relatively lower.

Nitrogen dioxide (in the form of HNO_3) along with SO_2 (as H_2SO_4) contributes to ***acid rain***.

Hydrocarbons

Many hydrocarbons polluting the environment affect human life. The aromatic hydrocarbons cause irritation to injuries.

Particulates

The ***solid dust particles*** suspended ***in the atmosphere*** constitute particulates. The sources of particulates are grinding, spraying, erosion, smoking etc.

The particulates have ill-affects on humans. These include interference in respiratory function (coughing, sneezing) and toxicity caused by the absorption particulate chemicals. Further, the dust particles carry microorganisms and other infective agents to spread diseases.

Ozone layer

Ozone is formed from atmospheric oxygen during high energy radiations of electrical discharges. This ozone forms a layer above the earth's surface (15-35 km). It ***absorbs harmful ultraviolet radiations of sun*** which would otherwise cause skin diseases and mutations, besides increasing the temperature of earth.

In recent years, a decrease in the ozone layer is observed due to chemical pollution in the air. Nitrogen oxides (released from engines of aeroplanes) and chlorofluoro carbons (used in refrigerators and air conditioners) deplete the ozone layer.

WATER POLLUTION

Water is the most predominant constituent of living matter. The very existence of life is unimaginable without water.

As such, pure water does not exist in nature. The available water contains dissolved gases, minerals and some suspended particles. Pollution of water occurs due to waste disposal from industries, agriculture and municipalities. The pollutants may be organic, inorganic, sediments, radioactive, thermal etc., in nature.

ORGANIC POLLUTANTS

The organic pollutants include agents carrying water borne diseases, oxygen demanding wastes and organic chemicals.

Water-borne disease agents

Several pathogenic organisms find their entry into water and cause diseases. The water borne disease include typhoid, paratyphoid, cholera, amoebiasis, giardiasis and infectious hepatitis. These diseases can be prevented by disinfection techniques employed for the treatment of water.

Oxygen demanding wastes

Sewage, and wastes from industries and agriculture provide good nutrients for algae. As the algae grow utilizing the wastes, oxygen depletion occurs. This phenomenon of ***water deoxygenation*** is technically referred to as ***eutrophication***. As a consequence of eutrophication, fish and other acquatic animals die (due to lack of O_2), causing foul smell.

Organic chemicals

The organic chemical pollutants of water include pesticides and several synthetic compounds (detergents, paints, plastics, pharmaceuticals, food additives etc.)

Pesticides

Pesticides is a broad term used for ***insecticides, herbicides, fungicides*** and ***rodenticides***. Based on their structure, pesticides are classified as follows.

(a) **Chlorinated hydrocarbons** : e.g. aldrin, dieldrin, endrin, dichlorodiphenyl trichloroethane (DDT).

(b) **Organophosphates** : e.g. malathion, diazinon.

(c) **Carbamates** e.g. baygon, carbaryl (sevin)

(d) **Chlorophenoxy** e.g. 2,4-dichlorophenoxy acetic acid.

The use of pesticides has helped in controlling certain diseases (malaria, typhus), besides boosting food production. However, pesticides pollute water and cause several health complications to humans, besides damaging acquatic life.

Dichloro-diphenyl trichloroethane (DDT) is a widely used pesticide to control cotton and peanut pests, besides malaria. However, continuous use of DDT leads to its accumulation in foods causing ill effects (hence banned in some countries like USA).

DDT, being fat soluble, ***accumulates in the adipose tissue*** and is not excreted. Thus, its concentration in the body goes on increasing. DDT causes nervous irritability, muscle twitching and convulsions.

Aldrin and dialdrin are also fat soluble and their effects on humans are comparable with that of DDT.

BIOMEDICAL / CLINICAL CONCEPTS

- *The body makes every effort to maintain its normal temperature, despite cold and heat surroundings, for optimal physiological and biochemical functions.*
- *Failure of heat regulatory system (thermoregulation) leads to heat stroke characterized by high body temperature, convulsions etc.*
- *Sulfur dioxide (SO_2) is the most dangerous industrial pollutant gas to man. It primarily affects the respiratory system, and may result in bronchitis, and even lung cancer.*
- *Carbon monoxide (CO) combines with hemoglobin to form carboxyHb. This reduces O_2 supply to tissues.*
- *Pollution of water with pathogenic organisms causes many diseases e.g. typhoid, cholera, amoebiasis.*
- *Lead toxicity affects central nervous system—learning disabilities, mental retardation etc.*
- *Radioactive pollution may lead to gene mutations, cancer etc.*

Organophosphates and carbamates are powerful ***neurotoxic agents***. They prevent the transmission of nerve impulse by inhibiting the enzyme cholinesterase.

INORGANIC POLLUTANTS

Heavy metals (lead, mercury, cadmium, aluminium, arsenic etc.) are the most dangerous among the inorganic pollutants.

Lead

Lead is the most common inorganic pollutant found in water, air, foods and soils. The sources of lead pollution include petrol, gasoline, paints, cigarettes, news papers, lead pipes and xerox copies. The plasma concentration of > 25 µg/dl in adults and > 10 µg/dl in children results in toxic manifestations.

The principal target of lead toxicity is ***central nervous system***. In the growing children, Pb causes learning disabilities, behavioural changes (hyperexcitability) and mental retardation. In adults, confusion, irritability, abdominal colic and severe anemia are associated with lead toxicity.

Lead inhibits several enzymes, particularly, δ-aminolevulinate (ALA) synthase, ALA dehydratase and ferrochelatase of heme synthesis (***Refer Chapter 10*** also). This results in severe anemia. There has been an increasing awareness worldover on the toxic manifestations of lead. This has lead to the supply of ***unleaded petrol*** in most countries.

Mercury

Mercury is a common industrial (plastic, paints, electrical apparatus, fungicides) pollutant. Acute mercuric poisoning causes gastritis, vomiting and pulmonary edema. Chronic manifestations of Hg include emotional changes, loss of memory and other neuropsychiatric disturbances. In addition, deposition of mercuric salts may cause renal failure.

Organic mercuric poisoning is commonly referred to as ***minamata disease*** (as it first occurred in Minamata, Japan in 1953-60 by consuming fish containing methyl mercury, as industrial pollutant).

Cadmium

The outbreak of cadmium toxicity was reported in Japan in the form of ***itai itai*** or ***ouch disease***. Cadmium poisoning causes fragile bones, anemia, bone marrow disorders and kidney damage. Biochemically, cadmium replaces zinc and adversely influences several metabolic reactions.

Aluminium

The sources of aluminium include cooking vessels, building materials, food additives and cosmetics. Aluminium toxicity is associated with Alzheimer's disease, anemia and osteomalacia.

Arsenic

Arsenic, commonly found in many insecticides and fungicides, is toxic to the body. Arsenic binds with–SH groups of several enzymes and inhibits biochemical reactions e.g. pyruvate dehydrogenase. Further, arsenic causes coagulation of proteins and blockage of ATP generation (functions as an uncoupler).

NOISE POLLUTION

The unwanted sound is noise, which is a major urban environmental pollutant. Man can tolerate noise upto 100 decibels (speaking – 60 decibels; telephone bell 70 decibels; motor cycle 110 decibels; rockets 170 decibels). A noise above 150 decibels is uncomfortable.

The affects of noise pollution include headache, increased blood pressure, irritability, neuromuscular tension, confusion, disturbed vision and digestion, depression and loss of hearing.

RADIOACTIVE POLLUTION

The pollution due to radioactive substances is the most dangerous to human life. The health hazards of radioactive pollution include ***gene mutations,*** cancer, destruction of living cells etc.

TOXIC COMPOUNDS IN FOODSTUFFS

The foodstuffs consumed by humans contain several toxic compounds which may be either normally present or enter foodstuffs during the course of cultivation, processing or storage.

Natural toxins in foodstuffs

Neurotoxins : Kesari dal (*Lathyrus sativus*) is a pulse grown in some parts of Madhya Pradesh, Bihar and Uttar Prodesh. Excessive consumption of kesari dal causes ***paralysis of lower limbs*** referred to as ***lathyrism***. This is due to a neurotoxin namely ***β-oxalylaminoalanine*** (***BOAA***). BOAA damages upper motor neurons, and inhibits the enzyme lysyl oxidase (reduces collagen cross-linking). Cooking of kesari dal 2-3 times and removal of the supernatant water will eliminate the toxin.

Protease inhibitors : Certain legumes (soya bean, peanut) contain inhibitors of protease enzymes particularly trypsin. Normally, protease inhibitors are destroyed by cooking. However, partial cooking does not totally destroy them. In such a case, protease inhibitors can inhibit digestion and proteins.

Goitrogens : These compounds prevent uptake and utilization of iodine by thyroid gland. Goitrogens are found in cabbage and turnips (thioglycosides), mustard and rape seed oils (thiocyanates), ground nuts and almonds (polyphenolic glycosides).

Biogenic amines : Bananas and cheese contain biogenic amines namely histamine, tryptamine, tyramine serotonin and epinephrine. In normal metabolism, they are degraded by monoamine oxidase (MAO). However, in persons taking MAO—inhibitors, the foodstuffs with amines may cause hypertension.

Anti-vitamins : ***Avidin*** of raw egg is a good example of anti-vitamin of biotin.

Toxic pollutants of foodstuffs

The foodstuffs may get polluted with several toxic chemicals which might occur during cultivation, processing or storage.

Cultivation : Pesticides and other unnatural chemicals used during cultivation do find an entry into the foodstuffs. It is fortunate that most of these chemicals can be removed by peeling the outer layers of vegetables and fruits, besides repeated washings.

Processing : Defects in freezing, and packing provide a suitable environment for the growth of several organisms which release toxic products e.g. milk contamination by *Salmonella*.

Several food additives are in use for preservation and enchancing flavour. Not all of them are safe e.g. aniline dyes used as colouring agents are carcinogenic; sweetening agent cyclamate may cause bladder cancer.

Storage : Contamination of stored foods occurs mostly due to fungal infections. ***Aflatoxins*** are produced by *Aspergillus favus* when ground nuts or coconuts are stored in moist conditions. Aflatoxins are hepatotoxic and carcinogenic.

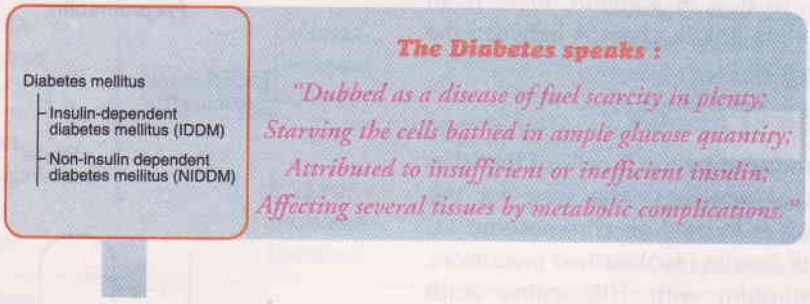
CARCINOGENS

The group of chemicals that cause cancer in man and animals are collectively referred to as carcinogens (***Refer Chapter 37***). Environmental pollution is undoubtedly associated with increased risk of cancer. The topic '***cancer***' may be considered as a part of environmental biochemistry for learning purpose.

SUMMARY

1. *Environmental biochemistry deals with the biochemical responses and adaptations in man (and other organisms) due to environmental factors.*
2. *The atmospheric (climatic) changes like cold and heat influence the body. Several metabolic adaptations occur to overcome the adverse affects.*
3. *The major chemical constituents of air pollution include SO_2, CO, CO_2 and oxides of nitrogen. Among these, sulfur dioxide is the most dangerous.*
4. *Water pollution occurs mainly due to waste disposal from industries, agriculture and municipalities. The pollutants may be organic (pathogenic organisms, pesticides), or inorganic (lead, mercury).*
5. *The foodstuffs consumed by humans may contain several toxic compounds. These may be normally present (e.g. BOAA causing lathyrism) or enter the foodstuffs during the course of cultivation (e.g. pesticides), or storage (e.g. aflatoxins).*

36 Insulin, Glucose Homeostasis, and Diabetes Mellitus



Diabetes mellitus is the ***third leading cause of death*** (after heart disease and cancer) in many developed countries. It affects about 2 to 3% of the general population. The complications of diabetes affect the eye, kidney and nervous system. Diabetes is a major cause of blindness, renal failure, amputation, heart attacks and stroke. (The term diabetes, whenever used, refers to diabetes mellitus. It should, however, be noted that diabetes insipidus is another disorder characterized by large volumes of urine excretion due to antidiuretic hormone deficiency).

Diabetes mellitus is a clinical condition characterized by ***increased blood glucose level (hyperglycemia)*** due to insufficient or inefficient (incompetent) insulin. In other words, insulin is either not produced in sufficient quantity or inefficient in its action on the target tissues. As a consequence, the blood glucose level is elevated which spills over into urine in diabetes mellitus (*Greek* : diabetes—a siphon or running through; mellitus—sweet).

An important feature of diabetes is that the body cells are starved of glucose despite its very high concentration around i.e. scarcity in plenty. For a comprehensive understanding of diabetes, the relevant hormones, namely insulin and glucagon, homeostasis of blood glucose, besides the biochemical aspects of diabetes, are discussed in this chapter.

INSULIN

Insulin is a polypeptide hormone ***produced by the β-cells of islets of Langerhans of pancreas***. It has profound influence on the metabolism of carbohydrate, fat and protein. Insulin is considered as anabolic hormone, as it promotes the synthesis of glycogen, triacylglycerols and proteins. This hormone has been implicated in the development of diabetes mellitus.

Insulin occupies a special place in the history of biochemistry as well as medicine. Insulin was the first hormone to be isolated, purified and

synthesized; first hormone to be sequenced; first hormone to be produced by recombinant DNA technology.

Structure of insulin

Human insulin (mol. wt. 5,734) contains ***51 amino acids***, arranged in two polypeptide chains. The chain A has 21 amino acids while B has 30 amino acids. Both are held together by two interchain disulfide bridges, connecting A_7 to B_7 and A_{20} to B_{19}. In addition, there is an intrachain disulfide link in chain A between the amino acids 6 and 11.

Biosynthesis of insulin

Insulin is produced by the β-cells of the islets of Langerhans of pancreas. The gene for this protein synthesis is located on chromosome 11. The synthesis of insulin involves two precursors, namely preproinsulin with 108 amino acids (mol. wt. 11,500) and proinsulin with 86 amino acids (mol. wt. 9,000). They are sequentially degraded (***Fig.36.1***) to form the active hormone insulin and a connecting peptide (C-peptide). Insulin and C-peptide are produced in equimolar concentration. ***C-peptide*** has no biological activity, however its estimation in the plasma serves as a ***useful index*** for the endogenous production of insulin.

In the β-cells, insulin (and also proinsulin) combines with zinc to form complexes. In this form, insulin is stored in the granules of the cytosol which is released in response to various stimuli (discussed below) by exocytosis.

Regulation of insulin secretion

About 40-50 units of insulin is secreted daily by human pancreas. The normal insulin concentration in plasma is 20-30 μU/ml. The important factors that influence the release of insulin from the β-cells of pancreas are discussed hereunder.

1. **Factors stimulating insulin secretion :** These include glucose, amino acids and gastrointestinal hormones.

- **Glucose** is the most important stimulus for insulin release. The effect is more predominant when glucose is administered orally (either direct or through a carbohydrate-rich meal). A rise in blood glucose level is a signal for insulin secretion.
- **Amino acids** induce the secretion of insulin. This is particularly observed after the ingestion of protein-rich meal that causes transient rise in plasma amino acid concentration. Among the amino acids, arginine and leucine are potent stimulators of insulin release.

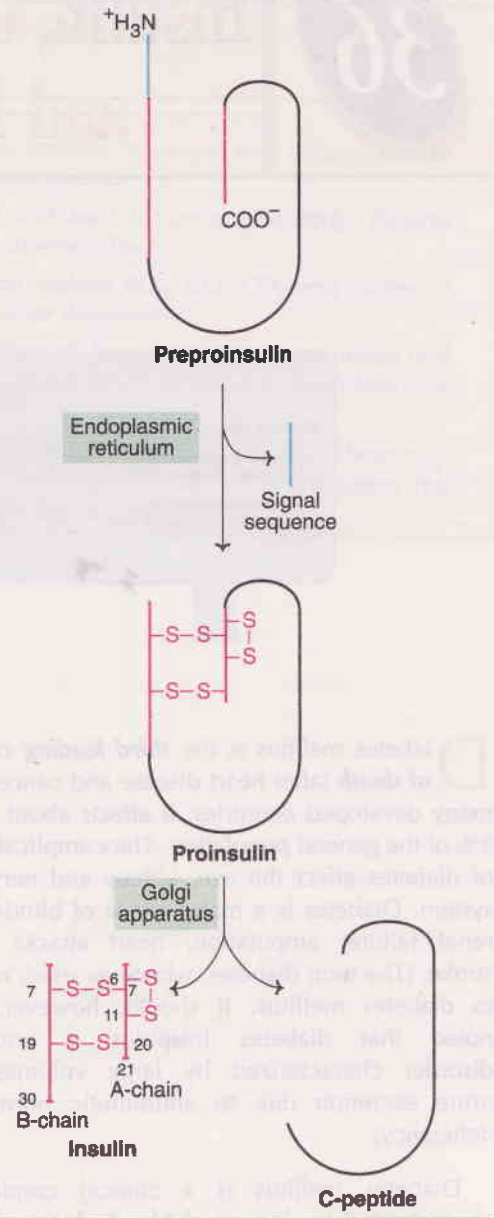


Fig. 36.1 : Formation of insulin from preproinsulin.

Table 36.1 Metabolic effects of insulin—a summary

Metabolism	*Net effect*	*Effect on important enzyme(s)*
Carbohydrate metabolism		
1. Glycolysis	Increased	Glucokinase ↑ Phosphofructokinase ↑ Pyruvate kinase ↑
2. Gluconeogenesis	Decreased	Pyruvate carboxylase ↓ Phosphoenol pyruvate carboxykinase ↓ Glucose 6-phosphatase ↓
3. Glycogenesis	Increased	Glycogen synthetase ↑
4. Glycogenolysis	Decreased	Glycogen phosphorylase ↓
5. HMP shunt	Increased	Glucose 6- phosphate dehydrogenase ↑
Lipid metabolism		
6. Lipogenesis	Increased	Acetyl CoA carboxylase ↑
7. Lipolysis	Decreased	Hormone sensitive lipase ↓
8. Ketogenesis	Decreased	HMG CoA synthetase ↓
Protein metabolism		
9. Protein synthesis	Increased	RNA polymerase ↑
10. Protein degradation	Decreased	Transaminases ↓ Deaminases ↓

- **Gastrointestinal hormones** (secretin, gastrin, pancreozymin) enhance the secretion of insulin. The GIT hormones are released after the ingestion of food.

2. **Factors inhibiting insulin secretion :** Epinephrine is the most potent inhibitor of insulin release. In emergency situations like stress, extreme exercise and trauma, the nervous system stimulates adrenal medulla to release epinephrine. ***Epinephrine*** suppresses insulin release and promotes energy metabolism by mobilizing energy-yielding compounds—glucose from liver and fatty acids from adipose tissue.

Degradation of insulin

In the plasma, insulin has a normal ***half-life*** of ***4-5 minutes.*** This short half-life permits rapid metabolic changes in accordance to the alterations in the circulating levels of insulin. This is advantageous for the therapeutic purposes. A protease enzyme, namely ***insulinase*** (mainly found in liver and kidney), degrades insulin.

Metabolic effects of insulin

Insulin plays a key role in the regulation of carbohydrate, lipid and protein metabolisms (***Table 36.1***). Insulin exerts anabolic and anticatabolic influences on the body metabolism.

1. **Effects on carbohydrate metabolism :** In a normal individual, about half of the ingested glucose is utilized to meet the energy demands of the body (mainly through glycolysis). The other half is converted to fat (~ 40%) and glycogen (~ 10%). This relation is severely impaired in insulin deficiency. Insulin influences glucose metabolism in many ways. The net effect is that insulin ***lowers blood glucose level (hypoglycemic effect)*** by promoting its utilization and storage and by inhibiting its production.

- **Effect on glucose uptake by tissues :** ***Insulin*** is ***required*** for the uptake of glucose **by muscle** (skeletal, cardiac and smooth), ***adipose tissue***, leukocytes and mammary glands. Surprisingly, about 80% of glucose uptake in the body is

not dependent on insulin. Tissues into which glucose can freely enter include brain, kidney, erythrocytes, retina, nerve, blood vessels and intestinal mucosa. As regards liver, glucose entry into hepatocytes does not require insulin. However, insulin stimulates glucose utilization in liver and, thus, indirectly promotes its uptake.

- **Effect on glucose utilization :** Insulin ***increases glycolysis*** in muscle and liver. The activation as well as the quantities of certain key enzymes of glycolysis, namely glucokinase (not hexokinase) phosphofructokinase and pyruvate kinase are increased by insulin. Glycogen production is enhanced by insulin by increasing the activity of glycogen synthetase.
- **Effect on glucose production :** Insulin ***decreases gluconeogenesis*** by suppressing the enzymes pyruvate carboxylase, phosphoenol pyruvate carboxykinase and glucose 6-phosphatase. Insulin also inhibits glycogenolysis by inactivating the enzyme glycogen phosphorylase.

2. **Effects on lipid metabolism :** The net effect of insulin on lipid metabolism is to reduce the release of fatty acids from the stored fat and decrease the production of ketone bodies. Among the tissues, adipose tissue is the most sensitive to the action of insulin.

- **Effect on lipogenesis :** Insulin ***favours*** the ***synthesis of triacylglycerols*** from glucose by providing more glycerol 3-phosphate (from glycolysis) and NADPH (from HMP shunt). Insulin increases the activity of acetyl CoA carboxylase, a key enzyme in fatty acid synthesis.
- **Effect on lipolysis :** Insulin decreases the activity of hormone-sensitive lipase and thus reduces the release of fatty acids from stored fat in adipose tissue. The mobilization of fatty acids from liver is also decreased by insulin. In this way, insulin keeps the circulating free fatty acids under a constant check.
- **Effect on ketogenesis :** Insulin ***reduces ketogenesis*** by decreasing the activity of HMG CoA synthetase. Further, insulin promotes the utilization of acetyl CoA for oxidation (Krebs cycle) and lipogenesis. Therefore, the availability of acetyl CoA for ketogenesis, in the normal circumstances, is very low.

3. **Effects on protein metabolism :** Insulin is an anabolic hormone. It stimulates the entry of amino acids into the cells, ***enhances protein synthesis*** and reduces protein degradation.

Besides the metabolic effects described above, insulin promotes cell growth and replication. This is mediated through certain factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF) and prostaglandins.

Mechanism of action of insulin

It is now recognized that ***insulin binds to*** specific plasma ***membrane receptors*** present on the target tissues, such as muscle and adipose. This results in a series of reactions ultimately leading to the biological action. Three distinct mechanisms of insulin action are known. One concerned with the induction of transmembrane signals (signal transduction), second with the glucose transport across the membrane and the third with induction of enzyme synthesis.

1. **Insulin receptor mediated signal transduction**

Insulin receptor : It is a tetramer consisting of 4 subunits of two types and is designated as $\alpha_2\beta_2$. The subunits are in the glycosylated form. They are held together by disulfide linkages. The α-subunit (mol. wt. 135,000) is extracellular and it contains insulin binding site. The β-subunit (mol. wt. 95,000) is a transmembrane protein which is activated by insulin. The cytoplasmic domain of β-subunit has tyrosine kinase activity.

The insulin receptor is synthesized as a single polypeptide and cleaved to α and β subunits which are then assembled. The insulin receptor has a half-life of 6-12 hours. There are about 20,000 receptors per cell in mammals.

Signal transduction : As the hormone insulin binds to the receptor, a conformational change is induced in the α-subunits of insulin receptor. This results in the generation of signals which

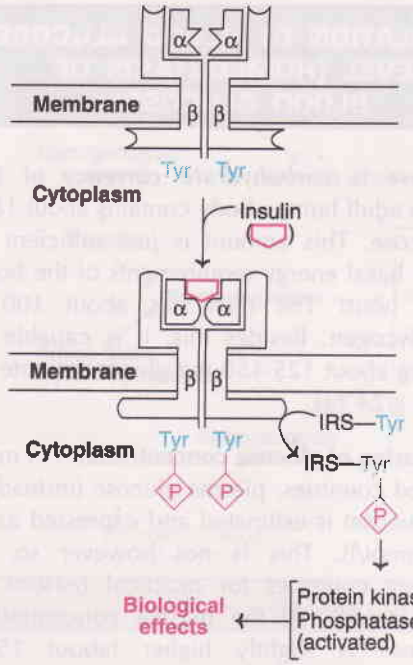


Fig. 36.2 : Insulin receptor mediated signal transduction (IRS—Insulin receptor substrate).

are transduced to β-subunits. The net effect is that insulin binding activates tyrosine kinase activity of intracellular β-subunit of insulin receptor. This causes the autophosphorylation of tyrosine residues on β-subunit. It is believed that receptor tyrosine kinase also phosphorylates insulin receptor substrate (IRS). The phosphorylated IRS, in turn, promotes activation of other protein kinases and phosphatases, finally leading to biological action (***Fig.36.2***).

2. **Insulin-mediated glucose transport :** The binding of insulin to insulin receptors signals the translocation of vesicles containing glucose transporters from intracellular pool to the plasma membrane. The vesicles fuse with the membrane recruiting the glucose transporters. The glucose transporters are responsible for the insulin–mediated uptake of glucose by the cells. As the insulin level falls, the glucose transporters move away from the membrane to the intracellular pool for storage and recycle (***Fig.36.3***).

3. **Insulin mediated enzyme synthesis :** Insulin promotes the synthesis of enzymes such as glucokinase, phosphofructokinase and pyruvate kinase. This is brought about by increased transcription (mRNA synthesis), followed by translation (protein synthesis).

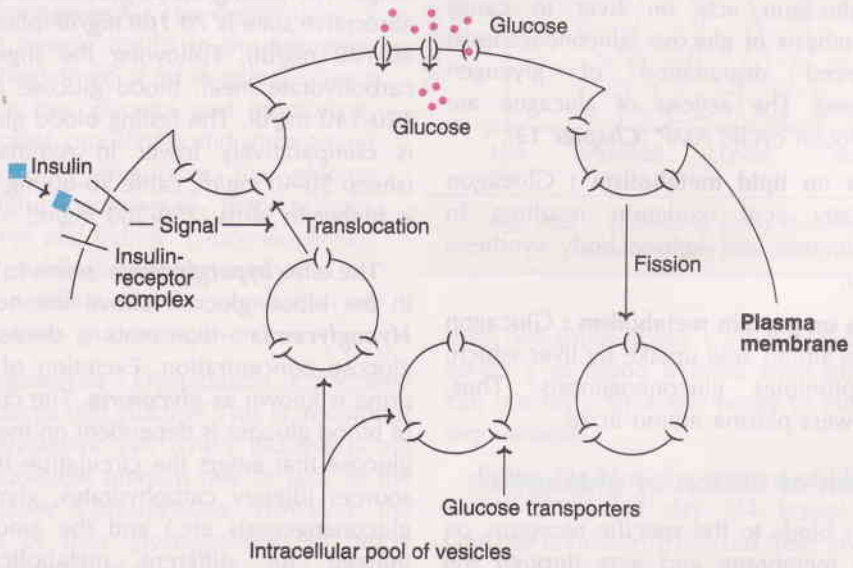


Fig. 36.3 : Insulin mediated glucose transport.

GLUCAGON

Glucagon, ***secreted by α-cells*** of the pancreas, opposes the actions of insulin. It is a polypeptide hormone composed of 29 amino acids (mol. wt. 3,500) in a single chain. Glucagon is actually synthesized as proglucagon (mol. wt. 9,000) which on sequential degradation releases active glucagon. Unlike insulin, the ***amino acid sequence*** of glucagon is the ***same in all mammalian species*** (so far studied). Glucagon has a short half-life in plasma i.e. about 5 minutes.

Regulation of glucagon secretion

The secretion of glucagon is ***stimulated by low blood glucose*** concentration, amino acids derived from dietary protein and low levels of epinephrine. Increased blood glucose level markedly inhibits glucagon secretion.

Metabolic effects of glucagon

Glucagon influences carbohydrate, lipid and protein metabolisms. In general, the effects of this hormone ***oppose that of insulin.***

1. **Effects on carbohydrate metabolism :** Glucagon is the most potent hormone that enhances the blood glucose level (hyperglycemic). Primarily, glucagon acts on liver to cause increased synthesis of glucose (gluconeogenesis) and enhanced degradation of glycogen (glycogenolysis). The actions of glucagon are mediated through cyclic AMP (***Chapter 13***).

2. **Effects on lipid metabolism :** Glucagon promotes fatty acid oxidation resulting in energy production and ketone body synthesis (ketogenesis).

3. **Effects on protein metabolism :** Glucagon increases the amino acid uptake by liver which, in turn, promotes gluconeogenesis. Thus, glucagon lowers plasma amino acids.

Mechanism of action of glucagon

Glucagon binds to the specific receptors on the plasma membrane and acts through the mediation of cyclic AMP, the second messenger. The details are given elsewhere (***Chapter 19***).

REGULATION OF BLOOD GLUCOSE LEVEL (HOMEOSTASIS OF BLOOD GLUCOSE)

Glucose is ***carbohydrate currency*** of the body. An adult human body contains about 18 g free glucose. This amount is just sufficient to meet the basal energy requirements of the body for one hour! The liver has about 100 g stored glycogen. Besides this, it is capable of producing about 125-150 mg glucose/minute or 180-220 g/24 hrs.

Expression of glucose concentration : In most developed countries, plasma glucose (instead of blood glucose) is estimated and expressed as SI units (mmol/l). This is not however so, in developing countries for practical reasons. It may be noted that the plasma concentration of glucose is slightly higher (about 15%) than blood glucose. Further, a glucose concentration of 180 mg/dl (plasma or blood) corresponds to 10 mmol/l. In this book, expression of blood glucose as mg/dl is more frequently used.

A healthy individual is capable of maintaining the blood glucose concentration within a narrow range. The ***fasting blood glucose*** level in a post-absorptive state is ***70-100 mg/dl*** (plasma glucose 80-120 mg/dl). Following the ingestion of a carbohydrate meal, blood glucose may rise to 120-140 mg/dl. The fasting blood glucose value is comparatively lower in ruminant animals (sheep 30-40 mg/dl; cattle 50-60 mg/dl), while it is higher in birds (250-300 mg/dl).

The term ***hyperglycemia*** refers to an increase in the blood glucose above the normal level. ***Hypoglycemia*** represents a decreased blood glucose concentration. Excretion of glucose in urine is known as ***glycosuria.*** The concentration of blood glucose is dependent on the quantity of glucose that enters the circulation from various sources (dietary carbohydrates, glycogenolysis, gluconeogenesis etc.) and the amount that is utilized for different metabolic purposes (glycolysis, glycogenesis, fat synthesis etc.) as illustrated in ***Fig.36.4***.

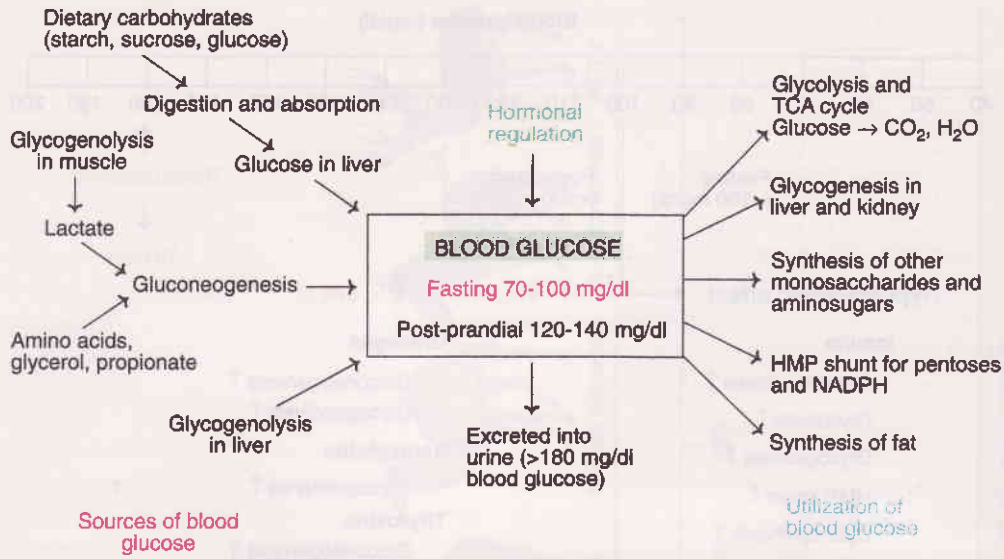


Fig. 36.4 : Overview of blood glucose homeostasis.

Sources of blood glucose

1. **Dietary sources :** The dietary carbohydrates are digested and ***absorbed as monosaccharides*** (glucose, fructose, galactose etc.). The liver is capable of ***converting*** fructose and galactose ***into glucose***, which can readily enter blood.

2. **Gluconeogenesis :** The degradation of glycogen in muscle results in the formation of lactate. Breakdown of fat in adipose tissue will produce free glycerol and propionate. Lactate, glycerol, propionate and some amino acids are good precursors for ***glucose synthesis*** (gluconeogenesis) that actively occurs in liver and kidney. Gluconeogenesis continuously adds glucose to the blood. Cori cycle is responsible for the conversion of muscle lactate to glucose in liver.

3. **Glycogenolysis :** Degradation of glycogen in liver produces free glucose. This is in contrast to muscle glycogenolysis where glucose is not formed in sufficient amount due to lack of the enzyme glucose 6-phosphatase. However, the contribution of liver glycogenolysis to blood glucose is rather limited and can meet only the short intervals of emergency. This is due to the limited presence of glycogen in liver. An adult liver (weighing about 1.5 kg) can provide only 40-50 g of blood glucose from glycogen, that can last only for a few hours to meet the body requirements.

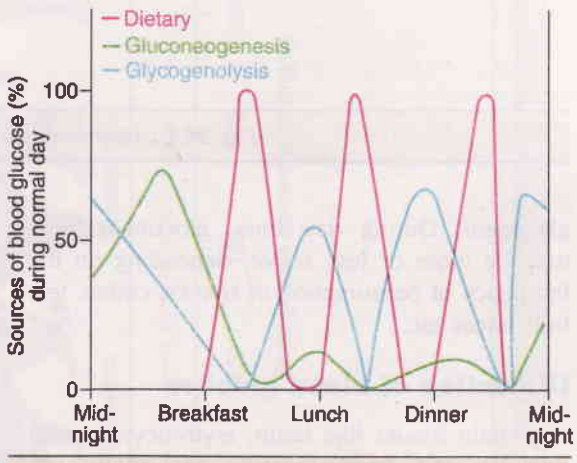


Fig. 36.5 : Sources of blood glucose during a normal day (24 hours).

In the ***Fig.36.5***, the sources of blood glucose during a normal day (24 hours) are given. Glucose is primarily derived from glycogenolysis (of hepatic glycogen) between the meals. Gluconeogenesis becomes a predominant source of glucose in late night (after depletion of hepatic

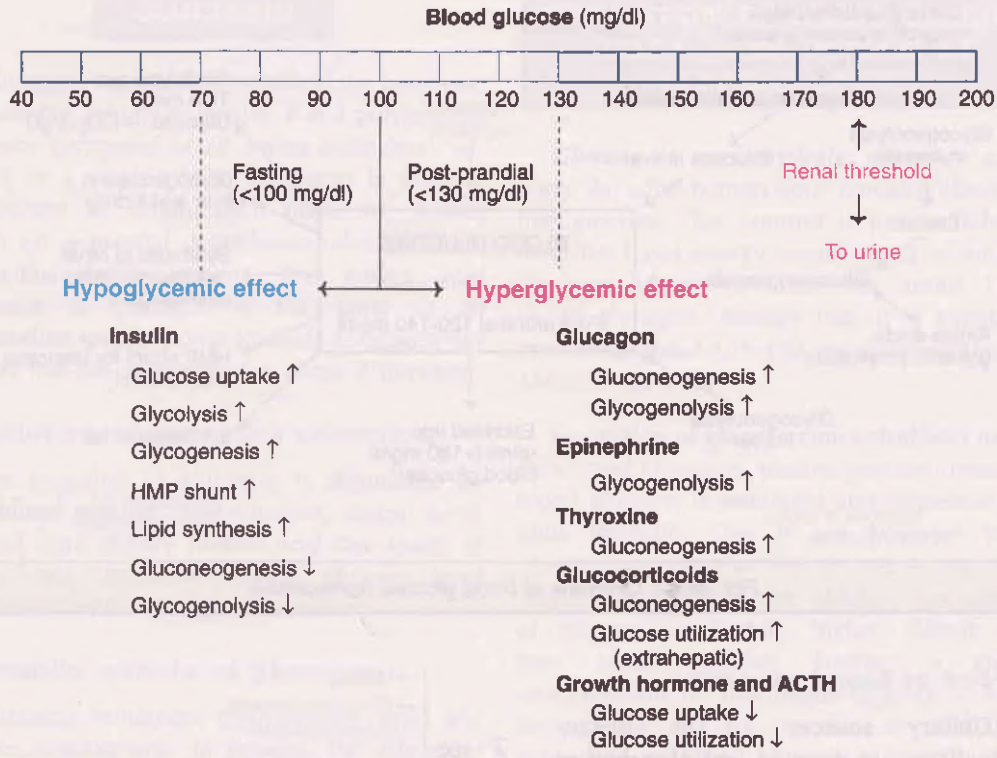


***Fig. 36.6** : Hormonal regulation of blood glucose.*

glycogen). During day time, gluconeogenesis may be more or less active, depending on the frequency of consumption of snacks, coffee, tea, fruit juices etc.

Utilization of blood glucose

Certain tissues like brain, erythrocytes, renal medulla and bone marrow are exclusively dependent on glucose for their energy needs. When the body is ***at total rest***, about ***two-thirds*** of the blood glucose is utilized ***by the brain***. The remaining one-third by RBC and skeletal muscle. A regular supply of glucose, by whatever means it may be, is absolutely required to keep the brain functionally intact.

The different metabolic pathways (glycolysis, glycogenesis, HMP shunt etc.) responsible for the utilization of blood glucose are already discussed (***Chapter 13***). The synthesis of fat from acetyl CoA and glycerol is described in lipid metabolism (***Chapter 14***).

Kidney plays a special role in the homeostasis of blood glucose. Glucose is continuously filtered by the glomeruli, reabsorbed and returned to the blood. If the level of glucose in blood is above 160-180 mg/dl, glucose is excreted in urine (glycosuria). This value (160-180 mg/dl) is referred to as ***renal threshold for glucose.*** The maximum ability of the renal tubules to reabsorb glucose per minute is known as tubular maximum for glucose (TmG). The value for ***TmG is 350 mg/minute.***

Role of hormones in blood glucose homeostasis

Hormones play a significant role in the regulation of blood glucose concentration (***Figs.36.6*** and ***36.7***). Primarily, ***insulin lowers blood glucose*** level (hypoglycemic) while the ***rest of the hormones oppose*** the actions of insulin (hyperglycemia).

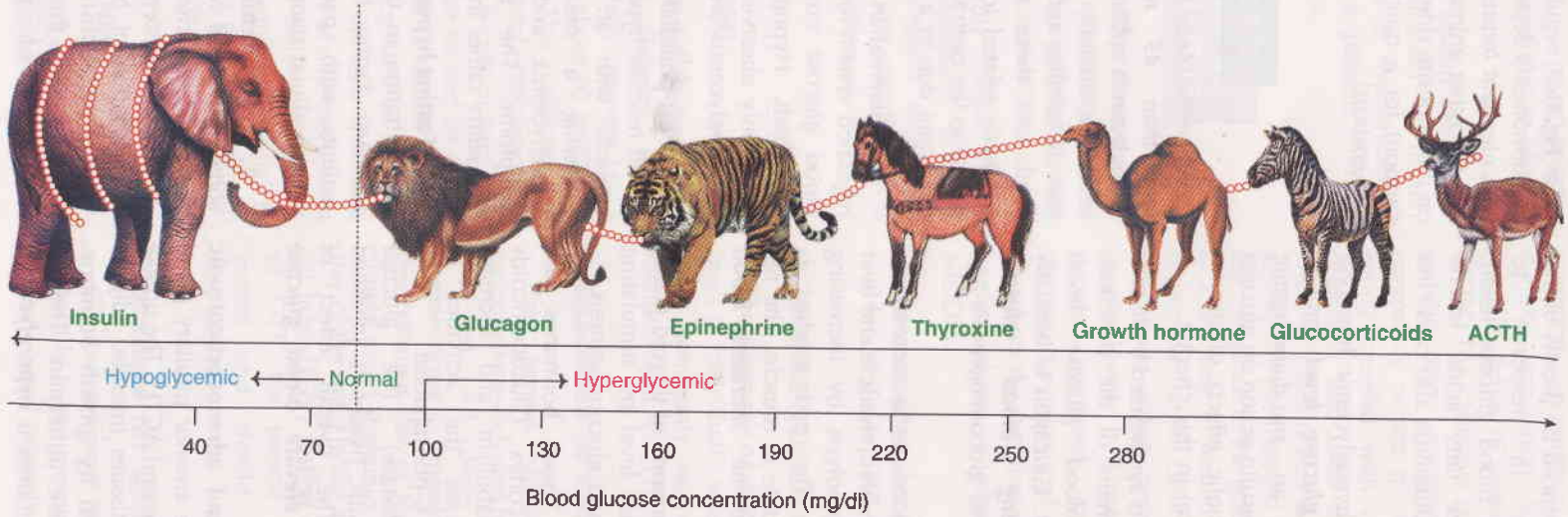


Fig. 36.7 : *A cartoon of tug of war illustrating hormonal action on blood glucose regulation.*

Insulin : Insulin is produced by β-cells of the islets of Langerhans in response to hyperglycemia (elevated blood glucose level). Some amino acids, free fatty acids, ketone bodies, drugs such as tolbutamide also cause the secretion of insulin.

Insulin is basically a hypoglycemic hormone that ***lowers*** in ***blood glucose level*** through various means. It is an anti-diabetogenic hormone. For details of insulin action on glucose homeostasis refer metabolic effects of insulin (carbohydrate metabolism) in this chapter.

Glucagon : Glucagon is synthesized by α-cells of the islets of Langerhans of the pancreas. Hypoglycemia (low blood glucose level) stimulates its production. Glucagon is basically involved in ***elevating blood glucose*** concentration. It enhances gluconeogenesis and glycogenolysis.

Epinephrine : This hormone is secreted by adrenal medulla. It acts both on muscle and liver to bring about glycogenolysis by increasing phosphorylase activity. The end product is glucose in liver and lactate in muscle. The net outcome is that epinephrine ***increases blood glucose level.***

Thyroxine : It is a hormone of thyroid gland. It elevates blood glucose level by stimulating hepatic glycogenolysis and gluconeogenesis.

Glucocorticoids : These hormones are produced by adrenal cortex. Glucocorticoids stimulate protein metabolism and increase gluconeogenesis (increase the activities of enzymes—glucose 6-phosphatase and fructose 1,6-bisphosphatase). The glucose utilization by extrahepatic tissues is inhibited by glucocorticoids. The overall effect of glucocorticoids is to ***elevate blood glucose concentration.***

Growth hormone and adrenocorticotropic hormone (ACTH) : The anterior pituitary gland secretes growth hormone and ACTH. The uptake of glucose by certain tissues (muscle, adipose tissue etc.) is decreased by growth hormone. ACTH decreases glucose utilization. The net effect of both these hormones is ***hyperglycemic.***

[In ***Fig.36.7,*** regulation of blood glucose level by hormones is depicted as a game of tug of war with elephant (representing insulin) on one side and the other animals (as rest of the hormones) on the opposite side. This is just an illustration (a cartoon) for a quick understanding of glucose homeostasis].

HYPOGLYCEMIA

When the blood glucose concentration falls to ***less than 45 mg/dl,*** the symptoms of hypoglycemia appear. The manifestations include headache, anxiety, confusion, sweating, slurred speech, seizures and coma, and, if not corrected, death. All these symptoms are directly and indirectly related to the deprivation of glucose supply to the central nervous system (particularly the brain) due to a fall in blood glucose level.

The mammalian body has developed a well regulated system for an efficient maintenance of blood glucose concentration (details already described). Hypoglycemia, therefore, is not commonly observed. The following three types of hypoglycemia are encountered by physicians.

1. **Post-prandial hypoglycemia :** This is also called reactive hypoglycemia and is observed in subjects with an elevated insulin secretion following a meal. This causes transient hypoglycemia and is associated with mild symptoms. The patient is advised to eat frequently rather than the 3 usual meals.

2. **Fasting hypoglycemia :** Low blood glucose concentration in fasting is not very common. However, fasting hypoglycemia is observed in patients with pancreatic β-cell tumor and hepatocellular damage.

3. **Hypoglycemia due to alcohol intake :** In some individuals who are starved or engaged in prolonged exercise, alcohol consumption may cause hypoglycemia. This is due to the accumulation of NADH (during the course of alcohol metabolism by alcohol dehydrogenase) which diverts the pyruvate and oxaloacetate (substrates of gluconeogenesis) to form,

respectively, lactate and malate. The net effect is that ***gluconeogenesis is reduced due to alcohol consumption.***

4. **Hypoglycemia due to insulin overdose :** The most common complication of insulin therapy in diabetic patients is hypoglycemia. This is particularly observed in patients who are on intensive treatment regime.

CLASSIFICATION OF DIABETES MELLITUS

Diabetes mellitus is a ***metabolic disease***, more appropriately a disorder of fuel metabolism. It is mainly characterized by ***hyperglycemia*** that leads to several long term complications.

Diabetes mellitus is broadly divided into 2 groups, namely insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). This classification is mainly based on the requirement of insulin for treatment.

Insulin-dependent diabetes mellitus (IDDM)

IDDM, also known as ***type I diabetes*** or (less frequently) ***juvenile onset diabetes,*** mainly occurs in childhood (particularly between 12-15 yrs age). IDDM accounts for about 10 to 20% of the known diabetics. This disease is characterized by almost total deficiency of insulin due to destruction of β-cells of pancreas. The b-cell destruction may be caused by drugs, viruses or autoimmunity. Due to certain genetic variation, the β-cells are recognized as non-self and they are destroyed by immune mediated injury. Usually, the symptoms of diabetes appear when 80-90% of the β-cells have been destroyed. The pancreas ultimately fails to secrete insulin in response to glucose ingestion. The patients of IDDM require insulin therapy.

Non-insulin dependent diabetes mellitus (NIDDM)

NIDDM, also called ***type II diabetes*** or (less frequently) ***adult-onset diabetes,*** is the most common, accounting for 80 to 90% of the diabetic population. NIDDM occurs in adults (usually above 35 years) and is less severe than IDDM. The causative factors of NIDDM include genetic and environmental. NIDDM more commonly occurs in obese individuals. Over-eating coupled with underactivity leading to obesity is associated with the development of NIDDM. Obesity acts as a diabetogenic factor in genetically predisposed individuals by increasing the resistance to the action of insulin. This is due to a decrease in insulin receptors on the insulin responsive (target) cells. The patients of NIDDM may have either normal or even increased insulin levels. It is suggested that over-eating causes increased insulin production but decreased synthesis of insulin receptors. This is based on the fact that weight reduction by diet control alone is often sufficient to correct NIDDM.

The comparison between IDDM and NIDDM is given in ***Table 36.2***.

GLUCOSE TOLERANCE TEST (GTT)

The ***diagnosis of diabetes*** can be made on the basis of individual's response to the oral glucose load, commonly referred to as oral glucose tolerance test (OGTT).

Preparation of the subject for GTT

The person should have been taking carbohydrate-rich diet for at least 3 days prior to the test. All drugs known to influence carbohydrate metabolism should be discontinued (for at least 2 days). The subject should avoid strenuous exercise on the previous day of the test. He/she should be in an ***overnight*** (at least 10 hr) ***fasting state***. During the course of GTT, the person should be comfortably seated and should refrain from smoking and exercise.

Procedure for GTT

Glucose tolerance test should be conducted preferably in the morning (ideal 9 to 11 AM). A fasting blood sample is drawn and urine

Table 36.2 Comparison of two types of diabetes mellitus

Character	*Insulin-dependent diabetes mellitus (IDDM)*	*Non-insulin dependent diabetes mellitus (NIDDM)*
General		
Prevalence	10-20% of diabetic population	80-90% of diabetic population
Age at onset	Usually childhood (<20 yrs)	Predominantly in adults (>30yrs)
Body weight	Normal or low	Obese
Genetic predisposition	Mild or moderate	Very strong
Biochemical		
Defect	Insulin deficiency due to destruction of β-cells	Impairment in the production of insulin by β-cells and/or resistance of target cells to insulin
Plasma insulin	Decreased or absent	Normal or increased
Auto antibodies	Frequently found	Rare
Ketosis	Very common	Rare
Acute complications	Ketoacidosis	Hyperosmolar coma
Clinical		
Duration of symptoms	Weeks	Months to years
Diabetic complications at diagnosis	Rare	Found in 10-20% cases
Oral hypoglycemic drugs	Not useful for treatment	Suitable for treatment
Administration of insulin	Always required	Usually not necessary

collected. The subject is given 75 g glucose orally, dissolved in about 300 ml of water, to be drunk in about 5 minutes. Blood and urine samples are collected at 30 minute intervals for at least 2 hours. All blood samples are subjected to glucose estimation while urine samples are qualitatively tested for glucose.

Interpretation of GTT

The graphic representation of the GTT results is depicted in ***Fig.36.8***. The fasting plasma glucose level is 75–110 mg/dl in normal persons. On oral glucose load, the concentration increases and the peak value (140 mg/dl) is reached in less than an hour which returns to normal by 2 hours. Glucose is not detected in any of the urine samples.

In individuals with ***impaired glucose tolerance***, the fasting (110-126 mg/dl) as well as 2 hour (140-200 mg/dl) plasma glucose levels are elevated. These subjects slowly develop frank

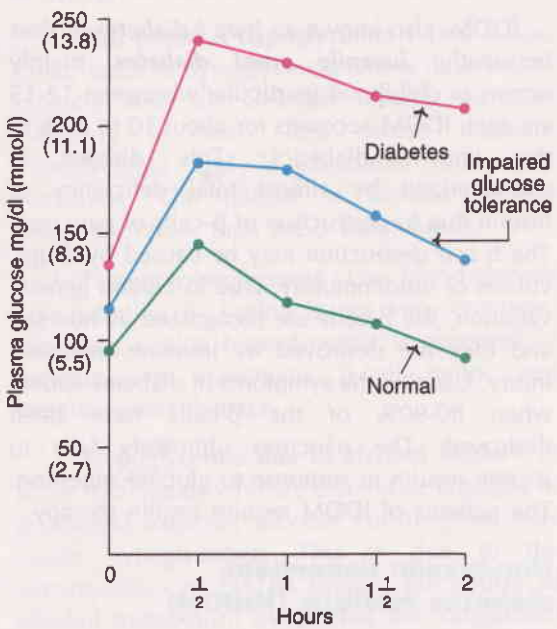


Fig. 36.8 : Oral glucose tolerance test.

Table 36.3 Diagnostic criteria for oral glucose tolerance test (WHO 1999)

Condition	Plasma glucose concentration as mmol/l (mg/dl)		
	Normal	Impaired glucose tolerance	Diabetes
Fasting	<6.1 (<110)	<7.0 (<126)	>7.0 (>126)
2 hours after glucose	<7.8 (<140)	<11.1 (<200)	>11.1 (>200)

diabetes at an estimated rate of 2% per year. Dietary restriction and exercise are advocated for the treatment of impaired glucose tolerance.

The WHO criteria for the diagnosis of diabetes by OGTT is presented in ***Table 36.3***. A person is said to be suffering from diabetes mellitus if his/her ***fasting plasma glucose exceeds*** 7.0 mmol/l (***126 mg/dl***) and, ***at 2 hrs.*** 11.1 mmol/l (***200 mg/dl***).

Other relevant aspects of GTT

1. For conducting GTT in children, oral glucose is given on the basis of weight (1.5 to 1.75 g/kg).

2. In case of pregnant women, 100 g oral glucose is recommended. Further, the diagnostic criteria for diabetes in pregnancy should be more stringent than WHO recommendations.

3. In the ***mini GTT*** carried out in some laboratories, fasting and 2 hrs. sample (instead of $^1/_2$ hr. intervals) of blood and urine are collected.

4. The GTT is rather unphysiological. To evaluate the glucose handling of the body under physiological conditions, fasting blood sample is drawn, the subject is allowed to take ***heavy breakfast***, blood samples are collected at ***1 hour*** and ***2 hrs*** (***post-prandial***—meaning after food). Urine samples are also collected. This type of test is commonly employed in established diabetic patients for monitoring the control.

5. For individuals with suspected mal-absorption, ***intravenous GTT is carried out.***

6. **Corticosteroid stressed GTT** is employed to detect latent diabetes.

Glycosuria

The commonest cause of glucose excretion in urine (glycosuria) is ***diabetes mellitus***. Therefore, glycosuria is the first line screening test for diabetes. Normally, glucose does not appear in urine until the plasma glucose concentration exceeds ***renal threshold (180 mg/dl)***. As age advances, renal threshold for glucose increases marginally.

Renal glycosuria : Renal glycosuria is a benign condition due to a ***reduced renal threshold for glucose***. It is unrelated to diabetes and, therefore, should not be mistaken as diabetes. Further, it is not accompanied by the classical symptoms of diabetes.

Alimentary glycosuria : In certain individuals, blood glucose level rises rapidly after meals resulting in its spill over into urine. This condition is referred to as ***alimentary (lag storage) glycosuria.*** It is observed in some normal people, and in patients of hepatic diseases, hyperthyroidism and peptic ulcer.

Metabolic changes in diabetes

Diabetes mellitus is associated with several metabolic alterations. Most important among them are hyperglycemia, ketoacidosis and hyper-triglyceridemia (***Fig.36.9***).

1. **Hyperglycemia :** Elevation of blood glucose concentration is the hallmark of uncontrolled diabetes. Hyperglycemia is primarily due to

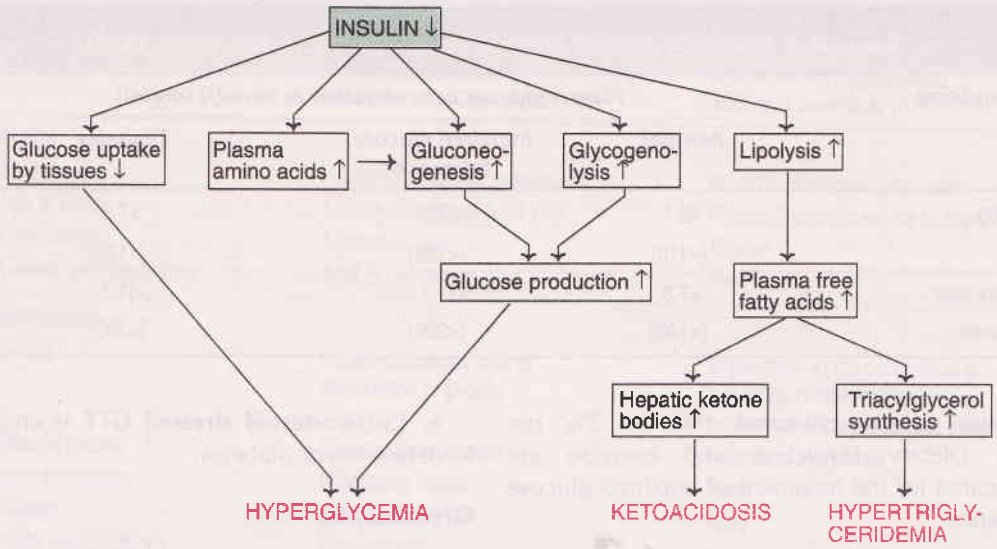


Fig. 36.9 : Major metabolic alterations in diabetes mellitus.

reduced glucose uptake by tissues and its increased production via gluconeogenesis and glycogenolysis. When the blood glucose level goes beyond the renal threshold, glucose is excreted into urine (glycosuria).

2. **Ketoacidosis :** Increased mobilization of fatty acids results in overproduction of ketone bodies which often leads to ketoacidosis.

3. **Hypertriglyceridemia :** Conversion of fatty acids to triacylglycerols and the secretion of VLDL and chylomicrons is comparatively higher in diabetics. Further, the activity of the enzyme lipoprotein lipase is low in diabetic patients. Consequently, the plasma levels of ***VLDL, chylomicrons*** and triacylglycerols are ***increased***. ***Hypercholesterolemia*** is also frequently seen in diabetics.

Long term effects of diabetes

Hyperglycemia is directly or indirectly associated with several complications. These include ***atherosclerosis, retinopathy, nephropathy*** and ***neuropathy***. The biochemical basis of these complications is not clearly understood. It is believed that at least some of them are related to microvascular changes caused by glycation of proteins.

Management of diabetes

Diet, exercise, drug and, finally, insulin are the management options in diabetics. Approximately, 50% of the new cases of diabetes can be adequately controlled by diet alone, 20-30% need oral hypoglycemic drugs while the remaining 20-30% require insulin.

Dietary management : A diabetic patient is advised to consume ***low calories*** (i.e. low carbohydrate and fat), ***high protein and fiber rich diet***. Carbohydrates should be taken in the form of starches and complex sugars. As far as possible, refined sugars (sucrose, glucose) should be avoided. Fat intake should be drastically reduced so as to meet the nutritional requirements of unsaturated fatty acids. ***Diet control and exercise*** will help to a large extent obese NIDDM patients.

Hypoglycemic drugs : The oral hypoglycemic drugs are broadly of two categories-***sulfonylureas*** and ***biguanides***. The latter are less commonly used these days due to side effects.

Sulfonylureas such as acetohexamide, tolbutamide and glibenclamide are frequently used. They promote the secretion of endogenous insulin and thus help in reducing blood glucose level.

Management with insulin : Two types of insulin preparations are commercially available—***short acting*** and ***long acting.*** The short acting insulins are unmodified and their action lasts for about 6 hours. The long acting insulins are modified ones (such as adsorption to protamine) and act for several hours, which depends on the type of preparation.

The advent of genetic engineering is a boon to diabetic patients since bulk quantities of insulin can be produced in the laboratory.

Biochemical indices of diabetic control

For a diabetic patient who is on treatment (drug or insulin therapy), ***periodical assessment*** of the efficacy of the ***treatment is essential.*** Urine glucose detection and blood glucose estimations are traditionally followed in several laboratories. In recent years, more reliable and long-term biochemical indices of diabetic control are in use.

Glycated hemoglobin : Glycated or glycosylated hemoglobin refers to the glucose derived products of normal adult hemoglobin (HbA). Glycation is a post-translational, non-enzymatic addition of sugar residue to amino acids of proteins. Among the glycated hemoglobins, the most abundant form is ***HbA_{1c}.*** ***HbA_{1c}*** is produced by the condensation of glucose with N-terminal valine of each β-chain of HbA.

Diagnostic importance of HbA_{1c} : The rate of synthesis of HbA_{1c} is directly related to the exposure of RBC to glucose. Thus, the concentration of HbA_{1c} serves as an indication of the blood glucose concentration over a period, approximating to the half-life of RBC (hemoglobin) i.e. 6–8 weeks. A close correlation between the blood glucose and HbA_{1c} concentrations have been observed when simultaneously monitored for several months.

Normally, HbA_{1c} concentration is about 3–5% of the total hemoglobin. In diabetic patients, HbA_{1c} is elevated (to as high as 15%). Determination of HbA_{1c} is used for monitoring of diabetes control. HbA_{1c} reflects the mean ***blood glucose level over 2 months period prior to its measurement.***

In the routine clinical practice, if the HbA_{1c} concentration is less than 7%, the diabetic patient is considered to be *in good control.*

Fructosamine : Besides HbA_{1c}, several other proteins in the blood are glycated. Glycated serum proteins (fructosamine) can also be measured in diabetics. As albumin is the most abundant plasma protein, glycated albumin largely contributes to plasma fructosamine measurements. Albumin has shorter half-life than Hb. Thus, glycated albumin represents glucose status over 3 weeks prior to its determination.

BIOMEDICAL / CLINICAL CONCEPTS

- *Diabetes affects about 2-3% of the population and is a major cause of blindness, renal failure, heart attack and stroke.*
- *The hormone insulin has been implicated in the development of diabetes.*
- *Diabetic ketoacidosis is frequently encountered in severe uncontrolled diabetics. The management includes administration of insulin, fluids and potassium.*
- *The hypoglycemic drugs commonly used in diabetic patients include tolbutamide, glibenclamide and acetohexamide.*
- *Measurement of glycated hemoglobin (HbA_{1c}) serves as a marker for diabetic control.*

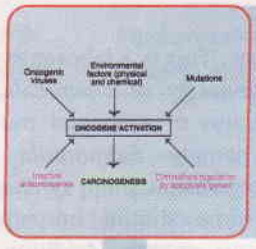
Microalbuminuria : Microalbuminuria is defined as the excretion of ***30-300 mg of albumin in urine per day***. It may be noted that microalbuminuria represents an intermediary stage between normal albumin excretion (2.5–30 mg/d) and macroalbuminuria (> 300 mg/d). The small increase in albumin excretion ***predicts impairment in renal function*** in diabetic patients. Microalbuminuria serves as a signal of early reversible renal damage.

Serum lipids : Determination of serum lipids (total cholesterol, HDL, triglycerides) serves as an index for overall metabolic control in diabetic patients. Hence, serum lipids should be frequently measured.

SUMMARY

1. *Diabetes mellitus is a common metabolic disorder, characterized by insufficient or inefficient insulin.*
2. *Insulin is a polypeptide hormone, secreted by the β-cells of pancreas. It has a profound influence on carbohydrate, fat and protein metabolisms. Insulin lowers blood glucose concentration (hypoglycemic effect).*
3. *Glucagon, secreted by the α-cells of pancreas, in general opposes the actions of insulin. The net effect of glucagon is to increase blood glucose concentration (hyperglycemic effect).*
4. *In a healthy person, the blood glucose level (fasting 70-100 mg/dl) is maintained by a well coordinated hormonal action regulating the sources that contribute to glucose (gluconeogenesis, glycogenolysis), and the utilization pathways (glycolysis, glycogenesis, lipogenesis). Insulin is hypoglycemic while other hormones (glucagon, epinephrine, thyroxine, glucocorticoids) are hyperglycemic.*
5. *In hypoglycemia (blood glucose <45 mg/dl), there is deprivation of glucose supply to brain resulting in symptoms such as headache, confusion, anxiety and seizures.*
6. *Diabetes mellitus is broadly classified into 2 categories—insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM).*
7. *The laboratory diagnosis of diabetes is frequently carried out by oral glucose tolerance test (GTT). As per WHO criteria, a person is said to be suffering from diabetes if his/her fasting blood glucose exceeds 126 mg/dl, and 2 hrs. after oral glucose load goes beyond 200 mg/dl.*
8. *Diabetes is associated with several metabolic derangements such as ketoacidosis and hypertriglyceridemia, besides hyperglycemia. The chronic complications of diabetes include atherosclerosis, retinopathy, nephropathy and neuropathy.*
9. *Diet, exercise, drug and insulin are the options for diabetic control. It is estimated that about half of the new diabetic patients can be adequately controlled by diet and exercise.*
10. *Estimation of glycated hemoglobin (HbA_{1C}), plasma fructosamine, microalbumin in urine, and serum lipids serve as biochemical indices to monitor diabetic control.*

37 Cancer



The dreaded disease cancer speaks :

"I am the world's second largest killer;
Characterized by uncontrolled proliferation of cells;
Implicating environmental and genetic factors;
Perhaps due to oncogene and antioncogene imblance."

In the normal circumstances, the proliferation of body cells is under strict control. The cells differentiate, divide and die in a sequential manner in a healthy organism. Cancer is characterized by ***loss of control of cellular growth*** and development leading to ***excessive proliferation and spread of cells***. Cancer is derived from a *Latin* word meaning *crab*. It is presumed that the word cancer originated from the character of cancerous cells which can migrate and adhere and cause pain (like a crab) to any part of the body.

Neoplasia literally means new growth. Uncontrolled growth of cells results in tumors (a word originally used to represent swelling). Oncology (*Greek : oncos—tumor*) deals with the study of tumors.

The tumors are of two types.

1. **Benign tumors :** They usually grow by expansion and remain encapsulated in a layer of connective tissue. Normally benign tumors are not life-threatening e.g. moles, warts. These types of benign tumors are not considered as cancers.

2. **Malignant tumors or cancers :** They are characterized by uncontrolled proliferation and spread of cells to various parts of the body, a process referred to as ***metastasis***. Malignant tumors are invariably life-threatening e.g. lung cancer, leukemia.

About 100 different types of human cancers have been recognized. Cancers arising from ***epithelial cells*** are referred to as ***carcinomas*** while that from ***connective tissues*** are known as ***sarcomas***. Methods for the early detection and treatment of cancers have been developed. However, little is known about the biochemical basis of cancer.

Incidence

Cancer is the ***second largest killer disease*** (the first being coronary heart disease) in the developed countries. It is estimated that cancer accounts for more than 20% of the deaths in United States. Based on the current rate of incidence, it is believed that one in every 3 persons will develop cancer at sometime during his life.

Although humans of all ages develop cancer, the incidence increases with advancement of age. More than 70% of the new cancer cases occur in persons over 60 years. Surprisingly, cancer is a leading cause of death in children in the age group 3-13 years, half of them die due to leukemia.

ETIOLOGY

In general, cancers are multifactorial in origin. The causative agents include ***physical***, ***chemical***, ***genetic*** and ***environmental factors***. A survey in USA has shown that about 90% of all cancer deaths are due to avoidable factors such as tobacco, pollution, occupation, alcohol and diet.

Most of the cancers are caused by chemical carcinogens, radiation energy and viruses. These agents may damage DNA or interfere with its replication or repair.

Chemical carcinogens

It is estimated that ***almost 80% of the human cancers are caused*** by chemical carcinogens in nature. The chemicals may be organic (e.g. dimethylbenzanthracene, benzo (a) pyrene, dimethyl nitrosamine) or inorganic (arsenic, cadmium) in nature. Entry of the chemicals into the body may occur by one of the following mechanisms.

1. Occupation e.g. asbestos, benzene.

2. Diet e.g. aflatoxin B produced by fungus (*Aspergillus flavus*) contamination of foodstuffs, particularly peanuts.

3. Drugs—certain therapeutic drugs can be carcinogenic e.g. diethylstibesterol.

4. Life style e.g. cigarette smoking.

Mechanism of action : Although a few of the chemicals are directly carcinogenic, majority of them require prior metabolism to become carcinogenic. The enzymes such as cytochrome P_{450} responsible for the metabolism of xenobiotics (***Chapter 31***) are involved in dealing with the chemical carcinogens. In general, a chemically non-reactive procarcinogen is converted to an ultimate carcinogen by a series of reactions.

The carcinogens can covalently bind to purines, pyrimidines and phosphodiester bonds of DNA, often causing unrepairable damage. The chemical carcinogens frequently ***cause mutations*** (a change in the nucleotide sequence of DNA) which may finally lead to the development of cancer, hence they are regarded as mutagens.

Ames assay : This is a laboratory test ***to check*** the ***carcinogenecity of chemicals***. Ames assay employs the use of a special mutant strain of bacterium, namely ***Salmonella typhimurium*** (His^-). This organism cannot synthesize histidine; hence the same should be supplied in the medium for its growth. Addition of chemical carcinogens causes mutations (reverse mutation) restoring the ability of the bacteria to synthesize histidine (His^+). By detecting the strain of *Salmonella* (His^+) in the colonies of agar plates, the chemical mutagens can be identified. The Ames assay can detect about 90% of the chemical carcinogens. This test is regarded as a ***preliminary screening procedure***. Animal experiments are conducted for the final assessment of carcinogenecity.

Promoters of carcinogenesis : Some of the chemicals on their own are not carcinogenic. Certain substances known as promoting agents make them carcinogenic. The application of benzo- (a)pyrene to the skin, as such, does not cause tumor development. However, if this is followed by the application of croton oil, tumors will develop. In this case, benzo(a)pyrene is the initiating agent while croton oil acts as a promoting agent or promoter. Several compounds that act as promoting agents in various organs of the body have been identified. These include saccharin and phenobarbital.

Radiation energy

Ultraviolet rays, X-rays and γ-rays have been proved to be mutagenic in nature causing cancers. These rays ***damage DNA*** which is the basic mechanism to explain the carcinogenicity

Table 37.1 Selected tumor viruses

Class	Members
DNA viruses	
Adenovirus	Adenovirus 12 and 18
Herpesvirus	Epstein-Barr virus, herpes simplex virus
Papovirus	Papilloma virus, polyoma virus
RNA viruses	
Retrovirus type B	Mammary tumor virus of mouse
Retrovirus type C	Leukemia, sarcoma.

of radiation energy. For instance, exposure to UV rays results in the formation of pyrimidine dimers in DNA while X-rays cause the production of free radicals. This type of molecular damages are responsible for the carcinogenic effects of radiations.

Carcinogenic viruses

The involvement of viruses in the etiology of cancer was first reported by ***Rous in 1911***. He demonstrated that the cell-free filtrates from certain chicken sarcomas (tumors of connective tissues) promote new sarcomas in chickens. Unfortunately, this epoch-making discovery of Rous was ignored for several years. This is evident from the fact that Rous was awarded the Nobel Prize in 1966 at the age of 85 for his discovery in 1911!

The presence of viral particles and the enzyme reverse transcriptase, besides the occurrence of base sequence in the DNA of malignant cells, complementary to tumor viruses indicate the involvement of viruses in cancer. The viruses involved in the development of cancer, commonly known as ***oncogenic viruses***, may contain either DNA or RNA. A selected list of tumor viruses is given in ***Table 37.1***.

DNA—the ultimate in carcinogenesis

DNA is the ultimate critical macromolecule in carcinogenesis. This fact is supported by several evidences.

1. Cancers are transmitted from mother to daughter cells. In other words, cancer cells beget cancer cells.

2. Chromosomal abnormalities are observed in many tumor cells.

3. Damage to DNA caused by mutations often results in carcinogenesis.

4. Laboratory experiments have proved that purified oncogenes can transform normal cells into cancer cells.

MOLECULAR BASIS OF CANCER

Cancer is caused by a genetic change in a single cell resulting in its uncontrolled multiplication. Thus, tumors are monoclonal. Two types of regulatory genes—***oncogenes*** and ***antioncogenes*** are involved in the development of cancer (carcinogenesis). In recent years, a third category of genes that control the cell death or ***apoptosis*** are also believed to be involved in carcinogenesis.

Oncogenes

The genes capable of causing cancer are known as oncogenes (*Greek* : oncos—tumor or mass). Oncogenes were originally discovered in tumor causing viruses. These viral oncogenes were found to be closely similar to certain genes present in the normal host cells which are referred to as protooncogenes. Now, about 40 viral and cellular protooncogenes have been identified. Protooncogenes encode for growth-regulating proteins. The activation of protooncogenes to oncogenes is an important step in the causation of cancer.

In the ***Table 37.2***, a selected list of oncoproteins, protooncogenes and the associated human cancers is given.

Activation of protooncogenes to oncogenes

There are several mechanisms for converting the protooncogenes to oncogenes, some of the important ones are described next.

Table 37.2 Selected oncoproteins, protooncogenes and associated cancers

Oncoproteins	*Protooncogene*	*Associated human cancer(s)*
Growth factors		
Platelet derived growth factor (PDGF)	*sis*	Osteosarcoma
Epidermal growth factor (EGF)	*hst*-1	Cancers of stomach, breast and bladder
Growth factor receptors	*erb*-B_1	Lung cancer
	erb-B_2	Stomach cancer
	erb-B_3	Breast cancer
Signal—transducing proteins		
GTP— binding proteins	*ras*	Leukemias, cancers of lung, pancreas and colon
Non-receptor tyrosine kinase	*abl*	Leukemia

1. **Viral insertion into chromosome :** When certain retroviruses (genetic material RNA) infect cells, a complementary DNA (***cDNA***) is made from their RNA by the enzyme ***reverse transcriptase***. The cDNA so produced gets inserted into the host genome (***Fig.37.1***). The integrated double-stranded cDNA is referred to as provirus. This pro-viral DNA takes over the control of the transcription of cellular chromosomal DNA and transforms the cells. Activation of protooncogene *myc* to oncogene by viral insertion ultimately causing carcinogenesis is well known (e.g. avian leukemia).

Some DNA viruses also get inserted into the host chromosome and activate the proto-oncogenes.

2. **Chromosomal translocation :** Some of the tumors exhibit chromosomal abnormalities. This is due to the rearrangement of genetic material (DNA) by chromosomal translocation i.e. splitting off a small fragment of chromosome which is joined to another chromosome. Chromosomal translocation usually results in ***overexpression of protooncogenes.***

Burkitt's lymphoma, a cancer of human B-lymphocytes, is a good example of chromosomal translocation. In this case, a fragment from chromosome 8 is split off and joined to chromosome 14 containing *myc* gene (***Fig.37.2***). This results in the activation of inactive *myc* gene leading to the increased synthesis of certain proteins which make the cell malignant.

3. **Gene amplification :** Severalfold amplifications of certain DNA sequences are observed in some cancers. Administration of anticancer drugs methotrexate (an inhibitor of the enzyme dihydrofolate reductase) is associated with gene amplification. The drug becomes inactive due to gene amplification resulting in a severalfold (about 400) increase in the activity of dihydrofolate reductase.

4. **Point mutation :** The *ras* protooncogene is the best example of activation by point mutation (change in a single base in the DNA). The mutated *ras* oncogene produces a protein

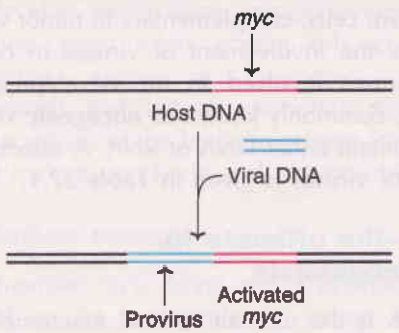


Fig. 37.1 : Integration of viral DNA into host DNA.

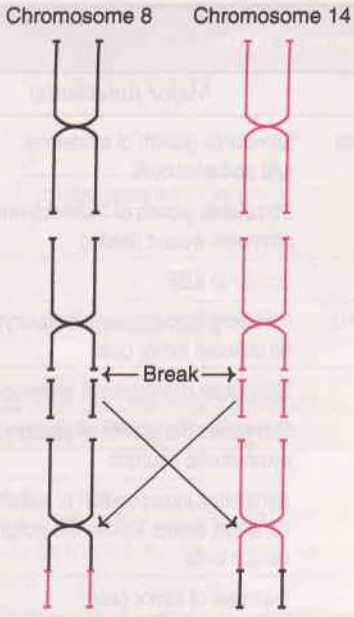


Fig. 37.2 : Diagrammatic representation of reciprocal translocation occurring in Burkitt's lymphoma.

(GTPase) which differs in structure by a single amino acid. This alteration diminishes the activity of GTPase, a key enzyme involved in the control of cell growth (details described later).

The presence of ras mutations is detected in several human tumors—90% of pancreatic, 50% of colon and 30% of lung. However, *ras* mutations have not been detected in the breast cancer.

Mechanism of action of oncogenes

Oncogenes encode for certain proteins, namely ***oncoproteins.*** These proteins are the altered versions of their normal counterparts and are involved in the transformation and multiplication of cells. Some of the products of oncogenes are discussed below.

Growth factors : Several growth factors stimulating the proliferation of normal cells are known. They regulate cell division by transmitting the message across the plasma membrane to the interior of the cell (transmembrane signal transduction). It is believed that growth factors play a key role in carcinogenesis.

A selected list of polypeptide growth factors, their sources and major functions is given in ***Table 37.3.***

The ***cell proliferation*** is ***stimulated by growth factors.*** In general, a growth factor binds to a protein receptor on the plasma membrane. This binding activates cytoplasmic protein kinases leading to the phosphorylation of intracellular target proteins. The phosphorylated proteins, in turn, act as intracellular messengers to stimulate cell division, the mechanism of which is not clearly known.

Transforming growth factor (TGF-α) is a protein synthesized and required for the growth of epithelial cells. TGF-α is produced in high concentration in individuals suffering from psoriasis, a disease characterized by excessive proliferation of epidermal cells.

Growth factor receptors : Some oncogenes encoding growth factor receptors have been identified. Overexpression and/or structural alterations in growth factor receptors are associated with carcinogenesis. For instance, the overexpression of gene *erb*-B, encoding EGF-receptor is observed in lung cancer.

GTP-binding proteins : These are a group of *signal transducing proteins. Guanosine triphosphate (GTP)-binding proteins are found in about 30% of human cancers. The mutation of ras* protooncogene is the single-most dominant cause of many human tumors.

The involvement of ras protein (product of *ras* gene) with a molecular weight 21,000 (P_{21}) in *cell multiplication is illustrated in* ***Fig.37.3.*** The inactive *ras* is in a bound state with GDP. When the cells are stimulated by growth factors, *ras* P_{21} gets activated by exchanging GDP for GTP. This exchange process is catalysed by guanine nucleotide releasing factor (GRF). The active *ras* P_{21} stimulates regulators such as cytoplasmic kinases, ultimately causing DNA replication and cell division. In normal cells, the activity of *ras* P_{21} is shortlived. The GTPase activity, which is an integral part (intrinsic) of *ras* P_{21}, hydrolyses GTP to GDP, reverting ras 21 to the original state. There are certain proteins, namely GTPase activating proteins (GAP), which accelerate the

Table 37.3 Selected polypeptide growth factors

Growth factor	*Source(s)*	*Major function(s)*
Epidermal growth factor (EGF)	Salivary gland, fibroblasts	Stimulates growth of epidermal and epithelial cells
Platelet derived growth factor (PDGF)	Platelets	Stimulates growth of mesenchymal cells, promotes wound healing
Transforming growth factor-α (TGF-α)	Epithelial cell	Similar to EGF
Transforming growth factor-β (TGF-β)	Platelets, kidney, placenta	Inhibitory (sometimes stimulatory) effect on cultured tumor cells
Erythropoietin	Kidney	Stimulates development erythropoietic cells
Nerve growth factor (NGF)	Salivary gland	Stimulates the growth of sensory and sympathetic neurons
Insulin like growth factors (IGF-I and IGF-II, respectively known as somatomedins C and A)	Serum	Stimulates incorporation of sulfates into cartilage; exerts insulin-like action on certain cells
Tumor necrosis factor (TNF-α)	Monocytes	Necrosis of tumor cells
Interleukin-1 (IL-1)	Monocytes, leukocytes	Stimulates synthesis of IL-2.
Interleukin-2 (IL-2)	Lymphocytes (mainly T-helper cells).	Stimulates growth and maturation of T-cells

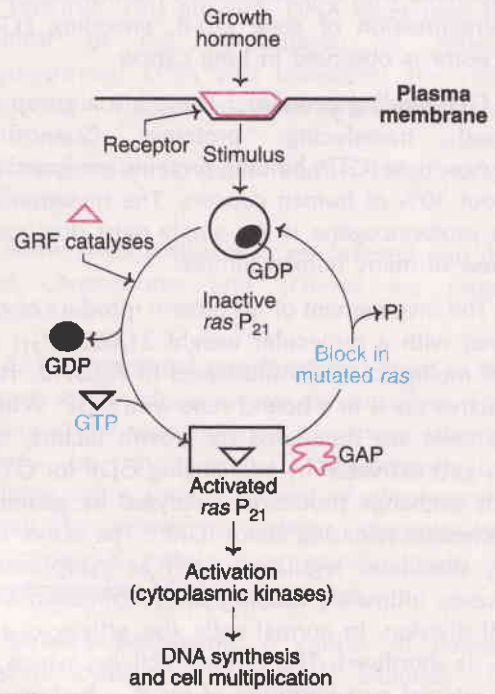


Fig. 37.3 : Model for the mechanism of action of ras P_{21} protein (GRF—Guanine nucleotide releasing factor; GAP—GTPase activating proteins).

hydrolysis of GTP of *ras* P_{21}. Thus, in normal cells, the activity of *ras* P_{21} is well regulated.

Point mutations in *ras* gene result in the production of altered *ras* P_{21}, lacking GTPase activity. This leads to the occurrence of *ras* P_{21} in a permanently activated state, causing uncontrolled multiplication of cells.

Non-receptor tyrosine kinases : These proteins are found on the interior of the inner plasma membrane. They phosphorylate the cellular target proteins (involved in cell division) in response to external growth stimuli. Mutations in the protooncogenes (e.g. *abl*) encoding non-receptor tyrosine kinases increase the kinase activity and, in turn, phosphorylation of target proteins causing unlimited cell multiplication.

Antioncogenes

A special category of genes, namely ***cancer suppressor genes*** (e.g. ***p^{53} gene***) or, more commonly, antioncogenes, have been identified. The products of these genes apply breaks and regulate cell proliferation. The loss of these

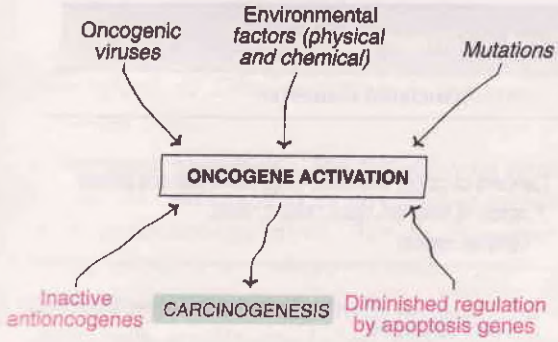


Fig. 37.4 : A simplified hypothesis for the development of cancer.

suppressor genes removes the growth control of cells and is believed to be a key factor in the development of several tumors, e.g. retinoblastoma, one type of breast cancer, carcinoma of lung, Wilms' kidney tumor.

With the rapid advances in the field of genetic engineering, introducing antioncogenes to a normal chromosome to correct the altered growth rate of cells may soon become a reality.

Genes that regulate apoptosis

A new category of genes that regulate ***programmed cell death*** (apoptosis) have been discovered. These genes are also important in the development of tumors.

The gene, namely *bcl*-2, causes B-cell lymphoma by preventing programmed cell death. It is believed that overexpression of *bcl*-2 allows other mutations of protooncogenes that, ultimately, leads to cancer.

Unified hypothesis of carcinogenesis

The multifactorial origin of cancer can be suitably explained by oncogenes. The physical and chemical agents, viruses and mutations all lead to the activation of oncogenes causing carcinogenesis. The antioncogenes and the genes regulating apoptosis are intimately involved in development of cancer. A simplification of a unified hypothesis of carcinogenesis is depicted in ***Fig.37.4.***

TUMOR MARKERS

The biochemical indicators employed to ***detect the presence of cancers*** are collectively referred to as tumor markers. These are the ***abnormally produced molecules of tumor cells*** *such as surface antigens, cytoplasmic proteins, enzymes and hormones.* Tumor markers can be measured in serum (or plasma). In theory, the tumor markers must ideally be useful for screening the population to detect cancers. In practice, however, this has not been totally true. As such, the tumor markers support the diagnosis of cancers, besides being useful for monitoring the response to therapy and for the early detection of recurrence.

A host of tumor markers have been described and the list is evergrowing. However, only a few of them have proved to be clinically useful. A selected list of tumor markers and the associated cancers are given in ***Table 37.4.***

A couple of the most commonly used tumor markers are discussed hereunder.

1. **Carcinoembryonic antigen** (***CEA***) **:** This is a complex glycoprotein, normally produced by the embryonic tissue of liver, gut and pancreas. The presence of CEA in serum is detected in several cancers (colon, pancreas, stomach, lung). In about 67% of the patients with ***colorectal cancer***, CEA can be identified. Unfortunately, serum CEA is also detected in several other disorders such as alcoholic cirrhosis (70%), emphysema (57%) and diabetes mellitus (38%). Due to this, CEA lacks specificity for cancer detection. However, in established cancer patients (particularly of colon and breast), the serum level of CEA is a useful indicator to detect the burden of tumor mass, besides ***monitoring the treatment.***

2. **Alpha-fetoprotein** (AFP) **:** It is chemically a glycoprotein, normally synthesized by yolk sac in early fetal life. Elevation in serum levels of AFP mainly indicates the cancers of liver and germ cells of testis and, to some extent, carcinomas of lung, pancreas and colon. As is the case with CEA, alpha-fetoprotein is not specific for the detection of cancers. Elevated

Table 37.4 Selected tumor markers and associated cancers

Tumor marker	*Associated cancer(s)*
Oncofetal antigens	
Carcinoembryonic antigen (CEA)	Cancers of colon, stomach, lung, pancreas and breast
Alpha fetoprotein (AFP)	Cancer of liver and germ cells of testis
Cancer antigen-125 (CA-125)	Ovarian cancer
Hormones	
Human chorionic gonadotropin (hCG)	Choriocarcinoma
Calcitonin	Carcinoma of medullary thyroid
Catecholamines and their metabolites (mainly vanillyl mandelic acid)	Pheochromocytoma and neuroblastoma
Enzymes	
Prostatic acid phosphatase	Prostate cancer
Neuron specific enolase	Neuroblastoma
Specific proteins	
Prostate specific antigen (PSA)	Prostate cancer
Immunoglobulins	Multiple myeloma

levels of AFP are observed in cirrhosis, hepatitis and pregnancy. However, measurement of serum AFP provides a sensitive ***index for tumor therapy and detection of recurrence***.

CHARACTERISTICS OF GROWING TUMOR CELLS

The morphological and biochemical changes in the growing tumor cells are briefly described here. These observations are mostly based on the *in vitro* culture studies. Knowledge on the alterations in the biochemical profile of tumor cells guides in the selection of chemotherapy of cancers.

1. General and morphological changes

- **Shape of cells :** The tumor cells are much rounder in shape compared to normal cells.
- **Alterations in cell structures :** The cytoskeletal structure of the tumor cells with regard to actin filaments is different.
- **Loss of contact inhibition :** The normal cells are characterized by contact inhibition i.e. they form monolayers. Further, they cannot move away from each other. The cancer cells form multilayers due to loss of contact inhibition (***Fig.37.5***). As a result, the cancer cells freely move and get deposited in any part of the body, a property referred to as ***metastasis***.
- **Loss of anchorage dependence :** The cancer cells can grow without attachment to the surface. This is in contrast to the normal cells which firmly adhere to the surface.
- **Alteration in permeability properties :** The tumor cells have altered permeability and transport.

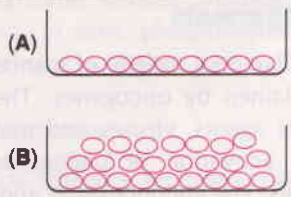


Fig. 37.5 : Growth cells in culture (A) Normal cells forming monolayer (exhibiting contact inhibition); (B) Cancer cells forming multilayers (loss of contact inhibition).

2. **Biochemical changes**

- **Increased replication and transcription :** The synthesis of DNA and RNA is increased in cancer cells.
- **Increased glycolysis :** The fast growing tumor cells are characterized by elevation in aerobic and anaerobic glycolysis due to increased energy demands of multiplying cells.
- **Reduced requirement of growth factors :** The tumor cells require much less quantities of growth factors. Despite this fact, there is an increased production of growth factors by these cells.
- **Synthesis of fetal proteins :** During fetal life, certain genes are active, leading to the synthesis of specific proteins. These genes are suppressed in adult cells. However, the tumor cells synthesize the fetal proteins e.g. carcinoembryonic antigen, alfa fetoprotein.
- **Alterations in the structure of molecules :** Changes in the structure of glycoproteins and glycolipids are observed.

Metastasis

Metastasis refers to the spread of cancer cells from the primary site of origin to other tissues of the body where they get deposited and grow as secondary tumors. Metastasis is the major cause of cancer related morbidity and mortality. The biochemical basis of metastasis is not clearly known. It is believed that the morphological changes in tumor cells, loss of contact inhibition, loss of anchorage dependence and alterations in the structure of certain macromolecules are among the important factors responsible for metastasis.

CANCER THERAPY

Chemotherapy, employing certain anticancer drugs, is widely used in the treatment of cancer. In the ***Table 37.5***, a selected list of the most commonly used drugs, and their mode of action is given. The effectiveness of anticancer drugs is inversely proportional to the size of the tumor i.e. the number of cancer cells. The major limitation of cancer chemotherapy is that the rapidly dividing normal cells (of hematopoietic system, gastrointestinal tract, hair follicles) are also affected. Thus, the use of anticancer drugs is associated with toxic manifestations.

For the treatment of solid tumors, surgery and radiotherapy are very effective.

PREVENTION OF CANCER

In recent years, certain precautionary measures are advocated to prevent or reduce the occurrence of cancer. The most important

BIOMEDICAL / CLINICAL CONCEPTS

- *About 80% of the human cancers are caused by chemical carcinogens.*
- *The products of oncogenes (growth factors, GTP-binding proteins) have been implicated in the development of cancer. Antioncogenes apply breaks and regulate the cell proliferation.*
- *The physical and chemical agents, viruses and mutations result in the activation of oncogenes causing carcinogenesis.*
- *The abnormal products of tumor cells, referred to as tumor markers (CEA, AFP, PSA) are useful for the diagnosis and prognosis of cancer.*
- *Anticancer drugs (e.g. methotrexate, cisplatin) are commonly used in the treatment of cancer. Antioxidants (vitamins E and C, β-carotene, Se) decrease the risk of carcinogenesis and hence their increased consumption is advocated.*

Table 37.5 A selected list of the most commonly used anticancer drugs and their mode of action

Anticancer drug	*Chemical nature*	*Mode of action*
Methotrexate	Folic acid analogue	Blocks the formatin of tetrahydrofolate (inhibits the enzyme dihydrofolate reductase). THF is required for nucleotide synthesis.
6-Mercaptopurine	Purine analogue	Inhibits the formation of AMP from IMP.
6-Thioguanine	Purine analogue	Blocks thymidylate synthase reaction.
Mitomycin C	Antibiotic	Results in the formation of cross bridges between DNA base pairs.
Actinomycin D	Antibiotic	Blocks transcription
Vinblastine and vincristine	Alkaloids	Inhibit spindle movement (of cell division) and interfere with cytoskeleton formation
Cisplatin	Platinum compound	Results in the formation of intrastrand DNA adducts.

among them, from the biochemical perspective, are the antioxidants namely vitamin E, β-carotene, vitamin C and selenium.

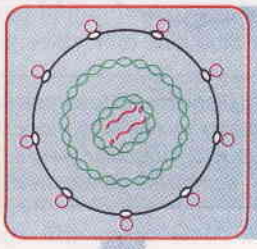
The antioxidants prevent the formation or detoxify the existing free radicals (free radicals are known to promote carcinogenesis). In addition, antioxidants stimulate body's immune system, and promote detoxification of various carcinogens.

In general, most of the vegetables and fruits are rich in antioxidants. Their increased consumption is advocated to prevent cancer. (For more details on free radicals and antioxidants, ***Refer Chapter 34***).

SUMMARY

1. *Cancer is characterized by uncontrolled cellular growth and development, leading to excessive proliferation and spread of cells. Cancer is the second largest killer disease (next to heart disease) in the developed world.*
2. *Regulatory genes—namely oncogenes, antioncogenes and genes controlling cell death—are involved in the development of cancer. Activation of oncogenes is a fundamental step in carcinogenesis. This may occur by insertion of viral DNA into host chromosome, translocation of chromosomes, gene amplification and point mutation.*
3. *The products of activated oncogenes such as growth factors, growth factor receptors, GTP-binding proteins, non-receptor tyrosine kinases have all been implicated in the development of cancer.*
4. *Tumor markers of cancers include carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), cancer antigen-125 and prostate specific antigen (PSA). They are mainly useful to support diagnosis, monitor therapy and detect recurrence.*
5. *There are several morphological and biochemical changes in the tumor cells which distinguish them from the normal cells. The cancer cells are characterized by loss of contact inhibition, altered membrane transport, increased DNA and RNA synthesis, increased glycolysis, alteration in the structure of certain molecules etc.*

38 Acquired Immunodeficiency Syndrome (AIDS)



AIDS speaks :

"I am the most feared disease of the world;
Due to a retrovirus, causing immunodeficiency;
With no cure in sight, except prevention;
I challenge the scientists worldwide to conquer me!"

Acquired immunodeficiency syndrome (AIDS) was first reported in 1981 in homosexual men. AIDS is a retroviral disease caused by human immunodeficiency virus (HIV). The disease is ***characterized by immunosuppression, secondary neoplasma*** and ***neurological manifestations***. AIDS is invariably fatal since there is no cure. In the USA, it is the fourth leading cause of death in men between the ages 15 to 55 years.

No other disease has attracted as much attention as AIDS by the governments, public and scientists. AIDS has stimulated an unprecedented amount of biomedical research which led to a major understanding of this deadly disease within a short period of time. So rapid is the research on AIDS (particularly relating to molecular biology), any review is destined to be out of date by the time it is published!

The isolation of human immunodeficiency virus (HIV) from lymphocytes of AIDS patients was independently achieved by Gallo (USA) and Montagnier (France) in 1984.

Epidemiology

AIDS was first described in USA and this country has the majority of reported cases. The prevalence of AIDS has been reported from almost every country. The number of people living with HIV worldwide is estimated to be around 40 million by the end of the year 2005. (India alone has about 5 million persons). At least 5 million deaths occurred in 2005, due to AIDS. AIDS is truely a global disease with an alarming increase in almost every country.

Transmission of HIV : Transmission of AIDS essentially requires the exchange of body fluids (semen, vaginal secretions, blood, milk) containing the virus or virus-infected cells. There are three major routes of HIV transmission—sexual contact, parenteral inoculation, and from infected mothers to their newborns.

The distribution of risk factors for AIDS transmission are as follows.

Sex between men (homosexuals)	— 60%
Sex between men and women	— 15%

Intravenous drug abusers	— 15%
Transfusion of blood and blood products	— 6%
All others	— 4%

The predominant methods of HIV transmission (about 75%) are through anal or vaginal intercourse. The risk for the transmission is much higher with anal than with vaginal intercourse. The practice of 'needle sharing' is mainly responsible for the transmission of HIV in drug abusers. Pediatric AIDS is mostly caused by vertical transmission (mother to infant).

It should, however, be noted that HIV cannot be transmitted by casual personal contact in the household or work place. Further, the transmission of AIDS from an infected individual to health personnel attending on him is extremely rare.

Virology of HIV

AIDS is caused by a retrovirus, namely ***human immunodeficiency virus*** (HIV), belonging to lentivirus family. Retroviruses contain RNA as the genetic material. On entry into the host cell, they transcribe DNA which is a complementary copy of RNA. The DNA, in turn is used, as a template to produce new viral RNA copies.

Two different forms of HIV, namely HIV-1 and HIV-2 have been isolated from AIDS patients. HIV-1 is more common, being found in AIDS patients of USA, Canada, Europe and Central Africa while HIV-2 is mainly found in West Africa. Both the viruses are almost similar except they differ in certain immunological properties.

HIV-1 is described in some detail.

Structure of HIV : The viruse is spherical with a diameter of about 110 nm. It contains a core, surrounded by a lipid envelop derived from the host plasma membrane (***Fig.38.1***). The core of the HIV has two strands of genomic RNA and four core proteins, p_{24}, p_{18}, reverse transcriptase (p_{66}/p_{51}) and endonuclease (p_{32}). Note that the naming of the proteins is based on the molecular weight. For instance, a protein with a molecular weight of 24,000 is designated as p_{24}.

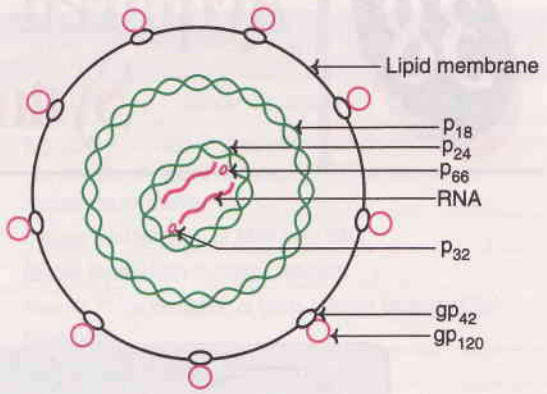


Fig. 38.1 : Diagrammatic representation of HIV (p represents protein with molecular weight e.g. p_{18} is with molecular weight 18,000; gp represents glycoprotein).

The lipid membrane of the virus is studded with two glycoproteins gp_{120} and gp_{41}. The surface antigen gp_{120} is very important for the viral infection and the detection of AIDS.

Genome and gene products of HIV : The HIV genome contains 3 structural genes–*gag*, *pol* and *env* that, respectively, code for *core proteins*, *reverse transcriptase* and *envelop proteins*. On either side of the HIV genome are long terminal repeat (LTR) genes which control transcription. Besides the structural genes, HIV contains several regulatory genes including *vif*, *vpr*, *tat*, *rev*, *vpu* and *nef* (***Fig.38.2***). These genes control the synthesis and assembly of infectious viral proteins. In fact, the regulatory genes of HIV play a key role in the development of AIDS.

Immunological abnormalities in AIDS

As is evident from the name AIDS, immunodeficiency (or immunosuppression) is the hallmark of this disease. AIDS ***primarily affects*** the ***cell-mediated immune system*** which protects the body from intracellular parasites such as viruses, protozoa and mycobacteria. This is caused by a reduction in CD_4 (cluster determinant antigen 4) cells of T-lymphocytes, besides impairment in the functions of surviving CD_4 cells.

CD_4 cells may be regarded as master cells of cell mediated immunity. They produce cytokines, macrophage chemotactic factors, hemopoietic growth factors, and others involved in the body immunity.

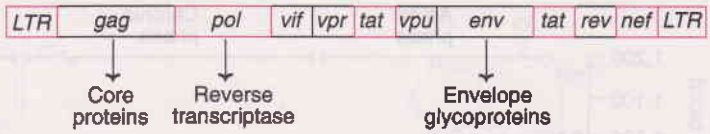


Fig. 38.2 : Genome of HIV.

Entry of HIV and lysis of CD_4 cells : The virus enters the CD_4 T-lymphocytes. HIV binds to the specific receptors on CD_4 cells by using its surface membrane glycoprotein (gp_{120}). Following the entry into the host cells, RNA of HIV is transcribed into DNA by the viral enzyme *reverse transcriptase. The viral DNA gets* incorporated into the host genomic DNA. The virus may remain locked in the host genome for months or years and this is considered as the latent period. The viral DNA may undergo replication, and translation, respectively, producing viral RNA and viral proteins. The latter two, on assembly, result in new viruses. The newly synthesized viruses leave the host cells by forming buds on plasma membrane. Extensive viral budding is associated with lysis and death of CD_4 cells (***Fig.38.3***). The new viral particles infect other host cells and repeat the whole process, ultimately resulting in a profound loss of CD_4 cells from the blood. ***Most of the immunodeficiency symptoms of AIDS are associated with the reduction in CD_4 cells.***

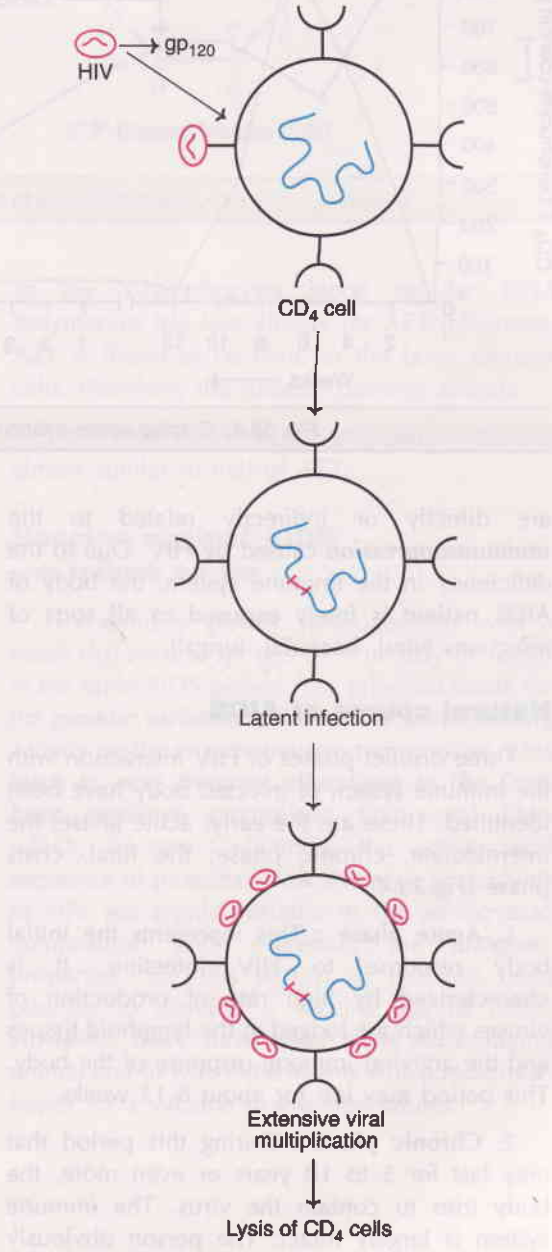


Fig. 38.3 : Immunological abnormalities in CD_4 cells on HIV infection.

Other immunological abnormalities

The viral membrane protein gp_{120} binds with normal T-helper cells and kills them. AIDS patients also display abnormalities in antibody production by B-lymphocytes (humoral immunity).

Abnormalities of central nervous system : HIV also infects the cells of central nervous system. It is believed that HIV infected monocytes enter the brain and cause damage, the mechanism of which remains obscure.

Consequences of immunodeficiency : The various clinical symptoms (fever, diarrhea, weight loss, neurological complications, multiple opportunistic infections, generalized lymphadenopathy, secondary neoplasma etc.) of AIDS

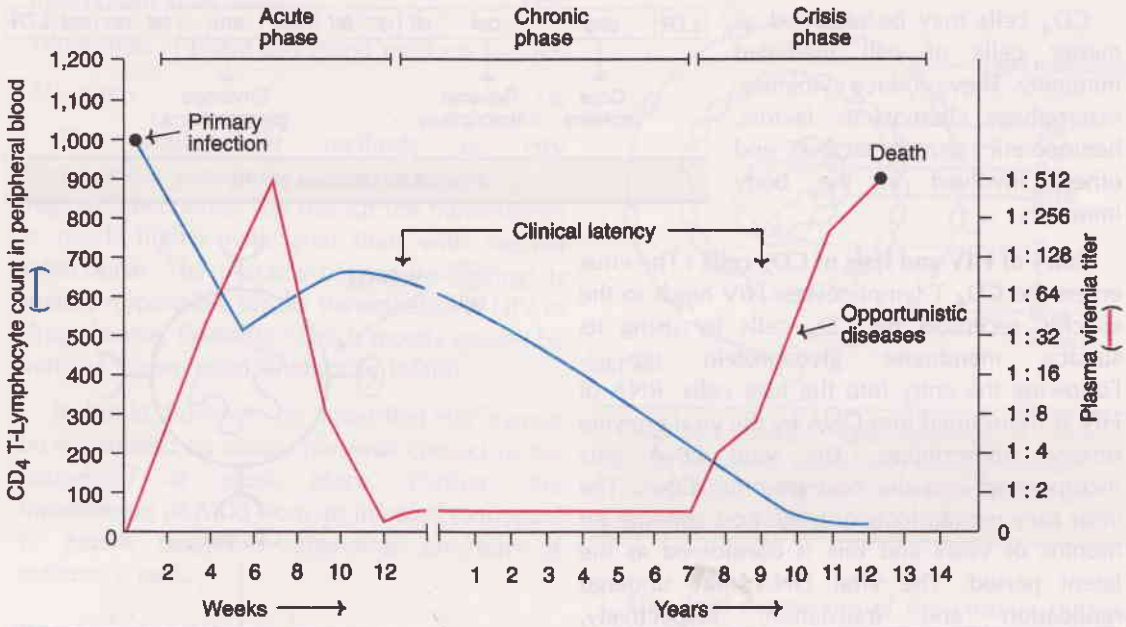


Fig. 38.4 : Graphic representation of a typical course of HIV infection.

are directly or indirectly related to the ***immunosuppression*** caused by HIV. Due to the deficiency in the immune system, the body of AIDS patient is freely exposed to all sorts of infections (viral, bacterial, fungal).

Natural course of AIDS

Three distinct phases of HIV interaction with the immune system of infected body have been identified. These are the early, acute phase; the intermediate, chronic phase; the final, crisis phase (***Fig.38.4***).

1. **Acute phase :** This represents the initial body response to HIV infection. It is characterized by high rate of production of viruses which are lodged in the lymphoid tissues and the antiviral immune response of the body. This period may last for about 8-12 weeks.

2. **Chronic phase :** During this period that may last for 5 to 10 years or even more, the body tries to contain the virus. The immune system is largely intact. The person obviously appears normal, although he/she is the carrier of HIV which can be transmitted to others. Antibodies to HIV are found in the circulation, hence this phase is also referred to as ***seropositive period.***

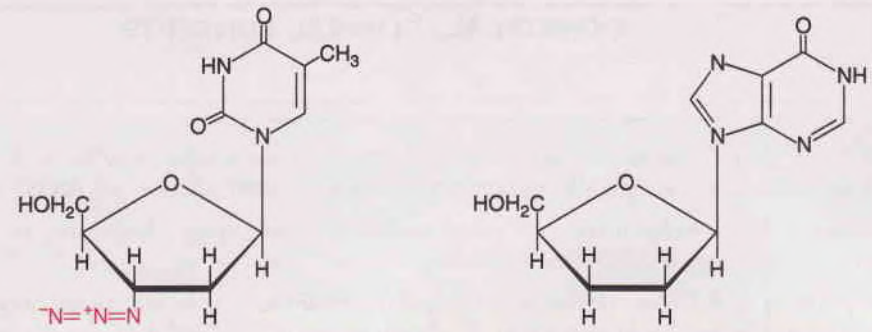
3. **Crisis phase :** A failure in the defense system of the body, caused by immunosuppression by HIV, represents the crisis phase. The plasma level of virus is tremendously increased. CD_4 T-lymphocyte concentration drastically falls. A patient with lower than 200 CD_4 T-lymphocytes/µl blood is considered to have developed AIDS. Crisis phase is characterized by opportunistic infections and the related clinical manifestations. In Western countries, a cancer—Kaposis sarcoma—is associated with AIDS.

In general, AIDS patients die between 5-10 years after HIV infection. Treatment may, however, prolong the life.

Laboratory diagnosis of AIDS

The following laboratory tests are employed to diagnose the HIV infection.

1. The detection of antibodies in the circulation by ELISA (enzyme-linked immunosorbant assay).



3′-Azido 2′,3′-dideoxythymidine (AZT) 2′,3′-Dideoxyinosine (DDI)

Fig. 38.5 : Structure of anti-AIDS drugs.

2. Western blot technique, a more specific test for the HIV antibodies, is employed for confirmation of ELISA positive cases.

3. A more recent and sophisticated PCR can be used to detect the presence of the HIV genome in the peripheral blood lymphocytes.

Drugs for the treatment of AIDS

Although there is no cure for AIDS, use of certain drugs can prolong the life of AIDS patients. ***Zidovudine*** or ***AZT*** (3′-azido 2′, 3′-dideoxy thymidine), a structural analog of deoxythymidine was the first drug used and continues to be the drug of choice for the treatment of AIDS. ***Didanosine*** (dideoxyionosine, DDI) is another drug employed to treat AIDS. The structures of AZT and DDI are shown in ***Fig.38.5.***

Mechanism of action : AZT is taken up by the lymphocytes and converted to AZT triphosphate which inhibits the enzyme HIV reverse transcriptase. AZT triphosphate competes with dTTP for the synthesis of DNA from viral RNA. Further, AZT is added to the growing DNA chain and the synthesis is halted. This drug is not toxic to the T-lymphocytes since cellular DNA polymerase has low affinity for AZT. However, AZT is found to be toxic to the bone marrow cells, therefore, the patients develop anemia.

The mechanism of action of dideoxyinosine is almost similar to that of AZT.

Vaccine against AIDS —a failure so far

HIV exhibits genetic heterogenecity with a result that several species of virus may be found in the same AIDS patient. The principal cause for the ***genetic variation*** is the lack of proof-reading activity by the enzyme reverse transcriptase. This leads to ***very frequent alterations in the DNA base sequence*** synthesized from viral RNA which, in turn, influences the amino acid sequence of proteins. Thus, the protein products of HIV are highly variable in the amino acid composition and, therefore, the antigenic properties. For this reason, it has not been possible to develop a vaccine against AIDS. However, there have been some encouraging animal and *in vitro* experiments which raise fresh hopes for a vaccine in the near future.

BIOMEDICAL / CLINICAL CONCEPTS

- *AIDS is a global disease with an alarming increase in the incidence of occurrence. By the year 2005, more than 40 million people were globally affected by AIDS.*
- *Homosexuality (predominantly in men) and intravenous drug abuse are the major factors in the risk of AIDS transmission.*
- *The patients of AIDS are destined to die (within 5–10 years after infection), since there is no cure. However, administration of certain drugs (AZT, DDI) prolongs life.*
- *The clinical manifestations of AIDS are directly or indirectly related to immunosuppression (mostly due to reduced CD_4 cells). AIDS patients are freely exposed to all sorts of infections (viral, bacterial, fungal).*

SUMMARY

1. *AIDS is a retorviral disease caused by human immunodeficiency virus (HIV). It is characterized by immunosuppression, secondary neoplasms and neurological manifestations. Transmission of HIV occurs by sexual contact (more in male homosexuals), parental inoculation (intravenous drug abusers) and from infected mothers to their newborns.*
2. *HIV enters CD_4 T-lymphocytes where its genetic material RNA is transcribed into DNA by the enzyme reverse transcriptase. The viral DNA gets incorporated into the host genome ultimately leading to the multiplication of the virus and the destruction of CD_4 cells. This is the root cause of immunosuppression leading to opportunistic infections in AIDS.*
3. *The natural course of AIDS has 3 distinct phases—acute, chronic and crisis. A patient with lower than 200 CD_4 T-lymphocytes/µl is considered to have developed AIDS. The sensitive laboratory tests for AIDS detection are—ELISA, Western blot technique and, recently PCR.*
4. *There is no cure for AIDS. The patients generally die within 5–10 years after HIV infection. Administration of drugs (zidovudine and didanosine), however, prolongs the life of AIDS patients. These drugs inhibit the viral enzyme reverse transcriptase and halt the multiplication of the virus.*
5. *The attempts to produce vaccine for AIDS have been unsuccessful due to the variations in the genome (and, therefore, the protein products) of the HIV.*