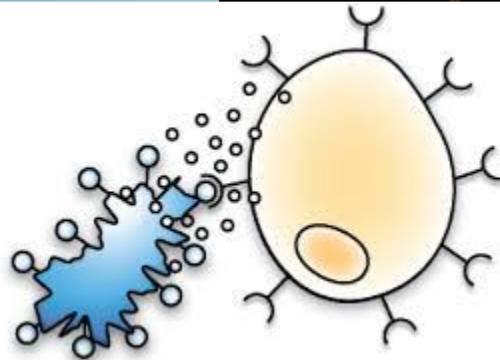
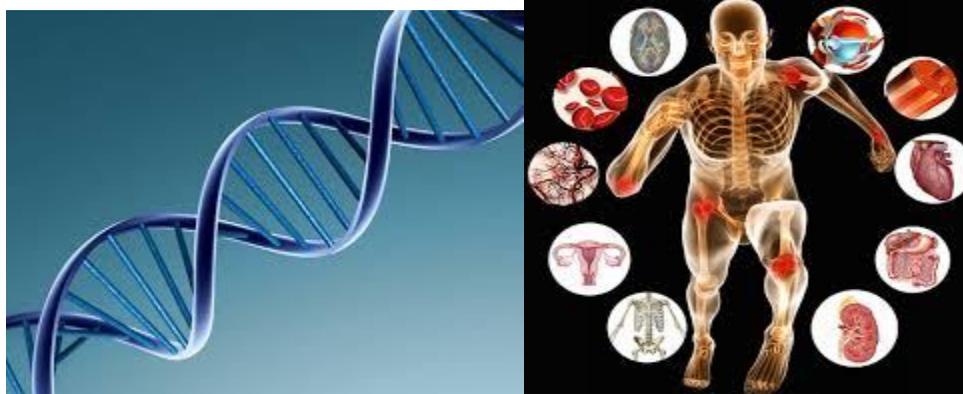




MZO-03

**Vardhman Mahaveer Open University, Kota**



**Biochemistry, Physiology and Immunology**



**MZO-03**

**Vardhman Mahaveer Open University, Kota**

**Biochemistry, Physiology and Immunology**

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## Vardhman Mahaveer Open University, Kota

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## Vardhman Mahaveer Open University, Kota

### Preface

The present book entitled “**Biochemistry, Physiology and Immunology**” has been designed so as to cover the unit-wise syllabus of MZO-03 course for M.Sc. Zoology (Previous) students of Vardhman Mahaveer Open University, Kota. The basic principles and theory have been explained in simple, concise and lucid manner. Adequate examples, diagrammes, photographs and self-learning exercises have also been included to enable the students to grasp the subject easily. The unit writers have consulted various standard books and internet on the subject and they are thankful to the authors of these reference books.

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## Unit -1

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# **Biocatalysts: classification and nomenclature of the enzymes; nature of enzymes, enzyme specificity, factors affecting enzyme activity, enzymatic and non-enzymatic catalysts, coenzymes and their functions, abzymes**

---

### Structure of the Unit

- 1.0 Objectives
- 1.1 Introduction
- 1.2 Structure of enzymes
- 1.3 How enzyme functions
  - 1.3.1 Lock and key hypothesis
  - 1.3.2 Induced fit hypothesis
- 1.4 Factors affecting catalytic activity of enzymes
  - 1.4.1 pH
  - 1.4.2 Temperature
  - 1.4.3 Concentration of enzyme and substrate
- 1.5 Enzyme Nomenclature and classification - Principles
- 1.6 Classification Types - Enzymes
  - 1.6.1 Trival name based
  - 1.6.2 Systematic Name based
  - 1.6.3 EC(Enzyme commission ) Number based
    - 1.6.3.1 Oxidoreductase
    - 1.6.3.2 Transferases
    - 1.6.3.3 Hydrolases
    - 1.6.3.4 Lyases

1.6.3.5 Isomerases

1.6.3.6 Ligases

1.7 Enzymatic and non enzymatic catalyst

1.8 Coenzyme and their function

1.8 Summary

1.9 Glossary

1.10 Self-Learning Exercise

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## 1.0 Objectives

---

After going through this unit you will be able to understand

- Biocatalyst and their structure.
- Mode of functioning of enzymes.
- Factors which affects the catalytical activities of enzymes.
- Classification of enzymes based on different criteria.
- Difference between enzymatic and non enzymatic catalyst.
- Cofactors, types and their function.
- Catalytical antibodies and their function.

---

## 1.1 Introduction

---

A living system controls its activity through enzymes. An enzyme is a protein molecule that is a biological catalyst with three characteristics.

1. The basic function of an enzyme is to increase the rate of a reaction. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme.
2. Most enzymes act specifically with only one reactant (called as Substrate) to produce products.
3. Enzymes are regulated from a state of low activity to high activity and vice versa.

Enzymes are very efficient catalysts for biochemical reactions. They speed up reactions by providing an alternative reaction pathway of lower activation energy. Like all catalysts, enzymes take part in the reaction - that is how they provide an alternative reaction pathway. But they do not undergo permanent

changes and so remain unchanged at the end of the reaction. They can only alter the rate of reaction, not the position of the equilibrium.

Most chemical catalysts catalyse a wide range of reactions. They are not usually very selective. In contrast enzymes are usually highly selective, catalysing specific reactions only. This specificity is due to the shapes of the enzyme molecules. Enzymes consist of a protein and a non-protein (called the cofactor). The proteins in enzymes are usually globular. The intra- and intermolecular bonds that hold proteins in their secondary and tertiary structures are disrupted by changes in temperature and pH. This affects shapes and so the catalytic activity of an enzyme is pH and temperature sensitive.

Gradually, you will appreciate that the individuality of a living cell is due in large part to the unique set of some 3,000 enzymes that it is genetically programmed to produce. If even one enzyme is missing or defective, the results can be disastrous. Much of the information about enzymes has been made possible because they can be isolated from cells and made to work in a test tube environment. Extensive work has also been done with X-Ray diffraction techniques to elucidate the three-dimensional structure of some enzymes.

---

## 1.2 Structure of enzymes

---

The activity of an enzyme depends, at the minimum, on a specific protein chain. In many cases, the enzyme consists of the protein and a combination of one or more parts called cofactors. This enzyme complex is usually simply referred to simply as the enzyme.

### **Apoenzyme:**

The polypeptide or protein part of the enzyme is called the **apoenzyme** and may be inactive in its original synthesized structure. The inactive form of the apoenzyme is known as **aproenzyme or zymogen**. The proenzyme may contain several extra amino acids in the protein which are removed, and allows the final specific tertiary structure to be formed before it is activated as an apoenzyme.

### **Cofactors:**

A cofactor is a non-protein substance which may be organic, and called a **coenzyme**. The coenzyme is often derived from a vitamin with specific examples discussed later. Another type of cofactor is an inorganic metal ion called a **metal ion activator**. The inorganic metal ions may be bonded through coordinate covalent bonds. The major reason for the nutritional requirement for

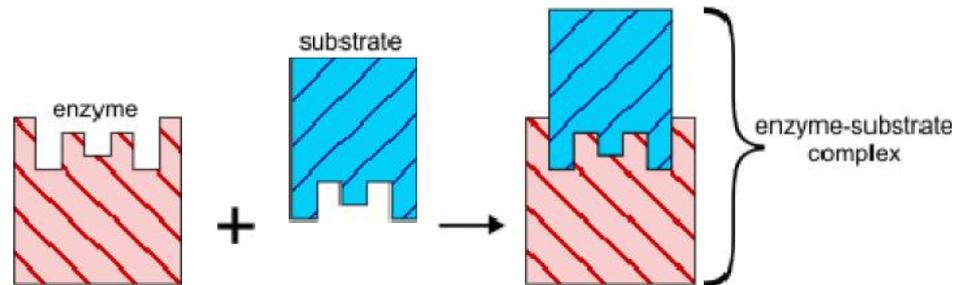




So the enzyme is used to form a reaction intermediate, but when this reacts with another reactant the enzyme reforms.

### 1.3.1 Lock and key hypothesis

This is the simplest model to represent how an enzyme works. The substrate simply fits into the active site to form a reaction intermediate.



### 1.3.2 Induced fit hypothesis

In this model the enzyme molecule changes shape as the substrate molecules gets close. The change in shape is 'induced' by the approaching substrate molecule. This more sophisticated model relies on the fact that molecules are flexible because single covalent bonds are free to rotate.

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## 1.4 Factor affecting the catalytic activity of enzyme

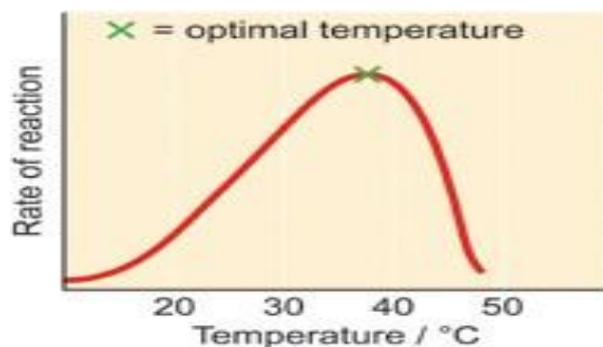
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### 1.4.1 Temperature

### 1.4.2 pH

### 1.4.3 Concentration of substrate and products

### 1.4.1 Temperature

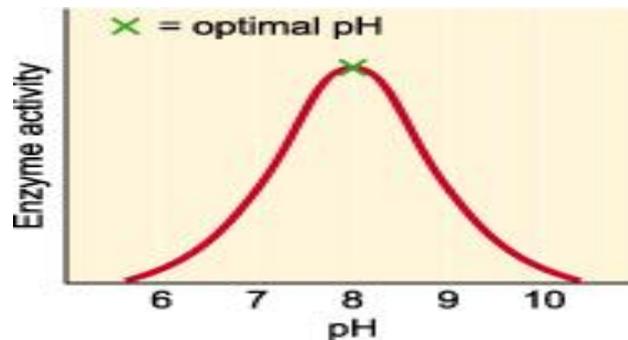


As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity

is at its greatest (see graph). This optimal temperature is usually around human body temperature (37.5 °C) for the enzymes in human cells.

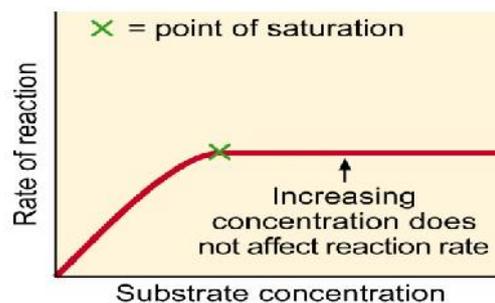
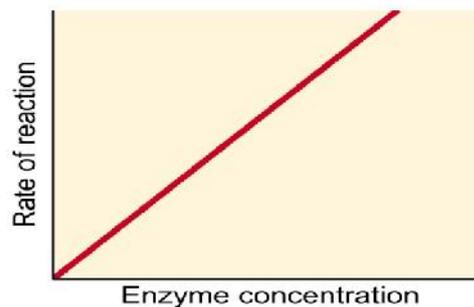
Above this temperature the enzyme structure begins to break down (denature) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy. Therefore as the temperature increases the rate of reaction increases but only to a particular temperature range because after that range enzyme get denatured.

### 1.4.2 pH



Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness or at optimum pH the shape of active site is such that it will readily recognizes the substrate thus increases the rate of reaction.

### 1.4.3 Concentration of enzyme and substrate



The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate. (See graph)

Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration. (See graph)

---

## **1.5 Enzyme Nomenclature and classification- Principles**

---

As their close interdependence, it is convenient to deal with the classification and nomenclature together.

### **1. first general principle**

Names purporting to be names of enzymes, especially those ending in *-ase*, should be used only for single enzymes, *i.e.* single catalytic entities. They should not be applied to systems containing more than one enzyme. When it is desired to name such a system on the basis of the overall reaction catalysed by it, the word *system* should be included in the name. For example, the system catalysing the oxidation of succinate by molecular oxygen, consisting of succinate dehydrogenase, cytochrome oxidase, and several intermediate carriers, should not be named *succinate oxidase*, but it may be called the *succinate oxidase system*.

### **2. Second general principle**

Enzymes are principally classified and named according to the reaction they catalyse. The chemical reaction catalysed is the specific property that distinguishes one enzyme from another, and it is logical to use it as the basis for the classification and naming of enzymes.

### **3. Third general principle**

Enzymes are divided into groups on the basis of the type of reaction catalysed, and this, together with the name(s) of the substrate(s) provides a basis for

naming individual enzymes. It is also the basis for classification and code numbers.

---

## **1.6 Classification Types -Enzyme**

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### **1.6.1 Trival name based**

### **1.6.2 systematic name based**

### **1.6.3 EC number based**

#### **1.6.1 Trival name based**

The first Enzyme Commission gave much thought to the question of a systematic and logical nomenclature for enzymes, and finally recommended that there should be two nomenclatures for enzymes, one systematic, and one working or trivial. The systematic name of an enzyme, formed in accordance with definite rules, showed the action of an enzyme as exactly as possible, thus identifying the enzyme precisely. The trivial name was sufficiently short for general use, but not necessarily very systematic; in a great many cases it was a name already in current use. The introduction of (often cumbersome) systematic names was strongly criticised. In many cases the reaction catalysed is not much longer than the systematic name and can serve just as well for identification, especially in conjunction with the code number.

#### **1.6.2 Systemic name based**

The Commission for Revision of Enzyme Nomenclature discussed this problem at length, and a change in emphasis was made. It was decided to give the trivial names more prominence in the Enzyme List; they now follow immediately after the code number, and are described as Common Name. Also, in the index the common names are indicated by an asterisk. Nevertheless, it was decided to retain the systematic names as the basis for classification for the following reasons:

- (i) The code number alone is only useful for identification of an enzyme when a copy of the Enzyme List is at hand, whereas the systematic name is self-explanatory;
- (ii) The systematic name stresses the type of reaction, the reaction equation does not.
- (iii) Systematic names can be formed for new enzymes by the discoverer, by application of the rules, but code numbers should **not** be assigned by individuals;

- (iv) Common names for new enzymes are frequently formed as a condensed version of the systematic name; therefore, the systematic names are helpful in finding common names that are in accordance with the general pattern.

It is recommended that for enzymes that are not the main subject of a paper or abstract, the common names should be used, but they should be identified at their first mention by their code numbers and source. Where an enzyme is the main subject of a paper or abstract, its code number, systematic name, or, alternatively, the reaction equation and source should be given at its first mention; thereafter the common name should be used. In the light of the fact that enzyme names and code numbers refer to reactions catalysed rather than to discrete proteins, it is of special importance to give also the source of the enzyme for full identification; in cases where multiple forms are known to exist, knowledge of this should be included where available.

### **1.6.3 Scheme for the classification of enzymes and the generation of EC numbers**

The first Enzyme Commission, in its report in 1961, devised a system for classification of enzymes that also serves as a basis for assigning code numbers to them. These code numbers, prefixed by EC, which are now widely in use, contain four elements separated by points, with the following meaning:

- (i) The first number shows to which of the six main divisions (classes) the enzyme belongs,
- (ii) The second figure indicates the subclass,
- (iii) The third figure gives the sub-subclass,
- (iv) The fourth figure is the serial number of the enzyme in its sub-subclass.

The subclasses and sub-subclasses are formed according to principles indicated below. The main divisions and subclasses are:

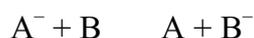
#### **1.6.3.1 Class 1. Oxidoreductases.**

In biochemistry, an oxidoreductase is an enzyme that catalyzes the transfer of electrons from one molecule, the reductant, also called the electron donor, to another, the oxidant, also called the electron acceptor. This group of enzymes usually utilizes NADP or NAD<sup>+</sup> as cofactors.

To this class belong all enzymes catalysing oxidoreduction reactions. The substrate that is oxidized is regarded as hydrogen donor. The systematic name is based on *donor:acceptor oxidoreductase*. The common name will be *dehydrogenase*, wherever this is possible; as an alternative, *reductase* can be used. *Oxidase* is only used in cases where O<sub>2</sub> is the acceptor. Substances called coenzymes, associated with the oxidoreductase enzymes and necessary for their activity, accept the hydrogen and electrons, which—in metabolic systems of animals—eventually are transferred to oxygen. Other enzymes of this group catalyze such reactions as the oxidation of aldehydes and ketones to carboxylic acids and the dehydrogenation of amino acids.

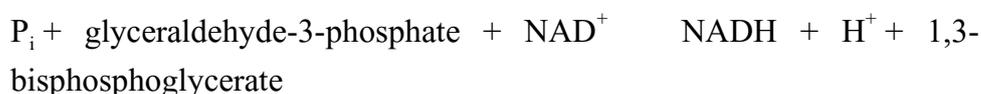
Reaction-:

For example, an enzyme that catalyzed this reaction would be an oxidoreductase:



In this example, A is the reductant (electron donor) and B is the oxidant (electron acceptor).

In biochemical reactions, the redox reactions are sometimes more difficult to see, such as this reaction from glycolysis:



In this reaction, NAD<sup>+</sup> is the oxidant (electron acceptor), and glyceraldehyde-3-phosphate is the reductant (electron donor).

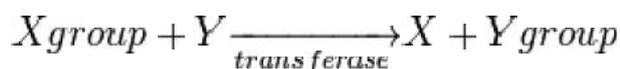
### 1.6.3.2 Class 2. Transferases.

In biochemistry, **transferase** is the general name for the class of enzymes that enact the transfer of specific functional groups (e.g. a methyl or glycosyl group) from one molecule (called the donor) to another (called the acceptor). They are involved in hundreds of different biochemical pathways throughout biology, and are integral to some of life's most important processes.

Transferases are involved in a myriad of reactions in the cell. Some examples of these reactions include the activity of CoA transferase, which transfers thiol esters,<sup>[3]</sup> the action of N-acetyltransferase is part of the pathway that metabolizes tryptophan and also includes the regulation of PDH, which converts pyruvate to Acetyl CoA. Transferases are also utilized during translation. In this case, an amino acid chain is the functional group transferred

by a Peptidyl transferase. The transfer involves the removal of the growing amino acid chain from the tRNA molecule in the A-site of the ribosome and its subsequent addition to the amino acid attached to the tRNA in the P-site.

Mechanistically, an enzyme that catalyzed the following reaction would be a transferase:



In the above reaction, X would be the donor, and Y would be the acceptor.<sup>[7]</sup> "Group" would be the functional group transferred as a result of transferase activity. The donor is often a coenzyme.

The common names are normally formed according to *acceptor grouptransferase* or *donor grouptransferase*. In many cases, the donor is a cofactor (coenzyme) charged with the group to be transferred.

### 1.6.3.3 Class 3. Hydrolases.

These enzymes catalyse the hydrolytic cleavage of C-O, C-N, C-C and some other bonds, including phosphoric anhydride bonds. Although the systematic name always includes *hydrolase*, the common name is, in many cases, formed by the name of the substrate with the suffix *-ase*. It is understood that the name of the substrate with this suffix means a hydrolytic enzyme.

In biochemistry, a **hydrolase** / haɪdr leɪz is an enzyme that catalyzes the hydrolysis of a chemical bond. For example, an enzyme that catalyzed the following reaction is a hydrolase:



A number of hydrolases acting on ester, glycosyl, peptide, amide or other bonds are known to catalyse not only hydrolytic removal of a particular group from their substrates, but likewise the transfer of this group to suitable acceptor molecules. In principle, all hydrolytic enzymes might be classified as transferases, since hydrolysis itself can be regarded as transfer of a specific group to water as the acceptor. Yet, in most cases, the reaction with water as the acceptor was discovered earlier and is considered as the main physiological function of the enzyme. This is why such enzymes are classified as hydrolases rather than as transferases.

#### 1.6.3.4 Class 4. Lyases.

Lyases are enzymes cleaving C-C, C-O, C-N, and other bonds by elimination, leaving double bonds or rings, or conversely adding groups to double bonds. The systematic name is formed according to the pattern *substrate group-lyase*. The hyphen is an important part of the name, and to avoid confusion should not be omitted, e.g. *hydro-lyase* not 'hydrolyase'. In the common names, expressions like *decarboxylase*, *aldolase*, *dehydratase* (in case of elimination of CO<sub>2</sub>, aldehyde, or water) are used. In cases where the reverse reaction is much more important, or the only one demonstrated, *synthase* (not synthetase) may be used in the name. Various subclasses of the lyases include pyridoxal-phosphate enzymes that catalyse the elimination of a - or -substituent from an -amino acid followed by a replacement of this substituent by some other group. In the overall replacement reaction, no unsaturated end-product is formed; therefore, these enzymes might formally be classified as *alkyl-transferases*. However, there is ample evidence that the replacement is a two-step reaction involving the transient formation of enzyme-bound , (or , )-unsaturated amino acids. According to the rule that the first reaction is indicative for classification, these enzymes are correctly classified as *lyases*. Examples are *tryptophan synthase* and *cystathionine -synthase*.

In biochemistry, a lyase is an enzyme that catalyzes the breaking (an "elimination" reaction) of various chemical bonds by means other than hydrolysis (a "substitution" reaction) and oxidation, often forming a new double bond or a new ring structure. The reverse reaction is also possible (called a "Michael addition"). For example, an enzyme that catalyzed this reaction would be a lyase:



Lyases differ from other enzymes in that they require only one substrate for the reaction in one direction, but two substrates for the reverse reaction.

#### 1.6.3.5 Class 5. Isomerases.

These enzymes catalyse geometric or structural changes within one molecule. According to the type of isomerism, they may be called *racemases*, *epimerases*, *cis-trans-isomerases*, *isomerases*, *tautomerases*, *mutases* or *cycloisomerases*.

In some cases, the interconversion in the substrate is brought about by an intramolecular oxidoreduction, since hydrogen donor and acceptor are the same

molecule, and no oxidized product appears, they are not classified as oxidoreductases, even though they may contain firmly bound NAD(P)<sup>+</sup>.

The subclasses are formed according to the type of isomerism, the sub-subclasses to the type of substrates.

**Isomerases** are a general class of enzymes which convert a molecule from one isomer to another. Isomerases can either facilitate intramolecular rearrangements in which bonds are broken and formed or they can catalyze conformational changes. The general form of such a reaction is as follows:



There is only one substrate yielding one product. This product has the same molecular formula as the substrate but differs in bond connectivity or spatial arrangements. Isomerases catalyze reactions across many biological processes, such as inglycolysis and carbohydrate metabolism.

#### 1.6.3.6 Class 6. Ligases.

Ligases are enzymes catalysing the joining together of two molecules coupled with the hydrolysis of a diphosphate bond in ATP or a similar triphosphate. The systematic names are formed on the system *X:Y ligase (ADP-forming)*. In earlier editions of the list the term *synthetase* has been used for the common names. Many authors have been confused by the use of the terms *synthetase* (used only for Group 6) and *synthase* (used throughout the list when it is desired to emphasis the synthetic nature of the reaction). Consequently NC-IUB decided in 1983 to abandon the use of *synthetase* for common names, and to replace them with names of the type *X-Y ligase*. In a few cases in Group 6, where the reaction is more complex or there is a common name for the product, a *synthase* name is used .

Isomerases catalyze changes within one molecule.<sup>[1]</sup> They convert one isomer to another, meaning that the end product has the same molecular formula but a different physical structure. Isomers themselves exist in many varieties but can generally be classified as structural isomers or stereoisomers. Structural isomers have a different ordering of bonds and/or different bond connectivity from one another, as in the case of hexane and its four other isomeric forms (2-methylpentane, 3-methylpentane, 2,2-dimethylbutane & 2,3dimethylbutane).

Stereoisomers have the same ordering of individual bonds and the same connectivity but the three-dimensional arrangement of bonded atoms differ. For

example, 2-butene exists in two isomeric forms: *cis*-2-butene and *trans*-2-butene.<sup>[2]</sup> The sub-categories of isomerases containing racemases, epimerases and cis-trans isomers are examples of enzymes catalyzing the interconversion of stereoisomers. Intramolecular lyases, oxidoreductases and transferases catalyze the interconversion of structural isomers.

From time to time, some enzymes have been deleted from the List, while some others have been renumbered. However, the old numbers have not been allotted to new enzymes; rather the place has been left vacant and cross-reference is made according to the following scheme:

### Top-level EC numbers

Group	Reaction catalyzed	Typical reaction	Enzyme example(s)
<u>EC-1</u> <u>Oxido-reductases</u>	To catalyze <u>oxidation</u> /reduction reactions; transfer of H and O atoms or <u>electrons</u> from one substance to another	$AH + B \rightarrow A + BH$ (reduced) $A + O \rightarrow AO$ (oxidized)	<u>Dehydrogenase</u> , <u>oxidase</u>
<u>EC-2</u> <u>Transferase</u>	Transfer of a <u>functional group</u> from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	$AB + C \rightarrow A + BC$	<u>Transaminase</u> , <u>kinase</u>
<u>EC-3</u> <u>Hydrolases</u>	Formation of two products from a substrate by <u>hydrolysis</u>	$AB + H_2O \rightarrow AOH + BH$	<u>Lipase</u> , <u>amylase</u> , <u>peptidase</u>
<u>EC-4</u> <u>Lyases</u>	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	$RCO_2COOH \rightarrow RCOH + CO_2$ or $[X-A-B-Y] \rightarrow [A=B + X-Y]$	<u>Decarboxylase</u>
<u>EC-5</u>	Intramolecule	$AB \rightarrow BA$	<u>Isomerase</u> ,

<u>Isomerases</u>	rearrangement, i.e. <u>isomerization</u> changes within a single molecule		<u>mutase</u>
<u>EC-6</u> <u>Ligases</u>	Join together two molecules by synthesis of new C-O, C- S, C-N or C-C <u>bonds</u> with simultaneous breakdown of <u>ATP</u>	X + Y + ATP XY + ADP + Pi	<u>Synthetase</u>

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## 1.7 Enzymatic and non enzymatic catalyst

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Enzymes and catalysts both affect the rate of a reaction. In fact, all known enzymes are catalysts, but not all catalysts are enzymes. The difference between catalysts and enzymes is that enzymes are largely organic in nature and are bio-catalysts, while non-enzymatic catalysts can be inorganic compounds. Neither catalysts nor enzymes are consumed in the reactions they catalyze.

For simplicity, *catalyst* in this article refers to non-enzymatic catalysts to easily differentiate from enzymes.

### Comparison chart

Criteria	Catalyst	Enzyme
Function	Catalysts are substances that increase or decrease the rate of a chemical reaction but remain unchanged.	Enzymes are proteins that increase rate of chemical reactions converting substrate into product.
Molecular weight	Low molecular weight compounds.	High molecular weight globular proteins.
Types	There are two types of catalysts – positive and negative catalysts.	There are two types of enzymes - activation enzymes and inhibitory enzymes.

Nature	Catalysts are simple inorganic molecules.	Enzymes are complex proteins.
Alternate terms	Inorganic catalyst.	Organic catalyst or bio catalyst.
Reaction rates	Typically slower	Several times faster
Specificity	They are not specific and therefore end up producing residues with errors	Enzymes are highly specific producing large amount of good residues
Conditions	High temp, pressure	physiological pH and temperature
C-C and C-H bonds	Absent	Present
Example	vanadium oxide	amylase, lipase

## 1.8 Coenzymes and their functions

Cofactors are the non proteinaceous part of the enzymes which is essential for the catalytic activities, mostly includes metal ions or coenzymes, are inorganic and organic chemicals that assist enzymes during the catalysis of reactions.

Coenzymes are non-protein organic molecules that are mostly derivatives of vitamins soluble in water by phosphorylation; they bind apoenzyme to proteins to produce an active holoenzyme. Apoenzymes are enzymes that lack their necessary cofactor(s) for proper functioning; the binding of the enzyme to a coenzyme forms a holoenzyme. Holoenzymes are the active form of an apoenzyme.

Cofactors can be metals or coenzymes, and their primary function is to assist in enzyme activity. They are able to assist in performing certain, necessary, reactions the enzyme cannot perform alone. They are divided into coenzymes and prosthetic groups. A holoenzyme refers to a catalytically active enzyme that consists of both apoenzyme (enzyme without its cofactor(s)) and cofactor. There are two groups of cofactors: metals and small organic molecules called coenzymes. Coenzymes are small organic molecules usually obtained from vitamins. Prosthetic groups refer to tightly bound coenzymes, while

cosubstrates refer to loosely bound coenzymes that are released in the same way as substrates and products. Loosely bound coenzymes differ from substrates in that the same coenzymes may be used by different enzymes in order to bring about proper enzyme activity.

Metal ions are known as the common cofactors. In some enzymes, the function as a catalyst cannot be carried out if a metal ion is not available to be bound the active site. In daily nutrition, this kind of cofactor plays a role as the essential trace elements such as: iron ( $\text{Fe}^{3+}$ ), manganese ( $\text{Mn}^{2+}$ ), cobalt ( $\text{Co}^{2+}$ ), copper ( $\text{Cu}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), selenium ( $\text{Se}^{2+}$ ), and molybdenum ( $\text{Mo}^{5+}$ ). For example,  $\text{Mg}^{2+}$  is used in glycolysis. In the first step of converting glucose to glucose 6-phosphate, before ATP is used to give ADP and one phosphate group, ATP is bonded to  $\text{Mg}^{2+}$  which stabilizing the other two phosphate groups so it is easier to release only one phosphate group without resonate with other two. In some bacteria such as genus *Azotobacter* and *Pyrococcus furiosus*, metal cofactors are also discovered to play an important role. An example of cofactors in action is the zinc-mediated function of carbonic anhydrase or the magnesium-mediated function of restriction endonuclease.

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## 1.9 Abenzymes

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Catalytic antibodies are antibodies that can enhance a couple of chemical and metabolic reactions in the body by binding a chemical group, resembling the transition state of a given reaction. Catalytic antibodies are produced when an antibody is immunized with a hapten molecule. The hapten molecule is usually designed to resemble the transition state of metabolic reaction.

Abyzymes are artificial catalytic antibodies and come from the words “antibody” and “enzyme” They are monoclonal antibodies that have catalytic properties, or carry out catalysis. The figure bellow shows the active of site of the abyzyme chorismate mutase and side-chain interactions with the transition state analog.

Antibodies act like soldiers to the body, fighting unwanted materials. They are secreted, for instance, when the body is infected with a bacterium or virus. The animal produces antibodies with binding sites that are exactly complementary to some molecular feature of the invader. The antibodies can thus recognize and bind only to the invader, identifying it as foreign and leading to its destruction by the rest of the immune system. Antibodies are also elicited in large quantity when an animal is injected with molecules, a process known as immunization. A small molecule used for immunization is called a hapten. Ordinarily, only

large molecules effectively elicit antibodies via immunization, so small-molecule haptens must be attached to a large protein molecule, called a carrier protein, prior to the actual immunization. Antibodies that are produced after immunization with the hapten-carrier protein conjugate are complementary to, and thus specifically bind, the hapten.

Ordinarily, antibody molecules simply bind; they do not catalyze reactions. However, catalytic antibodies are produced when animals are immunized with hapten molecules that are specially designed to elicit antibodies that have binding pockets capable of catalyzing chemical reactions. For example, in the simplest cases, binding forces within the antibody binding pocket are enlisted to stabilize transition states and intermediates, thereby lowering a reaction's energy barrier and increasing its rate. This can occur when the antibodies have a binding site that is complementary to a transition state or intermediate structure in terms of both three-dimensional geometry and charge distribution. This complementarity leads to catalysis by encouraging the substrate to adopt a transition-state-like geometry and charge distribution. Not only is the energy barrier lowered for the desired reaction, but other geometries and charge distributions that would lead to unwanted products can be prevented, increasing reaction selectivity.

Making antibodies with binding pockets complementary to transition states is complicated by the fact that true transition states and most reaction intermediates are unstable. Thus, true transition states or intermediates cannot be isolated or used as haptens for immunization. Instead, so-called transition-state analog molecules are used. Transition-state analog molecules are stable molecules that simply resemble a transition state (or intermediate) for a reaction of interest in terms of geometry and charge distribution. To the extent that the transition-state analog molecule resembles a true reaction transition state or intermediate, the elicited antibodies will also be complementary to that transition state or intermediate and thus lead to the catalytic acceleration of that reaction.

Catalytic antibodies bind very tightly to the transition-state analog haptens that were used to produce them during the immunization process. The transition-state analog haptens only bind and do not react with catalytic antibodies. It is the substrates, for example, the analogous ester molecules, that react. For this reason, transition-state analog haptens can interfere with the catalytic reaction by binding in the antibody binding pocket, thereby preventing any substrate molecules from binding and reacting. This inhibition by the transition-state

analog hapten is always observed with catalytic antibodies, and is used as a first level of proof that catalytic antibodies are responsible for any observed catalytic reaction.

The important feature of catalysis by antibodies is that, unlike enzymes, desired reaction selectivity can be programmed into the antibody by using an appropriately designed hapten. Catalytic antibodies almost always demonstrate a high degree of substrate selectivity. In addition, catalytic antibodies have been produced that have regioselectivity sufficient to produce a single product for a reaction in which other products are normally observed in the absence of the antibody.

Finally, catalytic antibodies have been produced by immunization with a single-handed version (only left- or only right-handed) of a hapten, and only substrates with the same handedness can act as substrates for the resulting catalytic antibodies. The net result is that a high degree of stereoselectivity is observed in the antibody-catalyzed reaction.

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## 1.8 Summary

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- A living system controls its activity through enzymes. An enzyme is a protein molecule that is a biological catalyst with three characteristics.
  1. The basic function of an enzyme is to increase the rate of a reaction.
  2. Most enzymes act specifically with only one reactant (called as Substrate) to produce products.
  3. Enzymes are regulated from a state of low activity to high activity and vice versa.
- The polypeptide or protein part of the enzyme is called the apoenzyme and may be inactive in its original synthesized structure.
- A cofactor is a non-protein substance which may be organic, and called a coenzyme.
- Temperature, pH and Concentration of substrate and products are affecting the catalytic activity of enzymes.
- Enzymes are divided into 6 classes i.e. oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

- **Abyzymes** are artificial catalytic antibodies and come from the words “antibody” and “enzyme” They are monoclonal antibodies that have catalytic properties, or carry out catalysis.

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## 1.9 Glossary

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- **Activated complex.** The highest free energy state of a complex in going from reactants to products.
- **Active site.** The region of an enzyme molecule that contains the substrate binding site and the catalytic site for converting the substrate(s) into product(s).
- **Catalyst.** A compound that lowers the activation energy of a reaction without itself being consumed.
- **Catalytic site.** The site of an enzyme involved in the catalytic process.
- **Coenzyme.** An organic molecule that associates with enzymes and affects their activity.
- **Cofactor.** A small molecule required for enzyme activity. It could be organic in nature, like a coenzyme, or inorganic in nature, like a metallic cation.
- **Enzyme.** A molecule, most often a protein, that contains a catalytic site for a biochemical reaction.
- **Induced fit.** A change in the shape of an enzyme that results from the binding of substrate.
- **Isomerase.** An enzyme that catalyzes an intramolecular rearrangement.

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## 1.10 Self-Learning Exercise

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### Section A : Very short answer type

1. Define enzyme.
2. What is coenzyme?
3. Which group of enzymes usually utilizes NADP or NAD<sup>+</sup> as cofactors?

### **Section B : Short answer type**

1. Explain induced fit hypothesis.
2. Write short notes on :
  - a) Hydrolases
  - b) Structure of enzyme
3. Write short notes on :
  - a) Ligases
  - b) Lock and key hypothesis

### **Section C : Long answer type**

1. Describe various classes of enzymes?
2. Write short notes on:
  - a) Abenzymes
  - b) Oxidoreductase
  - c) Isomerases
3. Explain affect of various factors on catalytic activity of enzymes.

## Unit-2

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# Metabolic pathways of protein, carbohydrates, lipids and nucleic acids (including sequence determination)

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### Structure of the Unit

- 2.0 Objectives
- 2.1 Introduction
- 2.2 Carbohydrate metabolism
  - 2.2.1 Photosynthesis
    - 2.2.1.1 CAM pathway.
    - 2.2.1.2 C<sub>3</sub> pathway.
    - 2.2.1.3 C<sub>4</sub> pathway.
  - 2.2.2 Respiration
    - 2.2.2.1 Glycolysis
    - 2.2.2.2 Krebs cycle
  - 2.2.3 Glycogenesis
  - 2.2.4 Glycogenolysis
  - 2.2.5 Gluconeogenesis
- 2.3 Protein metabolism
  - 2.3.1 Transamination reactions
  - 2.3.2 Glutamate:oxaloacetate transaminase [GOT]
  - 2.3.3 Glutamate pyruvate transaminase
  - 2.3.4 Glutamate dehydrogenase [GluDH]
  - 2.3.5 Transdeamination
  - 2.3.6 Urea cycle
- 2.4 Lipid metabolism
  - 2.3.1 beta- Oxidation
  - 2.3.2 Fatty acid biosynthesis

2.5	Nucleotide metabolism
2.5.1	Salvage pathway
2.5.2	Purine biosynthesis pathway
2.5.3	Purine degradation pathway
2.6	Summary
2.7	Glossary
2.8	Self-Learning Exercise

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## 2.0 Objectives

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After going through this unit you will be able to understand

- What is metabolism and the difference between the catabolism and anabolism.
- Different anabolic and catabolic pathway occurs within the cell.
- How these pathways of metabolism are linked with each other.
- The molecular level regulation of different pathways.
- The final fate of different metabolic intermediates.

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## 2.1 Introduction

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Metabolism is the set of life-sustaining chemical transformations within the cells of living organisms. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The word metabolism can also refer to all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells, in which case the set of reactions within the cells is called intermediary metabolism or intermediate metabolism.

Metabolism is usually divided into two categories.

1. Catabolism, that breaks down organic matter and harvests energy by way of cellular respiration
2. Anabolism that uses energy to construct components of cells such as proteins and nucleic acids.

The chemical reactions of metabolism are organized into metabolic pathways, in which one chemical is transformed through a series of steps into another chemical, by a sequence of enzymes. Enzymes are crucial for the

metabolism because they allow organisms to carry out desirable reactions that require energy that will not occur by themselves, by coupling them to spontaneous reactions that release energy. Enzymes act as catalysts that allow the reactions to proceed more rapidly. Enzymes also allow the regulation of metabolic pathways in response to changes in the cell's environment or to signals from other cells.

A striking feature of metabolism is the similarity of the basic metabolic pathways and components between even vastly different species. For example, the set of carboxylic acids that are best known as the intermediates in the citric acid cycle are present in all known organisms, being found in species as diverse as the unicellular bacterium *Escherichia coli* and huge multicellular organisms like elephants. These striking similarities in metabolic pathways are likely due to their early appearance in evolutionary history, and their retention because of their efficacy.

### **Basic Biomolecule**

Most of the structures that make up animals, plants and microbes are made from three basic classes of molecule: amino acids, carbohydrates and lipids (often called fats). As these molecules are vital for life, metabolic reactions either focus on making these molecules during the construction of cells and tissues, or by breaking them down and using them as a source of energy, by their digestion. These biomolecule can be joined together to make polymers such as DNA and proteins, essential macromolecules of life.

<b>Type of molecule</b>	<b><u>monomerforms</u></b>	<b><u>polymer for ms</u></b>	<b>Examples of polymer forms</b>
<u>Amino acids</u>	Amino acids	<u>Proteins</u> (polypeptides)	<u>Fibrous proteins</u> and <u>globular proteins</u>
<u>Carbohydrates</u>	<u>Monosaccharides</u>	<u>Polysaccharides</u>	<u>Starch, glycogen</u> and <u>cellulose</u>
<u>Nucleic acids</u>	<u>Nucleotides</u>	<u>Polynucleotides</u>	<u>DNA</u> and <u>RNA</u>

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## 2.2 Carohydrate metabolism

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2.2.1 Photosynthesis

2.2.2 Respiration

2.2.3 Pentose phosphate pathway

2.2.4 Glycogenesis

2.2.5 Glycogenolysis

2.2.6 Gluconeogenesis

**Carbohydrate metabolism** denotes the various biochemical processes responsible for the formation, breakdown and interconversion of carbohydrates in living organisms.

The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. Glucose and other carbohydrates are part of a wide variety of metabolic pathways across species: plants synthesize carbohydrates from carbon dioxide and water by photosynthesis storing the absorbed energy internally, often in the form of starch or lipids. Plant components are consumed by animals and fungi, and used as fuel for cellular respiration.

Oxidation of one gram of carbohydrate yields approximately 4 kcal of energy and from lipids about 9 kcal. Energy obtained from metabolism (e.g. oxidation of glucose) is usually stored temporarily within cells in the form of ATP. Organisms capable of aerobic respiration metabolize glucose and oxygen to release energy with carbon dioxide and water as byproducts.

Carbohydrates can be chemically divided into complex and simple. Simple carbohydrates consist of single or double sugar units (monosaccharides and disaccharides, respectively). Sucrose or table sugar (a disaccharide) is a common example of a simple carbohydrate. Complex carbohydrates contain three or more sugar units linked in a chain, with most containing hundreds to thousands of sugar units. They are digested by enzymes to release the simple sugars. Starch, for example, is a polymer of glucose units and is typically broken down to glucose. Cellulose is also a polymer of glucose but it cannot be digested by most organisms. Some bacteria that produce enzymes for cellulose live inside the gut of some mammals such as cows, and when cows eat plants, the cellulose is broken down by the bacteria and some of it is released into the gut.

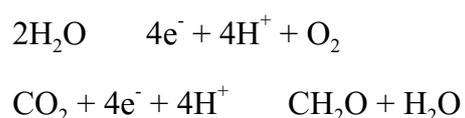
Carbohydrates are typically stored as long polymers of glucose molecules with glycosidic bonds for structural support (e.g. chitin, cellulose) or for energy storage (e.g. glycogen, starch). However, the strong affinity of most carbohydrates for water makes storage of large quantities of carbohydrates inefficient due to the large molecular weight of the solvated water-carbohydrate complex. In most organisms, excess carbohydrates are regularly catabolised to form acetyl-CoA, which is a feed stock for the fatty acid synthesis pathway; fatty acids, triglycerides, and other lipids are commonly used for long-term energy storage. The hydrophobic character of lipids makes them a much more compact form of energy storage than hydrophilic carbohydrates. However, animals, including humans, lack the necessary enzymatic machinery and so do not synthesize glucose from lipids, though glycerol can be converted to glucose.<sup>[8]</sup>

All carbohydrates share a general formula of approximately  $C_nH_{2n}O_n$ ; glucose is  $C_6H_{12}O_6$ . Monosaccharides may be chemically bonded together to form disaccharides such as sucrose and longer polysaccharides such as starch and cellulose.

### 2.2.1 Photosynthesis

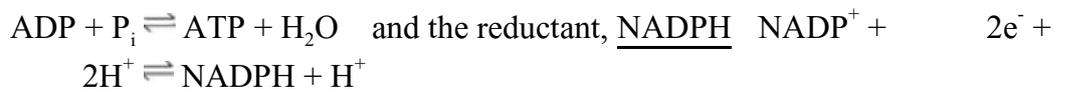
Carbon fixation or carbon assimilation refers to the conversion process of inorganic carbon (carbon dioxide) to organic compounds by living organisms. The most prominent example is photosynthesis, although chemosynthesis is another form of carbon fixation that can take place in the absence of sunlight. Organisms that grow by fixing carbon are called autotrophs. Autotrophs include photoautotrophs, which synthesize organic compounds using the energy of sunlight, and lithoautotrophs, which synthesize organic compounds using the energy of inorganic oxidation

In photosynthesis, energy from sunlight drives the carbon fixation pathway. *Oxygenic* photosynthesis is used by the primary producers—plants, algae, and cyanobacteria. They contain the pigment chlorophyll, and use the Calvin cycle to fix carbon autotrophically. The process works like this:

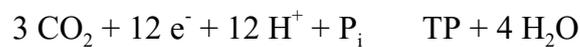


In the first step i.e photo part or light reaction water is dissociated into electrons, protons, and free oxygen. This allows the use of water, one of the most abundant substances on Earth, as an electron donor—as a source of

reducing power. The release of free oxygen is a side-effect of enormous consequence. The first step uses the energy of sunlight to oxidize water to O<sub>2</sub>, and, ultimately, to produce ATP



In the second step, called as dark reaction or synthesis part, the actual fixation of carbon dioxide is carried out. This process consumes ATP and NADPH. The Calvin cycle in plants accounts for the preponderance of carbon fixation on land. In algae and cyanobacteria, it accounts for the preponderance of carbon fixation in the oceans. The Calvin cycle converts carbon dioxide into sugar, as triose phosphate (TP), which is glyceraldehyde 3-phosphate (GAP) together with dihydroxyacetone phosphate(DHAP):



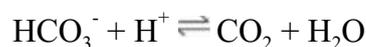
### **Evolutionary considerations**

Somewhere between 3.5 and 2.3 billion years ago, cyanobacteria evolved oxygenic photosynthesis.

### **Carbon concentrating mechanisms**

Many photosynthetic organisms have acquired inorganic carbon concentrating mechanisms (CCM), which increase the concentration of carbon dioxide available to the initial carboxylase of the Calvin cycle, the enzyme RuBisCO. The benefits of CCM include increased tolerance to low external concentrations of inorganic carbon, and reduced losses to photorespiration. CCM can make plants more tolerant of heat and water stress.

Carbon concentrating mechanisms use the enzyme carbonic anhydrase (CA), which catalyze both the dehydration of bicarbonate to carbon dioxide and the hydration of carbon dioxide to bicarbonate



Lipid membranes are much less permeable to bicarbonate than to carbon dioxide. To capture inorganic carbon more effectively, some plants have adapted theanaplerotic reactions



catalyzed by PEP carboxylase (PEPC), to carboxylate phosphoenolpyruvate (PEP) to oxaloacetate (OAA) which is a C<sub>4</sub> dicarboxylic acid.

## **CAM plants**

CAM plants that use Crassulacean acid metabolism as an adaptation for arid conditions. CO<sub>2</sub> enters through the stomata during the night and is converted into the 4-carbon compound, malic acid, which releases CO<sub>2</sub> for use in the Calvin cycle during the day, when the stomata are closed. The jade plant (*Crassula ovata*) and cacti are typical of CAM plants. Sixteen thousand species of plants use CAM.

## **C<sub>4</sub> plants**

C<sub>4</sub> plants preface the Calvin cycle with reactions that incorporate CO<sub>2</sub> into one of the 4-carbon compounds, malic acid or aspartic acid. C<sub>4</sub> plants have a distinctive internal leaf anatomy called as kranz anatomy. Tropical grasses, such as sugar cane and maize are C<sub>4</sub> plants, but there are many broadleaf plants that are C<sub>4</sub>. Overall, 7600 species of terrestrial plants use C<sub>4</sub> carbon fixation, representing around 3% of all species.

## **C<sub>3</sub> plants**

The large majority of plants are C<sub>3</sub> plants. They are so-called to distinguish them from the CAM and C<sub>4</sub> plants, and because the carboxylation products of the Calvin cycle are 3-carbon compounds. They lack C<sub>4</sub> dicarboxylic acid cycles, and therefore have higher carbon dioxide compensation points than CAM or C<sub>4</sub> plants.

### **1.2.1.1 Crassulacean acid metabolism(CAM)**

Crassulacean acid metabolism, also known as CAM photosynthesis, is a carbon fixation pathway that evolved in some plants as an adaptation to arid conditions. In a plant using full CAM, the stomata in the leaves remain shut during the day to reduce evapotranspiration, but open at night to collect carbon dioxide (CO<sub>2</sub>). The CO<sub>2</sub> is stored as the four-carbon acid malate, and then used during photosynthesis during the day. The pre-collected CO<sub>2</sub> is concentrated around the enzyme RuBisCO, increasing photosynthetic efficiency.

#### **During the night**

During the night, a plant employing CAM has its stomata open that is called as skoto-active stomata, allowing CO<sub>2</sub> to enter and be fixed as organic acids that are stored in vacuoles. During the day the stomata are closed (thus preventing water loss), and the carbon is released to the Calvin cycle so that photosynthesis may take place.

The carbon dioxide is fixed in the cytoplasm of mesophyll cells by a PEP reaction similar to that of C<sub>4</sub> pathway. But, unlike the C<sub>4</sub> mechanism, the resulting organic acids are stored in vacuoles for later use; that is, they are not immediately passed on to the Calvin cycle. The latter cannot operate during the night because the light reactions that provide it with ATP and NADPH cannot take place and the important enzyme i.e RUBISCO also requires light for their activation.

### **During the day**

During the day, the CO<sub>2</sub>-storing organic acids are released from the vacuoles of the mesophyll cells and enter the stroma of the chloroplasts where an enzyme called as malic enzyme acts and releases the CO<sub>2</sub>, which then enters into the Calvin cycle.

### **Benefits**

The most important benefit of CAM to the plant is the ability to leave most leaf stomata closed during the day. Plants employing CAM are most common in arid environments, where water comes at a premium. Being able to keep stomata closed during the hottest and driest part of the day reduces the loss of water through evapotranspiration, allowing such plants to grow in environments that would otherwise be far too dry. Plants using only C<sub>3</sub> carbon fixation, for example, lose 97% of the water they uptake through the roots to transpiration - a high cost avoided by plants able to employ CAM.

### **Biochemistry of CAM**

Plants with CAM must control storage of CO<sub>2</sub> and its reduction to branched carbohydrates in space and time. At low temperatures (frequently at night), plants using CAM open their stomata, CO<sub>2</sub> molecules diffuse into the spongy mesophyll's intracellular spaces and then into the cytoplasm. Here, they can meet phosphoenolpyruvate (PEP), which is a phosphorylated triose. During this time, the plants are synthesizing a protein called PEP carboxylase kinase (PEP-C kinase), whose expression can be inhibited by high temperatures (frequently at daylight) and the presence of malate. PEP-C kinase phosphorylates its target enzyme PEP carboxylase (PEP-C). Phosphorylation dramatically enhances the enzyme's capability to catalyze the formation of oxalacetate, which can be subsequently transformed into malate by NAD<sup>+</sup> malate dehydrogenase.

Malate is then transported via malate shuttles into the vacuole, where it is converted into the storage form malic acid. In contrast to PEP-C kinase, PEP-C

is synthesized all the time but almost inhibited at daylight either by dephosphorylation via PEP-C phosphatase or directly by binding malate. The latter is not possible at low temperatures, since malate is efficiently transported into the vacuole, whereas PEP-C kinase readily inverts dephosphorylation.

At daylight, plants using CAM close their guard cells and discharge malate that is subsequently transported into chloroplasts. There, depending on plant species, it is cleaved into pyruvate and CO<sub>2</sub> either by malic enzyme or by PEP carboxykinase. CO<sub>2</sub> is then introduced into the Calvin cycle, a coupled and self-recovering enzyme system, which is used to build branched carbohydrates. The by-product pyruvate can be further degraded in the mitochondrial citric acid cycle, thereby providing additional CO<sub>2</sub> molecules for the Calvin Cycle. Pyruvate can also be used to recover PEP via pyruvate phosphate dikinase, a high-energy step, which requires ATP and an additional phosphate.

#### **C4 Pathway**

##### **Rubisco evolution reprise**

The wastefulness of photorespiration is probably a consequence of just two factors. Early in the evolution of photosynthesis there was a higher carbon dioxide to oxygen gas ratio in the ancient atmosphere. Indeed the atmosphere was likely anaerobic in the earliest times on Earth. Rubisco evolved its active site when oxygen was rare and carbon dioxide was common. Since ancient times, rubisco has not yet evolved a mechanism to discriminate between the two similar substrates (O=C=O and O=O).

The reactions are similar too; the substrate is attached at a point along RuBP resulting in its splitting into organo-monophosphates. 3-phosphoglycerate is a common product of both reactions. So the difficulty of a protein to distinguish such similar molecules and to catalyze one reaction but not the other just has not happened yet. Photorespiration losses have not been intolerable either; the selection pressure is probably not severe in most environments. But in hot, dry, environments that are heavily-populated with plants, where the local carbon-dioxide content of the atmosphere is greatly reduced, selection should have resulted in a few adaptations to overcome photorespiration.

##### **2.2.1.2 The C<sub>4</sub> cycle**

It is no surprise that if O=C=O and O=O compete for the active site of rubisco, then mechanisms that would concentrate carbon dioxide around chloroplasts would evolve among plants competing for low carbon dioxide supplies in hot, dry climates (where it is already depleted!).

While some unicellular algae and cyanobacteria apparently use some membrane pumps to concentrate carbon dioxide in their cells (at an ATP cost), terrestrial plants evolved a Calvin-cycle add-on cycle. While the Calvin cycle produces 3-phosphoglycerate (a three-carbon sugar-phosphate) as its fixation product, this add-on cycle produces oxaloacetate (a four-carbon sugar-phosphate) as its fixation product. Thus the Calvin cycle is sometimes called the  $C_3$  cycle; the add-on is usually called the  $C_4$  cycle. Other names for the add-on include  $C_4$  photosynthesis, and the Hatch-Slack cycle (in honor of its discoverers). This pathway is found in 16 monocot and dicot families and has apparently evolved as three different specific pathways and two fundamentally-different separations.

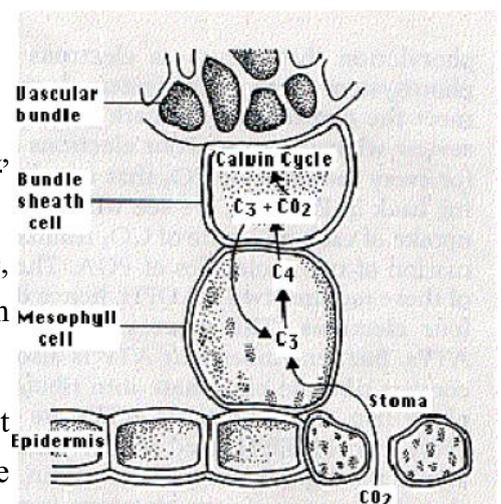
They all use a **supplementary** method of  $CO_2$  uptake which forms a 4-carbon molecule instead of the two 3-carbon molecules of the Calvin cycle. Hence these plants are called  $C_4$  plants. (Plants that have only the Calvin cycle are thus  $C_3$  plants.)

- Some  $C_4$  plants — called **CAM plants** — separate their  $C_3$  and  $C_4$  cycles by **time**. CAM plants are discussed below.
- Other  $C_4$  plants have structural changes in their leaf anatomy so that
  - their  $C_4$  and  $C_3$  pathways are separated in different parts of the leaf with
  - RUBISCO sequestered where the  $CO_2$  level is high; the  $O_2$  level low.

These adaptations are described now.

The details of the  $C_4$  cycle

- After entering through stomata,  $CO_2$  diffuses into a **mesophyll cell**.
  - Being close to the leaf surface, these cells are exposed to high levels of  $O_2$ , but
  - have no RUBISCO so cannot start photorespiration (nor the dark reactions of the Calvin



cycle).

- Instead the  $\text{CO}_2$  is inserted into a **3-carbon** compound ( $\text{C}_3$ ) called **phosphoenolpyruvic acid (PEP)** forming
- the **4-carbon** compound **oxaloacetic acid ( $\text{C}_4$ )**.
- Oxaloacetic acid is converted into malic acid or aspartic acid (both have 4 carbons), which is
- transported (by plasmodesmata) into a **bundle sheath cell**. Bundle sheath cells
  - are deep in the leaf so atmospheric oxygen cannot diffuse easily to them;
  - often have thylakoids with reduced photosystem II complexes (the one that produces  $\text{O}_2$ ).
  - Both of these features keep oxygen levels low.
- Here the 4-carbon compound is broken down into
  - **carbon dioxide**, which enters the Calvin cycle to form sugars and starch.
  - **pyruvic acid ( $\text{C}_3$ )**, which is transported back to a mesophyll cell where it is converted back into **PEP**.

These  $\text{C}_4$  plants are well adapted to (and likely to be found in) habitats with

- high daytime temperatures
- intense sunlight.

### **PEP-carboxylase fixes carbon dioxide**

In the  $\text{C}_4$  cycle, atmospheric carbon dioxide is taken into the fluid environment of the cells and enters into the typical bicarbonate equilibrium. The carbon is fixed (attached) to phosphoenolpyruvate by the enzyme phosphoenolpyruvate carboxylase. The product of this reaction is a four-carbon acid Malate. PEP carboxylase is a relatively-recently evolved cytosolic enzyme. The product of this fixation reaction is oxaloacetate, a four-carbon acid that gives the cycle its  $\text{C}_4$  name.

The 4-carbon transport material varies with species

Typically oxaloacetate is converted to a different 4-carbon acid for transport. In some species, NADP:malate dehydrogenase reduces the oxaloacetate to malate by using NADPH as its reducing power.

In other species, aspartate aminotransferase converts oxaloacetate to aspartate (with the glutamate/  $\alpha$ -ketoglutarate support shuttle). Whichever is produced, the 4-carbon acid is transported somewhere...in  $C_4$  plants this goes to an adjacent cell via plasmodesmata.

### **Biochemistry of $C_4$ Pathway**

The  $C_4$  pathway Plants that possess the  $C_4$  pathway have several specific enzymes which are located in two different cell types, the mesophyll cells and the bundle-sheath cells with transport of metabolites between the different compartments.

Atmospheric carbon is fixed at the 3-position of phosphoenolpyruvate (PEP) by the action of phosphoenolpyruvate carboxylase (PEPC), in the cytoplasm of mesophyll cells of  $C_4$  plants. The oxaloacetate so formed is then reduced to malate in the chloroplasts by NADP-malic dehydrogenase (NADP-MDH), or transformed to aspartate by transamination.

These acids are then exported to the bundle sheath cells, where a decarboxylation occurs (via malic enzyme or PEP carboxykinase) to yield  $CO_2$  that is refixed by the reductive pentose phosphate (RPP) pathway operative in these cells. The other three carbon atoms are recycled to the mesophyll cells in the form of pyruvate or alanine, where PEP is generated by the chloroplast enzyme pyruvate, Pi dikinase (PPDK).

The  $C_4$  pathway itself is not an alternative pathway for carbon fixation, but an additional one, since in the plants that possess it,  $CO_2$  is finally fixed by the RPP pathway (or Calvincycle) as in all other plants. Its function is to carry out  $CO_2$  fixation by the action of an enzyme that is not affected by high levels of  $O_2$  (PEPC) in the mesophyll cells and to release  $CO_2$  in the bundle-sheath cells, where the enzyme ribulose bis-phosphate carboxylase/oxygenase (RuBisCO) is located.

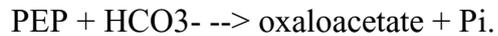
The elevated  $CO_2$  concentrations decrease the oxygenase activity of RuBisCO, and consequently the process of photorespiration, which may produce losses of around 30% in photosynthetic carbon fixation under normal atmospheric conditions.

One important feature of this mechanism is that PEPC is located only in mesophyll cells and RuBisCO only in bundle sheath cells. The differential expression in the genes for these enzymes in the two cell types is regulated at the level of translatable mRNA.

Thus, the C<sub>4</sub> cycle is also a good example of how regulation of a metabolic pathway may be achieved.

### **Regulatory aspects**

Phosphoenolpyruvate carboxylase (PEPC) catalyzes the irreversible carboxylation of PEP, using bicarbonate as substrate:



The enzyme requires Mg<sup>2+</sup> as an essential cofactor, and is composed of four subunits of identical molecular weight. Recent studies have shown that the amount of this protein is increased during the greening of etiolated leaves as a consequence of induction of mRNA synthesis.

Under these conditions, higher levels of C<sub>4</sub> acids are produced to keep the RPP pathway at full activity. Pyruvate, Pi dikinase This enzyme catalyzes the reaction



by a ping-pong mechanism that involves a phosphorylated enzyme intermediate. This reaction is thought to proceed in the direction of PEP formation since it is linked to the irreversible cleavage of PPi by inorganic pyrophosphatase.

### **Decarboxylation step**

The decarboxylation of the C<sub>4</sub> acids occurs in the bundle-sheath cells in different ways. The reactions that take place are



Reaction (1) is catalyzed by a chloroplast NADP-malic enzyme, reaction (2) by a mitochondrial NAD-malic enzyme, and reaction (3) by a cytoplasmic PEP carboxykinase. The kind of reaction that predominates at this stage is dependent on the species of C<sub>4</sub> plant. Thus three types are distinguished, which are named according to the decarboxylating enzyme. The most important C<sub>4</sub> plants, such as maize, sugar cane and sorghum, are of the NADP-malic enzyme type.

### **Comparison with C<sub>4</sub> metabolism**

The C<sub>4</sub> pathway bears resemblance to CAM; both act to concentrate CO<sub>2</sub> around RuBisCO, thereby increasing its efficiency. CAM

concentrates it in time, providing CO<sub>2</sub> during the day, and not at night, when respiration is the dominant reaction.

C<sub>4</sub> plants, in contrast, concentrate CO<sub>2</sub> spatially, with a RuBisCO reaction centre in a "bundle sheath cell" being inundated with CO<sub>2</sub>. Due to the inactivity required by the CAM mechanism, C<sub>4</sub> carbon fixation has a greater efficiency in terms of PGA synthesis.

### **2.2.1.3 The C<sub>3</sub> cycle**

The C<sub>3</sub> photosynthetic carbon reduction cycle was first elucidated by Calvin, Bassham and Benson in the 1950s in a series of experiments using the green alga *Chlorella* and radiolabelled carbon. This cycle, frequently referred to as the Calvin cycle, uses the products of the light reactions of photosynthesis, ATP and NADPH, to fix atmospheric CO<sub>2</sub> into carbon skeletons that are used directly for starch and sucrose biosynthesis .

The cycle can be divided into three stages.

1. The first of these is carboxylation, in which the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyses the fixation of CO<sub>2</sub> to the acceptor molecule ribulose 1,5-bisphosphate. This reaction results in the formation of the first stable compound in the cycle, 3-phosphoglycerate, a three carbon sugar that gives the cycle its name.
2. Carboxylation is followed by the reduction phase producing triose phosphates
3. Final, regenerative, phase resulting in the production of the CO<sub>2</sub> acceptor molecule, ribulose 1,5-bisphosphate.

The catalytic activities of a number of enzymes within the cycle are highly regulated, ensuring

that a balance is maintained between the carbon leaving the cycle and that remaining for synthesis of the CO<sub>2</sub> acceptor molecule.

### **Enzymes and Reactions of the Cycle**

Carboxylation of ribulose 1,5-bisphosphate The first reaction of the C<sub>3</sub> photosynthetic carbon

reduction cycle is the binding of CO<sub>2</sub> to the acceptor molecule, ribulose 1,5-bisphosphate, to form two molecules of 3-phosphoglycerate. This carboxylation reaction, catalysed by Rubisco, is unique to photosynthetic organisms.

In higher plants the functional 550 kDa Rubisco holoenzyme comprises eight identical large and eight identical small subunits, with one catalytic site located on each of the large subunits.

This enzyme is easily the most abundant protein on earth, constituting up to 50% of leaf soluble protein in C<sub>3</sub> plants. The reason for the high concentrations of Rubisco is that it has a poor affinity for its substrate, CO<sub>2</sub> and it also has an extremely low turnover number, so that large amounts of the enzyme are needed to catalyse the fluxes required for photosynthesis. Rubisco is a bifunctional enzyme and in addition to catalyzing the carboxylation reaction it can also catalyse the oxygenation of ribulose 1,5-bisphosphate at the same active site. This oxygenation reaction diverts carbon from the Calvin cycle to the photorespiratory pathway, releasing CO<sub>2</sub> and NH<sub>3</sub>.

The carboxylation and oxygenation reactions of Rubisco are competitive and the ratio of these reactions is determined by the relative concentrations of CO<sub>2</sub> and O<sub>2</sub>. As a consequence of this feature of the enzyme, present-day high atmospheric concentrations of oxygen relative to CO<sub>2</sub> favour photorespiration and are estimated to result in the loss of up to 40% of fixed carbon.

Reduction of 3-phosphoglycerate, the second phase of the cycle is the production of triose phosphates from the carboxylation product, 3-phosphoglycerate.

First, 3-phosphoglycerate is phosphorylated by the enzyme phosphoglycerate kinase, forming

1,3-bisphosphoglycerate, which is then reduced by glyceraldehyde 3-phosphate dehydrogenase to glyceraldehyde 3-phosphate, consuming ATP and NADPH.

Triose-phosphate isomerase catalyses the reversible isomerization of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate, although the equilibrium is strongly biased towards dihydroxyacetone phosphate.

Regeneration of ribulose 1,5-bisphosphate, in the third phase of the cycle, the CO<sub>2</sub> acceptor molecule ribulose 1,5-bisphosphate is regenerated from triose phosphates through a series of sugar condensation and carbon rearrangement reactions. Condensation of the triose phosphates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) by aldolase yields fructose 1,6-bisphosphate.

This six-carbon sugar is then irreversibly hydrolysed to the monophosphate form, fructose 6-phosphate, by fructose-1,6-bisphosphatase. The enzyme

transketolase then performs a two-carbon transfer from fructose 6-phosphate to glyceraldehyde 3-phosphate, forming xylulose 5-phosphate and erythrose 4-phosphate. Transketolase uses thiamin pyrophosphate as a prosthetic group to mediate the two-carbon transfer.

The result in erythrose 4-phosphate is combined with dihydroxyacetone phosphate, in a reaction again catalysed by aldolase, to form sedoheptulose 1,7-bisphosphate. This seven-carbon product is hydrolysed by sedoheptulose-1,7-bisphosphatase, yield in sedoheptulose 7-phosphate. Transfer of two carbons from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate by transketolase produces ribose 5-phosphate and xylulose 5-phosphate. Ribose 5-phosphate and xylulose 5-phosphate are converted to ribulose 5-phosphate by ribose-5-phosphate isomerase and ribulose-phosphate epimerase, respectively. The final step converts ribulose 5-phosphate to the CO<sub>2</sub> acceptor molecule ribulose 1,5-bisphosphate by the action of phosphoribulokinase in an irreversible reaction utilizing ATP.

### **Regulation of Enzymes**

The activity of a number of enzymes in the cycle, including Rubisco, phosphoglycerate kinase, transketolase, fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase, is regulated by large increases in the pH and Mg<sup>2+</sup> concentrations in the chloroplast stroma that occur on illumination of a previously darkened leaf. In addition to this general activation by light, the activity of a number of enzymes is regulated by further specific mechanisms described below.

### **Rubisco regulation**

The activity of the first enzyme in the C<sub>3</sub> carbon reduction cycle, Rubisco, is highly regulated at a number of different levels. First, it must be converted from an inactive to an active form before it is able to catalyse the fixation of CO<sub>2</sub>.

This process of carbamoylation involves the binding of CO<sub>2</sub> and Mg to a lysine residue adjacent to the catalytic site.

Another protein, Rubisco activase, is involved in mediating light activation and is itself subject to light regulation via the thioredoxin system described below (Zhang and Portis, 1999). Rubisco activase functions by removing inhibitors bound to the Rubisco active site on transition from darkness to light. These inhibitors prevent activation (carbamoylation) of the enzyme and may include the substrate ribulose 1,5-bisphosphate, the products of alternative reactions of Rubisco, and the potent inhibitor 2-carboxy arabinitol phosphate (although this

compound is not found in all species). Rubisco activase requires ATP to function, linking activation to the production of ATP and further coordinating carbon fixation with the light reactions of photosynthesis.

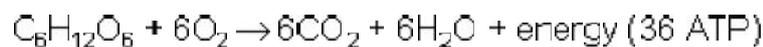
### **Thioredoxin regulation**

The activity of a number of Calvin cycle enzymes, glyceraldehyde-3-phosphate dehydrogenase, fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase and phosphoribulokinase, is modulated by light. Although the dark activity of these enzymes varies, the cycle is essentially inactive until illumination takes place, when there is a rapid increase in carbon flux through the cycle. Light activation utilizes the reducing power produced by the photosynthetic light reactions, which is transferred from ferredoxin to thioredoxin in a reaction catalysed by the enzyme ferredoxin/thioredoxin reductase .

Thioredoxin then binds to the inactive target enzyme and reduces the regulatory disulfide bond. The enzyme is activated by the associated change in conformation, and oxidized thioredoxin is released.

### **2.2.2 Respiration**

Cellular respiration allows organisms to use (release) energy stored in the chemical bonds of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>). The energy in glucose is used to produce ATP. Cells use ATP to supply their energy needs. Cellular respiration is therefore a process in which the energy in glucose is transferred to ATP.



In respiration, glucose is oxidized and thus releases energy. Oxygen is reduced to form water.

The carbon atoms of the sugar molecule are released as **carbon dioxide** (CO<sub>2</sub>).

The complete breakdown of glucose to carbon dioxide and water requires two major steps: 1) glycolysis and 2) aerobic respiration. Glycolysis produces two ATP. Thirty-four more ATP are produced by aerobic pathways if oxygen is present.

In the absence of oxygen, fermentation reactions produce alcohol or lactic acid but no additional ATP.

#### **2.2.2.1 Glycolysis**

During glycolysis, glucose (C<sub>6</sub>) is broken down to two molecules of pyruvate (C<sub>3</sub>). (Note that compounds that end in "\_\_\_ate" can be called "\_\_\_ic acid". For example, lactate is lactic acid and malate is malic acid.)

Glycolysis occurs in the **cytoplasm (cytosol)** and does not require oxygen.

There are ten steps in glycolysis and each one is catalyzed by a specific enzyme. A brief summary of these reactions is presented here.

2 ATP molecules are used to phosphorylate and activate compounds that will eventually become converted to **pyruvate** (or **pyruvic acid**) (see diagram below).

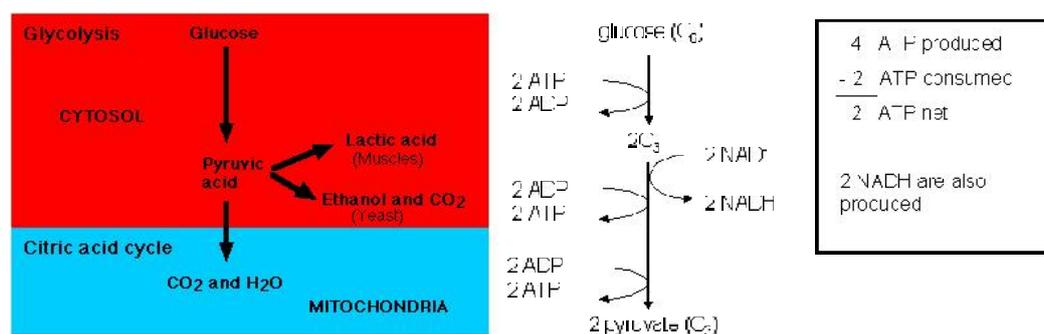
Two hydrogen atoms are removed by  $\text{NAD}^+$  forming 2 NADH (see diagram).

Additional phosphorylation results in intermediate 3-carbon molecules with 2 phosphate groups.

Four ATP are produced by **substrate-level phosphorylation**. Recall that substrate-level phosphorylation is the production of ATP using energy from other high-energy compounds but without the use of the electron transport system in the mitochondria.

Glycolysis is the anaerobic catabolism of glucose.

- It occurs in virtually all cells.
- In eukaryotes, it occurs in the cytosol.
- It converts a molecule of glucose into 2 molecules of pyruvic acid.
- $\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{NAD}^+ \rightarrow 2\text{C}_3\text{H}_4\text{O}_3 + 2\text{NADH} + 2\text{H}^+$
- The free energy stored in 2 molecules of pyruvic acid is somewhat less than that in the original glucose molecule.
- Some of this difference is captured in 2 molecules of ATP.



The net yield of ATP in glycolysis is 2 for each glucose molecule (2 are used but 4 are produced).

Some bacteria have alternative energy-producing reactions. Two of these are the pentose phosphate pathway and the Entner-Doudoroff pathway.

The Fates of Pyruvic Acid

### In Yeast

- Pyruvic acid is decarboxylated and reduced by NADH to form a molecule of carbon dioxide and one of ethanol.
- $C_3H_4O_3 + NADH + H^+ \rightarrow CO_2 + C_2H_5OH + NAD^+$
- This accounts for the bubbles and alcohol in, for examples, beer and champagne.
- The process is called alcoholic fermentation.
- The process is energetically wasteful because so much of the free energy of glucose (some 95%) remains in the alcohol (a good fuel!).

### In Red Blood Cells and active Muscles

- Pyruvic acid is reduced by NADH forming a molecule of lactic acid.
- $C_3H_4O_3 + NADH + H^+ \rightarrow C_3H_6O_3 + NAD^+$
- The process is called lactic acid fermentation.
- The process is energetically wasteful because so much free energy remains in the lactic acid molecule. (It can also be debilitating because of the drop in pH as the lactic acid produced in overworked muscles is transported out into the blood.)

### In Mitochondria

- Pyruvic acid is oxidized completely to form carbon dioxide and water.
- The process is called cellular respiration.
- Approximately 40% of the energy in the original glucose molecule is trapped in molecules of ATP.

### **Formation of Acetyl CoA**

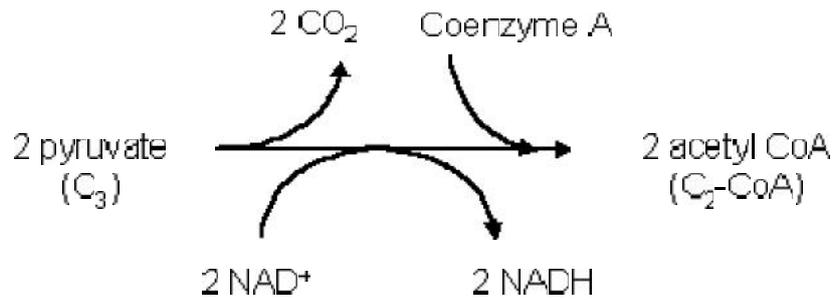
Pyruvate produced by glycolysis (see above) enters the *mitochondrion* by active transport and is converted to *acetyl CoA* as shown below. The remainder of the reactions of cellular respiration occur in the *mitochondrion*.

pyruvate (C<sub>3</sub>) --> acetyl CoA (C<sub>2</sub>) + CO<sub>2</sub>

A carbon atom is removed from each of the pyruvate molecules forming a two-carbon compound and CO<sub>2</sub>.

Each of the two-carbon compounds are oxidized forming NADH from NAD<sup>+</sup>.

Coenzyme A is attached to each of the two-carbon compounds producing two acetyl CoA molecules.



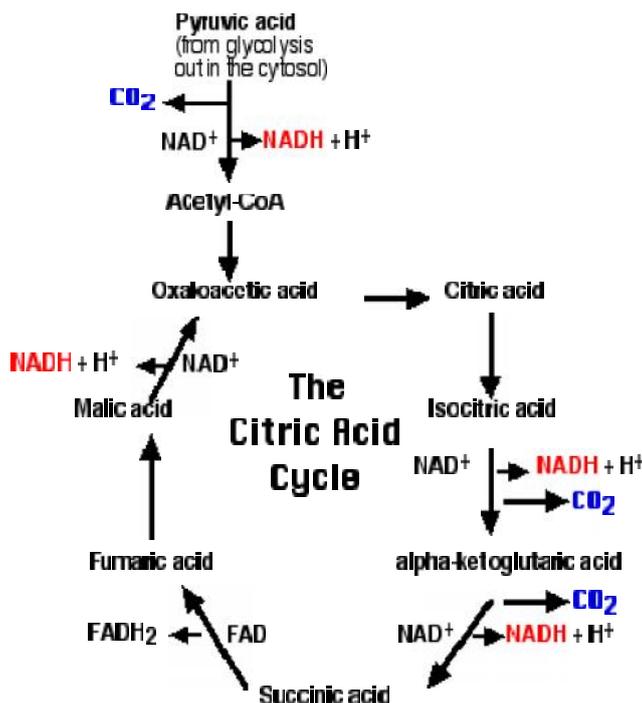
## Mitochondria

Mitochondria are membrane-enclosed organelles distributed through the cytosol of most eukaryotic cells. Their number within the cell ranges from a few hundred to, in very active cells, thousands. Their main function is the conversion of the potential energy of food molecules into ATP.

Mitochondria have:

- an outer membrane that encloses the entire structure
- an inner membrane that encloses a fluid-filled matrix
- between the two is the intermembrane space
- the inner membrane is elaborately folded with shelflike cristae projecting into the matrix.
- a small number (some 5–10) circular molecules of DNA

This electron micrograph (courtesy of Keith R. Porter) shows a single mitochondrion from a bat pancreas cell. Note the double membrane and the way the inner membrane is folded into cristae. The dark, membrane-bounded objects above the mitochondrion are lysosomes.



The number of mitochondria in a cell can

- increase by their fission (e.g. following mitosis);
- decrease by their fusing together.

(Defects in either process can produce serious, even fatal, illness.)

#### The Outer Membrane

The outer membrane contains many complexes of integral membrane proteins that form channels through which a variety of molecules and ions move in and out of the mitochondrion.

#### The Inner Membrane

The inner membrane contains 5 complexes of integral membrane proteins:

- NADH dehydrogenase (Complex I)
- succinate dehydrogenase (Complex II)
- cytochrome c reductase (Complex III; also known as the cytochrome b-c<sub>1</sub> complex)
- cytochrome c oxidase (Complex IV)
- ATP synthase (Complex V)

#### The Matrix

The matrix contains a complex mixture of soluble enzymes that catalyze the respiration of pyruvic acid and other small organic molecules.

Here pyruvic acid is

- oxidized by  $\text{NAD}^+$  producing  $\text{NADH} + \text{H}^+$
- decarboxylated producing a molecule of
  - carbon dioxide ( $\text{CO}_2$ ) and
  - a 2-carbon fragment of acetate bound to coenzyme A forming acetyl-CoA

#### 2.2.2.2 The Citric Acid Cycle

- This 2-carbon fragment is donated to a molecule of oxaloacetic acid.
- The resulting molecule of citric acid (which gives its name to the process) undergoes the series of enzymatic steps shown in the diagram.
- The final step regenerates a molecule of oxaloacetic acid and the cycle is ready to turn again.
- Each of the 3 carbon atoms present in the pyruvate that entered the mitochondrion leaves as a molecule of carbon dioxide ( $\text{CO}_2$ ).
- At 4 steps, a pair of electrons ( $2e^-$ ) is removed and transferred to  $\text{NAD}^+$  reducing it to  $\text{NADH} + \text{H}^+$ .

- At one step, a pair of electrons is removed from succinic acid and reduces the prosthetic group flavin adenine dinucleotide (FAD) to  $\text{FADH}_2$ .

The electrons of NADH and  $\text{FADH}_2$  are transferred to the electron transport chain.

### The Electron Transport Chain

The electron transport chain consists of 3 complexes of integral membrane proteins

- the NADH dehydrogenase complex (I)
- the cytochrome c reductase complex (III)
- the cytochrome c oxidase complex (IV)

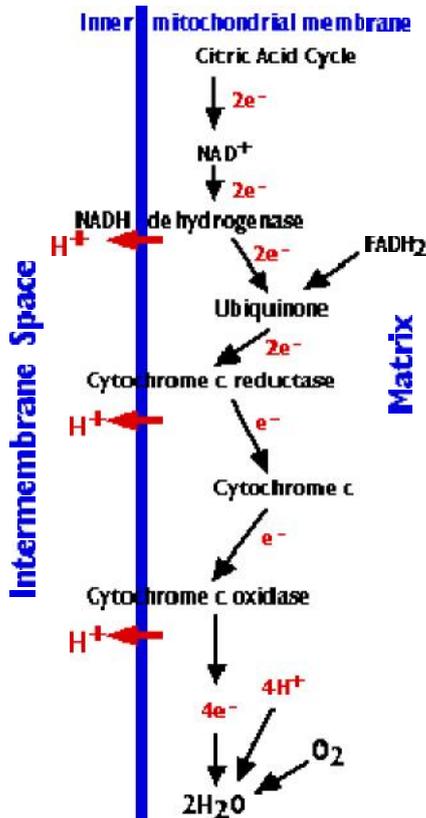
and two freely-diffusible molecules

- ubiquinone
- cytochrome c

that shuttle electrons from one complex to the next.

The electron transport chain accomplishes:

- the stepwise transfer of electrons from NADH (and  $\text{FADH}_2$ ) to oxygen molecules to form (with the aid of protons) water molecules ( $\text{H}_2\text{O}$ );



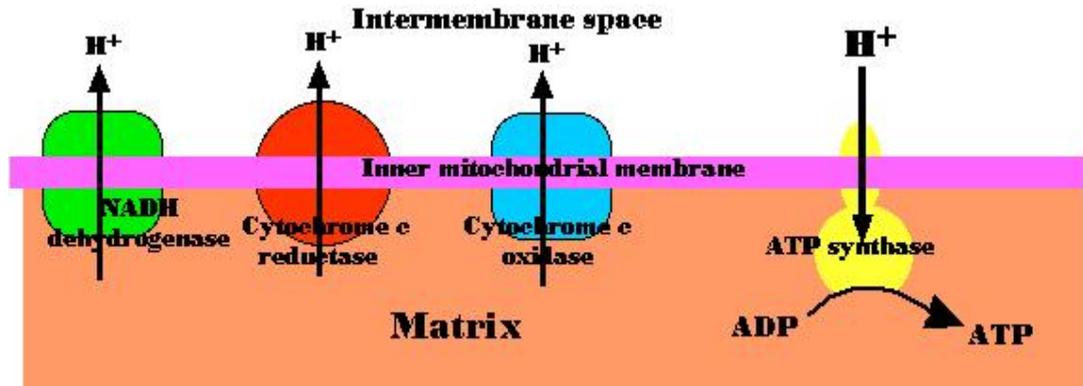
(Cytochrome c can only transfer one electron at a time, so cytochrome c oxidase must wait until it has accumulated 4 of them before it can react with oxygen.)

- harnessing the energy released by this transfer to the pumping of protons ( $\text{H}^+$ ) from the matrix to the intermembrane space.
- Approximately 20 protons are pumped into the intermembrane space as the 4 electrons needed to reduce oxygen to water pass through the respiratory chain.
- The gradient of protons formed across the inner membrane by this process of active transport forms a miniature battery.
- The protons can flow back down this gradient only by reentering the matrix through ATP synthase, another complex (complex V) of 16

integral membrane proteins in the inner membrane. The process is called chemiosmosis.

### Chemiosmosis in mitochondria

The energy released as electrons pass down the gradient from NADH to oxygen is harnessed by three enzyme complexes of the respiratory chain (I, III, and IV) to pump protons ( $H^+$ ) against their concentration gradient from the matrix of the mitochondrion into the intermembrane space (an example of active transport).



As their concentration increases there (which is the same as saying that the pH decreases), a strong diffusion gradient is set up. The only exit for these protons is through the ATP synthase complex. As in chloroplasts, the energy released as these protons flow down their gradient is harnessed to the synthesis of ATP. The process is called chemiosmosis and is an example of facilitated diffusion.

How many ATPs?

It is tempting to try to view the synthesis of ATP as a simple matter of stoichiometry (the fixed ratios of reactants to products in a chemical reaction). But (with 3 exceptions) it is not.

Most of the ATP is generated by the proton gradient that develops across the inner mitochondrial membrane. The number of protons pumped out as electrons drop from NADH through the respiratory chain to oxygen is theoretically large enough to generate, as they return through ATP synthase, 3 ATPs per electron pair (but only 2 ATPs for each pair donated by  $FADH_2$ ).

With 12 pairs of electrons removed from each glucose molecule,

- 10 by  $NAD^+$  (so  $10 \times 3 = 30$ ); and
- 2 by  $FADH_2$  (so  $2 \times 2 = 4$ ),

this could generate 34 ATPs.

Add to this the 4 ATPs that are generated by the 3 exceptions and one arrives at 38.

But

- The energy stored in the proton gradient is also used for the active transport of several molecules and ions through the inner mitochondrial membrane into the matrix.
- NADH is also used as reducing agent for many cellular reactions.

So the actual yield of ATP as mitochondria respire varies with conditions. It probably seldom exceeds 30.

### **Total ATP yield per glucose**

#### **Conversions**

NADH produced in the cytoplasm produces two to three ATP by the electron transport system.

NADH produced in the mitochondria produces approximately three ATP.

FADH<sub>2</sub> adds its electrons to the electron transport system at a lower level than NADH, so it produces approximately two ATP.

#### **Glycolysis**

2 ATP

2 NADH (= 4 ATP; these are converted to ATP in the mitochondria during cellular respiration)

#### **Formation of Acetyl CoA**

2 NADH (= 6ATP)

#### **Citric Acid Cycle**

6 NADH (= 18 ATP)

2 FADH<sub>2</sub> (= 4 ATP)

2 ATP

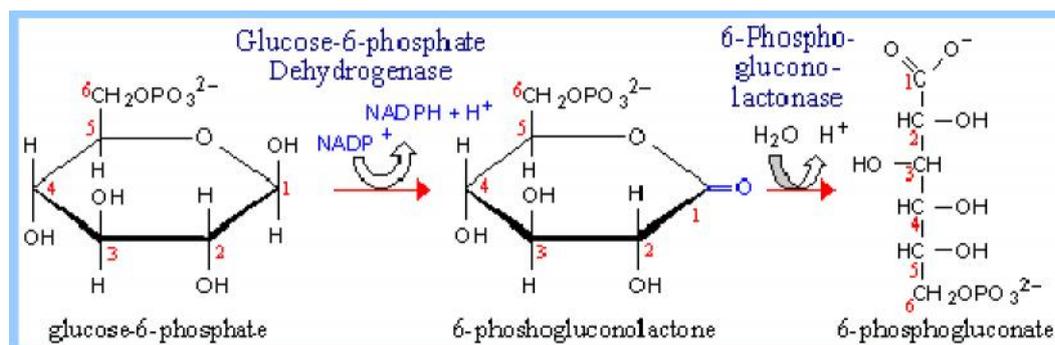
## Total Yield

Glycolysis produces 2 ATP; aerobic respiration produces 34 more ATP

Pathway	Substrate-Level Phosphorylation	Oxidative Phosphorylation	Total ATP
Glycolysis	2 ATP	2 NADH = 4 - 6 ATP	6 - 8
CoA		2 NADH = 6 ATP	6
Citric Acid Cycle	2 ATP	6 NADH = 18 ATP 2 FADH <sub>2</sub> = 4 ATP	24
<b>TOTAL</b>	4 ATP	32 ATP	36 - 38

### 2.2.3 Pentose Phosphate Pathway

The Pentose Phosphate Pathway (also called Phosphogluconate Pathway, or Hexose Monophosphate Shunt) is depicted with structures of intermediates in Fig. 23-25 p. 863 of Biochemistry, by Voet & Voet, 3rd Edition. The linear portion of the pathway carries out oxidation and decarboxylation of glucose-6-phosphate, producing the 5-C sugar ribulose-5-phosphate.

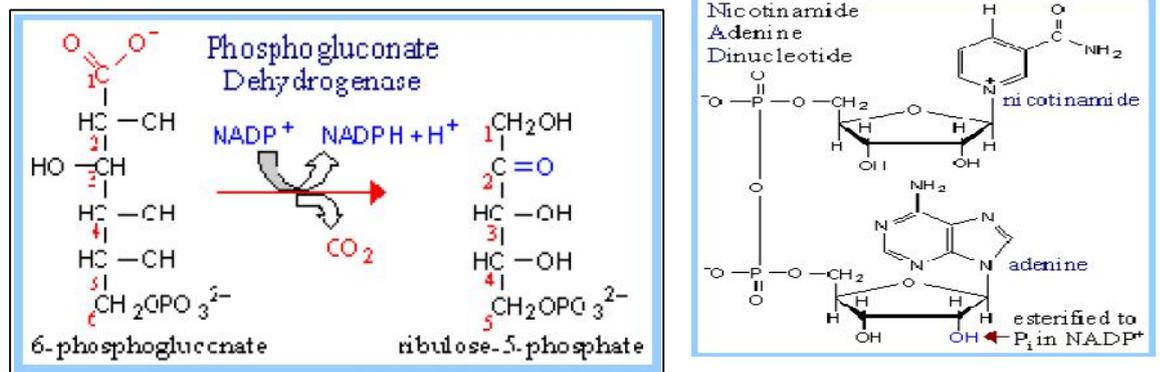


**Glucose-6-phosphate Dehydrogenase** catalyzes oxidation of the aldehyde (hemiacetal), at C1 of glucose-6-phosphate, to a carboxylic acid in ester linkage (lactone). NADP<sup>+</sup> serves as electron acceptor.

**6-Phosphogluconolactonase** catalyzes hydrolysis of the ester linkage (lactone) resulting in ring opening. The product is 6-phosphogluconate. Although ring opening occurs in the absence of a catalyst, 6-Phosphogluconolactonase speeds

up the reaction, decreasing the lifetime of the highly reactive, and thus potentially toxic, 6-phosphogluconolactone.

**Phosphogluconate Dehydrogenase** catalyzes **oxidative decarboxylation** of 6-phosphogluconate, to yield the 5-C ketose ribulose-5-phosphate. The hydroxyl at C3 (C2 of the product) is oxidized to a ketone. This promotes loss of the carboxyl at C1 as  $\text{CO}_2$ .  $\text{NADP}^+$  again serves as oxidant (electron acceptor).



Reduction of  $\text{NADP}^+$  (as with  $\text{NAD}^+$ ) involves transfer of  $2e^-$  plus  $1\text{H}^+$  to the nicotinamide moiety.  $\text{NAD}^+$  and  $\text{NADP}^+$  differ only in the presence of an extra phosphate on the adenosine ribose of  $\text{NADP}^+$ . This difference has little to do with redox activity, but is recognized by substrate-binding sites of enzymes. It is a mechanism for separation of catabolic and synthetic pathways.

$\text{NADPH}$ , a product of the Pentose Phosphate Pathway, functions as a reductant in various synthetic (anabolic) pathways, including fatty acid synthesis.

$\text{NAD}^+$  serves as electron acceptor in catabolic pathways in which metabolites are oxidized. The resultant  $\text{NADH}$  is reoxidized by the respiratory chain, producing ATP.

### Regulation:

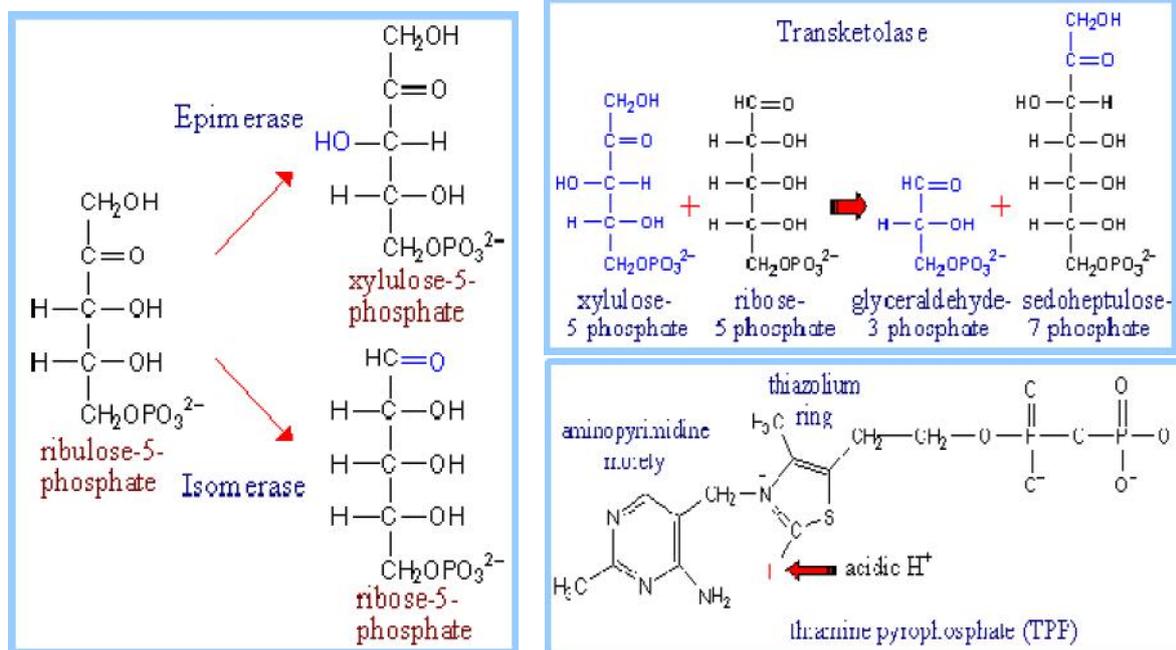
Glucose-6-phosphate Dehydrogenase is the committed step of the Pentose Phosphate Pathway. This enzyme is regulated by availability of the substrate  $\text{NADP}^+$ . As  $\text{NADPH}$  is utilized in reductive synthetic pathways, the increasing concentration of  $\text{NADP}^+$  stimulates the Pentose Phosphate Pathway, to replenish  $\text{NADPH}$ .

The remainder of the Pentose Phosphate Pathway accomplishes conversion of the 5-C ribulose-5-phosphate to the 5-C product ribose-5-phosphate, or to the 3-C glyceraldehyde-3-phosphate and the 6-C fructose-6-phosphate.

Additional enzymes include Ribulose-5-phosphate Epimerase, Ribulose-5-phosphate Isomerase, Transketolase, and Transaldolase.

- Epimerase interconverts the stereoisomers ribulose-5-phosphate and xylulose-5-phosphate.
- Isomerase converts the **ketose** ribulose-5-phosphate to the **aldose** ribose-5-phosphate.

Both reactions involve deprotonation to form an endiolate intermediate, followed by specific reprotonation to yield the product. Both reactions are reversible.



**Transketolase** and **Transaldolase** catalyze transfer of 2-C and 3-C molecular fragments respectively, in each case from a ketose donor to an aldose acceptor. D. E. Nicholson has suggested that the names of these enzymes should be changed, since Transketolase actually transfers an aldol moiety (glycoaldehyde) and Transaldolase actually transfers a ketol moiety (dihydroxyacetone). However the traditional enzyme names are used here.

Transketolase transfers a 2-C fragment from xylulose-5-phosphate to either ribose-5-phosphate or erythrose-4-phosphate.

Transketolase utilizes a prosthetic group thiamine pyrophosphate (TPP), derivative of vitamin B<sub>1</sub>.

H<sup>+</sup> readily dissociates from the C between N and S in the thiazolium ring of thiamine pyrophosphate.

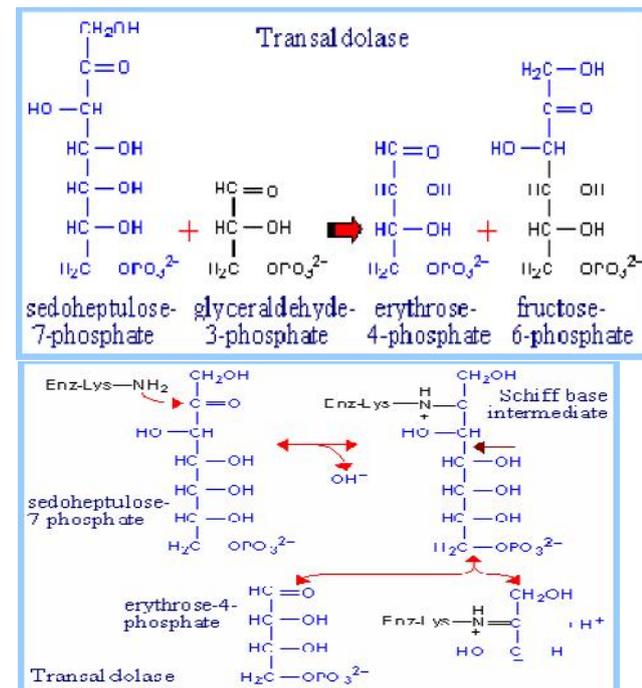
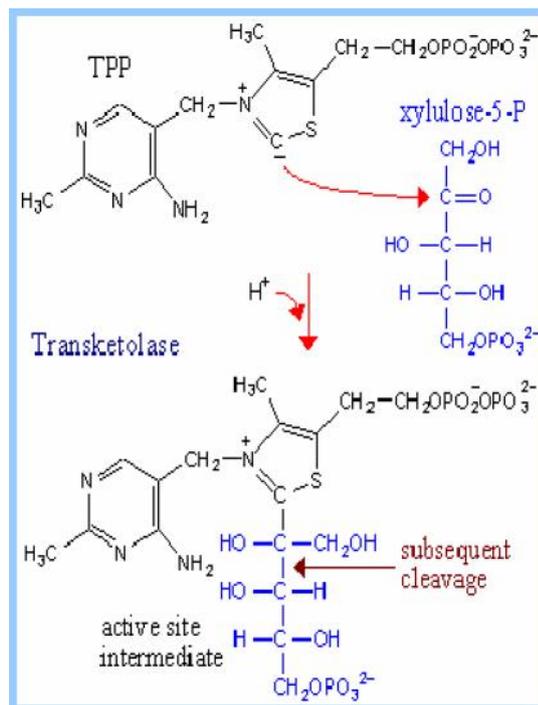
Thiamine pyrophosphate binds at the active sites of enzymes in a "V" conformation. The amino group of the aminopyrimidine moiety is close to the dissociable proton, and serves as the proton acceptor. This proton transfer is promoted by a glutamate residue adjacent to the pyrimidine ring.

The thiazolium **carbanion** that results from proton dissociation reacts with the carbonyl C of xylulose-5-P to form an addition compound.

The **positively charged N** in the thiazole ring acts as an **electron sink**, promoting C-C bond cleavage. The 3-C aldose glyceraldehyde-3-phosphate is released. A 2-C fragment remains on TPP.

Completion of the reaction is by reversal of these steps. The 2-C fragment condenses with one of the aldoses erythrose-4-phosphate (4-C) or ribose-5-phosphate (5-C) to form a 6-C or 7-C ketose-phosphate product.

Transfer of the 2-C fragment to the 5-C aldose ribose-5-phosphate yields sedoheptulose-7-phosphate. Transfer instead to the 4-C aldose erythrose-4-phosphate yields fructose-6-phosphate.



Explore at right the structure of *E. coli* Transaldolase, crystallized with a modified active site intermediate. The structure of human Transaldolase has also been determined, and it exhibits a similar  $\alpha$ ,  $\beta$  barrel structure.

Explore at right the structure of Transketolase with bound substrate erythrose-4-phosphate.

Transaldolase catalyzes transfer of a 3-C dihydroxyacetone moiety, from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate.

The -amino group of an active site lysine residue reacts with the carbonyl C of sedoheptulose-7-phosphate to form a protonated Schiff base intermediate.

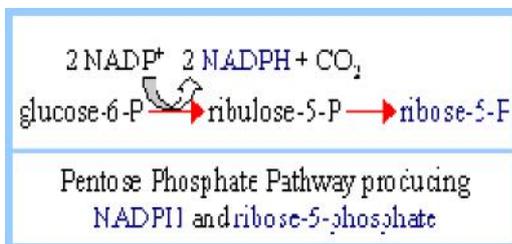
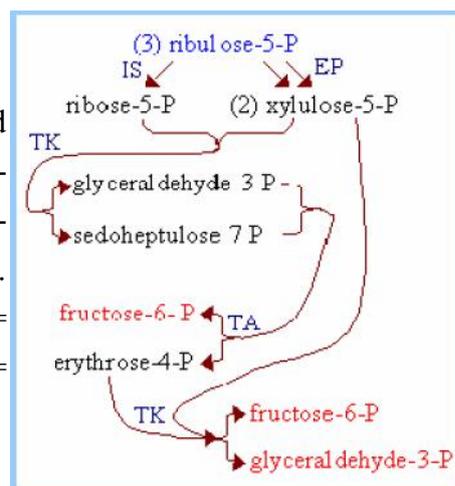
Aldol cleavage results in release of erythrose-4-phosphate. The Schiff base stabilizes the carbanion on C3.

Completion of the reaction occurs by **reversal**, as the carbanion attacks instead the aldehyde carbon of the 3-carbon aldose glyceraldehyde-3-phosphate to yield the 6-carbon fructose-6-phosphate.

The flow of intermediates containing 15 C atoms through Pentose Phosphate Pathway reactions by which 5-C sugars are converted to 3-C and 6-C sugars is summarized in the diagram. at right and balance sheet below.

Glucose-6-phosphate may be regenerated from either the 3-C product glyceraldehyde-3-phosphate or the 6-C product fructose-6-phosphate, via enzymes of Gluconeogenesis.

In the diagram at right, IS = Isomerase, EP = Epimerase, TK = Transketolase, TA = Transaldolase

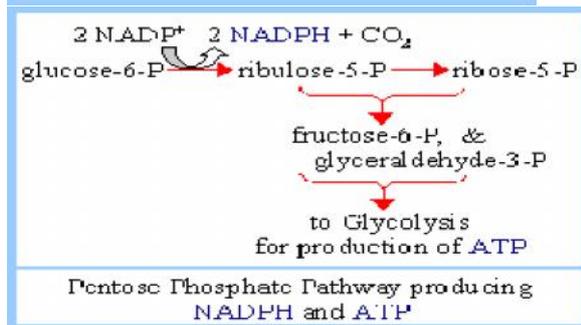
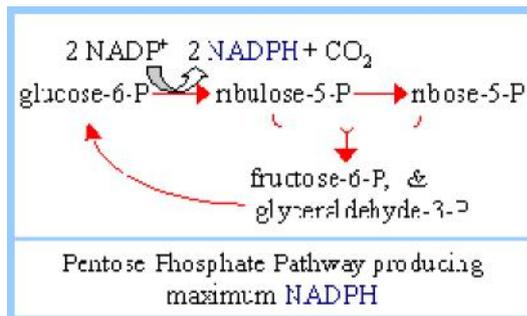


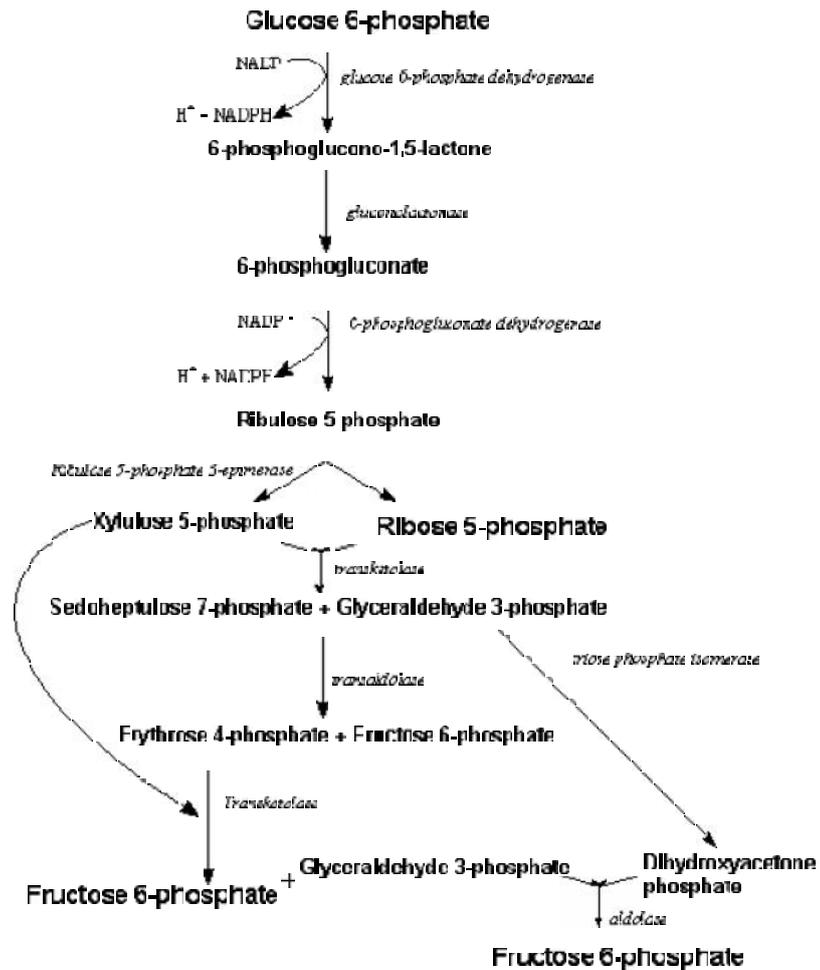
Depending on relative needs of a cell for **ribose-5-phosphate**, **NADPH**, and **ATP**, the Pentose Phosphate Pathway can operate in various modes, to maximize different products. There are three major scenarios:

1. Ribulose-5-phosphate may be converted to **ribose-5-phosphate**, a substrate for synthesis of nucleotides and nucleic acids. The pathway also produces some NADPH.

2. Glyceraldehyde-3-phosphate and fructose-6-phosphate, formed from the 5-carbon sugar phosphates, may be converted to glucose-6-phosphate for reentry into the linear portion of the Pentose Phosphate Pathway, maximizing formation of **NADPH**.

3. Glyceraldehyde-3-phosphate and fructose-6-phosphate, formed from the 5-carbon sugar phosphates, may enter Glycolysis, for synthesis of **ATP**. The pathway also produces some NADPH.





## 2.2.4 Glycogenesis

### Biosynthesis of Glycogen:

The goal of glycolysis, glycogenolysis, and the citric acid cycle is to conserve energy as ATP from the catabolism of carbohydrates. If the cells have sufficient supplies of ATP, then these pathways and cycles are inhibited. Under these conditions of excess ATP, the liver will attempt to convert a variety of excess molecules into glucose and/or glycogen.

### Glycogenesis:

Glycogenesis is the formation of glycogen from glucose. Glycogen is synthesized depending on the demand for glucose and ATP (energy). If both are present in relatively high amounts, then the excess of insulin promotes the glucose conversion into glycogen for storage in liver and muscle cells.

In the synthesis of glycogen, one ATP is required per glucose incorporated into the polymeric branched structure of glycogen. actually, glucose-6-phosphate is the cross-roads compound. Glucose-6-phosphate is synthesized directly from glucose or as the end product of gluconeogenesis.

Glycogenesis is the process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage. This process is activated during rest periods following the Cori cycle, in the liver, and also activated by insulin in response to high glucose levels, for example after a carbohydrate-containing meal<sup>[5]</sup>.

### **Steps of Glycogenesis**

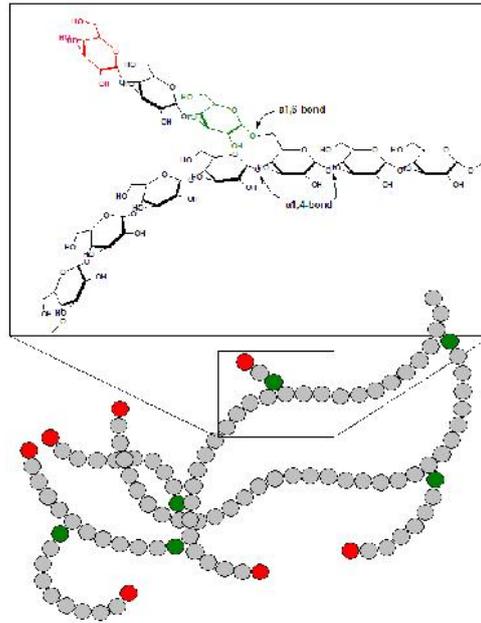
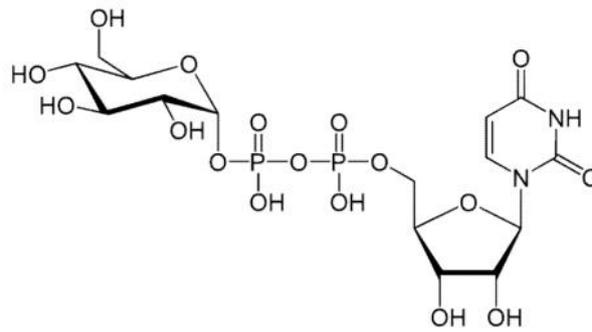
Glucose is converted into glucose-6-phosphate by the action of glucokinase or hexokinase.

Glucose-6-phosphate is converted into glucose-1-phosphate by the action of Phosphoglucomutase, passing through an obligatory intermediate step of glucose-1,6-bisphosphate. Phosphoglucomutase is an enzyme that transfers a phosphate group on a glucose monomer from the 1' to the 6' position in the forward direction or the 6' to the 1' position in the reverse direction. To be specific, it facilitates the interconversion of glucose 1-phosphate and glucose 6-phosphate. Phosphoglucomutase also acts in the opposite fashion when a large concentration of glucose-6-phosphate is present. In this case, it is the 1-carbon that is phosphorylated and the 6-carbon that is dephosphorylated. The resulting glucose-1-phosphate is then changed into UDP-glucose in a number of intermediate steps. If activated by insulin, glycogen synthase will proceed to clip the glucose from the UDP-glucose complex and on to the glycogen molecule.

Glucose-1-phosphate is converted into UDP-glucose by the action of Uridyl Transferase (also called UDP-glucose pyrophosphorylase) and pyrophosphate is formed, which is hydrolyzed by pyrophosphatase into 2 molecules of Pi.

UTP—glucose-1-phosphate uridylyltransferase also known as glucose-1-phosphate uridylyltransferase (or UDP—glucose pyrophosphorylase) is an enzyme associated with glycogenesis. It synthesizes UDP-glucose from glucose-1-phosphate and UTP; i.e.,

glucose-1-phosphate + UTP -- UDP-glucose + pyrophosphate



### Control and regulation

Glucose molecules are assembled in a chain by glycogen synthase, which must act on a pre-existing glycogen primer or glycogenin (small protein that forms the primer). The mechanism for joining glucose units is that glycogen synthase binds to UDPG, causing it to break down into an oxonium ion, also formed in glycogenolysis. This oxonium ion can readily add to the 4-hydroxyl group of a glucosyl residue on the 4 end of the glycogen chain. Branches are made by branching enzyme (also known as **amylo- (1:4)-> (1:6)transglycosylase**), which transfers the end of the chain onto an earlier part via  $\alpha$ -1:6 glucosidic bond, forming branches, which further grow by addition of more  $\alpha$ -1:4 glucosidic units.

Glycogenesis responds to hormonal control. One of the main forms of control is the varied phosphorylation of glycogen synthase and glycogen phosphorylase. This is regulated by enzymes under the control of hormonal activity, which is in turn regulated by many factors. As such, there are many different possible effectors when compared to allosteric systems of regulation.

### **Epinephrine (Adrenaline)**

Glycogen phosphorylase is activated by phosphorylation, whereas glycogen synthase is inhibited. Glycogen phosphorylase is converted from its less active b form to an active a form by the enzyme phosphorylase kinase. This latter enzyme is itself activated by protein kinase A and deactivated by phosphoprotein phosphatase-1.

Protein kinase A itself is activated by the hormone adrenaline. Epinephrine binds to a receptor protein that activates adenylate cyclase. The latter enzyme causes the formation of cyclic AMP from ATP; two molecules of cyclic AMP bind to the regulatory subunit of protein kinase A, which activates it allowing the catalytic subunit of protein kinase A to dissociate from the assembly and to phosphorylate other proteins. Returning to glycogen phosphorylase, the less active form (b) can itself be activated without the conformational change. 5'AMP acts as an allosteric activator, whereas ATP is an inhibitor, as already seen with phosphofructokinase control, helping to change the rate of flux in response to energy demand. Epinephrine not only activates glycogen phosphorylase but also inhibits glycogen synthase. This amplifies the effect of activating glycogen phosphorylase. This inhibition is achieved by a similar mechanism, as protein kinase A acts to phosphorylate the enzyme, which lowers activity. This is known as co-ordinate reciprocal control. Refer to glycolysis for further information of the regulation of glycogenesis.

### **Insulin**

Insulin has an antagonistic effect to adrenaline. When insulin binds on the G protein-coupled receptor, the alpha subunit of GDP in the G protein changes to GTP and dissociates from the inhibitory beta and gamma subunits. The alpha subunit binds on adenylyl cyclase to inhibit its activity. As a result, less cAMP then less protein kinase A will be produced. Thus, glycogen synthase, one of the targets of protein kinase A, will be in non-phosphorylated form, which is the active form of glycogen synthase. Active glycogen synthase can decrease the blood glucose level after a full meal

### **Calcium ions**

Calcium ions or cyclic AMP (cAMP) act as secondary messengers. This is an example of negative control. The calcium ions activate phosphorylase kinase. This activates glycogen phosphorylase and inhibits glycogen synthase.

## **Glycogen branching enzyme**

A glycogen branching enzyme is an enzyme that takes part in converting glucose to glycogen. It adds branches to the growing glycogen molecule. Glycogen is a branching polymer of large numbers of glucose units linked together. The structure is based on chains of glucose units with linkages between carbon atoms 1 and 4 of each pair of units (alpha 1, 4 linkages). These linkages are catalyzed by the enzyme glycogen synthase. Every 10 to 14 glucose units a side branch with an additional chain of glucose units occurs.

The side chain attaches at carbon atom 6 of a glucose unit, and the linkage is termed an alpha-1,6 glycosidic bond. To form this connection a separate enzyme known as a branching enzyme is used. A branching enzyme attaches a string of seven glucose units to the sixth carbon of a glucose unit, usually in an interior location of the glycogen molecule.

This enzyme belongs to the family of transferases, to be specific, those glycosyltransferases that transfer hexoses (hexosyltransferases). The systematic name of this enzyme class is 1,4-alpha-D-glucan:1,4-alpha-D-glucan 6-alpha-D-(1,4-alpha-D-glucano)-transferase. Other names in common use include branching enzyme, amylo-(1,4 1,6)-transglycosylase, Q-enzyme, alpha-glucan-branching glycosyltransferase, amylose isomerase, enzymatic branching factor, branching glycosyltransferase, enzyme Q, glucosan transglycosylase, 1,4-alpha-glucan branching enzyme, plant branching enzyme, alpha-1,4-glucan:alpha-1,4-glucan-6-glycosyltransferase, and starch branching enzyme. This enzyme participates in starch and sucrose metabolism.

### **2.2.4 Glycogenolysis**

In glycogenolysis, glycogen stored in the liver and muscles, is converted first to glucose-1-phosphate and then into glucose-6-phosphate. Two hormones which control glycogenolysis are a peptide, glucagon from the pancreas and epinephrine from the adrenal glands.

Glucagon is released from the pancreas in response to low blood glucose and epinephrine is released in response to a threat or stress. Both hormones act upon enzymes to stimulate glycogen phosphorylase to begin glycogenolysis and inhibit glycogen synthetase (to stop glycogenesis).

Glycogen is a highly branched polymeric structure containing glucose as the basic monomer. First individual glucose molecules are hydrolyzed from the chain, followed by the addition of a phosphate group at C-1. In the next step the

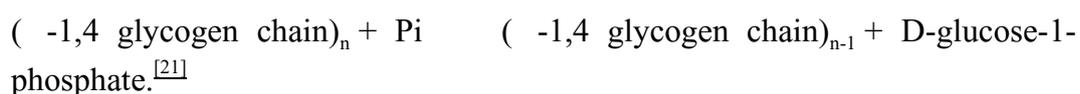
phosphate is moved to the C-6 position to give glucose 6-phosphate, a cross road compound.

Glucose-6-phosphate is the first step of the glycolysis pathway if glycogen is the carbohydrate source and further energy is needed. If energy is not immediately needed, the glucose-6-phosphate is converted to glucose for distribution in the blood to various cells such as brain cells.

Glycogen phosphorylase was the first allosteric enzyme to be discovered. This accomplishment was one of many landmark achievements made by Carl and Gerty Cori.

In mammals, the major isozymes of glycogen phosphorylase are found in muscle, liver, and brain. The brain type is predominant in adult brain and embryonic tissues, whereas the liver and muscle types are predominant in adult liver and skeletal muscle, respectively.

The overall reaction of Glycogen phosphorylase is written as:



Glycogen phosphorylase breaks up glycogen into glucose subunits. Glycogen is left with one fewer glucose molecule, and the free glucose molecule is in the form of glucose-1-phosphate. In order to be used for metabolism, it must be converted to glucose-6-phosphate by the enzyme phosphoglucomutase.

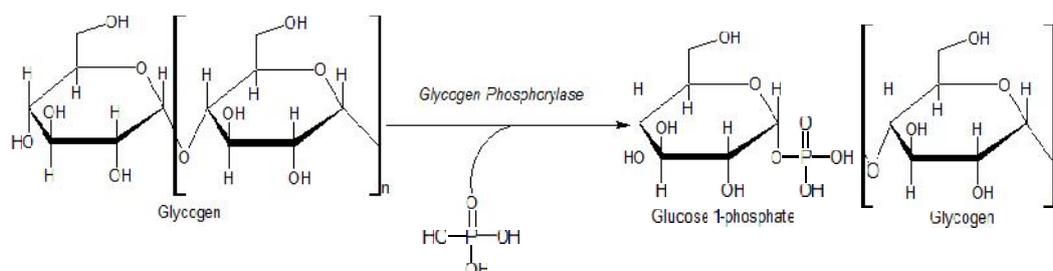
Although the reaction is reversible in solution, within the cell the enzyme only works in the forward direction as shown above because the concentration of inorganic phosphate is much higher than that of glucose-1-phosphate.

Glycogen phosphorylase can act only on linear chains of glycogen (1-4 glycosidic linkage). Its work will immediately come to a halt four residues away from 1-6 branch (which are exceedingly common in glycogen). In these situations, a debranching enzyme is necessary, which will straighten out the chain in that area. In addition, the enzyme transferase shifts a block of 3 glucosyl residues from the outer branch to the other end, and then a 1-6 glucosidase enzyme is required to break the remaining (single glucose) 1-6 residue that remains in the new linear chain. After all this is done, glycogen phosphorylase can continue.

Glycogen phosphorylase has a pyridoxal phosphate (PLP, derived from Vitamin B<sub>6</sub>) at each catalytic site. Pyridoxal phosphate links with basic

residues (in this case Lys680) and covalently forms a Schiff base. Once the Schiff base linkage is formed, holding the PLP molecule in the active site, the phosphate group on the PLP readily donates a proton to an inorganic phosphate molecule, allowing the inorganic phosphate to in turn be deprotonated by the oxygen forming the  $\alpha$ -1,4 glycosidic linkage. PLP is readily deprotonated because its negative charge is not only stabilized within the phosphate group, but also in the pyridine ring, thus the conjugate base resulting from the deprotonation of PLP is quite stable.

The protonated oxygen now represents a good leaving group, and the glycogen chain is separated from the terminal glycogen in an  $S_N1$  fashion, resulting in the formation of a glucose molecule with a secondary carbocation at the 1 position. Finally, the deprotonated inorganic phosphate acts as a nucleophile and bonds with the carbocation, resulting in the formation of glucose-1-phosphate and a glycogen chain shortened by one glucose molecule.



## 2.2.4 Gluconeogenesis

### Biosynthesis of Glucose:

**Gluconeogenesis** is the process of synthesizing glucose from non-carbohydrate sources. The starting point of gluconeogenesis is pyruvic acid, although oxaloacetic acid and dihydroxyacetone phosphate also provide entry points. Lactic acid, some amino acids from protein and glycerol from fat can be converted into glucose. Gluconeogenesis is similar but not the exact reverse of glycolysis, some of the steps are the identical in reverse direction and three of them are new ones. Without going into detail, the general gluconeogenesis sequence is given in the graphic on the left.

Notice that oxaloacetic acid is synthesized from pyruvic acid in the first step. Oxaloacetic acid is also the first compound to react with acetyl CoA in the citric acid cycle. The concentration of acetyl CoA and ATP determines the fate of oxaloacetic acid. If the concentration of acetyl CoA is low and concentration of ATP is high then gluconeogenesis proceeds. Also notice that ATP is required for a biosynthesis sequence of gluconeogenesis.

Gluconeogenesis occurs mainly in the liver with a small amount also occurring in the cortex of the kidney. Very little gluconeogenesis occurs in the brain, skeletal muscles, heart muscles or other body tissue. In fact, these organs have a high demand for glucose. Therefore, gluconeogenesis is constantly occurring in the liver to maintain the glucose level in the blood to meet these demands.

Gluconeogenesis (abbreviated GNG) is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids. It is one of the two main mechanisms humans and many other animals use to keep blood glucose levels from dropping too low (hypoglycemia). The other means of maintaining blood glucose levels is through the degradation of glycogen (glycogenolysis). Gluconeogenesis is a ubiquitous process, present in plants, animals, fungi, bacteria, and other microorganisms. In animals, gluconeogenesis takes place mainly in the liver and, to a lesser extent, in the cortex of kidneys. This process occurs during periods of fasting, starvation, low-carbohydrate diets, or intense exercise and is highly endergonic. For example, the pathway leading from phosphoenolpyruvate to glucose-6-phosphate requires 6 molecules of ATP. Gluconeogenesis is often associated with ketosis. Gluconeogenesis is also a target of therapy for type II diabetes, such as metformin, which inhibits glucose formation and stimulates glucose uptake by cells.

Lactate is transported back to the liver where it is converted into pyruvate by the Cori cycle using the enzyme lactate dehydrogenase. Pyruvate, the first designated substrate of the gluconeogenic pathway, can then be used to generate glucose. All citric acid cycle intermediates, through conversion to oxaloacetate, amino acids other than lysine or leucine, and glycerol can also function as substrates for gluconeogenesis.

Transamination or deamination of amino acids facilitates entering of their carbon skeleton into the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle. Whether fatty acids can be converted into glucose in animals has been a longstanding question in biochemistry. It is known that odd-chain fatty acids can be oxidized to yield propionyl CoA, a precursor for succinyl CoA, which can be converted to pyruvate and enter into gluconeogenesis. In plants, to be specific, in seedlings, the glyoxylate cycle can be used to convert fatty acids (acetate) into the primary carbon source of the organism. The glyoxylate cycle produces four-carbon dicarboxylic acids that can enter gluconeogenesis. In 1995, researchers identified the glyoxylate cycle in nematodes. In addition, the glyoxylate enzymes malate synthase and

isocitrate lyase have been found in animal tissues. Genes coding for malate synthase gene have been identified in other [metazoans] including arthropods, echinoderms, and even some vertebrates. Mammals found to possess these genes include monotremes (platypus) and marsupials (opossum) but not placental mammals.

Genes for isocitrate lyase are found only in nematodes, in which, it is apparent, they originated in horizontal gene transfer from bacteria. The existence of glyoxylate cycles in humans has not been established, and it is widely held that fatty acids cannot be converted to glucose in humans directly. However, carbon-14 has been shown to end up in glucose when it is supplied in fatty acids. Despite these findings, it is considered unlikely that the 2-carbon acetyl-CoA derived from the oxidation of fatty acids would produce a net yield of glucose via the citric acid cycle. However, it is possible that, with additional sources of carbon via other pathways, glucose could be synthesized from acetyl-CoA. In fact, it is known that Ketone bodies,  $\beta$ -hydroxybutyrate in particular, can be converted to glucose at least in small amounts ( $\beta$ -hydroxybutyrate to acetoacetate to acetone to propanediol to pyruvate to glucose). Glycerol, which is a part of the triacylglycerol molecule, can be used in gluconeogenesis. In humans, gluconeogenesis is restricted to the liver and to a lesser extent the kidney. In all species, the formation of oxaloacetate from pyruvate and TCA cycle intermediates is restricted to the mitochondrion, and the enzymes that convert PEP to glucose are found in the cytosol. The location of the enzyme that links these two parts of gluconeogenesis by converting oxaloacetate to PEP, PEP carboxykinase, is variable by species: it can be found entirely within the mitochondria, entirely within the cytosol, or dispersed evenly between the two, as it is in humans. Transport of PEP across the mitochondrial membrane is accomplished by dedicated transport proteins; however no such proteins exist for oxaloacetate. Therefore species that lack intra-mitochondrial PEP, oxaloacetate must be converted into malate or aspartate, exported from the mitochondrion, and converted back into oxaloacetate in order to allow gluconeogenesis to continue.

Gluconeogenesis is a pathway consisting of eleven enzyme-catalyzed reactions. The pathway can begin in the mitochondria or cytoplasm, depending on the substrate being used. Many of the reactions are the reversible steps found in glycolysis.

Gluconeogenesis begins in the mitochondria with the formation of oxaloacetate through carboxylation of pyruvate. This reaction also requires one molecule of ATP, and is catalyzed by pyruvate carboxylase. This enzyme is stimulated by high levels of acetyl-CoA (produced in  $\beta$ -oxidation in the liver) and inhibited by high levels of ADP. Oxaloacetate is reduced to malate using NADH, a step required for transport out of the mitochondria.

Malate is oxidized to oxaloacetate using NAD<sup>+</sup> in the cytoplasm, where the remaining steps of gluconeogenesis occur.

Oxaloacetate is decarboxylated and phosphorylated to produce phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. One molecule of GTP is hydrolyzed to GDP during this reaction. The next steps in the reaction are the same as reversed glycolysis. However, fructose-1,6-bisphosphatase converts fructose-1,6-bisphosphate to fructose 6-phosphate, requiring one water molecule and releasing one phosphate. This is also the rate-limiting step of gluconeogenesis.

Glucose-6-phosphate is formed from fructose 6-phosphate by phosphoglucosomerase. Glucose-6-phosphate can be used in other metabolic pathways or dephosphorylated to free glucose. Whereas free glucose can easily diffuse in and out of the cell, the phosphorylated form (glucose-6-phosphate) is locked in the cell, a mechanism by which intracellular glucose levels are controlled by cells.

The final reaction of gluconeogenesis, the formation of glucose, occurs in the lumen of the endoplasmic reticulum, where glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase to produce glucose. Glucose is shuttled into the cytosol by glucose transporters located in the membrane of the endoplasmic reticulum.

While most steps in gluconeogenesis are the reverse of those found in glycolysis, three regulated and strongly exergonic reactions are replaced with more kinetically favorable reactions. Hexokinase/glucokinase, phosphofruktokinase, and pyruvate kinase enzymes of glycolysis are replaced with glucose-6-phosphatase, fructose-1,6-bisphosphatase, and PEP carboxykinase. This system of reciprocal control allow glycolysis and gluconeogenesis to inhibit each other and prevent the formation of a futile cycle. The majority of the enzymes responsible for gluconeogenesis are found in the cytoplasm; the exceptions are mitochondrial pyruvate carboxylase and, in animals, phosphoenolpyruvate carboxykinase. The latter exists as an isozyme

located in both the mitochondrion and the cytosol. The rate of gluconeogenesis is ultimately controlled by the action of a key enzyme, fructose-1,6-bisphosphatase, which is also regulated through signal transduction by cAMP and its phosphorylation. Most factors that regulate the activity of the gluconeogenesis pathway do so by inhibiting the activity or expression of key enzymes. However, both acetyl CoA and citrate activate gluconeogenesis enzymes (pyruvate carboxylase and fructose-1,6-bisphosphatase, respectively).

Due to the reciprocal control of the cycle, acetyl-CoA and citrate also have inhibitory roles in the activity of pyruvate kinase.

### **Pyruvate carboxylase**

During gluconeogenesis, pyruvate carboxylase is involved in the synthesis of phosphoenolpyruvate (PEP) from pyruvate. Pyruvate is first converted by pyruvate carboxylase to oxaloacetate (OAA) in the mitochondrion requiring hydrolysis of one molecule of ATP. The OAA is then decarboxylated and simultaneously phosphorylated, which is catalyzed by one of two isoforms of phosphoenolpyruvate carboxykinase (PEPCK) either in the cytosol or in the mitochondria to produce PEP. Under ordinary gluconeogenic condition, OAA is converted into PEP by mitochondrial PEPCK; the resultant PEP is then transported out of the mitochondria via the Citric acid cycle carrier system, and converted into glucose by cytosolic gluconeogenic enzymes. However, during starvation when cytosolic NADH concentration is low and mitochondrial NADH levels are high oxaloacetate can be used as a shuttle of reducing equivalents. As such OAA is converted into malate by mitochondrial Malate dehydrogenase (MDH). After export into the cytosol, malate is converted back into OAA, with concomitant reduction of NAD<sup>+</sup>; OAA is subsequently converted to PEP which is available for gluconeogenesis in the cytosol along with the transported reducing equivalent NADH. Very high levels of PC activity, together with high activities of other gluconeogenic enzymes including PEPCK, fructose-1,6-bisphosphatase and glucose-6-phosphatase in liver and kidney cortex, suggest that a primary role of PC is to participate in gluconeogenesis in these organs. During fasting or starvation when endogenous glucose is required for certain tissues (brain, white blood cells and kidney medulla), expression of PC and other gluconeogenic enzymes is elevated. In rats and mice, alteration of nutrition status has been shown to affect hepatic PC activity. Fasting promotes hepatic glucose production sustained by an increased pyruvate flux, and increases in PC activity and protein concentration; Diabetes similarly increases gluconeogenesis through enhanced uptake of substrate and

increased flux through liver PC in mice and rats. Similarly to other gluconeogenic enzymes, PC is positively regulated by glucagon and glucocorticoids while negatively regulated by insulin. Further supporting the key role of PC in gluconeogenesis, in dairy cattle, which have hexose absorption ability at adequate nutrition levels, PC and the associated gluconeogenic enzyme PEPCK are markedly elevated during the transition to lactation in proposed support of lactose synthesis for milk production.

Aside from the role of PC in gluconeogenesis, PC serves an anaplerotic role (an enzyme catalyzed reaction that can replenish the supply of intermediates in the citric acid cycle) for the tricarboxylic acid cycle (essential to provide oxaloacetate), when intermediates are removed for different biosynthetic purposes.

### **Phosphoenolpyruvate carboxykinase (PEPCK)**

Phosphoenolpyruvate carboxykinase (PEPCK) is an enzyme in the lyase family used in the metabolic pathway of gluconeogenesis. It converts oxaloacetate into phosphoenolpyruvate and carbon dioxide. It is found in two forms, cytosolic and mitochondrial. It has been shown that PEPCK catalyzes the rate-controlling step of gluconeogenesis, the process whereby glucose is synthesized. The enzyme has therefore been thought to be essential in glucose homeostasis, as evidenced by laboratory mice that contracted diabetes mellitus type 2 as a result of the overexpression of PEPCK. A recent study suggests that the role that PEPCK plays in gluconeogenesis may be mediated by the citric acid cycle, the activity of which was found to be directly related to PEPCK abundance. PEPCK levels alone were not found to be highly correlated with gluconeogenesis in the mouse liver, as previous studies have suggested. Therefore, the role of PEPCK in gluconeogenesis may be more complex and involve more factors than was previously believed.

### **Glucose-6-phosphate isomerase**

This gene belongs to the GPI family whose members encode multifunctional phosphoglucose isomerase proteins involved in energy pathways. The protein encoded by this gene is a dimeric enzyme that catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate.

glucose 6-phosphate  $\rightleftharpoons$  fructose 6-phosphate

The protein has different functions inside and outside the cell. In the cytoplasm, the protein is involved in glycolysis and gluconeogenesis, while outside the cell it functions as a neurotrophic factor for spinal and sensory neurons. The same

protein is also secreted by cancer cells, where it is called autocrine motility factor and stimulates metastasis. Defects in this gene are the cause of nonspherocytic hemolytic anemia and a severe enzyme deficiency can be associated with hydrops fetalis, immediate neonatal death and neurological impairment.

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## 2.3 Protein metabolism

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Nitrogen metabolism is no less important than carbohydrate and lipid metabolism. Proteins make up the structural tissue for muscles and tendons, transport oxygen or hemoglobin, catalyze all biochemical reactions as enzymes, and regulate reactions as hormones. Our bodies must be able to synthesize the many proteins, amino acids, and other non-protein nitrogen containing compounds needed for growth, replacement, and repair. Proteins in excess are used to supply energy or build reserves of glucose, glycogen, or lipids.

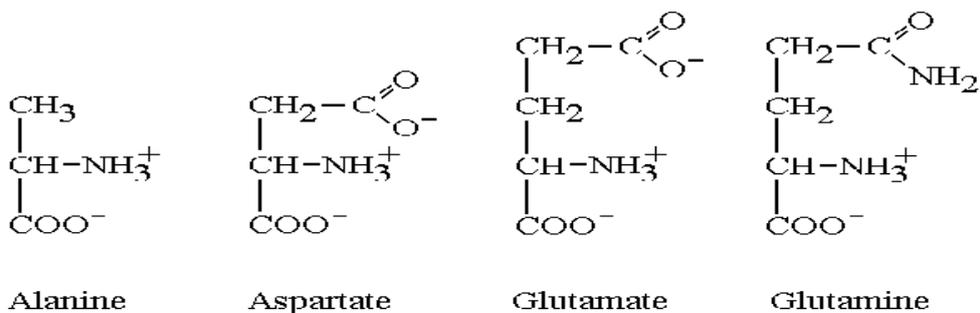
Protein metabolism denotes the various biochemical processes responsible for the synthesis of proteins and amino acids, and the breakdown of proteins (and other large molecules, too) by catabolism. Dietary proteins are first broken down to individual amino acids by various enzymes and hydrochloric acid present in the gastro-intestinal tract.<sup>[1]</sup> These amino acids are further broken down to  $\alpha$ -keto acids which can be recycled in the body for generation of energy, and production of glucose or fat or other amino acids. This break-down of amino acids to  $\alpha$ -keto acids occurs in the liver by a process known as transamination, which follows a bimolecular ping pong mechanism.

Protein digestion is largely completed in the small intestine at a slightly alkaline pH. The pancreatic proteases trypsin, chymotrypsin and elastase divide the proteins into short peptides. These are attacked from both ends by aminopeptidase and carboxypeptidase, and the fragments are finished off by dipeptidases secreted from the gut wall. Amino acid uptake from the gut lumen into enterocytes is driven by the sodium gradient. There is a relatively high sodium concentration in the gut (regardless of dietary intake, as a result of the pancreatic secretion of sodium bicarbonate) and a low concentration in the enterocytes, as a result of the sodium pump in the basolateral membrane. A multiplicity of sodium-linked amino acid carriers operate within the intestinal brush border, balanced by sodium-independent export carriers on the serosal surface (i.e. the opposite side) of the cells.

## Central role of glutamate

Four of the amino acids: glutamate, aspartate, alanine and glutamine are present in cells at much higher concentrations than the other 16.

All four have major metabolic functions in addition to their roles in proteins, but glutamate occupies the prime position.



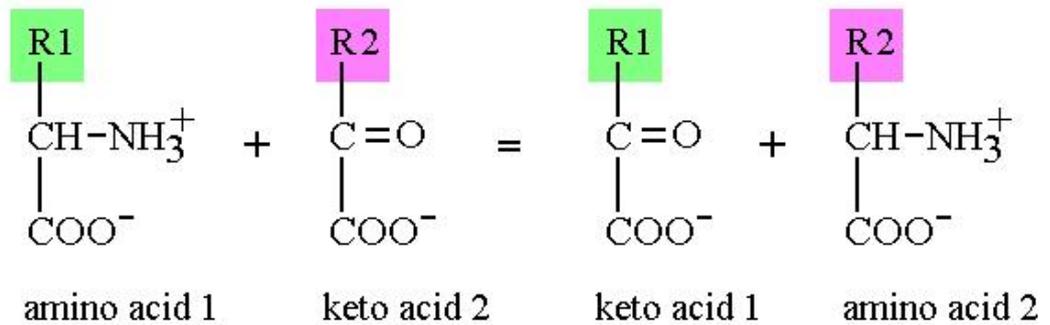
Glutamate and aspartate function as excitatory neurotransmitters in the central nervous system, and glutamate is partly responsible for the flavour of food. (It is the mono sodium glutamate listed on processed food labels.) However, glutamate also occupies a special position in amino acid breakdown, and most of the nitrogen from dietary protein is ultimately excreted from the body via the glutamate pool.

Glutamate is special because it is chemically related to 2-oxoglutarate (= alpha keto glutarate) which is a key intermediate in the citric acid (Krebs) cycle. Glutamate can be reversibly converted into oxoglutarate by transaminases or by glutamate dehydrogenase. In addition, glutamate can be reversibly converted into glutamine, an important nitrogen carrier, and the most common free amino acid in human blood plasma.

### 2.3.1 Transamination reaction

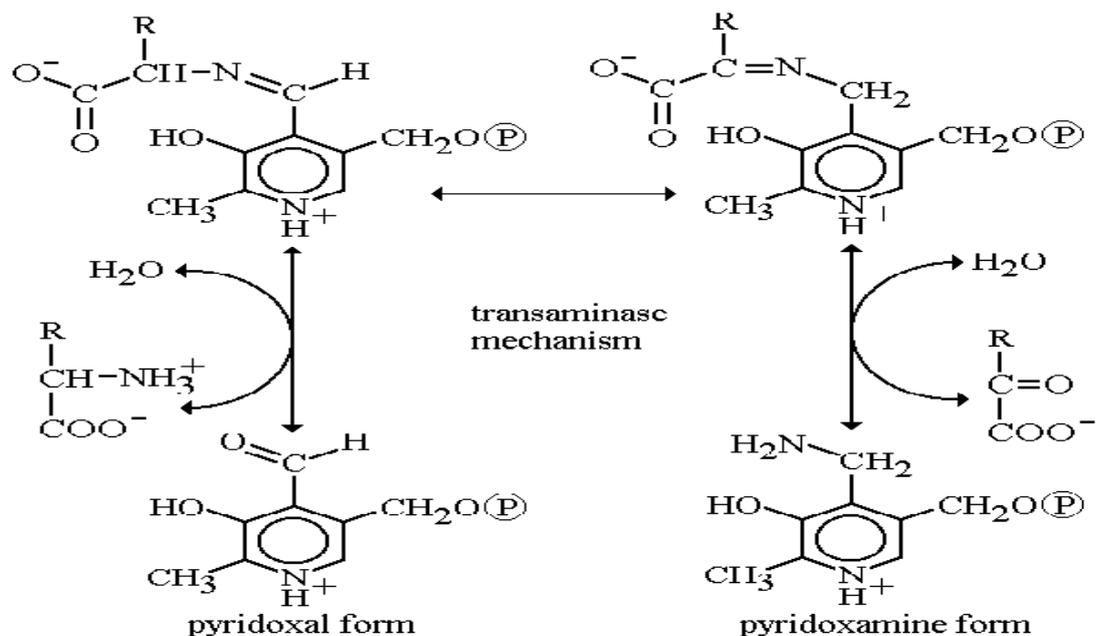
Most common amino acids can be converted into the corresponding keto acid by transamination. This reaction swaps the amino group from one amino acid to a different keto acid, thereby generating a new pairing of amino acid and keto

acid. There is no overall loss or gain of nitrogen from the system .



Transamination reactions are readily reversible, and the equilibrium constant is close to 1. One of the two pairs is almost invariably glutamate and its corresponding keto acid oxoglutarate, although there are a few exceptions to this rule. All transaminases require pyridoxal phosphate (derived from vitamin b6) as a cofactor.

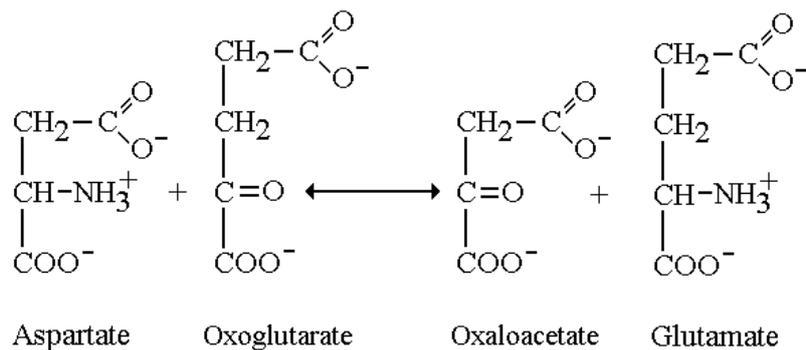
The substrates bind to the active centre one at a time, and the function of the pyridoxal phosphate is to act as a temporary store of amino groups until the next substrate comes along. In the process the pyridoxal phosphate is converted into pyridoxamine phosphate, and then back again. Enzymologists call this a "ping pong" mechanism, and it leads to a characteristic pattern in the reaction kinetics.



The condensation between the alpha amino group and the aromatic aldehyde to form a "Schiff base" makes the alpha carbon atom chemically reactive, so the isomerisation of the Schiff base takes place very easily. In practice the pyridoxal form of the coenzyme condenses with the epsilon amino group of a lysine residue in the enzyme protein when no amino acid is bound, and the free aldehyde form of the coenzyme has only a transitory existence. Many of the enzymes that metabolise amino acids require pyridoxal phosphate as a cofactor. Unexpectedly, this compound also serves in a different manner in the active centre of glycogen phosphorylase.

### 2.3.2 Glutamate:oxaloacetate transaminase [GOT]

This enzyme is also known as aspartate aminotransferase and is one of the most active enzymes in the cell. It exists in mitochondrial and cytosolic variants, and the detailed iso-enzyme pattern is tissue-specific. It escapes in large amounts from dead or dying tissues and enters the bloodstream, so GOT is often measured in blood samples for medical diagnostic purposes.



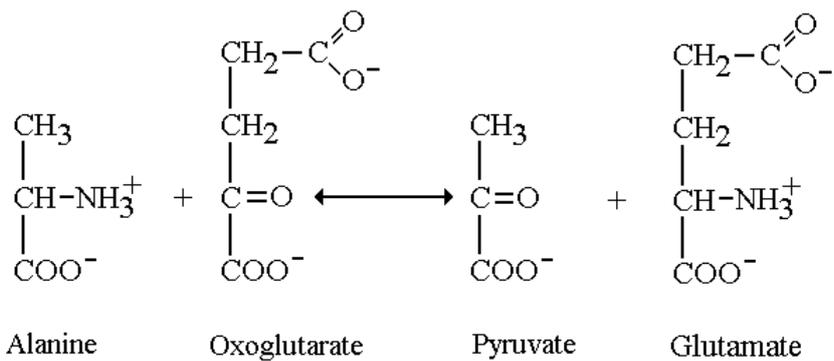
The metabolic importance of this enzyme is that it brings about a free exchange of amino groups between glutamate (which is the most common amino acid) and aspartate which is a second major amino acid pool. Glutamate and aspartate are each required for separate but essential steps in the urea cycle, which is responsible for ammonia detoxication and nitrogen excretion. The free movement of nitrogen between the glutamate and aspartate pools is an important balancing process that is vital for normal cellular metabolism.

This reaction is close to equilibrium in both the cytosol and the mitochondrial compartments. It forms an integral part of the malate - aspartate shuttle which is effectively responsible for the "transport" of NADH across the inner mitochondrial membrane.

### 2.3.3 Glutamate:pyruvate transaminase[GPT]

This very active enzyme is also known as alanine aminotransferase and exists in mitochondrial and cytosolic variants. The detailed iso-enzyme pattern is tissue-specific.

It escapes in large amounts from dead or dying tissues and GPT may be measured in blood samples for medical diagnostic purposes.

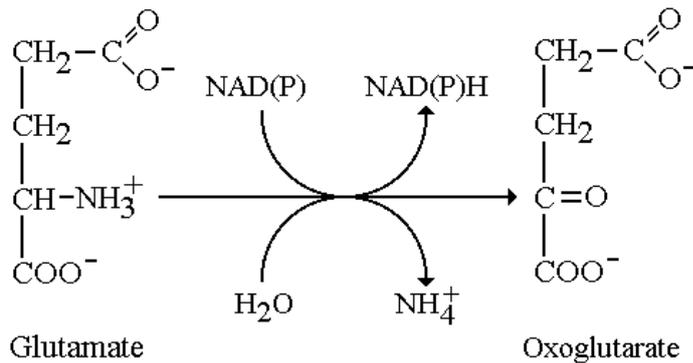


Alanine is the principal amino acid released from muscle tissue during starvation. It is an important substrate for hepatic gluconeogenesis, and alanine transamination is required for the proper maintenance of fasting blood glucose concentrations.

### 2.3.4 Glutamate:dehydrogenase[GluDH]

This enzyme is the first committed step on the final common pathway for mammalian nitrogen excretion, leading eventually to urea. A few of the amino acids have specific deamination pathways, but about 75% of ingested protein nitrogen follows the glutamate route. Glutamate dehydrogenase in mammals is almost entirely confined to the liver mitochondrial matrix space, where it accounts for a significant proportion of the total protein. In contrast to the transamination reactions which merely swap amino groups from one compound to another, GluDH catalyses a net loss of nitrogen from the amino acid pool. The process is therefore termed "oxidative deamination". It is the only common dehydrogenase which is non-specific for

NAD or NADP, and this may be important for its overall regulation.



NADH / NAD and NADPH / NADP have the same standard redox potential of -420mV when the oxidised and reduced forms are present in equal concentrations. In practice these coenzymes have different effective redox potentials and perform specialised functions within cells. The NADPH / NADP pool operates almost entirely in the reduced form, but the NADH / NAD pool is rarely more than 30% reduced. In general NADPH is used to drive reductive biosynthetic reactions, whereas NAD is the coenzyme for the oxidative energy-yielding pathways.

The dual coenzyme specificity is a potential source of difficulty for the cell, since in theory this readily reversible enzyme could catalyse a futile cycle, proceeding first in the oxidative direction with NAD, followed by a reductive step using NADPH. The effect would be to "short circuit" the two coenzyme pools, which normally require considerable investment in substrates and cellular equipment to keep them separate. If this futile cycle happens to any significant extent then it must be an *advantage* for the cell, because it has persisted unchanged for 2,000,000,000 years of evolutionary development.

The most likely explanation at present is that this futile cycle takes place, but for various reasons it does not place an excessive burden on its owner. The  $K_m$  of GluDH for ammonia is quite high, and the free ammonia concentration is kept very low by the next enzyme in the pathway, carbamyl phosphate synthetase. This will severely reduce the rate of the synthetic reaction, and allow the enzyme to catalyse a net glutamate oxidation at a slow controlled rate that provides the maximum opportunity for regulatory interference.

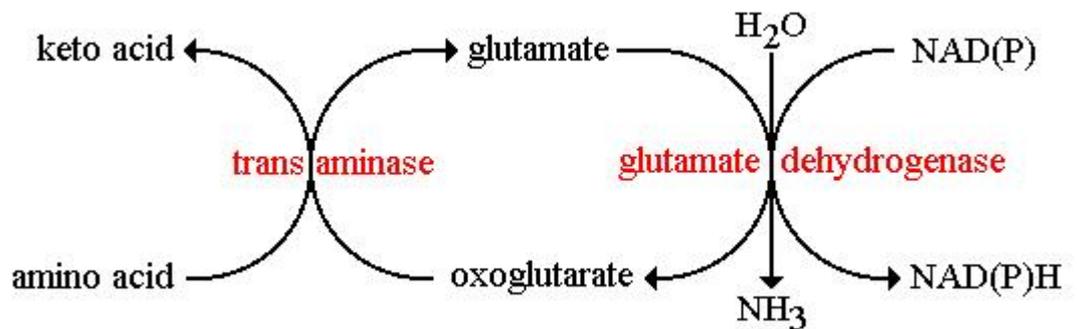
Regulation is plainly critical at this point, since GluDH and carbamyl phosphate synthetase jointly control the overall rate of nitrogen excretion and determine whether a particular individual will be in positive, neutral or negative nitrogen

balance. Control of unwanted nitrogen losses remains an important unsolved problem after major surgery, burns or other serious traumatic injuries.

The enzyme is indeed modulated by adenine and guanine nucleotides, although it is difficult to make much sense of the observed effects. GluDH has all the hallmarks of a large multimeric allosteric enzyme, although the true nature of the regulation remains to be identified. The situation is in some ways similar to the parallel NAD and NADP linked oxidation pathways for malate and isocitrate, although the competing reactions for these substrates are separately regulated and catalysed by different proteins.

### 2.3.5 Trans-deamination

Most transaminases share a common substrate and product (glutamate and oxoglutarate) with glutamate dehydrogenase, and this permits a combined nitrogen excretion pathway for individual amino acids that is commonly described as "trans-deamination".



This process underlines the central role of glutamate in the overall control of nitrogen metabolism.

### 2.3.6 Urea Cycle

Ammonium ions are in equilibrium with about 1% free ammonia at physiological pH. Ammonium salts are toxic compounds, causing vomiting, convulsions and ultimately coma and death when the blood concentration exceeds approximately 0.25mM. It is not entirely clear why this should be so: it may be that ammonium ions mimic potassium ions, but gain access as uncharged ammonia to areas from which they should be excluded. Alternatively, they may favour the synthesis of excessive amounts of glutamate and glutamine which have excitatory effect on the neural tissue. It is therefore necessary to have an efficient means to remove ammonia from the body. Water-living species commonly excrete free ammonia through their

gills [ammonotelism], but this easy option is not available to land dwellers which produce a variety of less toxic nitrogenous end products.

Urea synthesis and excretion [ureotelism] first evolved in lungfish and primitive amphibia about 400 million years ago. The process is replicated today when ammonotelic tadpoles leave the water and metamorphose into ureotelic frogs. Urea is also used in humans, and in all placental mammals, which start to express the urea cycle genes around the time of birth. Urea is very soluble, but still requires appreciable quantities of water for its removal via the kidneys. This imposes a minimum daily water requirement and limits the range of environments that these species can exploit.

Urea is not the only possible solution to the problem: spiders excrete guanine, which packs no less than 5 surplus nitrogen atoms into a single small molecule, while reptiles and birds excrete mainly uric acid [uricotelism]. Uric acid is an extremely insoluble purine compound that readily forms supersaturated solutions. This has been turned to advantage in uricotelic species, which can survive in extremely arid environments. They regurgitate concentrated urine, supersaturated with uric acid, from the cloaca into the hindgut where the uric acid crystallises and the residual water is resorbed. The uric acid forms the fine pasty mass of white crystals that is familiar to us in bird droppings.

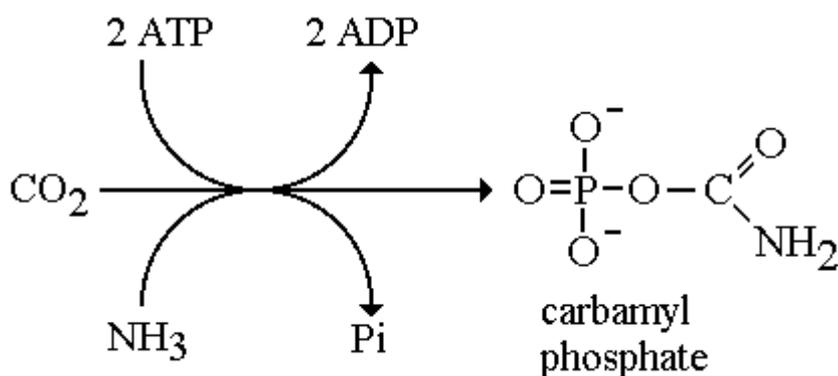
Uricotelism is also an advantage to animals that lay shelled eggs, which of necessity have a zero water intake. The uric acid crystallises within the allantois, part of which eventually becomes incorporated into the lower gut as the embryo develops. In humans the insolubility of uric acid is a considerable nuisance, since it gives rise to the extremely painful deposits of small crystals [called "tophi"] within the joints of patients suffering from gout.

Urea is synthesised via the urea cycle, which is confined to mammalian liver. Individual enzymes from the urea cycle are present in other tissues, and may be important for arginine biosynthesis, but the complete cycle does not occur. Extra-hepatic tissues export their surplus nitrogen to the liver by other routes, principally as the amino acids alanine and glutamine. In addition, the cleavage of arginine by nitric oxide synthetase generates citrulline, which is a urea cycle intermediate. Citrulline is recycled to arginine, and in tissues which use the nitric oxide signalling system the relevant urea cycle enzymes have sufficient activity to maintain cellular arginine supplies.

The urea cycle takes place partly in the cytosol and partly in the mitochondria, and the individual reactions are as follows.

### carbamyl phosphate synthetase 1 [CPS1]

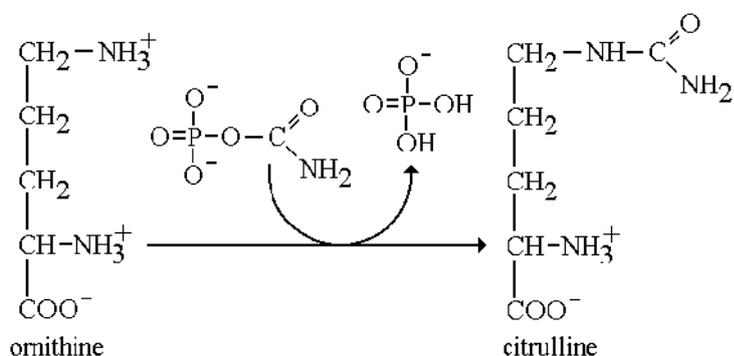
This mitochondrial enzyme converts the ammonia produced by glutamate dehydrogenase into carbamyl phosphate (=carbamoyl phosphate) which is an unstable high energy compound. It is the mixed acid anhydride of carbamic acid and phosphoric acid, and requires two molecules of ATP to drive its synthesis.



CPS1 is strongly activated by N-acetyl glutamate, which controls the overall rate of urea production. This bizarre method of regulation is not fully understood: N-acetyl glutamate is an intermediate in the bacterial synthesis of ornithine, but this feature has been lost from mammals and only the regulatory system has survived. There is a futile cycle catalysed by the enzymes N-acetylglutamate synthetase and N-acetylglutamate hydrolase. This is plainly important for the control of nitrogen metabolism, but we do not yet know how it works.

### ornithine transcarbamylase [OTCase]

The next reaction also takes place in the liver mitochondrial matrix space, where ornithine is converted into citrulline.



### ornithine and citrulline porters

The remainder of the urea cycle takes place in the cytosol. This requires the continuous export of citrulline and the uptake of ornithine across the inner

mitochondrial membrane. These processes are catalysed by specific amino acid porters, which are present only in liver mitochondria. A very rare deficiency state has been described.

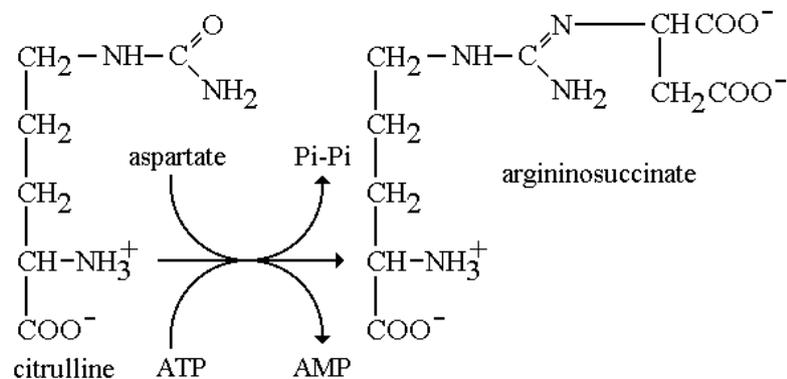
### Glutamate and glutamate:aspartate porters

Urea production requires continuous mitochondrial glutamate uptake, to replenish the substrate for the glutamate dehydrogenase reaction. This process is catalysed by a specific electroneutral glutamate / hydroxyl antiporter, which is largely confined to liver mitochondria.

In addition, depending on the diet, mitochondria may also need to export aspartate in exchange for glutamate in order to balance the supplies of nitrogen to the mitochondrial and cytosolic segments of the urea cycle. This electrical process is driven by the mitochondrial membrane potential and is discussed more fully in connection with the malate - aspartate cycle.

### arginino-succinate synthetase

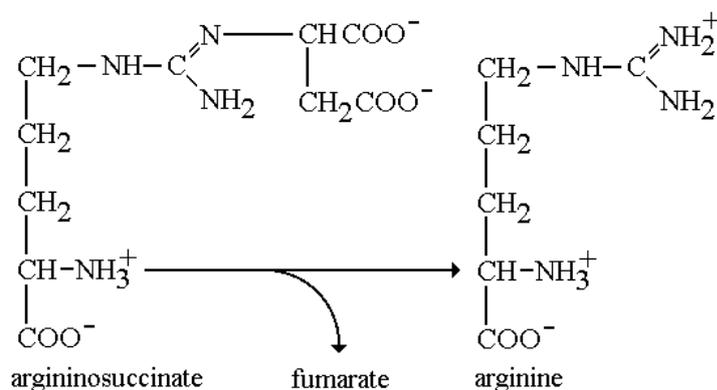
Once in the cytosol, citrulline condenses with aspartate and the reaction is driven by ATP. In this way aspartate contributes the second nitrogen atom to urea, the first having come from glutamate.



Production of arginino-succinate is an energetically expensive process, since the ATP is split to AMP and pyrophosphate. The pyrophosphate is then cleaved to inorganic phosphate using pyrophosphatase, so the overall reaction costs two equivalents of high energy phosphate per mole.

### arginino-succinate lyase

Elimination of fumarate from arginino-succinate then yields arginine.

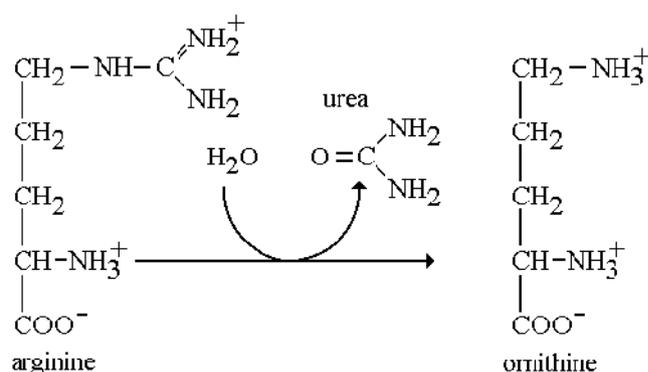


This reaction sequence is very similar to the conversion of IMP to AMP in the purine biosynthetic pathway. In each case fumarate is formed as a by-product. Fumarate is not transported by mitochondria, so this requires the presence of cytosolic fumarase to form malate.



The reaction is readily reversible, and the equilibrium slightly favours malate. The cytosolic and mitochondrial fumarase isoenzymes are extremely similar and derived from the same gene through alternative mRNA splicing reactions.

Cleavage of arginine by arginase to produce urea regenerates ornithine, which is then available for another round of the cycle.



### Nitric Oxide

In addition to its metabolic functions in the urea cycle, arginine is also the immediate precursor for nitric oxide [NO], an important signalling molecule involved in the local regulation of blood flow. Nitric oxide synthase uses



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## 2.4 Lipid metabolism

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### Oxidation of Long-Chain Fatty Acids

ACTIVATION OF FATTY ACIDS: Fats are delivered to cells as free fats. They must be activated before they can be burned.

- **Acyl-CoA Synthetase:** Free Fat -----> Acyl-CoA Thioester, which has a high-energy bond.
  - ATP is required in the synthesis.
  - This step is fully reversible, as ATP and the Acyl-CoA Thioester product both have equivalent energy levels.

TRANSLOCATION OF FATTY ACYL-CoA THIOESTER: The Acyl-CoA must get into the mitochondrial matrix.

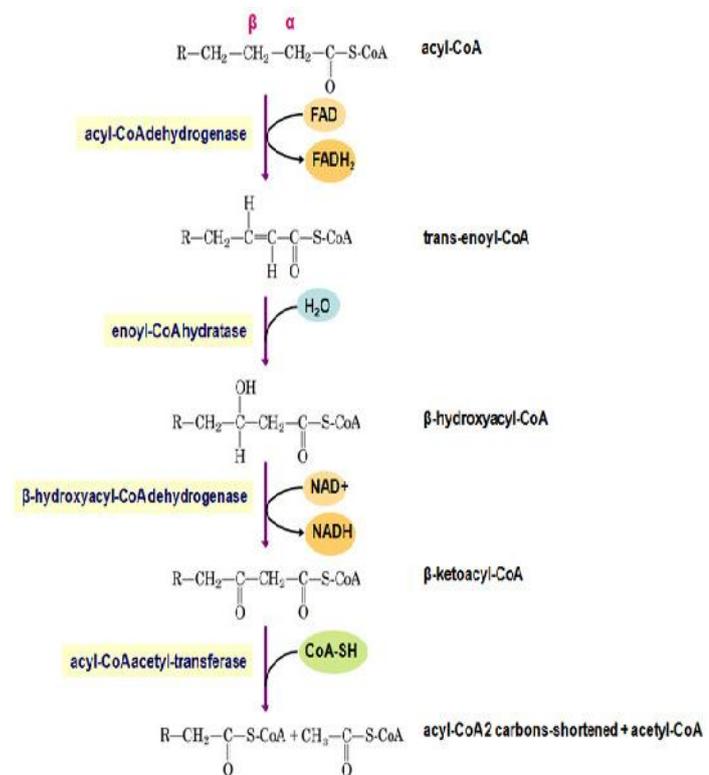
- Once activated, the Acyl-CoA can get through the out mitochondrial membrane by traversing through a Porin protein.
- Carnitine Intermediate: Only Long-chain fatty acids are converted to carnitine as an intermediate. Short-chain fats can traverse the inner membrane directly:
  - Intermembrane space have an enzyme i.e **Carnitine Acyl Transferase I** which converts Acyl-CoA -----> Acyl Carnitine.
  - Translocase: Only recognizes Acyl-Carnitine. It translocated the carnitine structure through the inner membrane to the matrix.
  - Mitochondrial matrix contains another enzyme : **Carnitine Acyl Transferase II**: which converts Acyl-Carnitine -----> Acyl-CoA
  - In the matrix the fat is esterified back to Coenzyme-A.

#### 2.4.1 beta- Oxidation

beta-OXIDATION: A four-step process. Called beta-Oxidation because most of the chemistry occurs on the beta-Carbon (beta to the carbonyl) per turn of the cycle.

- The Four Steps: Ultimately we are oxidizing the beta-Carbon from most reduced to most oxidized state.
  - OXIDATION: Acyl-CoA Dehydrogenase catalyzes an elimination of hydrogens on the alpha-carbon, to create the alpha,beta-Unsaturated Acyl-CoA.

- FAD -----> FADH<sub>2</sub> is the corresponding reduction.
- HYDRATION: Add water across the double bond, creating an OH group on the beta-Carbon.
- OXIDATION: Oxidize the OH group to a carbonyl function. Now we have a beta-keto-acid
  - NAD<sup>+</sup> -----> NADH is the corresponding reduction.
- THIOLYSIS: Cut the end-acid off, and add CoA to the newly created keto-group.
  - An additional mole of Coenzyme-A is esterified to the beta-Keto function, leaving Acetyl-CoA and an Acyl-CoA of two less carbons



- The Ultimate Products: Every cycle of beta-Oxidation (1) reduces the fat-chain by two carbons and (2) yields a free Acetyl-CoA (which can then be further metabolized as directed).
  - Acyl-CoA<sub>(n-2)</sub>
  - Acetyl-CoA
- Odd Chain Fats: Most fats are even-numbered. But beta-Oxidation can occur with odd chains, at which point the products are Acetyl-CoA (2C) and

Propanoyl-CoA (3C). Propanoyl-CoA is then metabolized by a different mechanism.

#### **ENERGETICS OF beta-OXIDATION:**

- COST: -2 ATP, but we only have to invest that once!
  - -1 ATP for the Acyl-CoA Ligase
  - -1 ATP net for the Pyrophosphatase, since we actually end up with AMP.
- BENEFIT: Per turn of beta-Oxidation (i.e. per two carbons).
  - Acetyl-CoA +12 ATP
  - FADH<sub>2</sub> +2 ATP
  - NADH +3 ATP
  - Total +17 ATP per 2 carbon, or about 8 per carbon
- By Comparison, Glucose gives us about +36 ATP per 6 carbons, or about 6 per carbon

#### **REGULATION OF beta-OXIDATION:**

- Positive Effectors: Starvation and a general low-energy level
  - Low insulin and high glucagon (i.e. low insulin:glucagon ratio)
  - ADP
- Malonyl-CoA inhibits it because it is a reactant of fat-synthesis.
  - It inhibits the Carnitine Acetyltransferase, preventing the transport of fats into the mitochondria and thereby effectively slowing beta-oxidation.
  - This occurs in conjunction with fat-synthesis, so that newly synthesized fats are not immediately broken down again.
- Negative Effectors: General indicators of sufficient energy in the cell:
  - High insulin and low glucagon
  - High ATP

#### **2.4.2 Fatty acid Biosynthesis**

ACETYL-CoA CARBOXYLASE. This is the enzyme we use to get Malonyl-CoA for fat-synthesis.

- Acetyl-CoA + HCO<sub>3</sub><sup>-</sup> + ATP -----> Malonyl-CoA

- A simple carboxylation reaction -- adding a carboxylate group onto the alpha carbon.
- Biotin is an intermediate. First biotin is carboxylated, forming Carboxybiotin -- This is the step that requires ATP.
- Then Carboxybiotin transfers the carboxy group to the Acetyl-CoA, requiring no additional energy.

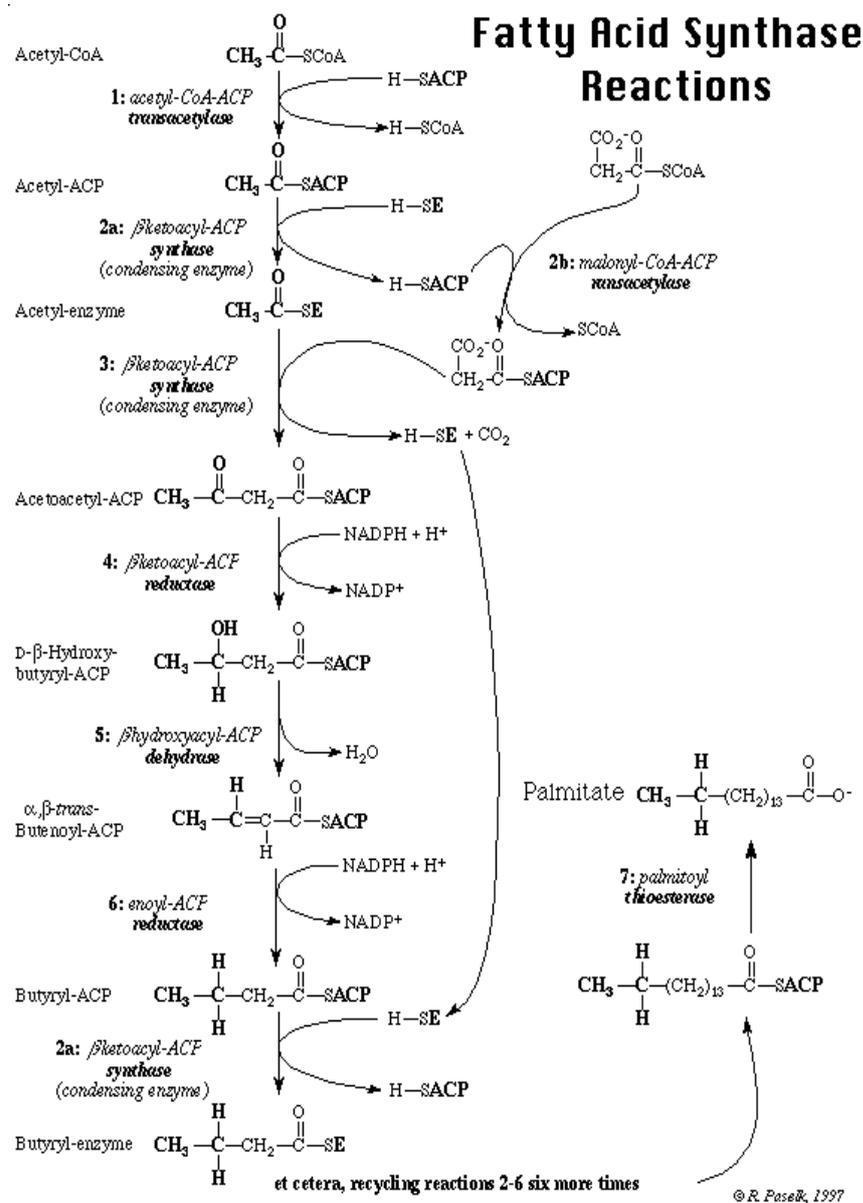
STEPS OF FATTY-ACID SYNTHESIS: Fatty-Acid Synthesis occurs in the cytosol.

- CONDENSATION:
  - Acetyl Unit (2C) + Malonyl Unit (3C) -----> 4-carbon chain + CO<sub>2</sub>
  - CO<sub>2</sub> is lost in order to provide the energy for the reaction. It is the same CO<sub>2</sub> that was just put on!!
- REDUCTION:
  - The beta-Carbonyl from above is reduced to OH.
  - NADPH -----> NADP<sup>+</sup> is concurrent oxidation. Remember NADPH is the most common biosynthetic cofactor.
- DEHYDRATION
  - The OH group is eliminated creating a double bond -- alpha,beta-unsaturated species.
  - Loss of H<sub>2</sub>O
- REDUCTION
  - Add H across the double-bond, fully saturating it.
  - NADPH -----> NADP<sup>+</sup> is concurrent oxidation.
- FINAL PRODUCT: Butyryl Unit -- a 4-carbon acid.

FATTY ACID SYNTHASE: A single multi-functional enzyme is used to synthesize fatty acids. It has multiple catalytic domains, similar to the ribosomal complex (A-Site and P-Site) in translation of proteins.

- Condensation Reaction:
  - Cys Residue binds Acetyl-CoA.
  - Pantetheine cofactor has sulfur groups that bind Malonyl-CoA.

- These two parts of the big enzyme then bring the constituents close enough together to undergo condensation.
- Then the other reactions occur at distinct sites on the protein.
- Elongation:
  - The butyryl unit is then translocated back to the Cysteine site.
  - The now-free Pantetheine site can now accept another malonyl unit to continue elongation.
- Termination: The process stops by a hydrolysis reaction, always at 16-carbons, at Palmitate for some reason.



**Regulation of Fatty Acid Synthesis:** Fat synthesis is an anabolic process, so it is promoted by dephosphorylation.

- Positive Effectors: General surplus of energy
  - High ATP
  - High insulin:glucagon ratio.
  - INSULIN -- STIMULATES Acetyl-CoA Carboxylase by dephosphorylating it.
    - Insulin activates a Phosphoprotein Phosphatase -----> Dephosphorylate the enzyme
  - CITRATE in the cytosol allosterically stimulates Acetyl-CoA Carboxylase.
    - Citrate can also break down to Oxaloacetate and Acetyl-CoA, and Acetyl-CoA can then be used as a fatty-acid building block.
- Negative Effectors: General lack of energy
  - High ADP
  - Low insulin:glucagon ratio
  - GLUCAGON, EPINEPHRINE -- INHIBITS Acetyl-CoA Carboxylase by phosphorylating it directly.
- Inhibited by Long-Chain Acyl-CoA, i.e. intermediates of beta-oxidation.

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## 2.5 Nucleotide biosynthesis

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Nucleotides and nucleosides can be supplied to an organism by either a salvage reaction or by synthesis from smaller precursors. Salvage reactions convert free purine and pyrimidine bases into nucleotides. Additionally, free purines and pyrimidines can be degraded, the purines to the oxidized ring compound uric acid and the pyrimidines to smaller compounds ( -amino acids, not the -amino acids found in proteins). Finally, purines and pyrimidines can be synthesized from smaller precursors ( de novo synthesis). Thus three interacting pathways for nucleotides, nucleosides, and the free bases exist:

1. salvage
2. degradation
3. biosynthesis

This complexity is due to the central role of nucleotides as energy currency, signaling molecules, and precursors to informational macromolecules in the

cell. If the supply of nucleotides becomes limiting, cells couldn't make DNA or RNA, for example. Likewise, cells need to have a balanced supply of nucleotides, because A and T, as well as C and G, occur at the same proportions in DNA and in similar amounts in RNA. Thus the cell must ensure the availability of an adequate supply of precursors. On the other hand, more ATP is needed in energy storage relative to the other nucleoside triphosphates. Finally, the purine bases themselves and the purine nucleosides are toxic to humans (for a variety of reasons), so they must be readily eliminated.

### 2.5.1 Salvage pathway

The nucleotide and nucleosides of a cell are continually in flux. For example, DNA and RNA chains are being synthesized in the cell. Even though the overall DNA content of a cell is constant, small stretches are continually being repaired. Part of the repair process is the breakdown of one strand of the DNA double helix into nucleotides, nucleosides, and free bases. Free purines and pyrimidines are converted back into nucleoside triphosphate monomers to be reincorporated into DNA. A common step in this pathway is the reaction of free bases with phosphoribosyl pyrophosphate (PRPP) to yield nucleotides. PRPP is a general activator of nitrogen ring compounds. For example, PRPP is added to anthranilate during the biosynthesis of tryptophan in bacteria. PRPP is made by the activation of ribose 5-phosphate. Ribose-5-phosphate can be made through the pentose phosphate pathway. Apparently, two enzymes exist in all systems—one for purines and one for pyrimidines. The synthesis of the glycosidic bond uses the 1-pyrophosphate of PRPP as an energy source, and either enzyme transfers the free base to the 1 position of the ribose, making a nucleotide.

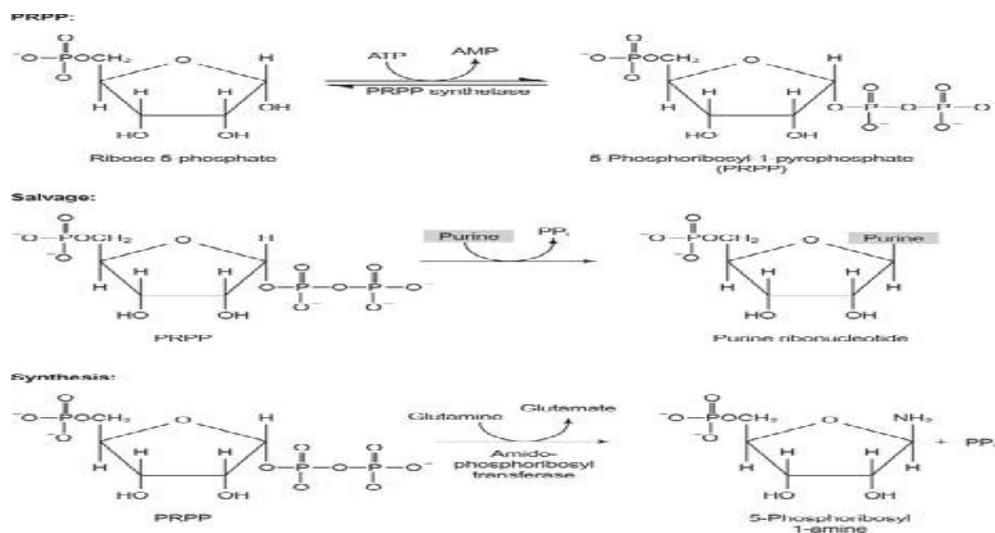
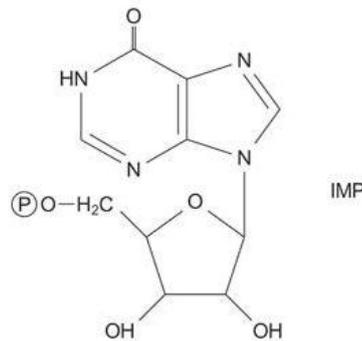


Figure-One enzyme uses either guanine or hypoxanthine (adenine with the

amino group replaced by an OH). A second enzyme uses free adenine. A third enzyme is specific for uracil and thymine. All the enzymes carry out the same reaction: transfer of the free base to the ribose-5 -monophosphate of PRPP, forming a nucleoside-5 -monophosphate (NMP).

### 2.5.2 Purine Biosynthesis

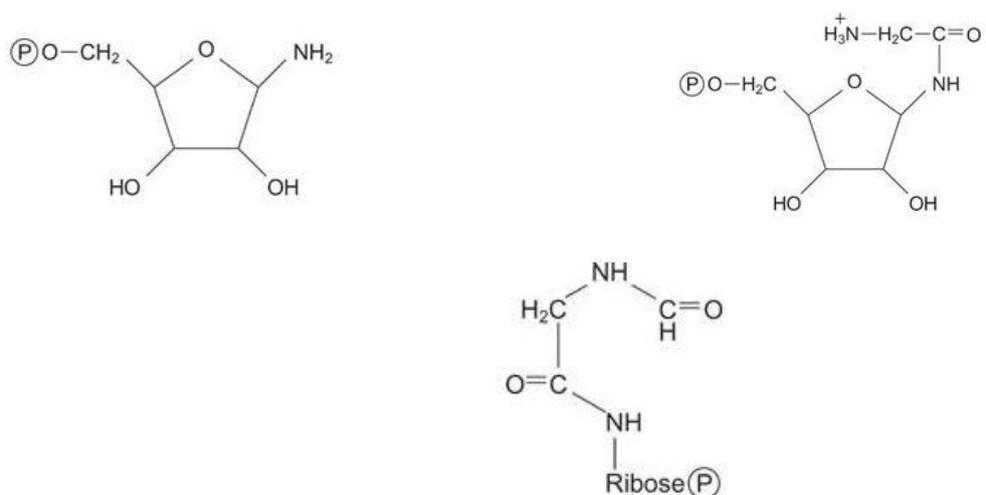
Purine synthesis uses a PRPP “handle” where the ring is assembled to make a 5 NMP, **inosine monophosphate (IMP)**.



IMP is the common intermediate in purine biosynthesis, and can be converted to GMP or AMP as needed.

The first reaction in purine biosynthesis is the transfer of the amide from glutamine to PRPP with release of pyrophosphate. The product is phosphoribosylamine (PRA).

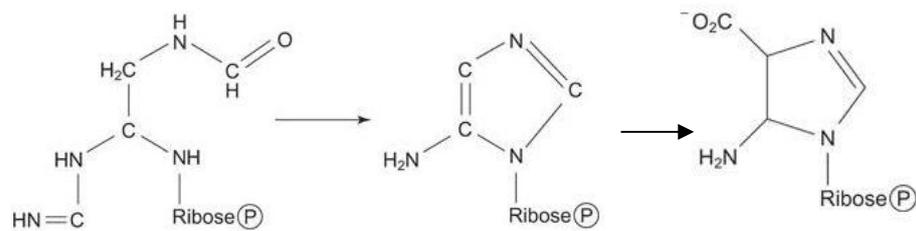
Then the amino acid glycine is transferred to PRA, making glycinamide mononucleotide.



PRA glycinamide mononucleotide

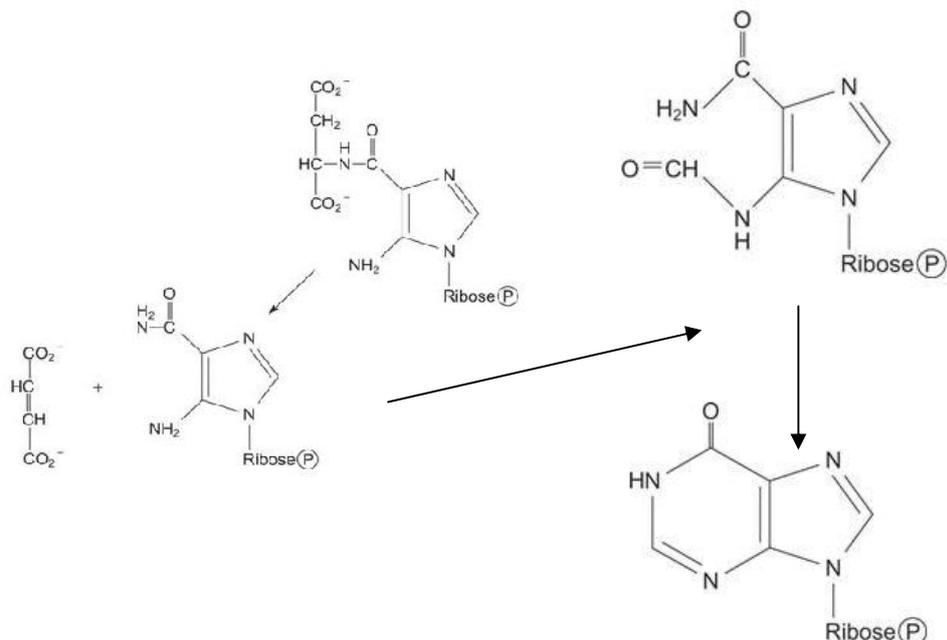
The amino group of glycine is formylated, with the formyl group being donated by N<sup>10</sup>-formyl-tetrahydrofolate.

Now the amino NH<sub>2</sub> is transferred to the carboxyl carbon of glycine from glutamin, with ATP as an energy source. This compound, formylglycineamidine ribonucleotide, closes to make the “smaller” (imidazole) ring of the purine. Again, ring closure uses ATP energy.



Now the larger ring is built on the smaller one. A carboxylation reaction with CO<sub>2</sub> starts synthesis of the 6-membered ring.

Then the amino group of aspartate is transferred to the carboxyl, making an amide. This condensation uses ATP and the amide is cleaved to release fumarate, leaving behind the imidazole with a 5-amino group (left from the amidation of glycine four steps earlier) and a 4-carboxamide. (Note how this reaction is similar to the formation of arginine during the urea cycle.)



Eight of the nine components of the ring are now present. The last ring component comes from a 1-carbon transfer of a formyl group from  $N^{10}$ -formyltetrahydrofolate.

Finally, the ring is closed by dehydration to yield IMP.

IMP is the key intermediate of purine nucleotide biosynthesis. IMP can react along two pathways that yield either GMP or AMP. Oxidation of the 2 position makes xanthine monophosphate, which is transamidated to GMP. Alternatively, the  $\alpha$ -amino group of aspartate can replace the ring oxygen of IMP to make AMP. (Note again how this reaction is similar to the synthesis of arginine from citrulline.)

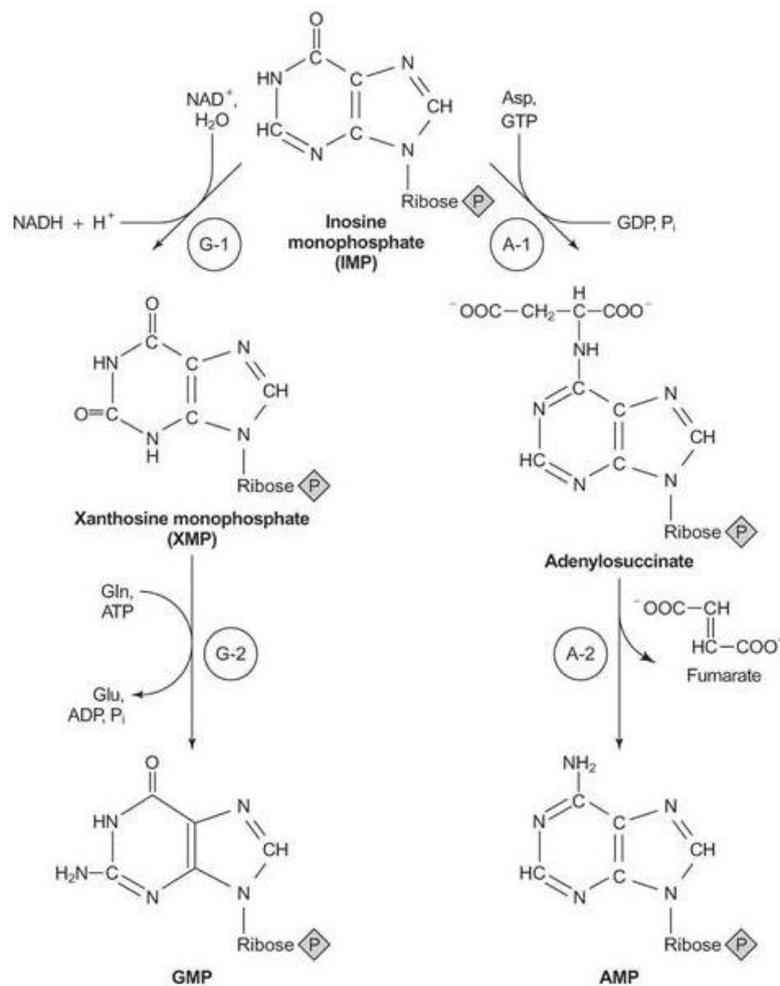


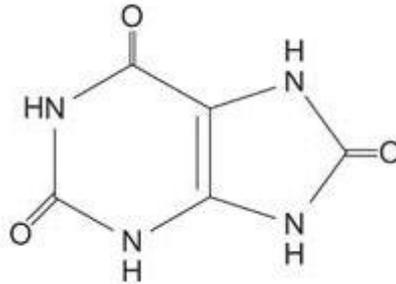
Figure 2

The rates of these two complementary reactions can control the amount of either AMP or GMP present in the cell. Each of these reactions is

feedback-inhibited by its nucleotide product. Thus, if more adenosine nucleotides exist than guanosine nucleotides, the synthesis of AMP slows down until the purine nucleotides balance.

### 2.5.3 Degradation of purine nucleotides

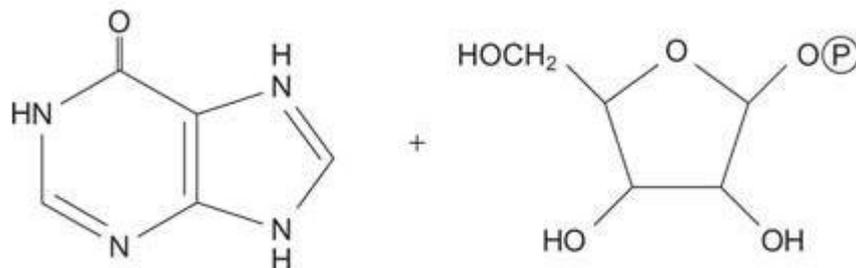
Extra purines in the diet must be eliminated. In mammals, the product of purine breakdown is a weak acid, **uric acid**, which is a purine with oxygen at each of three carbons.



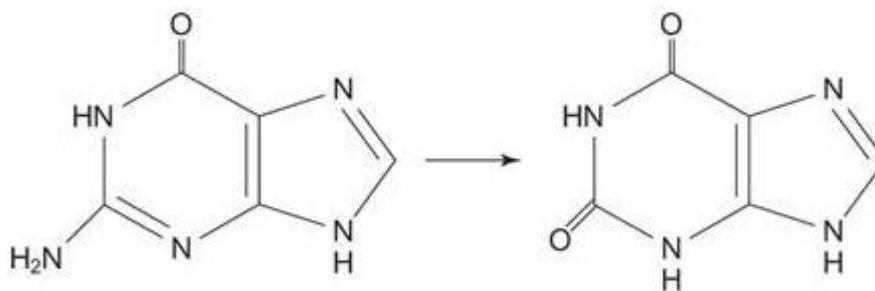
Uric acid is the major nitrogen excretion product in birds and reptiles, where it is responsible for the white, chalky appearance of these droppings. Uric acid is poorly soluble in water, and in humans, formation of uric acid crystals is responsible for the painful symptoms of gout. These crystals are deposited in joints (recall that the classic symptom of gout is an inflamed toe).

Adenosine is degraded in a two-step reaction. First, the enzyme adenosine deaminase acts on AMP or adenosine nucleoside to yield IMP or inosine.

IMP is cleaved by phosphorolysis of the nucleoside to yield hypoxanthine and ribose-1-phosphate. (This reaction is similar to the phosphorolysis of glycogen by glycogen phosphorylase.)

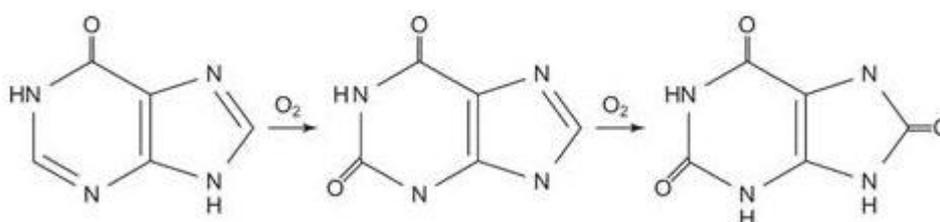


Guanosine is degraded in a two-step reaction sequence. First, guanosine phosphorylase phosphorolytically cleaves the nucleoside to free guanine and ribose-1-phosphate.



The next reaction is the deamination of guanosine to xanthine. Xanthine needs only one more oxygen to form uric acid.

Xanthine oxidase oxidizes hypoxanthine and xanthine to uric acid, using molecular oxygen,  $O_2$ .



As mentioned earlier, uric acid is only slightly soluble and individuals with impaired secretion or excess production of uric acid are subject to the pain of gout as uric acid precipitates in the joints. Most cases of gout are probably due to impaired excretion of uric acid because of poor kidney function. Because the concentration of uric acid in the blood is near the solubility limit, only a slight impairment of elimination can push the concentration high enough to precipitate uric acid. More frequently nowadays, gout appears in persons whose kidney function is impaired with age, although it is also found in individuals with genetic deficiencies in the level of hypoxanthine-guanine phosphoribosyl transferase. In the latter case, the salvage pathway does not function well, and more purines must be eliminated through their conversion to uric acid.

The drug allopurinol, which is an inhibitor of xanthine oxidase, effectively treats gout. Allopurinol is structurally similar to hypoxanthine, except that the 5-membered ring has the positions of the carbon and nitrogens reversed.

Xanthine oxidase is able to bind allopurinol and catalyze one oxidation, converting it to a compound that is similar to xanthine. However, after that conversion, the enzyme is trapped in an inactive oxidation state and can't carry out its normal function of forming uric acid. Additionally, allopurinol inhibits

the de novo (new, from other compounds; not recycled) synthesis of purines, further decreasing the amount of uric acid formed in the blood.

beta-OXIDATION: A four-step process. Called beta-Oxidation because most of the chemistry occurs on the beta-Carbon (beta to the carbonyl) per turn of the cycle.

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## 2.6 Summary

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- Metabolism is usually divided into two categories.
  1. Catabolism, that breaks down organic matter and harvests energy by way of cellular respiration
  2. Anabolism that uses energy to construct components of cells such as proteins and nucleic acids.
- Most of the structures that make up animals, plants and microbes are made from three basic classes of molecule: amino acids, carbohydrates and lipids (often called fats).
- Carbon fixation or carbon assimilation refers to the conversion process of inorganic carbon (carbon dioxide) to organic compounds by living organisms. Ex. photosynthesis, chemosynthesis
- CAM plants (jade plant (*Crassula ovata*) and cacti) that use Crassulacean acid metabolism as an adaptation for arid conditions.
- C4 plants preface the Calvin cycle with reactions that incorporate CO<sub>2</sub> into one of the 4-carbon compounds, malic acid or aspartic acid. C4 plants have a distinctive internal leaf anatomy called as kranz anatomy. Ex. sugar cane and maize
- The large majority of plants are C3 plants. They are so-called to distinguish them from the CAM and C4 plants, and because the carboxylation products of the Calvin cycle are 3-carbon compounds.
- Cellular respiration allows organisms to use (release) energy stored in the chemical bonds of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>). The energy in glucose is used to produce ATP.

- The complete breakdown of glucose to carbon dioxide and water requires two major steps: 1) glycolysis and 2) aerobic respiration. Glycolysis produces two ATP. Thirty-four more ATP are produced by aerobic pathways if oxygen is present.
- Gluconeogenesis is the process of synthesizing glucose from non-carbohydrate sources.
- Urea is synthesised via the urea cycle, which is confined to mammalian liver. The urea cycle takes place partly in the cytosol and partly in the mitochondria of liver.
- Nucleotides and nucleosides can be supplied to an organism by either a salvage reaction or by synthesis from smaller precursors. Salvage reactions convert free purine and pyrimidine bases into nucleotides.

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## 2.7 Glossary

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- **Acetyl CoA.** Acetyl-coenzyme A, a high-energy ester of acetic acid that is important both in the tricarboxylic acid cycle and in fatty acid biosynthesis.
- **Adenine.** A purine base found in DNA or RNA.
- **Adenosine.** A purine nucleoside found in DNA, RNA, and many cofactors.
- **Beta-oxidation (b-oxidation).** Oxidative degradation of fatty acids that occurs by the successive oxidation of the b-carbon atom.
- **Biochemical pathway.** A series of enzyme-catalyzed reactions that results in the conversion of a precursor molecule into a product molecule.
- **Carbohydrate.** A polyhydroxy aldehyde or ketone.
- **Catabolism.** That part of metabolism that is concerned with degradation reactions.
- **Cytosine.** A pyrimidine base found in DNA and RNA.

- **Deamination.** The enzymatic removal of an amine group, as in the deamination of an amino acid to an alpha keto acid.
- **Dehydrogenase.** An enzyme that catalyzes the removal of a pair of electrons (and usually one or two protons) from a substrate molecule.
- **Fatty acid.** A long-chain hydrocarbon containing a carboxyl group at one end. Saturated fatty acids have completely saturated hydrocarbon chains. Unsaturated fatty acids have one or more carbon-carbon double bonds in their hydrocarbon chains.
- **Glycolysis.** The catabolic conversion of glucose to pyruvate with the production of ATP.
- **Guanosine.** A purine nucleoside found in DNA and RNA.
- **Ketone bodies.** Refers to acetoacetate, acetone, and b-hydroxybutyrate made from acetyl-CoA in the liver and used for energy in nonhepatic tissue.
- **Ketosis.** A condition in which the concentration of ketone bodies in the blood or urine is unusually high.
- **Nucleoside.** An organic molecule containing a purine or pyrimidine base and a five-carbon sugar (ribose or deoxyribose).
- **Nucleotide.** An organic molecule containing a purine or pyrimidine base, a five-carbon sugar (ribose or deoxyribose), and one or more phosphate groups. A phosphoester of a nucleoside.

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## 2.8 Self-Learning Exercise

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### Section A : Very short answer type

1. Define metabolism?
2. What is gluconeogenesis?
3. Kranz anatomy found in :
  - a) C3 plants
  - b) C4 plant
  - c) a and b both
  - d) None of these
4. How many ATP produced in aerobic respiration?

5. Where is urea cycle takes place in mammals?

**Section B : Short answer type**

1. What is transamination?
2. Explain - oxidation in short.
3. What is salvage pathway?
4. Describe glycolysis.
5. Explain C4 cycle.

**Section C : Long answer type**

1. Describe protein metabolism.
2. Write short notes on :
  - a) Pentose phosphate pathway
  - b) Glycogenolysis
  - c) CAM
  - d) Urea cycle
3. Explain citric acid cycle ?
4. Explain nucleotide metabolism in detail?

## Unit - 3

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# Physiology of the Nervous System: Molecular Physiology of nerve impulse, synapse physiology and integration of information

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### Structure of the unit

- 3.0 Objective.
- 3.1 Introduction.
- 3.2 Molecular Physiology of Nerve Impulse:
  - 3.2.1 Membrane potential caused by diffusion.
  - 3.2.2 Relation of the diffusion potential to the concentration Difference - the Nernst Equation.
  - 3.2.3 Resting membrane potential of Nerves.
  - 3.2.4 Nerve action potential.
  - 3.2.5 Voltage Gated sodium and potassium channels.
  - 3.2.6 Propagation of action potential as an impulse.
- 3.3 Synapse: Physiology and integration of information:
  - 3.3.1 Synapses.
  - 3.3.2 Electrical Synapses.
  - 3.3.3 Chemical Synapses.
  - 3.3.4 Excitatory postsynaptic potential (EPSP).
  - 3.3.5 Inhibitory postsynaptic potential (IPSP).
  - 3.3.6 Ionic Basis of Inhibition postsynaptic potential (IPSP).
  - 3.3.7 Properties of Synapse.
- 3.4 Summary
- 3.5 Glossary
- 3.6 Self-Learning Exercises

### **3.0 Objective**

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The purpose of the chapter is to present, a general outline of the overall mechanisms by which the nervous system performs such functions. Then the Molecular physiology of Nerve impulse, the role of synapses will be discussed, which will include the physiology of integration of information.

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### **3.1. Introduction**

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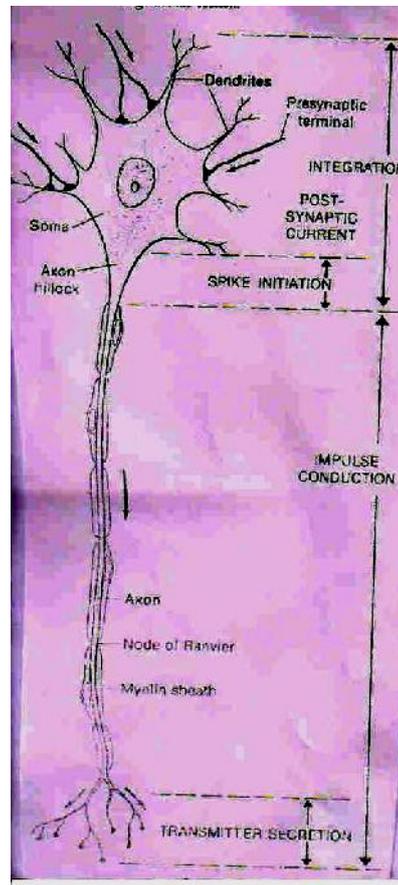
The Nervous System is unique in the vast complexity of the control actions it can perform. It receives millions of bits of information from the different sensory organs and then integrates all these to determine the response to be made by the body. For any physiological function, it is imperative that it should be performed in a controlled way different physiological functions are not independent events. All functional components need to be controlled and put together so that they operate harmoniously. This ensures integration of all the systems into a smoothly operating organism. In animals, physiological functions are coordinated by Nervous System.

The human nervous system consists of two contrasting functional subsystems, the central nervous system and the peripheral nervous system. Together, brain and spinal cord make the central nervous system (CNS). It is the site of information processing within the nervous system. The peripheral nervous system (PNS) includes all the nerve pathways of the body outside the brain and spinal cord. These pathways are divided into two groups: the sensory or afferent pathways, which transmit information to the CNS and the motor or efferent pathways, which transmit commands from the CNS. The motor pathways, in turn, are partitioned into somatic (voluntary) nervous system, which relay commands to skeletal muscles and autonomic (Involuntary) nervous system (ANS) that stimulates the glands and other muscles of the body. In addition, there is the neuroendocrine system, which is a network of endocrine glands whose hormone production is controlled by commands from the CNS. If the neurons are clustered into groups within the CNS, these are called ganglia. Within the CNS the bundles of nerve fibres are called tracts, whereas in the PNS they are called nerves.

A typical nerve has a tough outer covering, the epineurium. Inside are the long fibres or axons of individual nerve cells, gathered into bundles called fascicles,

wrapped in the perineurium. Each nerve has its own supply of small blood vessels.

Nerves are the functional units of the nervous system. They occur in a variety of shapes and sizes. A vertebrate motoneuron (motor neuron), which originates in the spinal cord and innervates skeletal muscle fibres.



(Fig.1)

**(Fig.1) A vertebrate spinal motoneuron, with the functions of different parts indicated. The flow of information is indicated by arrows. Axon and surrounding myelin sheaths shown in longitudinal section.**

In these neurons, the surface membrane of the dendrites and the soma is innervated by the terminals of other nerve cells. The axon (nerve fiber) carries action potentials from the spike-initiating zone in the axon hillock to the axon terminals, which innervate muscle cells. The dendrites and the axon are processes that grow out from the soma during development and into which there is a slow but steady flow of proteins and other constituents synthesized in the soma.

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## 3.2 Molecular Physiology of Nerve Impulse

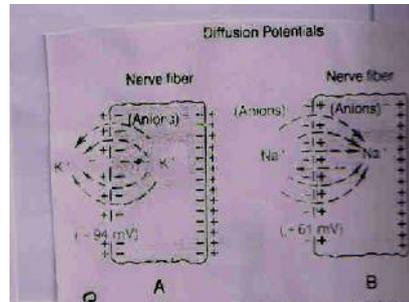
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- 3.2.1. Membrane potential caused by diffusion.
- 3.2.2. Relation of the diffusion potential to the concentration Difference - the Nernst Equation.
- 3.2.3. Resting membrane potential of Nerves.
- 3.2.4. Nerve action potential.
- 3.2.5. Voltage Gated sodium and potassium channels.
- 3.2.6. Propagation of action potential as an impulse.

Electrical potentials exist across the membranes of essentially all cells of the body. In addition, some cells, such as nerve and muscle cells, are "excitable" - that is, capable of self-generation of electrochemical impulses at their membranes.

### 3.2.1. Membrane Potentials Caused By Diffusion

In a nerve fiber when there is no active transport of sodium or potassium ions, the potassium concentration is great inside the membrane, whereas that outside is very low.



(Fig.2A)

**(Fig.2A) A, Establishment of a diffusion potential across a cell membrane, caused by potassium ions diffusing from inside the cell to the outside through a membrane that is selectively permeable only to potassium. B, Establishment of a diffusion potential when the membrane is permeable only to sodium ions. Note that the internal membrane potential is negative when potassium ions diffuse and positive when sodium ions diffuse because of opposite concentration gradients of these two ions.**

The membrane in this instance is permeable to the potassium ions but not to any other ions. Because of large potassium concentration gradient from the

inside toward the outside, there is a strong tendency for potassium ions to diffuse outward. As they do so, they carry positive charge to the outside, thus creating a state of electropositivity outside the membrane and electronegativity on the inside because of the negative action that remain behind and do not diffuse outward along with the potassium.

At the same time there a high concentration of sodium ions outside the membrane and a low sodium concentration inside Fig.2B. These ions are also positively charged and this time the membrane is highly permeable to the sodium ions but impermeable to all other ions. Diffusion of the sodium ions to the inside creates a membrane potential now of opposite polarity, with negativity outside and positivity inside.

Thus, in both parts of Fig.2, we see that a concentration difference of ions across a selectively permeable membrane can, under appropriate conditions, cause the creation of a membrane potential.

### 3.2.2. Relation of The Diffusion Potential To The Concentration

Difference - The Nernst Equation:

The potential level across the membrane that prevents net diffusion of an ion in either direction through the membrane is called the Nernst potential. The magnitude of this potential is determined by the ratio of the ion concentrations on the two sides of the membrane - the greater this ratio, the greater the tendency for the ions to diffuse in one direction, and therefore the greater is the Nernst potential. The following equation, called the Nernst equation, can be used to calculate the Nernst potential for any univalent ion at normal body temperature of 98.6°F (37°C):

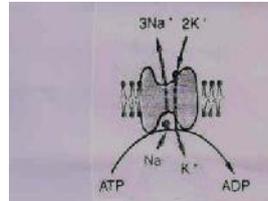
$$\text{EMF (millivolts)} = \pm 61 \log \frac{\text{Conc. Inside}}{\text{Conc. Outside}}$$

### 3.2.3. Resting Membrane Potential Of Nerves

The membrane potential of large nerve fibers when they are not transmitting nerve signals is about - 90 millivolts. That is, the potential inside the fiber is 90 millivolts more negative than the potential in the extracellular fluid on the outside of the fiber.

Active transport of sodium and potassium ions through the membrane - The sodium-potassium pump. All cell membranes of the body have a powerful sodium-potassium pump that continually pumps sodium to the outside of the

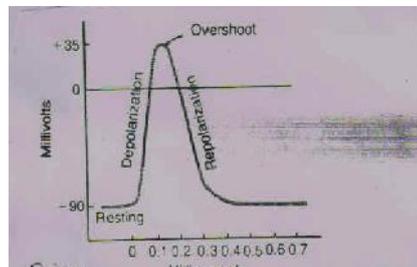
fiber and potassium to the inside, as illustrated on the left-hand side in Figure-3. Further, let us remember that this is an electrogenic pump because more positive charges are pumped to the outside than to the inside (three  $\text{Na}^+$  ions to the outside for each two  $\text{K}^+$  ions to the inside), leaving a net deficit of positive ions on the inside, this causes a negative charge inside the cell membrane.



**Fig.3 Functional characteristics of the  $\text{Na}^+$ - $\text{K}^+$  pump**

### 3.2.4. NERVE ACTION POTENTIAL:

Nerve signals are transmitted by action potentials, which are rapid changes in the membrane potential. Each action potential begins with a sudden change from the normal resting negative potential to a positive membrane potential and then ends with an almost equally rapid change back to the negative potential. To conduct a nerve signal, the action potential moves along the nerve fiber until it comes to the fiber's end.



**(Fig.4) Typical action potential recorded by the method shown in the upper panel of the figure.**

**The successive stages of the action potential are as follows.**

**Resting Stage.** This is the resting membrane potential before the action potential occurs. The membrane is said to be "polarized" during this stage because of the large negative membrane potential that is present.

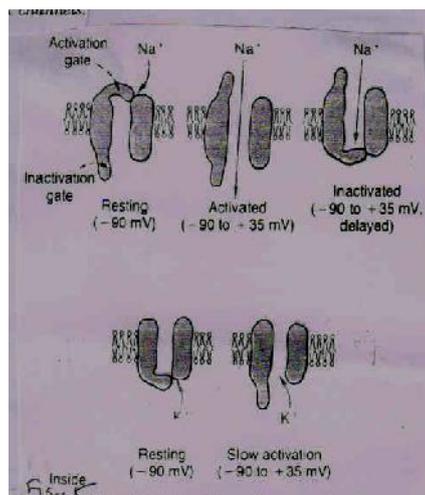
**Depolarization Stage.** At this time, the membrane suddenly becomes permeable to sodium ions, allowing tremendous numbers of positively charged sodium ions to flow to the interior of the axon. The normal "polarized" state of - 90 millivolts is lost, with the potential rising rapidly in the positive direction. This is called depolarization. In large

nerve fibers, the membrane potential "overshoots" beyond the zero level and becomes somewhat positive.

Repolarization Stage. Within a few 10,000ths of a second after the membrane becomes highly permeable to sodium ions, the sodium ions, the sodium channels begin to close and the potassium channels open more than they normally do. Then, rapid diffusion of potassium ions to the exterior re-establishes the normal negative resting membrane potential. This is called repolarization of the membrane.

### 3.2.5. Voltage-Gated Sodium And Potassium Channels

The necessary action in causing both depolarization and repolarization of the nerve membrane during the action potential is the voltage-gated sodium channel. The voltage-gated potassium channel also plays an important role in increasing the rapidity of repolarization of the membrane. These two voltage-gated channels are in addition to the  $\text{Na}^+ - \text{K}^+$  pump and the  $\text{Na}^+ - \text{K}^+$  leak channels.



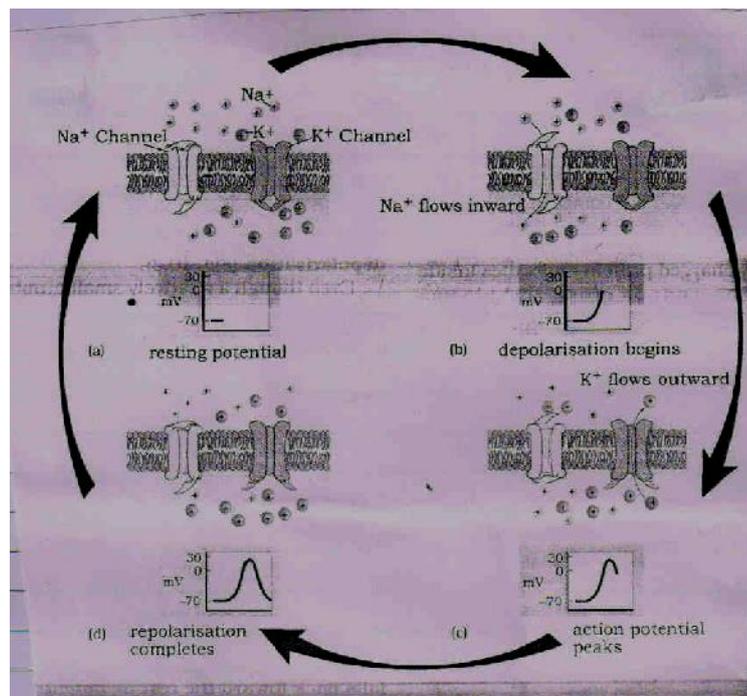
**Fig.5.**

**Fig.5. Characteristics of the voltage-gated sodium and potassium channels, showing both activation and inactivation of the sodium channels but activation of the potassium channels only when the membrane potential is changed from the normal resting negative value to a positive value.**

During the resting state, before the action potential begins, the conductance for potassium ions is shown to be 50 to 100 times as great as the conductance for sodium ions.

However, at the onset of the action potential, the sodium channels instantaneously become activated and allow up to a 5000-fold increase in sodium conductance. Then the inactivation process close the sodium channels within another few fractions of a millisecond. The onset of the action potential also causes voltage gating of the potassium channels, causing them to begin opening more slowly, a fraction of a millisecond after the sodium channels open. At the end of the action potential, the return of the membrane channels to close back to their original status, but again only after a delay.

The ratio of sodium conductance to potassium conductance at each instant during the action potential, and above this is shown the action potential itself. During the early portion of the action potential, the ratio of sodium to potassium conductance increases more than a thousandfold. Therefore, far more sodium ions now flow to the interior of the fiber than the potassium ions to the exterior. This is what causes the membrane potential to become positive. Then the sodium channels begin to close and, at the same time, the potassium channels open, so that the ratio of conductance now shifts far in favor of high potassium conductance but low sodium conductance. This allows extremely rapid loss of potassium ions to the exterior, whereas essentially no sodium ions flow to the interior. Consequently, the action potential quickly returns to its baseline level.



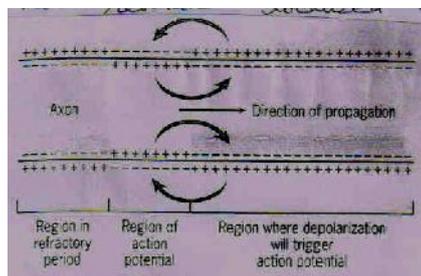
**Fig.6.**

**Fig.6. Stages of axon membrane during resting, depolarization, action potential and repolarization showing changes in membrane potentials and molecular events. (a) Resting state: voltage gated  $\text{Na}^+$  channels are in resting state and voltage-gated  $\text{K}^+$  channels are closed. (b) When stimulated, depolarization opens  $\text{Na}^+$  channel activation gates,  $\text{K}^+$  channels still close (c) Action potential leaks and repolarization begins.  $\text{K}^+$  channels open and  $\text{Na}^+$  channel gates close. (d) Repolarization complete,  $\text{K}^+$  ions exit and  $\text{Na}^+$  channels begin to open again.**

### **3.2.6. Propagation Of Action Potential As An Impulse**

Up to this point, we have discussed the events occurring at a particular site on the nerve cell membrane where experimental depolarization has triggered an action potential.

Once an action potential has been initiated, it does not remain localized at a particular site but it propagated as a nerve impulse down the length of the cell to the nerve terminals.



**Fig.7.**

**Figure7. Propagation of an impulse results from the local flow of ions. An action potential at one site on the membrane depolarizes an adjacent region of the membrane, triggering an action potential at the second site. The action potential can only flow in the forward direction because the portion of the membrane that has just experienced an action potential remains in a refractory period.**

Nerve impulses are propagated along a membrane because an action potential at one site has an effect on the adjacent site. The large depolarization that accompanies an action potential creates a difference in charge along the inner and outer surfaces of the plasma membrane (Figure 7). As a result, positive ions move toward the site of depolarization on the outer surface of the membrane and away from that site on the inner surface (Figure 7). This local flow of

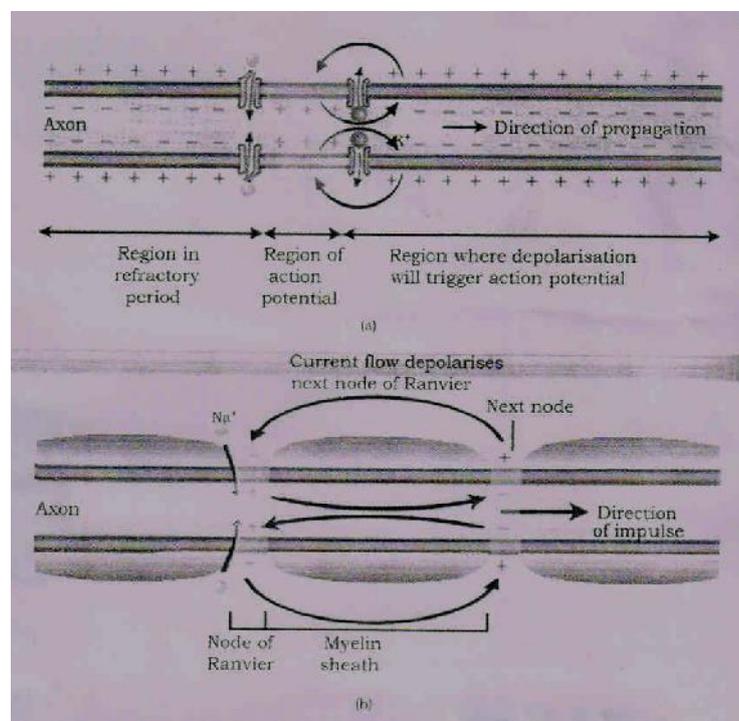
current causes the membrane in the region just ahead of the action potential to become depolarized. Because the depolarization accompanying the action potential is very large, the membrane in the adjacent region is readily depolarized to a level greater than the threshold value, which opens the sodium channels in this adjacent region, generating another action potential. Thus, once triggered, a succession of action potentials passes down the entire length of the neuron without any loss of intensity, arriving at its target cell with the same strength it had at its point of origin.

**Direction Of Propagation.** An excitable membrane has no single direction of propagation, but the action potential will travel in both directions away from the stimulus and even along all branches of a nerve fiber, until the entire membrane has become depolarized.

**All Or Nothing Principle:** Once an action potential has been elicited at any point on the membrane of a normal fiber, the depolarization process will travel over the entire membrane if conditions are right, or it might not travel at all if conditions are not right. This is called the all or nothing principle, and it applies to all normal excitable tissues. Occasionally, the action potential will reach a point on the membrane at which it does not generate sufficient voltage to stimulate the next area of the membrane. When this occurs, the spread of depolarization stops. Therefore, for continued propagation of an impulse to occur, the ratio of action potential to threshold for excitation must at all times be greater than 1. This is called the safety factor for propagation.

**Saltatory Conduction:** Many vertebrate neurons possess axons with sheath at intervals by Schwann cells (e.g., spinal and cranial nerves). These lipid-rich cells envelop the axon, wrapping spirally their plasma membrane around it many times to produce a series of layers, called myelin sheath. It acts as a biological electrical insulation, creating a region of high electrical resistance on the axon. Schwann cells are spaced along such an axon one after the other, with nodes of Ranvier separating each Schwann cell from the next. These nodes are critical to the propagation of the nerve impulse in these cells. Within the small gap represented by each node the surface of the axon is exposed to the fluid surrounding the nerve. The ion channels and transport pumps that move ions across the axons, are concentrated in this zone. The direct fluid contact permits ion transport to occur through the channels and an action potential to be generated. The action potential is not propagated by a wave of membrane not depolarization travelling down the axon since the insulating Schwann cells prevent this instead, the action potential jumps as an electrical current from one

node to the next. When the current reaches a node, it opens  $\text{Na}^+$  ion channels. In doing so, it generates a potential difference large enough to create a current that reaches the next node. The arrival of the current at that node opens its  $\text{Na}^+$  channels, creating another current that passes on to the next node, and so on. This very fast form of nerve impulse conduction is known as saltatory conduction (L. saltare: a jump), since the action potential effectively "jumps" from node to node. An impulse conducted in this fashion moves very rapidly, up to 120 metres per second for large-diameter neurons. Energetically, saltatory conduction is also very economical for the cell, since there is far less membrane depolarization for the ion pumps to deal with; only the nodes are undergoing depolarization, instead of the entire nerve surface.



**Fig.8.**

**Fig.8. Difference in the local circuits produced in (a) non-myelinated axon (continuous) (b) myelinated axon (saltatory).**

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### **3.3. Synapse: Physiology And Integration Of Information**

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3.3.1. Synapses.

3.3.2. Electrical Synapses.

3.3.3. Chemical Synapses.

3.3.4. Excitatory postsynaptic potential (EPSP).

3.3.5. Inhibitory postsynaptic potential (IPSP).

3.3.6. Ionic Basis of Inhibition postsynaptic potential (IPSP).

3.3.7. Properties of Synapse.

### **3.3.1. Synapses**

An action potential, passing down an axon, eventually reaches the end of the axon that is often branched. It may be associated either with several dendrites, or an axon or a soma of other nerve cells, or with sites on muscle or secretory cells. Nerve signals traverse from neuron to neuron all around the body. These associations are called synapses (Fig.9). In a synapse, there is a narrow intercellular gap, 10 to 20 nanometres across, separating the axon tip and the target cell. This gap is called a synaptic cleft. The number of synapses is usually very large, providing a large surface area for the transfer of information. For instance, over 1000 synapses may be found on the dendrites and the cell body of a motor neuron in the spinal cord.

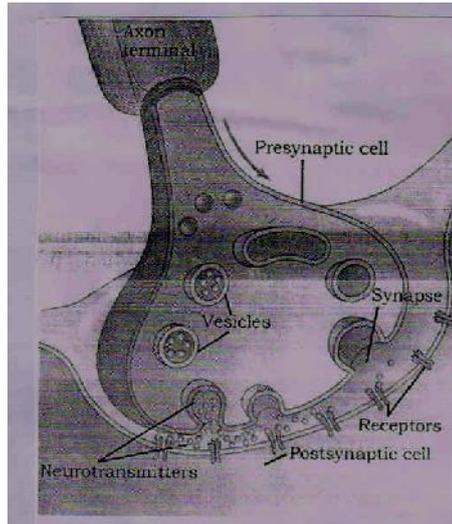
There are mainly two types of synapses: (i) electrical, and (ii) chemical, depending upon the nature of transfer of information across the synapse.

### **3.3.2. Electrical Synapses**

In electrical synapses, which are specialised for rapid signal transmission, the cells are separated by a gap, the synaptic cleft, of only 0.2 nm, so that an action potential arriving at the presynaptic side of cleft, can sufficiently depolarize the postsynaptic membrane to directly trigger its action potential. However, more than 20 nm gap of most synapses is too great a distance for such direct electrical coupling.

### **3.3.3. Chemical Synapses**

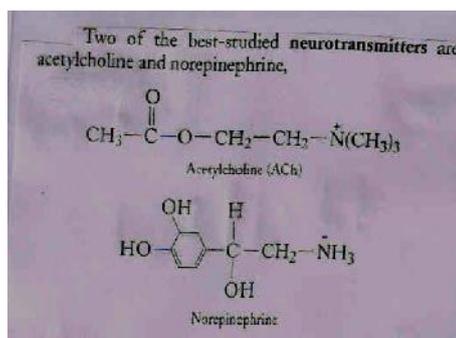
The commonest type of synapse, consist of a bulbous expansion of a nerve terminal, called synaptic knob, lying in close proximity to the membrane of a dendrite. The cytoplasm of the synaptic knob contains numerous tiny, round sacs, called synaptic vesicles. Each vesicle has a diameter of approximately 50 nm, and contains as many as 10,000 molecules of a neurotransmitter substance responsible for the transmission of nerve impulse across the synapse.



**Fig.9. NEURAL SYNAPSE**

The membrane of the synaptic knob on the axon side, thickened as a result of cytoplasmic condensation, is called presynaptic membrane. When a wave of depolarization reaches the presynaptic membrane, voltage gated calcium channels, concentrated at the synapse open. Because they are 10,000 times more concentrated outside cells,  $\text{Ca}^{2+}$  ions then diffuse into the terminal from the surrounding fluid. The  $\text{Ca}^{2+}$  ions, in some way, stimulate synaptic vesicles in the terminal to move to the terminal membrane, fuse with it and then rupture thereby of neurotransmitter chemicals from vesicles at the tip by exocytosis into the cleft. These neurotransmitters rapidly pass to the other side of the gap. They then combine with specific receptor molecules on the membrane of the target cell, which is called the postsynaptic membrane. By doing so, they cause a second electrical current, passing on its signal. To end the signal, the synaptic bulbs reabsorb some neurotransmitters and enzymes in the synapse neutralise others. The great advantage of a chemical synapse, compared with the direct electrical synapse, is that the nature of the messenger neurotransmitter can be different in different synapses, permitting different kinds of responses, either excitatory or inhibitory in nature. At least over 30 biochemicals (biogenic amines and derivatives of amino acids) and over 60 neuropeptides have been discovered and identified so far, that act as specific neurotransmitters.

Two of the best-studied neurotransmitters are acetylcholine and norepinephrine, which transmit impulses to the body skeletal and cardiac muscles.



## NOREPINEPHRINE

### 3.3.4. Excitatory Postsynaptic Potential (Epsp)

When an excitatory volley of impulses excites a motor neurone, depolarization of the cell membrane occurs. This is known as excitatory postsynaptic potential (EPSP). It is of brief duration. When the stimulus is stronger, excitatory postsynaptic potential (EPSP) reaches the threshold level and the nerve impulse is set up. The ionic events underlying the development of excitatory postsynaptic potential is presumably due to increased  $\text{Na}^+$  permeability to the postsynaptic membrane. If more excitatory synaptic knobs become active by propagated action potential then the liberation of excitatory transmitter material from synaptic vesicles is maximum. This state enhances the  $\text{Na}^+$  permeability to postsynaptic membrane, producing excitatory postsynaptic potential.

### 3.3.5. Inhibitory Postsynaptic Potential (Ipsp)

When a neuron receives an inhibitory volley of impulses, hyperpolarization of the cell membrane occurs. This is called inhibitory postsynaptic potential (IPSP). It has a longer latency. The hyperpolarization exerts an inhibitory effect on excitatory postsynaptic potential (EPSP) and depolarization of the cell membrane at the axon hillock and causes inhibition in setting up the nerve impulse.

### 3.3.6. Ionic Basis Of Inhibitory Postsynaptic Potential (Ipsp): is

presumably due to increased permeability of postsynaptic membrane to  $\text{K}^+$  and it but not of  $\text{Na}^+$ . Under such state  $\text{K}^+$  from the postsynaptic cell begins to come out (efflux) and  $\text{Cl}^-$  begins to enter producing negativity within the postsynaptic cell. This negativity is hyperpolarization of the membrane and membrane potential becomes  $-90$  mV. The decreased excitability of the nerve cell during IPSP is due to hyperpolarization which links the membrane potential to reach its firing level.

### 3.3.7. Properties of Synapse:

**Synaptic Response.** At the synaptic junction, impulses are received and discharged. But there is no relationship between the receipt and discharge of impulses. Sometimes many impulses are received from different sources but the neurone discharges its own. So it may be said that the synapse not only acts as a relay station but it may also act as an integrator. The integrating mechanism of the synapse is found in the cerebral cortex.

**Law Of Forward Conduction (Sherrington).** An impulse is, allowed to pass through a synapse in one direction only, viz., from the axon of one neurone to the dendrite of the next. But some synapses can transit impulses in both directions. They are bidirectional and usually electrical in nature, where presynaptic and postsynaptic membranes are in close apposition and often fused at several points.

**Synaptic Delay.** The impulse while passing through a synapse takes a certain length of time. The time between the arrival of the impulse and causing initial depolarization is called synaptic latency. The depolarization gradually rises to a spike height. So the synaptic delay is the sum of the synaptic latency and the time taken for depolarization leading to a spike height in the neurone. Synaptic delay in chemical synapses is less than 0.5 millisecond whereas in electrical junctions they are extremely short as there is no release of chemicals.

**Seat Of Fatigue.** The physiological seat of fatigue is in the control nervous system, probably at the synapses. The mechanism underlying the synaptic fatigue is presumably due to exhaustion of transmitter material from the synaptic vesicle following repeated presynaptic stimulation at a faster rate.

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### 3.4 Summary

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The nervous system has three main functions: sensory input, integration of data and motor output. The human nervous system consists of two functional subsystems, the central nervous system and the peripheral nervous system. The central nervous system (CNS) includes brain and spinal cord. The peripheral nervous system (PNS) includes all the nerve pathways of the body outside the brain and spinal cord. The PNS is then subdivided into the autonomic nervous system and the somatic nervous system. A neuron is a cell with an excitable membrane. An excitable membrane is created when the sodium-potassium pumps  $\text{Na}^+$  ions out across the membrane and  $\text{K}^+$  ions in. Because this membrane is only slightly permeable to  $\text{Na}^+$  or negatively charged anions, but is permeable to  $\text{K}^+$  ions, a net negative charge within the cell results.

A nerve impulse is an electrical signal that travels along an axon. When the nerve is activated, there is a sudden change in the voltage across the wall of the axon, caused by the movement of ions in and out of the neuron. This triggers a wave of electrical activity that passes from the cell body along the length of the axon to the synapse. Neurons that need to transmit electrical signals quickly are sheathed by a fatty substance called myelin. Myelin acts as an electrical insulator, and signals travel 20 times faster when it is present. This type of nerve impulse is known as saltatory conduction. An action potential, passing down an axon, eventually reaches the end of the axon that is often branched. It may be associated either with several dendrites or an axon or a soma of other nerve cells. The associations, where one neuron ends and the other begins, are called synapses. Messenger chemicals, called neurotransmitters and neuropeptides, pass across a synaptic cleft and interact with channels and receptors in the membrane of another neuron or of muscle cells. They either open  $\text{Na}^+$  ion channels and depolarize the postsynaptic membranes, an “excitatory” response or open  $\text{K}^+$  channels and hyperpolarize membranes, an “inhibitory” response.

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### 3.5. GLOSSARY

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- **Acetylcholine (ACh):** Chemical neurotransmitter in the brain and PNS; dominant neurotransmitter in the PNS, released at neuromuscular junctions and synapses of the parasympathetic division.
- **Acetylcholinesterase (AChE):** Enzyme found in the synaptic cleft, bound to the postsynaptic membrane and in tissue fluids; breaks down and inactivates ACh molecules.
- **Action potential:** A conducted change in the transmembrane potential of excitable cells, initiated by a change in the membrane permeability to sodium ions; see also nerve impulse.
- **Axolemma:** The cell membrane of an axon, continuous with the cell membrane of the soma and dendrites and distinct from any glial cell coverings.
- **Axon hillock:** Portion of the neural soma adjacent to the initial segment.
- **Axon terminals:** The network of fine branches at the end of the axon, each branch ending at a synaptic or neuroeffector junction.

- **Axon transport:** A process involving intracellular filamentous structures by which materials are moved from one end of an axon to the other and.
- **Axon:** Elongate extension of a neuron that conducts an action potential away from the soma and toward the synaptic terminals.
- **Axoplasm:** Cytoplasm within an axon.
- **'Command' neurons:** Neurons or groups of neurons whose activity initiates the series of neural events resulting in a voluntary action.
- **Central nervous system (CNS):** The brain and spinal cord.
- **Cholinergic synapse:** Synapse where the parasynaptic membrane releases ACh on stimulation.
- **Efferent fiber:** An axon that carries impulses away from the CNS.
- **Endoneurium:** A delicate network of connective tissue fibers that surrounds individual nerve fibers.
- **Excitatory postsynaptic potential:** The depolarization of a postsynaptic membrane by a chemical neurotransmitter released by the presynaptic cell.
- **Excitatory synapse:** A synapse which, when activated either increase the likelihood than the membrane potential of the postsynaptic neuron will reach threshold and undergo action potentials or increase the firing frequency of existing action potentials.
- **Gamma-aminobutyric acid:** A neurotransmitter of the CNS whose effects are usually inhibitory.
- **Ganglion/ganglia:** A collection of nerve cell bodies outside of the CNS.
- **Glial cells:** Supporting cells in the neural tissue of the CNS and PNS.
- **Glossopharyngeal nerve:** Cranial Nerve IX.
- **Interneuron:** An association neuron; neurons inside the CNS that are interposed between sensory and motor neurons.
- **Memory:** The ability to recall information on sensations can be divided into short-term and long-term memories.

- **Myelin:** Insulating sheath around an axon consisting of multiple layers of glial cell membrane; significantly increase conduction rate along the axon.
- **Nerve impulse:** An action potential in a nerve cell membrane.
- **Neurilemma:** The outer surface of a glial cell that encircles an axon.
- **Neuroeffector junction:** A synapse between a motor neuron and a peripheral effector, such as a muscle, gland cell or fat cell.
- **Neuroglia:** Non-neural cells of the CNS and PNS that support and protect the neurons.
- **Neuron:** A nerve cell.
- **Neurotransmitter:** Chemical compound released by one neuron to affect the transmembrane potential of another.
- **Node of Ranvier:** Area between adjacent glial cells where the myelin covering of an axon is incomplete.
- **Neuromuscular junction:** A specific type of neuroeffector junction.
- **Perineurium:** Connective tissue partition that separates adjacent bundles of nerve fibers in a peripheral nerve.
- **Peripheral nervous system (PNS):** All neural tissue outside of the CNS.
- **Saltatory conduction:** Relatively rapid conduction of a nerve impulse between successive nodes of a myelinated axon.
- **Schwann cells:** Glial cells responsible for the neurilemma that surrounds axons in the PNS.
- **Synapse:** Site of communication between a nerve cell and some other cell; if the other cell is not a neuron, neuroeffector junction is often used.
- **Synaptic delay:** The period between the arrival of an impulse at the presynaptic membrane and the initiation of an action potential in the postsynaptic membrane.
- **Transmembrane potential:** The potential difference, in million volts, measured across the cell membrane; a potential difference that results from the uneven distribution of positive and negative ions across a cell membrane.

- **Unipolar neuron:** A sensory neuron whose soma lies in a dorsal root ganglion or a sensory ganglion of a cranial nerve.
- **Unmyelinated axon:** Axon whose neurilemma does not contain myelin and where continuous conduction occurs.
- **White matter:** Regions inside the CNS that are dominated myelinated axons.

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### 3.6 Self-Learning Exercises:

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#### Section -A (Very Short Answer Type)

##### Fill in the blanks:

1. Schwann cells are located in the \_\_\_\_\_ (CNS/PNS).
2. Excessive polarization due to GABA is created due to the opening of \_\_\_\_\_ channels ( $\text{Cl}^-/\text{Na}^+$ ).
3. Multiple sclerosis is a disease that attacks the \_\_\_\_\_ of neurons in the CNS (Myelin sheath/ Axon terminals).
4. The progression of a nerve impulse with the nodes of Ranvier is called \_\_\_\_\_ (Saltatory Conduction/ Transmission).
5. Supporting cells located within the CNS are collectively called \_\_\_\_\_ (neuroglial cell/ Perikaryon).
6. A dendrite conducts nerve impulses \_\_\_\_\_ the cell body (Away from/towards).
7. Gaps in the myelin sheath are called \_\_\_\_\_ (Nodes of Ranvier/ the synapse)
8. The resting potential indicates that the inside of the neuron is \_\_\_\_\_ compared to the outside (positive/negative).
9. The "sodium-potassium pump" pumps \_\_\_\_\_ (sodium ions out and potassium ions in/ sodium ions in and potassium ions out).
10. Excitatory signals have a \_\_\_\_\_ effect (hyperpolarizing/ depolarizing).

#### Section -B (Short Answer Type)

1. What are the functional differences between neurons and glial cells?
2. What are the three main parts into which a neuron can be divided? What are their respective functions?

3. What are synapses?
4. What are ganglia?
5. What is meant by the peripheral nervous system (PNS)?
6. What is the function of the myelin sheath? Do all axons present a myelin sheath?
7. What are the cells that produce the myelin sheath? Of which substance is the myelin sheath formed?

### **Section -C (Long Answer Type)**

#### **Describe in Detail.**

1. How is the depolarization of the neuronal plasma membrane generated? How does the cell return to its original rest?
2. What is the excitation threshold of a neuron? How does this threshold relate to the “all-or-nothing” rule of the neural transmission?
3. What is a neural impulse? What is the mechanism by which the neural impulse is transmitted along the axon?
4. How does synaptic transmission between neurons take place?

#### **Answer Key for Section - A**

1. PNS
2.  $\text{Cl}^-$
3. Myelin sheath
4. Saltatory Conduction
5. Neuroglial cell
6. Towards
7. Nodes of Ranvier
8. Negative
9. Sodium ions out and potassium ions in
10. Depolarizing

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### **3.7. Reference**

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## Unit - 4

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# Neurotransmitters, Reflex Action : Various Types Of Central And Peripheral Reflexes In Mammalian Nervous System

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- 4.1 Neurotransmitters I
    - Types
    - Functions
  - 4.2 Reflex Action
    - Structure
    - Component of Reflex Action
  - 4.3 Mechanism of Reflex Action
  - 4.4 Types of Reflex Action
    - Monosynaptic Reflex
    - Polysynaptic Reflex
    - Simple Reflex
    - Acquired Reflex
  - 4.5 Transmission of Nerve Impulse along the Nerve Fibre
  - 4.6 Structure of Synapse
  - 4.7 Mechanism of Transmission of Nerve Impulse at a Synapse
  - 4.8 Self Learning Exercise
  - 4.9 Reference
- 

### 4.1 Neurotransmitters I

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#### Definition

**Neurotransmitters** are endogenous chemicals that transmit signals across a synapse from one neuron (brain cell) to another 'target' neuron.

Neurotransmitters are released from synaptic vesicles in synapses into the synaptic cleft, where they are received by receptors on other synapses. Many Neurotransmitters are synthesized from plentiful and simple precursors such as amino acids, which are readily available from the diet and only require a small number of biosynthetic steps to convert them. Neurotransmitters play a major role in shaping everyday life and functions. Scientists do not yet know exactly how many neurotransmitters exist, but more than 100 chemical messengers have been identified.

Neurotransmitters are stored in a synapse in synaptic vesicles, clustered beneath the membrane in the axon terminal located at the presynaptic side of the synapse. Neurotransmitters are released into and diffused across the synaptic cleft, where they bind to specific receptors in the membrane on the postsynaptic side of the synapse.

Most neurotransmitters are about the size of a single amino acid, however, some neurotransmitters may be the size of larger proteins or peptides.

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#### **4.4 Types of neurotransmitters.**

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There are many different ways to classify neurotransmitters. Dividing them into amino acids, peptides, and monoamines is sufficient for some classification purposes.

##### **Major neurotransmitters:**

**Amino acids:** glutamate, aspartate, D-serine,  $\gamma$ -aminobutyric acid (GABA), glycine

**Monoamines:** dopamine (DA), norepinephrine (noradrenaline; NE), epinephrine (adrenaline), histamine, serotonin (SER, 5-HT)

- **Trace amines:** phenethylamine,
- *N*-ethylphenethylamine, tyramine,
- 3-iodothyronamine, octopamine, tryptamine, etc.

**Peptides:** somatostatin, substance P, cocaine and amphetamine regulated transcript, opioid peptides

Gasotransmitters: nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H<sub>2</sub>S)

Others: acetylcholine (ACh), adenosine, etc.

In addition, over 50 neuroactive peptides have been found, and new ones are discovered regularly. Many of these are "co-released" along with a small-molecule transmitter. Nevertheless, in some cases a peptide is the primary transmitter at a synapse.  $\beta$ -endorphin is a relatively well known example of a peptide neurotransmitter because it engages in highly specific interactions with opioid receptors in the central nervous system.

Single ions (such as synaptically released zinc) are also considered neurotransmitters by some, as well as some gaseous molecules such as nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H<sub>2</sub>S). The gases are produced in the neural cytoplasm and are immediately diffused through the cell membrane into the extracellular fluid and into nearby cells to stimulate production of second messengers. Soluble gas neurotransmitters are difficult to study, as they act rapidly and are immediately broken down, existing for only a few seconds.

The most prevalent transmitter is glutamate, which is excitatory at well over 90% of the synapses in the human brain. The next most prevalent is Gamma-Aminobutyric Acid, or GABA, which is inhibitory at more than 90% of the synapses that do not use glutamate. Although other transmitters are used in fewer synapses, they may be very important functionally: the great majority of psychoactive drugs exert their effects by altering the actions of some neurotransmitter systems, often acting through transmitters other than glutamate or GABA. Addictive drugs such as cocaine and amphetamines exert their effects primarily on the dopamine system. The addictive opiate drugs exert their effects primarily as functional analogs of opioid peptides, which, in turn, regulate dopamine levels.

### **Here are a few examples of important neurotransmitter actions:**

#### **Glutamate**

Glutamate is used at the great majority of fast excitatory synapses in the brain and spinal cord. It is also used at most synapses that are "modifiable", i.e. capable of increasing or decreasing in strength. Modifiable synapses are thought to be the main memory-storage elements in the brain. Excessive glutamate release can over stimulate the brain and lead to excitotoxicity causing cell death resulting in seizures or strokes. Excitotoxicity has been implicated in certain chronic diseases including ischemic stroke, epilepsy, Amyotrophic lateral sclerosis, Alzheimer's disease, Huntington disease, and Parkinson's disease.

## **GABA**

GABA is used at the great majority of fast inhibitory synapses in virtually every part of the brain. Many sedative/tranquilizing drugs act by enhancing the effects of GABA. Correspondingly, glycine is the inhibitory transmitter in the spinal cord.

## **Acetylcholine**

Acetylcholine was the first neurotransmitter discovered in the peripheral and central nervous systems. It activates skeletal muscles in the somatic nervous system and may either excite or inhibit internal organs in the autonomic system. It is distinguished as the transmitter at the neuromuscular junction connecting motor nerves to muscles. The paralytic arrow-poison curare acts by blocking transmission at these synapses. Acetylcholine also operates in many regions of the brain, but using different types of receptors, including nicotinic and muscarinic receptors.

## **Dopamine**

Dopamine has a number of important functions in the brain; this includes regulation of motor behavior, pleasures related to motivation and also emotional arousal. It plays a critical role in the reward system; people with Parkinson's disease have been linked to low levels of dopamine and people with schizophrenia have been linked to high levels of dopamine.

## **Serotonin**

Serotonin is a monoamine neurotransmitter. Most is produced by and found in the intestine (approximately 90%), and the remainder in central nervous system neurons. It functions to regulate appetite, sleep, memory and learning, temperature, mood, behaviour, muscle contraction, and function of the cardiovascular system and endocrine system. It is speculated to have a role in depression, as some depressed patients are seen to have lower concentrations of metabolites of serotonin in their cerebrospinal fluid and brain tissue.<sup>[19]</sup>

**Norepinephrine** which focuses on the central nervous system, based on patients sleep patterns, focus and alertness. It is synthesized from tyrosine.

**Epinephrine** which is also synthesized from tyrosine takes part in controlling the adrenal glands. It plays a role in sleep, with ones ability to stay become alert, and the fight-or-flight response.

**Histamine** works with the central nervous system (CNS), specifically the hypothalamus (tuberomammillary nucleus) and CNS mast cells.

## 4.2 Reflex Action

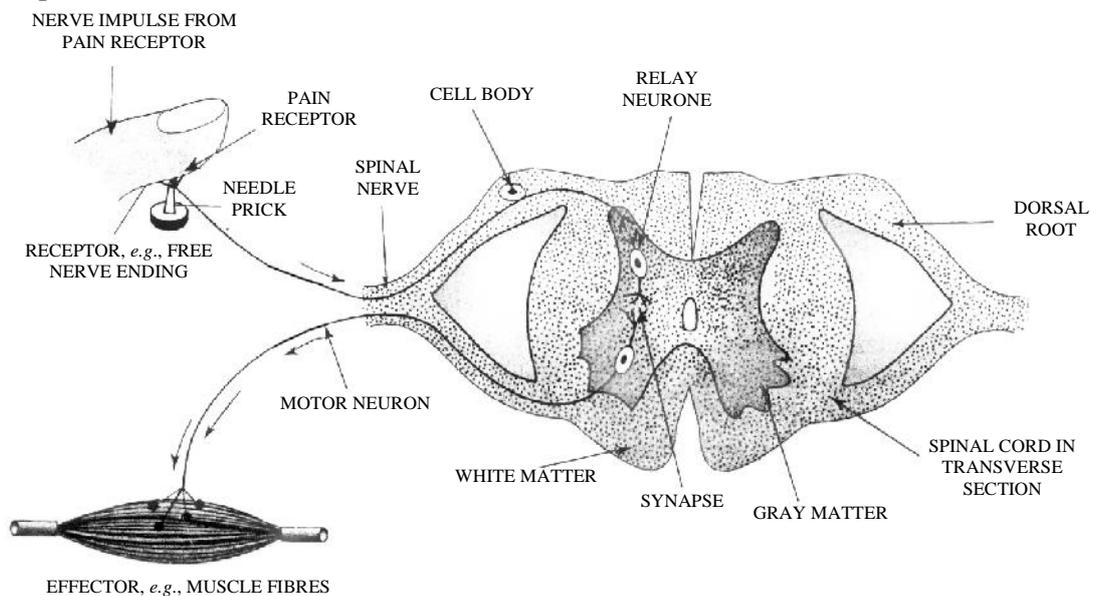
First of all Marshal Hall (1833) studied the reflex action. *Best* and *Taylor* defined reflex action “simplest form of irritability associated with the nervous system is reflex actions or a reflex reaction is an immediate involuntary response to a stimulus.” The reflex actions are involuntary actions because these are not under the conscious control of the brain. The spinal cord and brain stem are responsible for most of the reflex movements. A few examples of the reflex actions are withdrawal of hand or leg if pricked by a pin, secretion of saliva as soon as one thinks of delicious food or mere its sight causes salivation, if the body part is touched with acid or hot object it is automatically, without thinking and planning is withdrawn, cycling, motor driving etc. Central nervous system is responsible for the control of reflex action.

Reflex arc is formed by the neurons forming the pathway taken by the nerve impulses in reflex action. The simplest reflexes are found in animals involving a single neuron and the following pathway —

Stimulus → Receptor  $\xrightarrow{\text{Neuron}}$  Effector → Response

The reflex areas in all the higher animals than coelenterates, include at least two neurons, an afferent or sensory neuron carrying impulses from a receptor towards aggregation of nervous tissue which may be a ganglion, nerve cord or central nervous system and an efferent or motor neuron carrying impulses away from the aggregation to an effector.

(a) **Component of reflex action** : The whole of the reflex are includes six parts –



**Fig. Reflex arc**

(1) **Receptor organs** : Receptors are windows of the body or guards of the body. These are situated on all, important organs, for example – eyes, nose, ear, tongue, integument etc. These perceive the stimuli from outside the body.

(2) **Sensory neurons** : These are also termed afferent neurons. These carry the stimuli from receptors to spinal cord. These neurons are situated in the ganglion on the dorsal side of spinal cord.

(3) **Nerve centre** : Spinal cord is termed as nerve centre. Synaptic connections are formed in it.

(4) **Association neurons** : These are also called intermediate neurons or interstitial neurons. These are found in spinal cord. They transfer the impulses from sensory neurons to motor neurons.

(5) **Motor neurons** : These are situated in the ventral horn of spinal cord. These carry the impulses to effector organs.

(6) **Effector organs** : These are the organs, which react and behave in response to various stimuli, for example – muscles and glands.

(b) **Mechanism of reflex action** : The time taken by a reflex action is too short, for example – in frog it is 0.3 meter per second and in man 5-120 meter per second. Whenever, a part of the body is stimulated by any stimulus, for example – pin pricking, then the stimulus is converted into impulse. This impulse is perceived by the dendrites of sensory neurons. From here, the stimulus reaches the spinal cord through axonic fibres. In the spinal cord, this stimulus passes through synaptic junctions and reaches the intermediate neurons, from where this stimulus reaches the effector organs through visceral motor nerve fibres. As soon as the stimulus reaches the effector organs, it is stimulated and that part of the body is immediately withdrawn. The whole reflex action takes place so rapidly and quickly that we know it when it is completed.

(c) **Type of reflex** : The reflexes are of following types –

(1) Monosynaptic reflex

(2) Polysynaptic Spinal Reflex

(3) Polysynaptic Spinal/Brain Reflexes

(4) Unconditioned or Simple reflex

(5) Conditioned or Acquired reflexe

(1) **Monosynaptic reflex** : This is the simplest reflex found in vertebrates. The simplest reflex found in vertebrates. The sensory neuron synapses directly on to

the motor neuron cell body. In this case the reflex action takes place without the involvement of brain.

(2) **Polysynaptic spinal reflex** : This has at least two synapses situated within the spinal cord. It involves a third type of neuron also – the internuncial or inter-mediate relay neuron. The synapses take place between the sensory neuron and intermediate neuron, and between intermediate neuron and the motor neuron. These two reflex arcs allow the body to make automatic, involuntary, homeostatic adjustments, to changes in the external environment, such as the iris pupil reflex and balance during locomotion, and also in the internal environment such as breathing rate and blood pressure.

(3) **Polysynaptic spinal/brain reflexes** : In this case the sensory neuron synapses in the spinal cord with a second sensory neuron, which passes to the brain. The latter sensory neurons are part of the ascending nerve fibre tract and have their origin in preintermediate neuron synapse. The brain is capable of identifying this sensory information and stores it for further use. The motor activity may be initiated by the brain anytime and the impulses are transmitted down the motor neurons in descending nerve fibre tract, to synapse directly with spinal motor neurons in the postintermediate synaptic region.

(4) **Simple reflex** : Simple reflex is also known as **unconditioned reflex**. It is inborn, unlearned, reflex to a stimulus. Simple reflex is mostly protective in function. Example of simple reflex are

- (a) **Knee jerk** – Tendon of patella tapped.
- (b) **Corneal reflex** (blinking reflex) – closing of eyelids.
- (c) Rapid withdrawal of hand while burned or pricked.
- (d) Quick recovery of balance while falling.
- (e) **Scratch reflex** of frog – in pitched frog with acetic acid.
- (f) Coughing, sneezing and yawning.

(5) **Acquired reflex** : Acquired reflex is also known as conditioned reflex. It is not inborn, but acquired and dependent on past experience, training and learning. Demonstration of conditioned reflex was first made by Russian physiologist Ivan Petrovitch Pavlov (1846-1936) in hungry dog. Pavlov rang the bell while feeding dog, thus associated the unconditioned response with additional stimulus. Examples of conditioned reflex are learning of dancing, cycling, swimming, singing,, driving, etc. These actions are under cerebral control during learning.

**Main properties of nervous tissue :** The nervous tissue has two outstanding properties excitability and conductivity.

(1) **Excitability :** It is the ability of the nerve cells and fibres to enter into an active state called the **state of excitation** in response to a stimulus. Excitation arises at the receptors on account of various stimuli such as light, temperature, chemical, electrical or pressure which constantly act on the organisms.

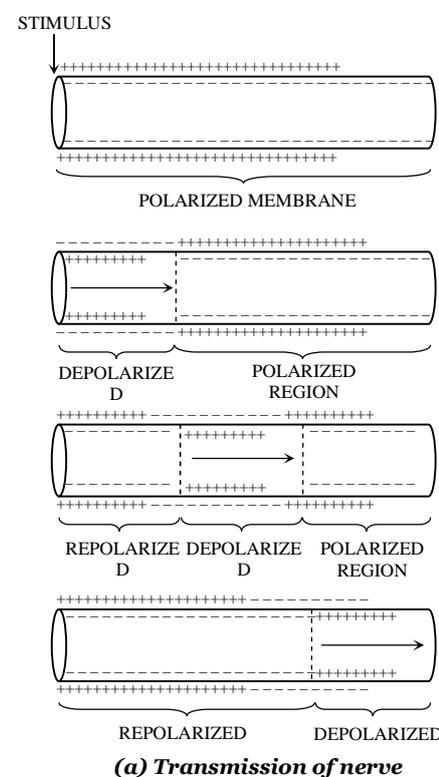
(2) **Conductivity :** The excitation does not remain at the site of its origin. It is transmitted along nerve fibres. The transmission of excitation in a particular direction is called conductivity.

**Definition of nerve impulse :** A wave of reversed polarity or depolarization (action potential) moving down an axon is called a nerve impulse.

**Mechanism of conduction of nerve impulse :** Most accepted mechanism of nerve impulse conduction is ionic theory proposed by Hodgkin and Huxley. This theory states that nerve impulse is an electro-chemical even governed by differential permeability of neurilemma to  $Na^+$  and  $K^+$  which in turn is regulated by the electric field.

**(i) Transmission of nerve impulse along the nerve fibre**

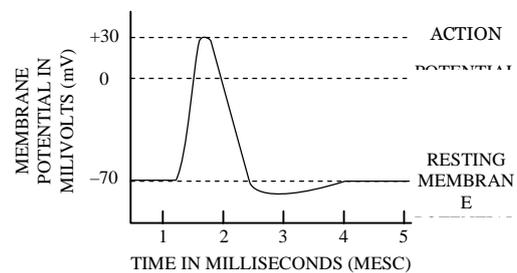
**(a) Polarization (Resting membrane potential-RMP) :** In a resting nerve fibre (a nerve fibre that is not conducting an impulse), sodium ions ( $Na^+$ ) predominate in the extracellular fluid, whereas potassium ions ( $K^+$ ) predominate in the intracellular fluid (within the fibre). Intracellular fluid also contains large number of negatively charged (anions) protein molecules.  $Na^+$  are 10 times more outside the neuron and  $K^+$  ions are 25 times more inside the cell. Thus it makes a considerable difference between the ion concentration outside and inside the plasma membrane. It also causes a difference in electrical charges on either side of the membrane. The plasma membrane is electrically positive outside and negative inside. This difference is called potential difference. The potential difference across the plasma membrane is known as resting potential. This potential averages  $-70\text{ mv}$  ( $-60$  to  $-90\text{ mv}$ ) in inner side of membrane in respect to outer side.



Due to different concentrations of ions on the two sides of the membrane, sodium ions tend to diffuse into the nerve fibre and potassium ions tend to diffuse out of the nerve fibre. The membrane of a resting nerve fibre is more permeable to potassium than to sodium. So potassium leaves the nerve fibre faster than sodium enters it. This results in a higher concentration of cations outside the membrane compared to the concentration of cations inside it. This state of the resting membrane is called polarised state and makes its inner side electronegative to its outside.

(b) **Depolarization (Action membrane potential or AMP)** : When the nerve fibre is stimulated mechanically, electrically, thermally or chemically a disturbance is felt at the point of stimulation which gives rise to a local excitatory state. The membrane becomes permeable to sodium ions. Suddenly sodium ions rush inside the nerve fibre and potassium ions diffuse out of the axon membrane. Due to the diffusion of ions, more sodium ions enter the axon than potassium ions leave it, so that the positive and negative charges on the outside and inside of the axon membrane are reversed. The membrane is negatively charged on the outside and positively charged on the inside. The membrane with reversed polarity is said to be depolarized. The depolarization of the membrane suddenly passes as a wave along the nerve fibre. Thus the impulse is propagated as a wave of depolarization (reversed polarity). This wave of depolarization travelling down a nerve fibre is called **action potential**. Infact, the action potential “moves” in the manner of a spark moving along a fuse. This “moving” action potential constitutes the **nerve impulse**. The action potential (impulse) is the basic means of communication within the nervous system. The action potential of + 45 *mv* on inner side of axolemma in respect to its outer side is also called spike potential.

(c) **Repolarization** : With the increase of sodium ions inside the nerve cell, the membrane becomes less permeable to sodium ions whereas the permeability membrane to potassium ions increases. The sodium ions are pumped out of the cell and potassium

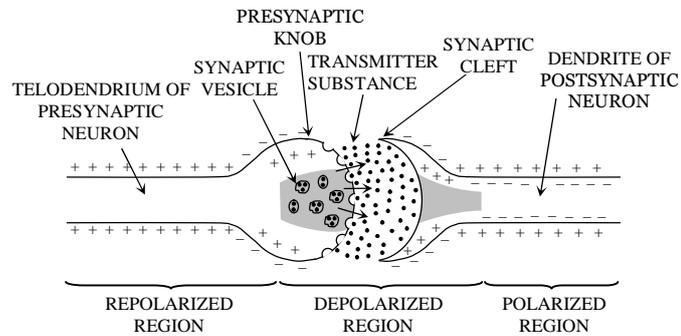


(b) Record of potential

ions are pumped into the cell until the original resting state of ionic concentration is achieved. Thus this makes the membrane negative on inside and positive on outside. This process is called repolarization.

The last movement of ions is thought to take place by an active transport mechanism called sodium potassium pump (also called sodium potassium exchange pump or sodium pump). The sodium-potassium pump is a process of expelling out sodium ions and drawing in potassium ions against concentration and electrochemical gradient. The entire process of repolarization requires some time during which the nerve cannot be stimulated again. This period is called **refractory period**.

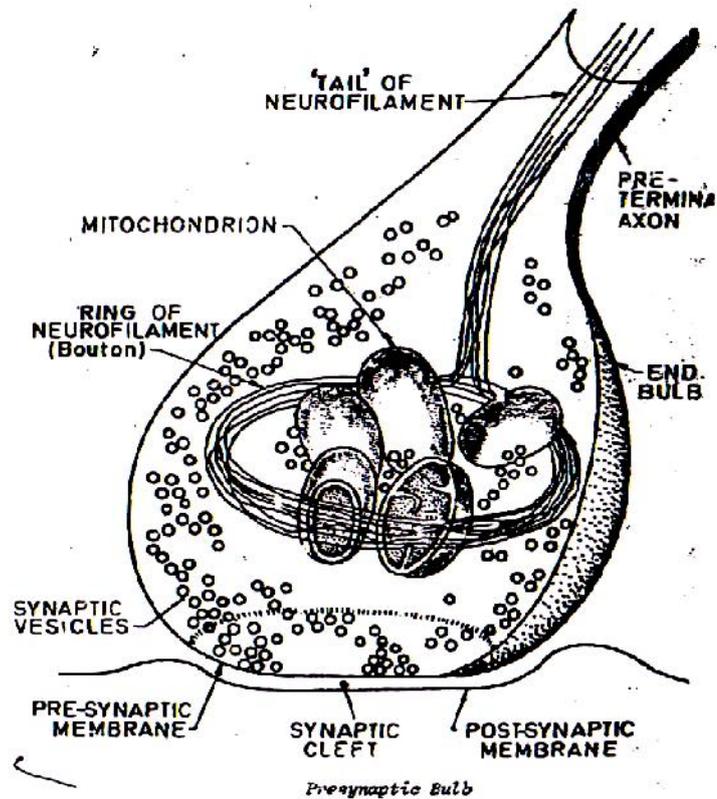
During repolarization, as the cell returns to its resting potential, the neuron is ready to receive another stimulus.



**Fig. Impulse conduction at**

(ii) **The synapse :** The synapse is an area of functional contact between one neuron and another for the purpose of transferring information. Synapses are usually found between the fine terminal branches of the axon of one neuron and the dendrites or cell body of another. This type of neuron is called axo-dendrite synapse. Sir Charles Sherrington (1861-1954) was the first person who used the term 'synapse' to the junctional points between two neurons.

**Structure of synapse :** A typical (generalized synapse) consists of a bulbous expansion of a nerve terminal called a pre-synaptic knob lying close to the membrane of a dendrite. The cytoplasm of the synaptic knob contains mitochondria, smooth endoplasmic reticulum, microfilaments and numerous synaptic vesicles. Each vesicle contains neurotransmitter (chemical substance) responsible for the transmission of the nerve impulse across the synapse. The membrane of the synaptic knob nearest the synapse is thickened and forms the presynaptic membrane. The membrane of the dendrite is also thickened and is called the post synaptic membrane. These membranes are separated by a gap, the synaptic cleft. It is about  $200 \text{ \AA}$  across. The post synaptic membrane contains large protein molecules which act as receptor sites for neurotransmitter and numerous channels and pores.



The two main neurotransmitters in vertebrate nervous system are acetylcholine (ACh) and noradrenaline although other neurotransmitters also exist. Acetylcholine (ACh) was the first neurotransmitter to be isolated and obtained by Otto Loewi in 1920 from the endings of parasympathetic neurons of the vagus nerve in frog heart. Neurons releasing acetylcholine are described as cholinergic neurons and those releasing noradrenaline are described as adrenergic neurons.

**Mechanism of transmission of nerve impulse at a synapse :** The process of chemical transmission across synapses was discovered by Henry Dale (1936). The physiological importance of synapse for the transmission of nerve impulses was established by McLennan in 1963. A brief description of the mechanism of synaptic transmission is given below

- (i) When an impulse arrives at a presynaptic knob, calcium ions from the synaptic cleft enter the cytoplasm of the presynaptic knob.
- (ii) The calcium ions cause the movement of the synaptic vesicles to the surface of the knob. The synaptic vesicles are fused with the presynaptic membrane and

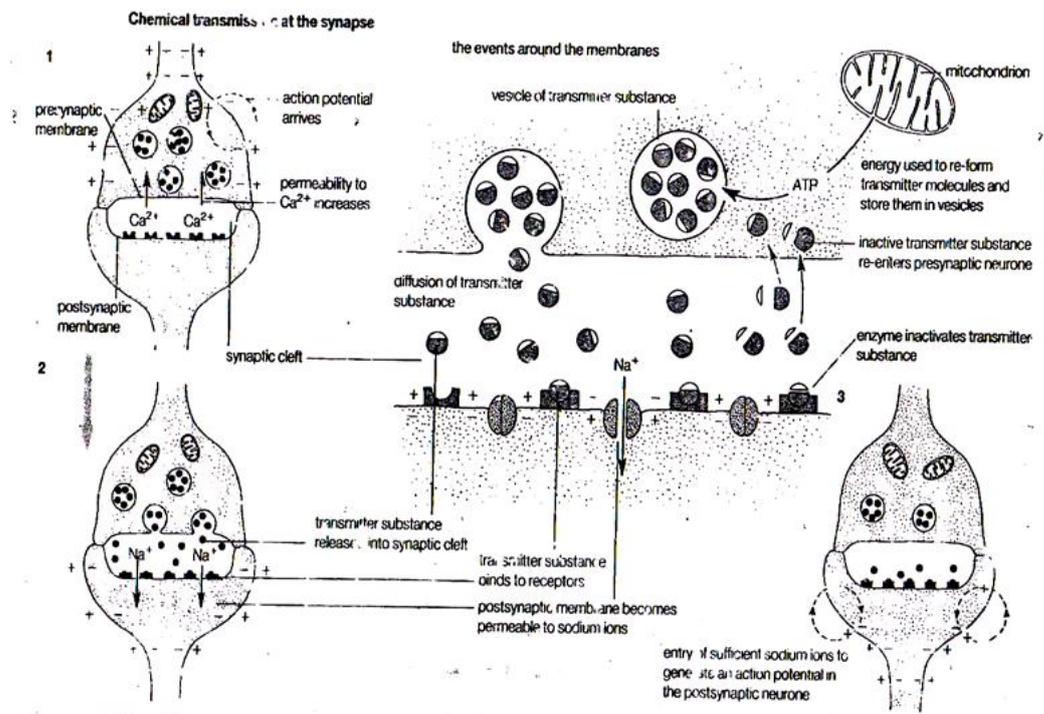
get ruptured (exocytosis) to discharge their contents (neurotransmitter) into the synaptic cleft.

(iii) The synaptic vesicles then return to the cytoplasm of the synaptic knob where they are refilled with neurotransmitter.

(iv) The neurotransmitter of the synaptic cleft binds with protein receptor molecules on the post synaptic membrane. This binding action changes the membrane potential of the postsynaptic membrane, opening channels in the membrane and allowing sodium ions to enter the cell. This causes the depolarization and generation of action potential in the post-synaptic membrane. Thus the impulse is transferred to the next neuron.

(v) Having produced a change in the permeability of the postsynaptic membrane the neurotransmitter is immediately lost from the synaptic cleft. In the case of cholinergic synapses, acetylcholine (ACh) is hydrolysed by an enzyme acetylcholinesterase (AChE) which is present in high concentration at the synapse.

(vi) The products of the hydrolysis are acetate and choline which are reabsorbed into the synaptic knob where they are resynthesized into acetylcholine, using energy from ATP.



**Neuromuscular junction :** Impulses are conducted from a neuron to a muscle cell across an area of contact called neuromuscular junction. When a nerve fibre ends on a muscle fibre, it forms motor end plate. The motor end plates have vesicles and mitochondria. The vesicles secrete neurotransmitter. When the motor impulse from the nerve is received on the motor end plates, a local depolarization occurs there resulting in the excitation of the muscle fibre.

**Neuroglandular junction :** It is an area of contact between a neuron and glandular cells. There is also a gap which is bridged at the time of the transmission of the impulse by a neurotransmitter.

**Synapse, A one-way valve :** The synapse cannot transmit an impulse in the reverse direction as the dendrites cannot secrete a neurotransmitter. Thus, the synapse acts as a one-way valve, allowing the conduct of impulse from axon to dendron only.

**Synaptic delay :** Transmission of an impulse across a synapse is slower than its conduction along a neuron. This is because of the time needed for the release of a neurotransmitter, its diffusion through the synaptic cleft, and its action on the postsynaptic membrane. The difference in the rate is called **synaptic delay**. It amounts to about half a millisecond at body temperature ( $37^{\circ}C$ ).

**Synaptic fatigue :** Repeated stimulation of the presynaptic knob may deplete the neurotransmitter, and this may fail to stimulate the postsynaptic membrane. This condition of the synapse is termed synaptic fatigue. It lasts for several seconds during which the neurotransmitter is resynthesized. Synaptic fatigue is the only fatigue that affects the nervous tissue. Conduction of the nerve impulse along the neurons is not subject to fatigue.

**“All or None law” (Keith Lucas, 1905) :** When stimulated, the axon membrane (= axolemma) does not respond for a moment due to its resistance or threshold to stimulation. However, when its threshold is broken, the stimulation is conducted through its whole length as a strong impulse. If the stimulation is too weak to break the axon’s threshold, impulse is not established, but if the intensity of stimulation is much more than the threshold value, impulse conduction remains normal. Thus, the action potential obeys “all or none law”. In other words, impulse conduction is such a triggered phenomenon which, though occurs in a twinkling, like an explosion, but only when it reaches “ignition point” or firing level”.

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## 4.7 Glossary

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- **Neurotransmitters** are endogenous chemicals that transmit signals across a synapse from one neuron (brain cell) to another 'target' neuron. Neurotransmitters are released from synaptic vesicles in synapses into the synaptic cleft, where they are received by receptors on other synapses. There are many different ways to classify neurotransmitters. Dividing them into amino acids, peptides, and monoamines is sufficient for some classification purposes. The most prevalent transmitter is glutamate, which is excitatory at well over 90% of the synapses in the human brain. The next most prevalent is Gamma-Aminobutyric Acid, or GABA, which is inhibitory at more than 90% of the synapses that do not use glutamate.
- The **reflex actions** are involuntary actions because these are not under the conscious control of the brain. The spinal cord and brain stem are responsible for most of the reflex movements. reflexes are of following types- (1) Monosynaptic reflex (2) Polysynaptic Spinal Reflex (3) Polysynaptic Spinal/Brain Reflexes (4) Unconditioned or Simple reflex (5) Conditioned or Acquired reflex
- The **synapse** is an area of functional contact between one neuron and another for the purpose of transferring information. Synapses are usually found between the fine terminal branches of the axon of one neuron and the dendrites or cell body of another.

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## 4.8 Self learning exercise

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### Section -A (Very Short Answer Type)

1. Who studied reflex action (Marshall Hall)
2. Write example of simple reflex ? (Knee Jerk)
3. what is value of spike potential ? (+45 mv)
4. What is value of membrane potential (-70 mv)
5. Which Scientist use the term synapse (Charles Sherrington)
6. Which chemical substances are found in synaptic knobs ?  
(Neurotransmitter)
7. Which enzymes is present in the high concentration at the synapse ?  
(Acetylcholinesterase)
8. Which type of neuron is found in vertebrates ? (Multipolar neuron)

9. Which rule is followed by nerve impulse ? (All or none)
10. Write example of polysynaptic spinal reflex. (Inis-pupil reflex)

### **Section -B (Short Answer Type)**

1. What is synaptic delay ?
2. What is all or NONE LAW ?
3. Define synaptic fatiuge.?
4. Write difference between action and membrane potential ?
5. What is polysynaptic reflexes ?
6. Draw a labeled diagram of Neuron.
7. What is repolarisation.
8. Define refractory period.
9. What is condition reflex. Give two examples.
10. What are Adenergetic and cholinergic nerve fibres.

### **Section -C (Long Answer Type)**

- 1 Describe Mechanism of Reflex action
- 2 Write detain account of synaptic transmission
- 3 Write Mechanism of nerve impulse transmission.
- 4 Describe Pavlov experiment.what is habbit formation .

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## **4.9 References**

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## Unit -5

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# **Physiology of the receptor system: general mechanism involved in stimulus transduction at receptor sites, functional architecture and stimulus processing in retina, organ of corti and olfactory epithelium**

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### **Structure of the Unit**

- 5.0 Objectives
- 5.1 Introduction
- 5.2 Physiology of the receptor system
  - 5.2.1. Synapses
  - 5.2.2. Reflex Arc
  - 5.2.3. Receptor cells
    - 5.2.3.1. Introduction
    - 5.2.3.2. Classification of Receptors
- 5.3. General mechanism involved in stimulus transduction at receptor sites
  - 5.3.1. Transduction
  - 5.3.2. The basic process of transduction
  - 5.3.3. Transduction in the gustatory (taste) system
- 5.4. Functional architecture and stimulus processing in retina
  - 5.4.1. Photoreceptors (Structure of Mammalian Eye)
  - 5.4.2. The Retina
    - 5.4.2.1 Functional architecture of the Retina
    - 5.4.2.2. Function (Stimulus processing in retina)

## 5.5 The Ear

- a) External ear
- b) Middle ear
- c) Inner ear

### 5.5.1. Organ of Corti

- a) Structure
- b) Function
- c) Auditory transduction
- d) Cochlear amplification
- e) Hearing loss

## 5.6 Olfactory epithelium

### 5.6.1. Odour

### 5.6.2. Anatomy of Olfactory Sensory Cells

#### 5.6.2.1. Olfactory epithelium

#### 5.6.2.2. Structure of Olfactory epithelium

#### 5.6.2.3. Neural Pathways for Olfactory Signals

#### 5.6.2.4. Electrophysiology of Olfactory Sensory Cells

#### 5.6.2.5. Possible Mechanisms of Odour Discrimination

#### 5.6.2.6. Reflex Olfactory Pathways

## 5.7 Summary

## 5.8 Self Assessment Questions

## 5.9 Reference Books

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## 5.0. Objectives

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After completing the unit, you will be able to understand about-

- Synapses
- Reflex Arc
- Different types of receptor cells
- The basic process of transduction
- Transduction in the gustatory (taste) system
- Photoreceptors (Structure of Mammalian Eye)
- Stimulus processing and functional architecture of the Retina

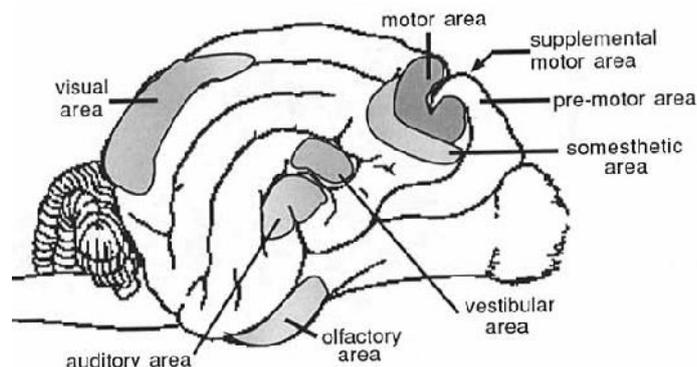
- Basic structure of the Ear
- Structure, function and auditory transduction of Organ of Corti
- Odour
- Anatomy of Olfactory Sensory Cells
- Structure, Neural Pathways, Electrophysiology of Olfactory epithelium

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## 5.1. Introduction

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Without sensory receptors in the human body, the race would eventually become extinct. A sensation from the outside environment is detected by one of the body receptors. That information is transmitted to the brain, and a reaction occurs. Through these receptors, we eat, love, avoid danger and move about. Sensory receptors bring information to the body and convert it to messages received by the brain where it is processed



**Figure: Sensory regions of human brain**

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## 5.2 Physiology of the receptor system

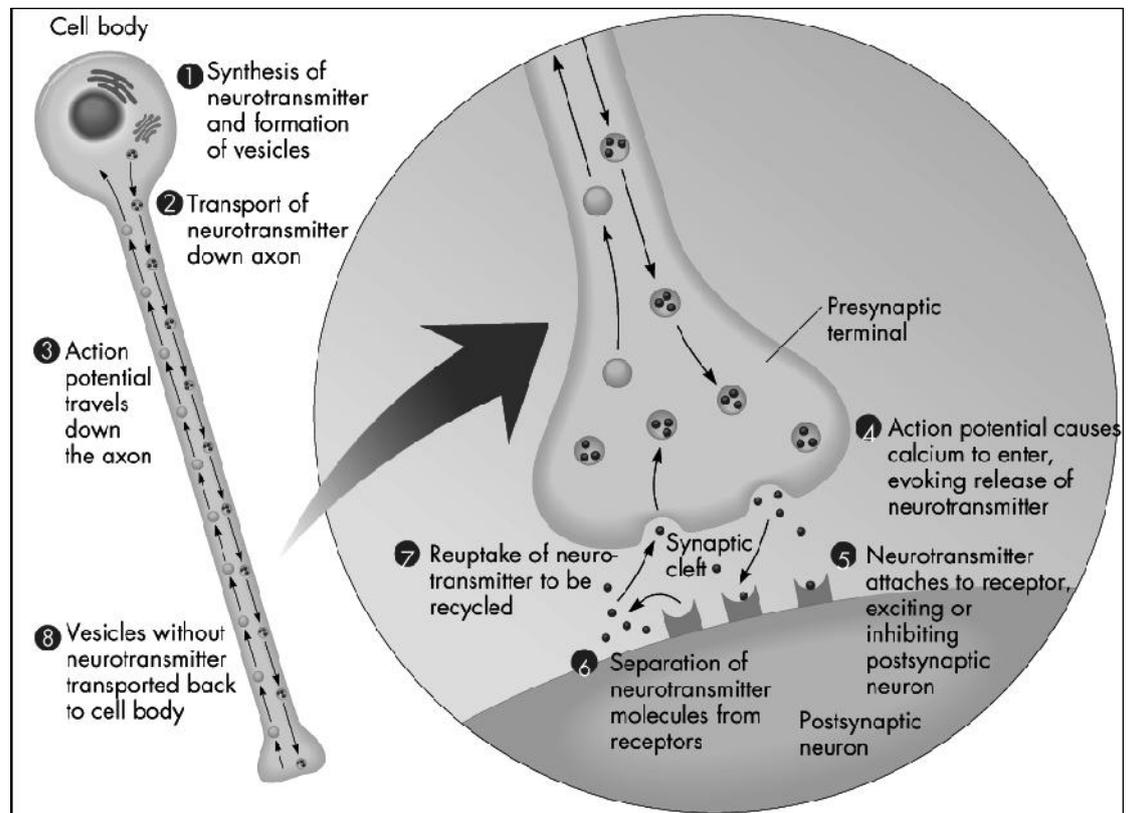
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### 5.2.1. Synapses

The function of the synapse is to transfer electric activity (information) from one cell to another. The transfer can be from nerve to nerve (neuro-neuro), or nerve to muscle (neuro-myo). The region between the pre- and postsynaptic membrane is very narrow, only 30-50 nm. It is called the synaptic cleft (or synaptic gap). Direct electric communication between pre- and post junctional cells does not take place; instead, a chemical mediator is utilized. The sequence of events is as follows:

1. An action pulse reaches the terminal endings of the presynaptic cell.
2. A neurotransmitter is released, which diffuses across the synaptic gap to bind to receptors in specialized membranes of the postsynaptic cell.

- The transmitter acts to open channels of one or several ion species, resulting in a change in the transmembrane potential. If depolarizing, it is an excitatory postsynaptic potential (EPSP); if hyperpolarizing, an inhibitory postsynaptic potential (IPSP).



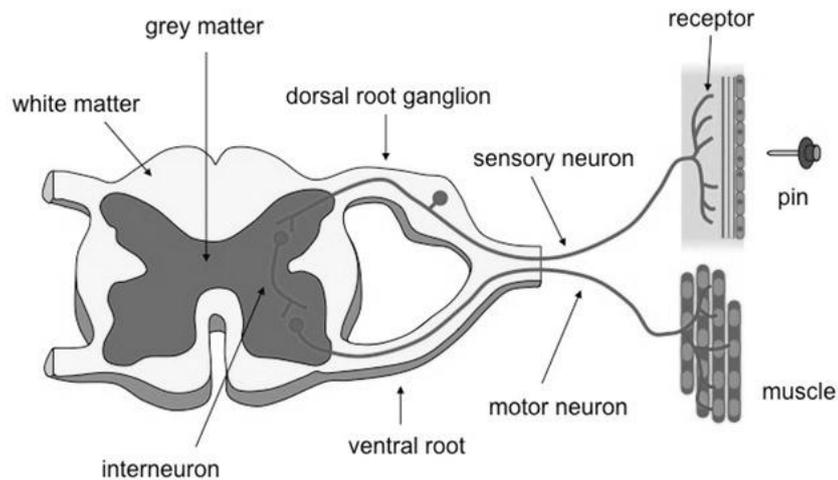
**Figure: The synapse function.**

The presynaptic nerve fiber endings are generally enlarged to form terminal buttons or synaptic knobs. Inside these knobs are the vesicles that contain the chemical transmitters. The arrival of the action pulse opens voltage-gated  $\text{Ca}^{2+}$  channels that permit an influx of calcium ions. These in turn trigger the release into the synaptic gap, by exocytosis, of a number of the "prepackaged" vesicles containing the neurotransmitter.

On average, each neuron divides into perhaps 1000 synaptic endings. On the other hand, a single spinal motor neuron may have an average of 10,000 synaptic inputs. In neuro-neuro synapses, the postjunctional site may be a dendrite or cell body, but the former predominates.

### 5.2.2. Reflex Arc

If our hand is mistakenly brought in contact to a hot surface, a set of signals to the hand and arm muscles resulting in a simpler reflex. In fact, a great deal of reflex activity is taking place *at all times* of which we are unaware. For example, input signals are derived from internal sensors, such as blood pressure, or oxygen saturation in the blood, and so on, leading to an adjustment of heart rate, breathing rate, etc.



**Figure: Reflex Arc**

When we touch the hot pot, a response is created in the body. At the point of contact with the hot pot, skin receptors quickly send nerve impulses (electrical) to the spinal cord (central nervous system) via sensory neurons.

In the spinal cord the nerve impulses move from sensory neurons to the interneurons. The impulses are then sent to motor neurons which project out of the spinal cord to stimulate our muscles (effector) to contract hence snatching our hand away from the hot pot. This is known as a 'reflex arc'. This process happens so fast that the response occurs before the message reaches the brain or the message may not be sent to the brain at all.

The *reflex arc* is considered to be the basic unit of integrated neural activity. It consists essentially of a sensory receptor, an afferent neuron, one or more synapses, an efferent neuron, and a muscle or other effector. The connection between afferent and efferent pathways is found, generally, in the spinal cord or the brain. The simplest reflex involves only a single synapse between afferent and efferent neurons (a monosynaptic reflex); an example is the familiar knee jerk reflex.

### 5.2.3. Receptor cells

#### 5.2.3.1. Introduction

To begin the overview of the physiology of the receptor system, we will discuss and try to understand some specialized sensory inputs of our body and how they are initiated. The central nervous system is kept continually informed of the ever-changing external and internal environment of the body by way of centrally directed signals which arise in its many and varied receptors. Scientists have recognized for almost 130 years that certain afferent nerve fibers of the peripheral nervous system are in contact with specialized non neural receptive structures which detect and transmit sensory information from the periphery to the CNS. The nonneural receptive structure together with its afferent nerve fiber is often called a receptor. Nature has evolved a variety of morphological structures which function as receptors.

There are many specialized receptor cells, each characterized by a modality to which it is particularly sensitive and to which it responds by generating a train of action pulses.

### **5.2.3.2. Classification of Receptors**

Sensory receptors may be classified as (1) extroreceptors, which sense stimuli arising external to the body; (2) introreceptors, which respond to physical or chemical qualities within the body; and (3) proprioceptors, which provide information on the body's position. Examples in each of these categories include the following:

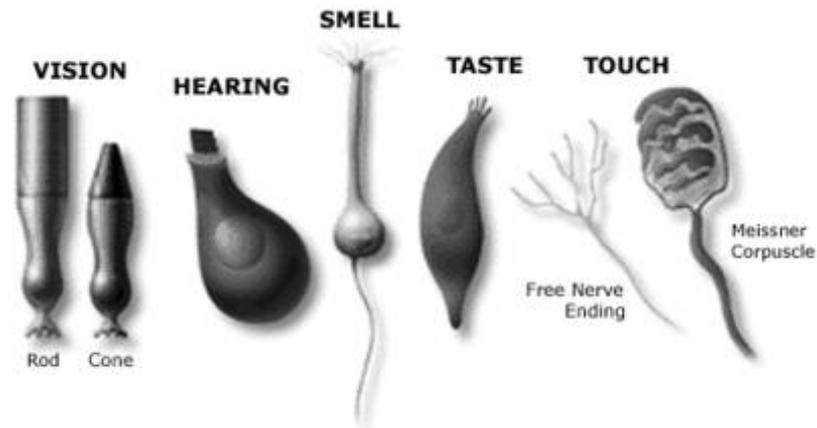
#### **1. Extroreceptors**

- a) Photoreceptors in the retina for, vision
- b) Chemoreceptors for sensing of smell and taste (gustatory)
- c) Mechanoreceptors for sensing sound, in the cochlea, or in the skin, for touch sensation
- d) Thermoreceptors (i.e., Krause and Ruffini cells), for sensing cold and heat

#### **2. Introreceptors**

- a) Chemoreceptors in the carotid artery and aorta, responding to the partial pressure of oxygen, and in the breathing center, responding to the partial pressure of carbon dioxide, taste cells in tongue.
- b) Mechanoreceptors in the labyrinth
- c) Osmoreceptors in the hypothalamus, registering the osmotic pressure of the blood
- d) Proprioceptors

- a. Muscle spindle, responding to changes in muscle length
- b. Golgi tendon organ, measuring muscle tension
- e) Nociceptors



**Figure: Structure of five different types of sensory receptors in brain**

Mechanoreceptors, thermoreceptors, and nociceptors in cutaneous, subcutaneous, and deep connective tissue are collectively called somatosensory receptors. While the morphological endings of many of these are unknown, the remainder are classified as either free endings, endings with expanded tips, or encapsulated endings.

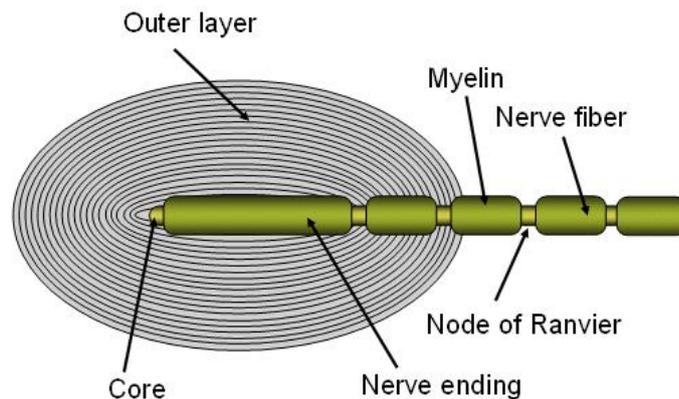
Nociceptors are the receptors which respond primarily to injurious or painful stimulation are called nociceptors. Within this general category are four subgroups: mechanonociceptors, mechano-heat nociceptors, mechano-cold nociceptors, and poly modal nociceptors. Nociceptors are found in skin, muscles, joints, and the viscera

The sensory receptor contains membrane regions that respond to one of the various forms of incident stimuli by a depolarization (or hyperpolarization). In some cases the receptor is actually part of the afferent neuron but, in others it consists of a separate specialized cell. All receptor cells have a common feature: They are *transducers* - that is, they change energy from one form to another. For instance, the sense of touch in the skin arises from the conversion of mechanical and/or thermal energy into the electric energy (ionic currents) of the nerve impulse.

Chemoreceptors located in the arteries measure carbon dioxide and oxygen in the circulating blood. When the concentration of either is too high or too low, the nervous system is notified and signals are sent out to the circulatory and

respiratory systems to adjust the beating of the heart and the rate of breathing in the appropriate direction.

The Pacinian corpuscle is a touch receptor which, under the microscope, resembles an onion. It consists of several concentric layers. The center of the corpuscle includes the core, where the unmyelinated terminal part of the afferent neuron is located. The first node of Ranvier is also located inside the core. Several mitochondria exist in the corpuscle, indicative of high energy production.



**Figure: The Pacinian corpuscle consists of a myelinated sensory neuron whose terminal portion is unmyelinated. The unmyelinated nerve ending and the first node lie within a connective tissue capsule, as shown.**

The taste cell is the chemically sensitive element for the sense of taste. Taste cells cluster together in small units called taste buds. Taste buds are chiefly located in raised areas of the tongue known as papillae. Four basic taste modalities are generally recognized. These are sweet, salty, sour, and bitter.

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## **5.3 General mechanism involved in stimulus transduction at receptor sites**

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### **5.3.1. Transduction**

In physiology, sensory transduction is the conversion of a sensory stimulus from one form to another. Transduction in the nervous system typically refers to stimulus alerting events wherein a physical stimulus is converted into an action potential, which is transmitted along axons towards the central nervous system where it is integrated.

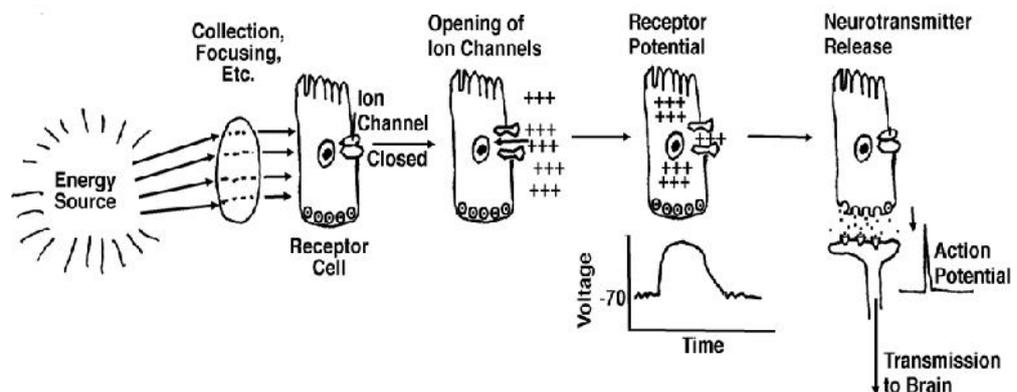
A receptor cell converts the energy in a stimulus into a change in the electrical potential across its membrane. It causes the depolarization of the membrane to allow the action potential to be transduced to the brain for integration.

### 5.3.2. The basic process of transduction

There must be some means of detecting the presence of information in the environment, before any neural processing can take place, for collecting the different forms and patterns of energy that represent this information, and converting the physical energy into a form that can be acted upon and utilized by the nervous system. The process through which a specific pattern of information like light, vibrations, dissolved chemicals or airborne chemicals from the environment is converted to a pattern of electrical activity in the nervous system is called transduction. Beginning with the physical energy of an environmental stimulus, there are several processes that must occur before a pattern of neural activity is generated. The basic steps leading to transduction are as follows:

The stimulus energy must reach specialized receptor cells. In some cases (e.g., taste, touch) this process is relatively simple and straightforward. In other systems (e.g., hearing, vision), it is quite complicated. The receptor cells must be activated. The activation process involves opening of ion channels to cause a change in the cell's membrane potential. In different sensory systems, different mechanisms cause ion channels to open in response to a stimulus.

The opening of ion channels creates a receptor potential. In a neuron, the receptor potential size reflects in some way the properties of the stimulus. In general, the larger the magnitude of the stimulus, the larger the receptor potential. The receptor potential causes release of neurotransmitter onto the dendrite of the *primary afferent* (nerve fiber projecting to the central nervous system). The larger the receptor potential, the greater the quantity of neurotransmitter released. If enough excitatory neurotransmitter is released to bring the primary afferent neuron to threshold, it will fire an action potential

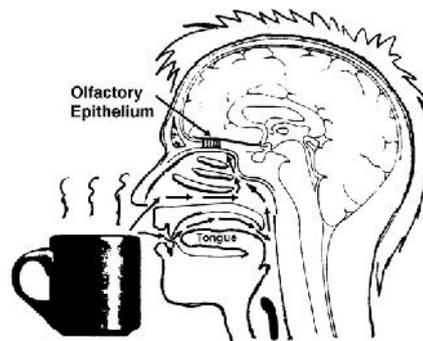


**Figure Diagram summarizing the events that take place in transduction.**

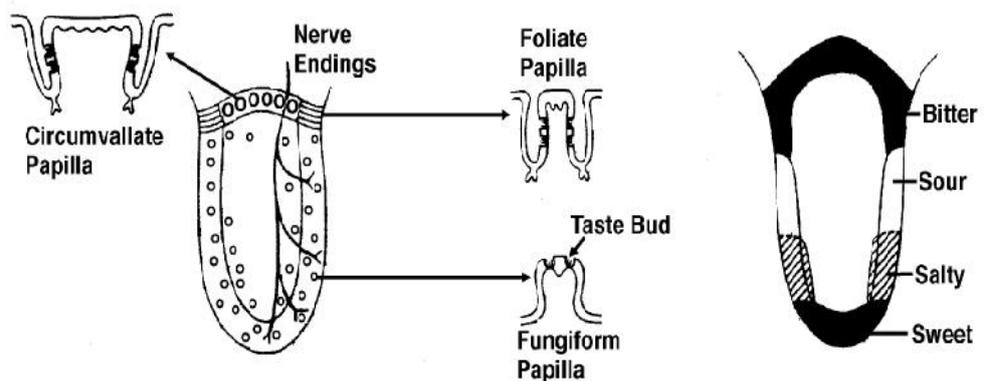
### 5.3.3. Transduction in The Gustatory (Taste) System.

#### a) Peripheral mechanisms.

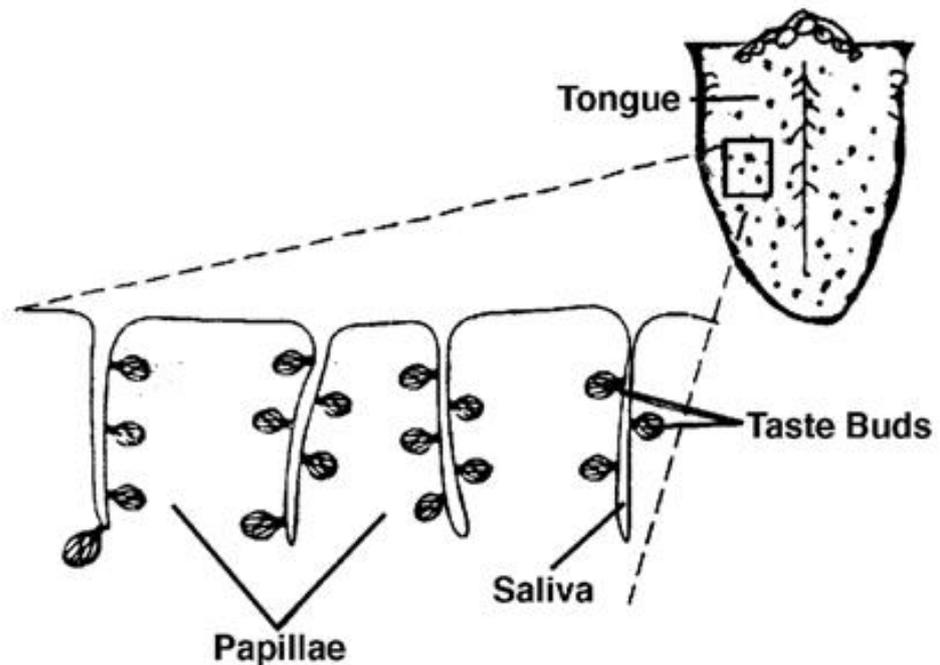
In order for us to *taste* a substance, it must be in the mouth, contacting the tongue. The experience that we commonly think of as "taste" is actually a complex interaction resulting from stimulation of several sensory systems including gustatory (sweet, salty, etc), olfactory (e.g. coffee, apple, or onion aroma), tactile (e.g., smooth or rough texture) temperature (hot or cold), and even pain (e.g., hot chili peppers). The tongue is covered with small structures called papillae. Different types of papillae are concentrated on different parts of the tongue. The transduction process takes place in the taste buds, specialized concentrations of receptor cells located on the papillae.



**Figure:** Interaction between taste and smell occurs when the liquid in the cup is taken into the mouth. The liquid that contacts the tongue stimulates taste receptors; the vapours that enter the nasal cavity through the nostrils and/or through the back of the throat (arrows) stimulate olfactory receptors.



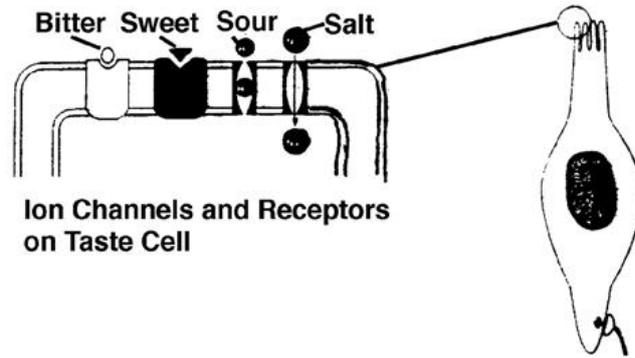
**Figure:** There are several different types of papillae on the surface of the tongue. The large circumvallate papillae are located at the rear of the tongue, the foliate papillae at the sides, and the small fungiform papillae on the middle to front of the tongue. **Right:** Although all parts of the tongue have some sensitivity to all taste qualities, it is possible to identify certain parts of the tongue that have the highest sensitivity to each taste quality.



**Figure:** The taste buds are located on the tops and sides of the papillae. Each papilla contains multiple taste buds. Each taste bud contains receptor cells which are contacted by nerve fibers of the chorda tympani nerve (a branch of the facial nerve) or by fibers of the hypoglossal nerve. Gustatory receptor cells, unlike most other sensory receptor cells, die and are replaced every few weeks.

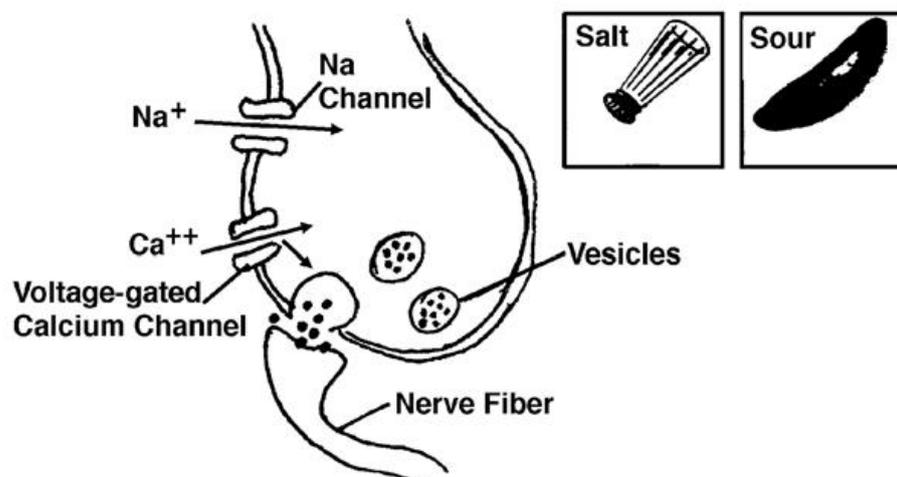
#### **b) The transduction process**

The membranes of taste receptor cells contain a variety of ion channels, many of which are associated with receptor sites for specific types of molecules. Nearly every taste cells has a variety of different ion channels and receptor sites, but these are mixed together in slightly different proportions.



**Figure:** The cell membrane of every taste receptor cell contains a variety of ion channels. Each type is activated by a different category of taste stimulus.

Transduction occurs when molecules that enter the mouth pass through ion channels or bind to receptor sites on ion channels. Some ion channels operate by allowing ions to pass through and depolarize the cell directly. For example, we perceive a salty taste when sodium ions are present in high concentration in the mouth. Because the sodium concentration is higher outside the taste receptor cell than inside it, sodium enters the cell. Because sodium has a positive charge, the inside of the cell becomes more positive with respect to the outside, i.e., it is depolarized. This ultimately results in neurotransmitter release. Sour stimuli contain an abundance of positively charged hydrogen ions ( $H^+$ ) that act in a manner similar to sodium ions to depolarize the taste receptor cell.



**Figure: Sodium ions and hydrogen ions pass through specific classes of ion channels to depolarize the taste receptor cell. These channels can be blocked by "drugs" such as amiloride. Depolarization activates voltage-gated calcium channels and calcium causes neurotransmitter release from the vesicles.**

Many ion channels operate through binding of a relatively large taste stimulus molecule to a specific site on the ion channel, causing the pore in the channel to undergo a conformational change (a change in shape) that admits a small ion (e.g., sodium), thereby depolarizing the cell.

For example, we perceive a sweet taste when sugar molecules bind to specific receptor sites on ion channels. The sugar does not enter the cell itself; instead, it opens channels that allow a small positive ion (probably sodium) to enter the cell. Bitter substances (e.g., quinine) and amino acids (e.g., glutamate) probably stimulate

taste receptor cells through specific receptor sites, in much the same way that sugars do. There are probably many different types of receptor sites for bitter substances. Amino acids or their salts (e.g., monosodium glutamate) elicit a taste that is unique, often called "umami".

#### **5.3.4. Transduction of some other senses**

##### **1. The visual system**

In the visual system, sensory cells called rod and cone cells in the retina convert the physical energy of light signals into electrical impulses that travel to the brain. The light causes a conformational change in a protein called rhodopsin. This conformational change sets in motion a series of molecular events that result in a reduction of the electrochemical gradient of the photoreceptor. The decrease in the electrochemical gradient causes a reduction in the electrical signals going to the brain. Thus, in this example, more light hitting the photoreceptor results in the transduction of a signal into fewer electrical impulses, effectively communicating that stimulus to the brain. A change in neurotransmitter release is mediated through a second messenger system. The change in neurotransmitter release is due to rods.

##### **2. The auditory system**

In the auditory system, sound vibrations (mechanical energy) are transduced into electrical energy by hair cells in the inner ear. Sound

vibrations from an object cause vibrations in air molecules, which in turn, vibrate our ear drum. The movement of the eardrum causes the bones of our middle ear (the ossicles) to vibrate. These vibrations then pass in to the cochlea, the organ of hearing. Within the cochlea, the hair cells on the sensory epithelium of the organ of Corti bend and cause movement of the basilar membrane. The membrane undulates in different sized waves according to the frequency of the sound. Hair cells are then able to convert this movement (mechanical energy) into electrical signals (action potentials) which travel along auditory nerves to hearing centres in the brain.

### 3. The olfactory system

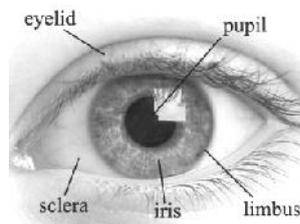
In the olfactory system, odorant molecules in the mucus bind to G-protein receptors on olfactory cells. The G-protein activates a downstream signalling cascade that causes increased level of cyclic-AMP (cAMP), which trigger neurotransmitter release.

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## 5.4 Functional architecture and stimulus processing in retina

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### 5.4.1. Photoreceptors (Structure of Mammalian Eye)



**Figure: Basic parts of human eye**

The structure of the mammalian eye can be divided into three main layers or *tunics* whose names reflect their basic functions: the fibrous tunic, the vascular tunic, and the nervous tunic.

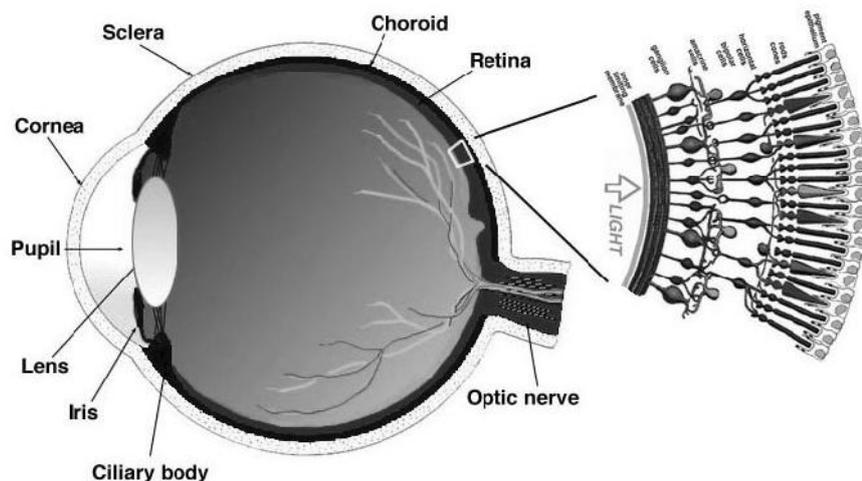
The fibrous tunic, also known as the *tunica fibrosa oculi*, is the outer layer of the eyeball consisting of the cornea and sclera. The sclera gives the eye most of its white color. It consists of dense connective tissue filled with the protein collagen to both protect the inner components of the eye and maintain its shape.

The vascular tunic, also known as the *tunica vasculosa oculi*, is the middle vascularized layer which includes the iris, ciliary body, and choroid. The

choroid contains blood vessels that supply the retinal cells with necessary oxygen and remove the waste products of respiration. The choroid gives the inner eye a dark color, which prevents disruptive reflections within the eye. The pupil (central aperture of iris) is black because there is no light reflected out of the interior eye.

The nervous tunic, also known as the *tunica nervosa oculi*, is the inner sensory layer which includes **the retina**. Contributing to vision, the retina contains the photosensitive rod and cone cells and associated neurons. To maximise vision and light absorption, the retina is a relatively smooth (but curved) layer. It has two points at which it is different; the fovea and optic disc. The fovea is a dip in the retina directly opposite the lens, which is densely packed with cone cells. It is largely responsible for color vision in humans, and enables high acuity, such as is necessary in reading. The optic disc, sometimes referred to as the anatomical blind spot, is a point on the retina where the optic nerve pierces the retina to connect to the nerve cells on its inside. No photosensitive cells exist at this point, it is thus "blind". Continuous with the retina are the ciliary epithelium and the posterior epithelium of the iris.

In addition to the rods and cones, a small proportion (about 1-2% in humans) of the ganglion cells in the retina are themselves photosensitive through the pigment melanopsin. They are generally most excitable by blue light, about 470–485 nm. Their information is sent to the SCN (suprachiasmatic nuclei), not to the visual center, through the retinohypothalamic tract which is formed as melanopsin-sensitive axons exit the optic nerve.



**Fig. A drawing of a section through the human eye with a schematic enlargement of the retina.**

The structure of the mammalian eye owes itself completely to the task of focusing light onto the retina. This light causes chemical changes in the photosensitive cells of the retina, the products of which trigger nerve impulses which travel to the brain. In the human eye, light enters the pupil and is focused on the retina by the lens. Light-sensitive nerve cells called rods (for brightness), cones (for color) and non-imaging ipRGC (intrinsically photosensitive retinal ganglion cells) react to the light. They interact with each other and send messages to the brain. The rods and cones enable vision. The ipRGCs enable entrainment to the Earth's 24-hour cycle, resizing of the pupil and acute suppression of the pineal hormone melatonin.

#### **5.4.2. The Retina**

The vertebrate **retina** is a light-sensitive layer of tissue, lining the inner surface of the eye. The optics of the eye create an image of the visual world on the retina (through the cornea and lens), which serves much the same function as the film in a camera. Light striking the retina initiates a cascade of chemical and electrical events that ultimately trigger nerve impulses. These are sent to various visual centres of the brain through the fibres of the optic nerve.

In vertebrate embryonic development, the retina and the optic nerve originate as outgrowths of the developing brain, so the retina is considered part of the central nervous system (CNS) and is actually brain tissue. It is the only part of the CNS that can be visualized non-invasively.

The retina is a layered structure with several layers of neurons interconnected by synapses. The only neurons that are directly sensitive to light are the photoreceptor cells.

##### **5.4.2.1 Functional architecture of the Retina**

The retina is a light-sensitive layer of nerve tissue lining the inner surface of the eye. The retina creates an image projected on its surface with help of the cornea and crystalline lens, and transforms it into nerve impulses sent to the brain.

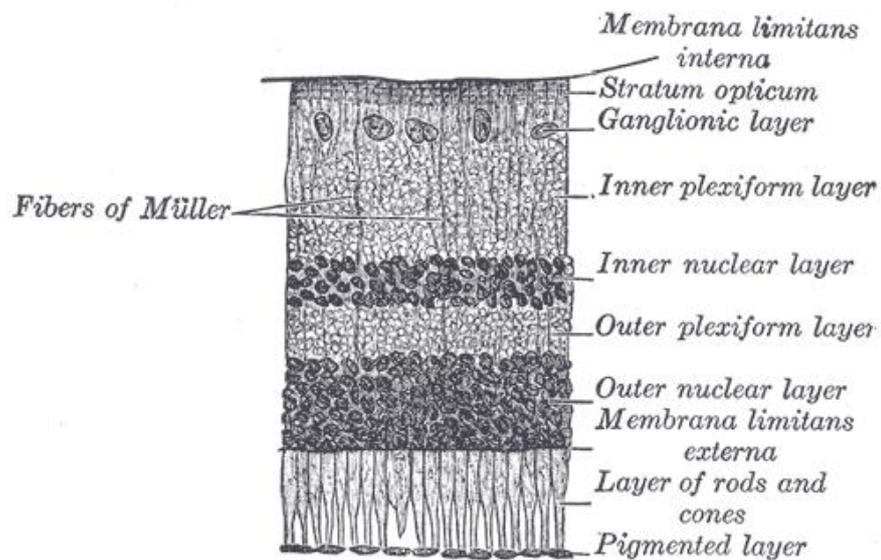
The retina is most closely linked with the underlying layers of the eyeball along the edge of the optic nerve head. The retina has varying thickness in different sections: 0.4 – 0.5 mm at the edge of the optic nerve head; 0.2 – 0.25 mm at the central fovea; only 0.07 – 0.08 mm at the foveal pit; about 0.1 mm at the ora serrata.

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The macula (macular zone, yellow spot) is the most important part of the retina. The macula ensures central vision since it contains numerous photo receptors – namely the cones. They allow good vision in daylight. Macula disorder may considerably reduce the vision.

The retina has a complex structure. The microscope discerns 10 layers in the retina



**Figure: Section of Retina**

**a) Section of retina**

The vertebrate retina has ten distinct layers. From closest to farthest from the vitreous body - that is, from closest to the front exterior of the head towards the interior and back of the head:

1. Inner limiting membrane – basement membrane elaborated by Müller cells
2. Nerve fibre layer – axons of the ganglion cell nuclei. Thin layer of Müller cell footplates exists between this layer and the inner limiting membrane.
3. Ganglion cell layer – contains nuclei of ganglion cells, the axons of which become the optic nerve fibres for messages and some displaced amacrine cells.

4. Inner plexiform layer – contains the synapse between the bipolar cell axons and the dendrites of the ganglion and amacrine cells.
5. Inner nuclear layer – contains the nuclei and surrounding cell bodies of the amacrine cells, bipolar cells and horizontal cells.
6. Outer plexiform layer – projections of rods and cones ending in the rod spherule and cone pedicle, respectively. These make synapses with dendrites of bipolar cells. In the macular region, this is known as the *Fiber layer of Henle*.
7. Outer nuclear layer – cell bodies of rods and cones
8. External limiting membrane – layer that separates the inner segment portions of the photoreceptors from their cell nucleus
9. Photoreceptor layer – rods/cones
10. Retinal pigment epithelium - single layer of cuboidal cells. This is closest to the choroid.

These can be simplified into 4 main processing stages: photoreception, transmission to bipolar cells, transmission to ganglion cells which also contain photoreceptors, the photosensitive ganglion cells, and transmission along the optic nerve. At each synaptic stage there are also laterally connecting horizontal and amacrine cells.

The optic nerve is a central tract of many axons of ganglion cells connecting primarily to the lateral geniculate body, a visual relay station in the diencephalon (the rear of the forebrain). It also projects to the superior colliculus, the suprachiasmatic nucleus, and the nucleus of the optic tract. It passes through the other layers creating the optic disc in primates.

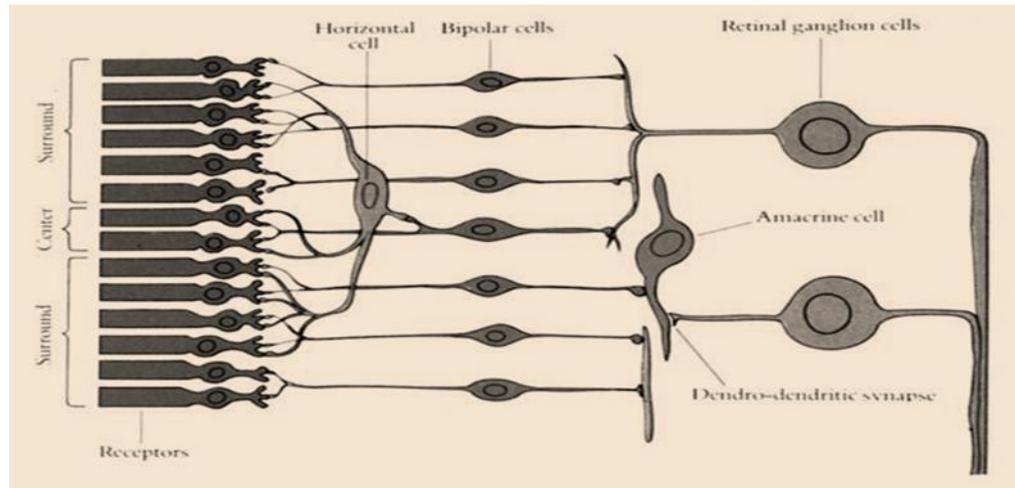
The retina contains three forms of photosensitive cells, two of them important to vision, **rods** and **cones**, in addition to the subset of **ganglion cells** involved in adjusting circadian rhythms and pupil size but probably not involved in vision.

Rods function mainly in dim light and provide black-and-white vision, while cones support daytime vision and the perception of colour. A third, much rarer type of photoreceptor, the intrinsically photosensitive ganglion cell, is important for reflexive responses to bright daylight.

Neural signals from the rods and cones undergo processing by other neurons of the retina. The output takes the form of action potentials in retinal ganglion

cells whose axons form the optic nerve. Several important features of visual perception can be traced to the retinal encoding and processing of light.

The entire retina contains about 7 million cones and 75 to 150 million rods. The optic disc, a part of the retina sometimes called "the blind spot" because it lacks photoreceptors, is located at the optic papilla, a nasal zone where the optic-nerve fibres leave the eye.



**Figure: Neural Structure of Retina**

Rods, cones and nerve layers in the retina. A chemical change in the rods and cones send a signal back to the nerves. The signal goes first to the bipolar and horizontal cells (yellow layer), then to the amacrine cells and ganglion cells, then to the optic nerve fibres. The signals are processed in these layers. First, the signals start as raw outputs of points in the rod and cone cells. Then the nerve layers identify simple shapes, such as bright points surrounded by dark points, edges, and movement.

In section the retina is no more than 0.5 mm thick. It has three layers of nerve cells and two of synapses, including the unique ribbon synapses. The optic nerve carries the ganglion cell axons to the brain and the blood vessels that open into the retina. The ganglion cells lie innermost in the retina while the photoreceptive cells lie outermost. Because of this counter-intuitive arrangement, light must first pass through and around the ganglion cells and through the thickness of the retina, before reaching the rods and cones.

Between the ganglion cell layer and the rods and cones there are two layers of neuropils where synaptic contacts are made. The neuropil layers are the outer plexiform layer and the inner plexiform layer. In the outer the rods and cones connect to the vertically running bipolar cells, and the horizontally oriented horizontal cells connect to ganglion cells.

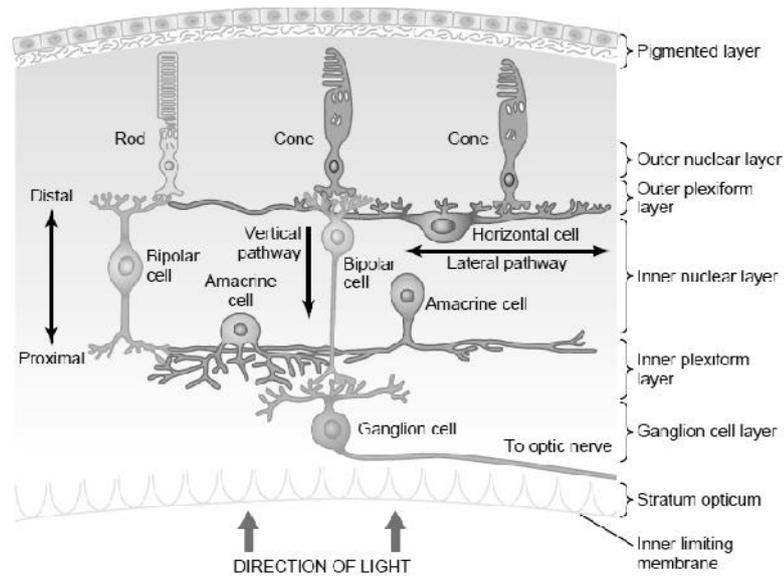
The central retina is cone-dominated and the peripheral retina is rod-dominated. At the centre of the macula is the foveal pit where the cones are smallest and in a hexagonal mosaic, the most efficient and highest density. Below the pit the other retina layers are displaced, before building up along the foveal slope until the rim of the fovea or parafovea which is the thickest portion of the retina. The macula has a yellow pigmentation from screening pigments and is known as the macula lutea. The area directly surrounding the fovea has the highest density of rods converging on single bipolars. Since the cones have a much lesser power of merging signals, the fovea allows for the sharpest vision the eye can attain.

#### **5.4.2.2. Function (Stimulus processing in retina)**

Around 1950, Stephen Kuffler became the first to record the responses of retinal ganglion cells to spots of light in a mammal, the cat. He was then working at the Wilmer Institute of Ophthalmology at the Johns Hopkins Hospital.

An image is produced by the patterned excitation of the cones and rods in the retina. The excitation is processed by the neuronal system and various parts of the brain working in parallel to form a representation of the external environment in the brain. The cones respond to bright light and mediate high-resolution colour vision during daylight illumination. The response of cones to various wavelengths of light is called their spectral sensitivity.

In normal human vision, the spectral sensitivity of a cone falls into one of three subgroups. These are often called blue, green, and red cones but more accurately are short, medium, and long wavelength sensitive cone subgroups. If any one or more is lacking than it causes individuals to have deficiencies in colour vision or various kinds of colour blindness.



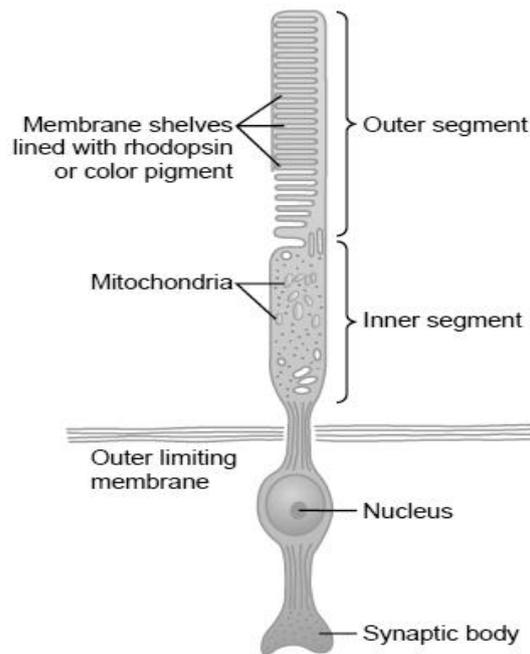
**Figure: Neural organization of the retina**

### 1. Role of Rods and cones

The outer segment which are light-sensitive photochemical.

- a) Rhodopsin- occur in the rod cell of the retina which are responsible for vision in poor light.
- b) Color pigment- occur in cones, that function almost exactly the same as rhodopsin except for differences in spectral sensitivity.

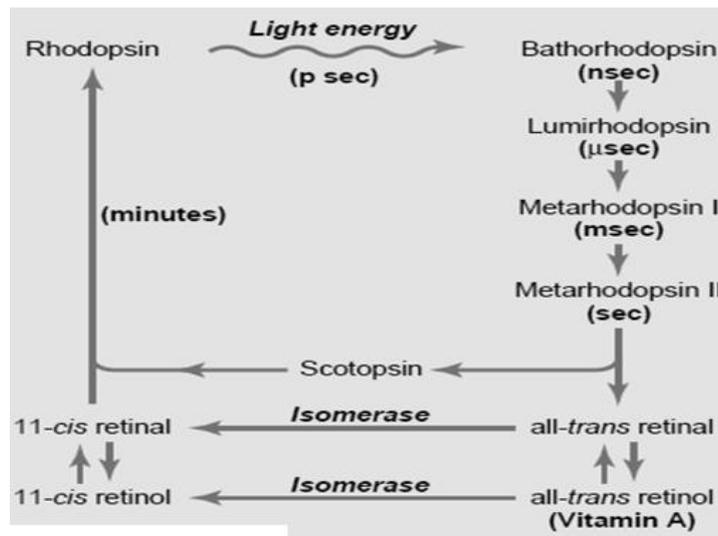
The inner segment of the rod or cone contains cytoplasmic organelles. Particularly important are the mitochondria; play the important role of providing energy for function of the photoreceptors. The synaptic body is the portion of the rod or cone that connects with subsequent neuronal cells.



**Figure: Functional parts of Rods and Cones**

## **2. Rhodopsin and Its Decomposition by Light Energy**

When light energy is absorbed by rhodopsin, the rhodopsin begins to decompose within a very small fraction of a second and immediate product is bathorhodopsin, which is a partially split combination of the all-trans retinal and scotopsin. Bathorhodopsin is extremely unstable and decays in nanoseconds to lumirhodopsin. This then decays in microseconds to metarhodopsin I, then in about a millisecond to metarhodopsin II, and finally, much more slowly (in seconds), into the completely split products scotopsin and all-trans retinal. It is the metarhodopsin II, also called activated rhodopsin, that excites electrical changes in the rods, and the rods then transmit the visual image into the central nervous system in the form of optic nerve action potential.



**Figure: Rhodopsin-Retinal Visual Cycle, and Excitation of the Rods**

### 3. Role of Vitamin A

- Vitamin A is present both in the cytoplasm of the rods and in the pigment layer of the retina.
- Role in the physiologic mechanism of vision, rhodopsin occur in the rod cells of the retina, which are responsible for vision in poor light.
- Large quantities of vitamin A are normally stored in the liver and can be made available to the eyes to avoid night blindness to occur

#### 1. Mechanism by Which Rhodopsin Decomposition decreases Membrane Sodium Conductance—The Excitation “Cascade.”

- The photon activates an electron in the 11-cis retinal portion of the rhodopsin; this leads to the formation of metarhodopsin II, which is the active form of rhodopsin.
- The activated rhodopsin functions as an enzyme to activate many molecules of transducin, a protein present in an inactive form in the membranes of the discs and cell membrane of the rod.
- The activated transducin activates many more molecules of phosphodiesterase.
- Activated phosphodiesterase is another enzyme; it immediately hydrolyzes many molecules of cyclic guanosine monophosphate (cGMP), thus destroying it. Before being destroyed, the cGMP had been bound with the sodium channel protein of the rod’s outer membrane in a way that “splints” it in the open state.
- Within about a second, another enzyme, rhodopsin kinase, which is always present in the rod, inactivates the activated rhodopsin (the

metarhodopsin II), and the entire cascade reverses back to the normal state with open sodium channels

## **2. Neuronal cell functioning**

- a) The photoreceptors themselves—the rods and cones—which transmit signals to the outer plexiform layer, where they synapse with bipolar cells and horizontal cells
- b) The horizontal cells, which transmit signals horizontally in the outer plexiform layer from the rods and cones to bipolar cells
- c) The bipolar cells, which transmit signals vertically from the rods, cones, and horizontal cells to the inner plexiform layer, where they synapse with ganglion cells and amacrine cells
- d) The amacrine cells, which transmit signals in two directions, either directly from bipolar cells to ganglion cells or horizontally within the inner plexiform layer from axons of the bipolar cells to dendrites of the ganglion cells or to other amacrine cells
- e) The ganglion cells, which transmit output signals from the retina through the optic nerve into the brain

## **3. Neurotransmitters Released by Retinal Neurons**

1. Cones release glutamate at their synapses with the bipolar cells.
2. Amacrine cells secreting;
  - a. Gamma-Aminobutyric Acid
  - b. Glycine
  - c. Dopamine
  - d. Acetylcholine
  - e. and Indolamine, all of which normally function as inhibitory transmitters.

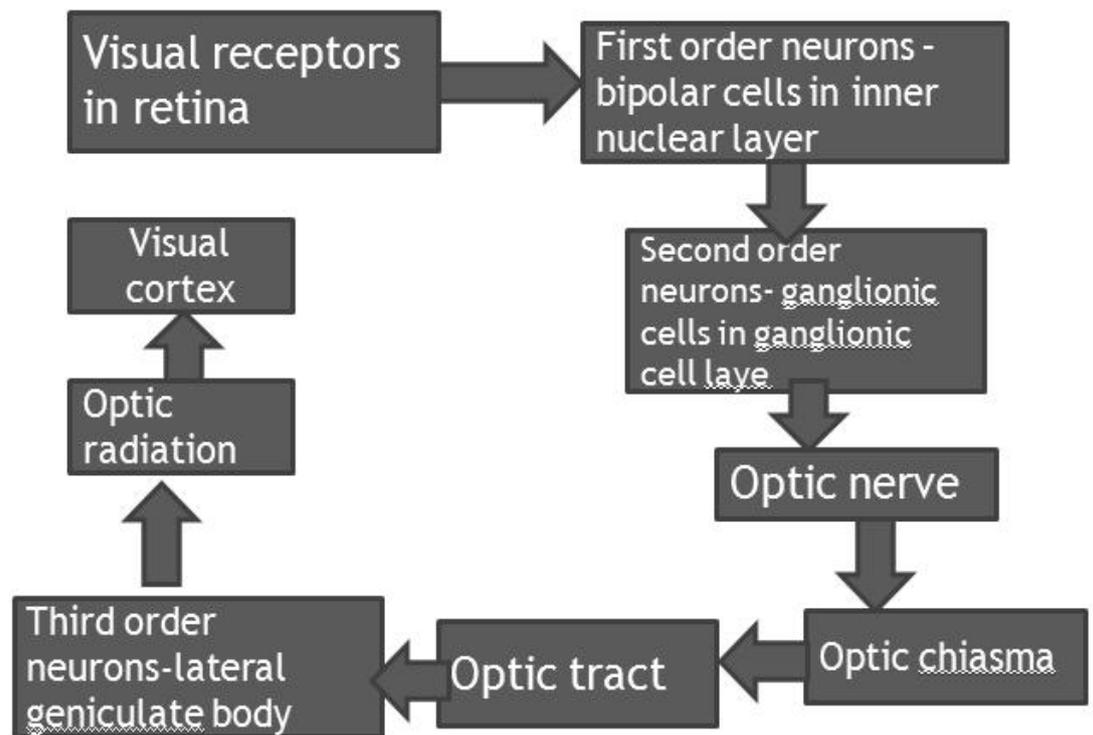
The Horizontal Cells plays a main role of lateral Inhibition to Enhance Visual Contrast. Horizontal cells connect laterally between the synaptic bodies of the rods and cones, as well as connecting with the dendrites of the bipolar cells. The outputs of the horizontal cells are always inhibitory.

Bipolar cells provide opposing excitatory and inhibitory signals in the visual pathway: They are of two types- the depolarizing bipolar cell and the hyperpolarizing bipolar cell. That is, some bipolar cells depolarize when the rods and cones are excited, and others hyperpolarize when they are inhibited.

Amacrine cells respond strongly at the onset of a continuing visual signal, but the response dies rapidly. They respond strongly at the offset of visual signals and when a light is turned either on or off, signalling simply a change in illumination. They also respond to movement of a spot across the retina in a specific direction; therefore, these amacrine cells are said to be directional sensitive.

In the transfer of visual signals to the brain, the visual pathway, the retina is vertically divided in two, a temporal (nearer to the temple) half and a nasal (nearer to the nose) half. The axons from the nasal half cross the brain at the optic chiasma to join with axons from the temporal half of the other eye before passing into the lateral geniculate body.

Several rod cells are connected to a single bipolar cell, which then connects to a single ganglion cell called as synaptic convergence, by which information is relayed to the visual cortex. This convergence is in direct contrast to the situation with cones, where each cone cell is connected to a single bipolar cell. This divergence results in the high visual acuity, or the high ability to distinguish detail, of cone cells compared to rods. If a ray of light were to reach just one rod cell, the cell's response may not be enough to hyperpolarize the connected bipolar cell. But because several "converge" onto a bipolar cell, enough transmitter molecules reach the synapses of the bipolar cell to hyperpolarize it.



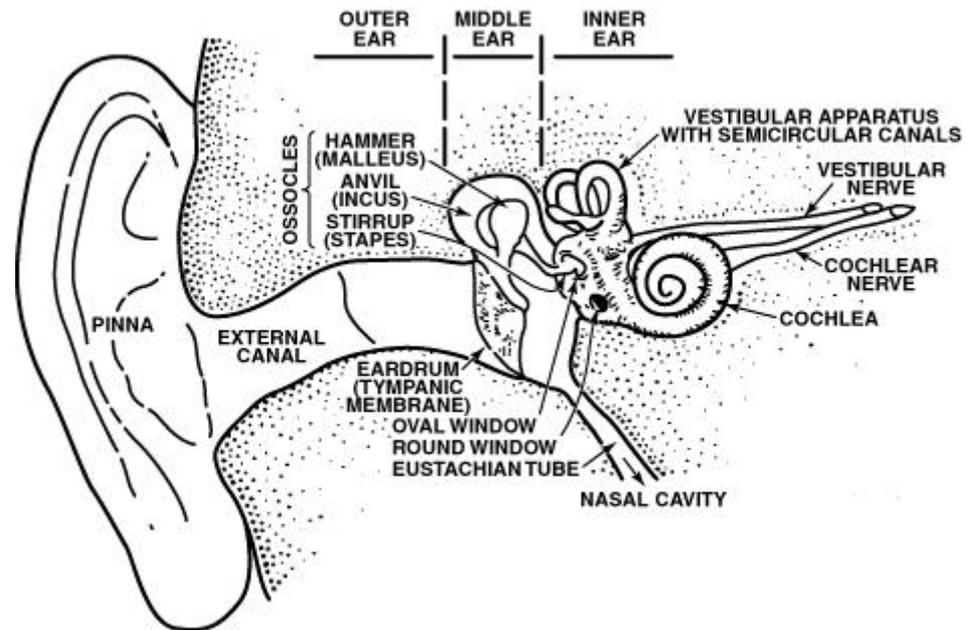
**Figure: Schematic representation of visual pathways**

## 5.5 The Ear

The ear is a three-chambered sensory structure that functions in the perception of sound (**auditory system**) and in the maintenance of balance (**vestibular system**). Each of the three divisions of the ear, the **external ear**, the **middle ear**, and the **inner ear**, is an essential part of the auditory system. The external and middle ear collect and conduct sound energy to the inner ear, where auditory sensory receptors transduce that energy into the electrical energy of nerve impulses. The sensory receptors of the vestibular system are also located in the inner ear. These receptors respond to gravity and movement of the head.

### a) External ear

The external ear is composed of an **auricle** and an **external auditory meatus**. The auricle (pinna) is the appendage that projects from the lateral surface of the head, i.e., the "ear." Thin skin with hair follicles, sweat glands, and sebaceous glands covers the auricle. The **external auditory canal** (meatus) follows a slightly S-shaped course for about 25 mm to the tympanic membrane (eardrum). The lateral one-third of the canal is cartilage and is continuous with the elastic cartilage of the auricle, the medial two-thirds of the canal is contained within the temporal bone. The lateral part of the canal is lined by skin that contains hair follicles, sebaceous glands, and ceruminous (wax) glands.



**Figure: Structure of human ear**

### **b) Middle ear**

The middle ear is an air-filled mucus-membrane-lined space in the temporal bone, the **tympanic cavity**. It is spanned by three small bones, the auditory **ossicles** that are connected by two movable joints. The middle ear also contains the **internal auditory canal (Eustachian canal)** as well as the muscles that move the ossicles. The primary function of the middle ear is to convert sound waves (air vibrations) arriving from the external auditory meatus into mechanical vibrations that are transmitted to the inner ear. Two openings in the medial wall of the middle ear, the **vestibular (oval) window** and the **cochlear (round) window**, are essential components in this conversion process.

The **tympanic membrane** (Eardrum) separates the external auditory canal from the middle ear. The three small bones known as the **ossicles**, the **malleus**, the **incus**, and the **stapes**, cross the space of the middle ear in series and connect the tympanic membrane to the oval window. These bones help to convert sound waves,

The **internal auditory canal**, commonly known as the **eustachian canal**, a narrow flattened channel lined with ciliated pseudostratified columnar epithelium, is approximately 3.5 cm long and connects to the nasopharynx. It allows pressure in the middle ear to equilibrate with atmospheric pressure.

### **c) Inner ear**

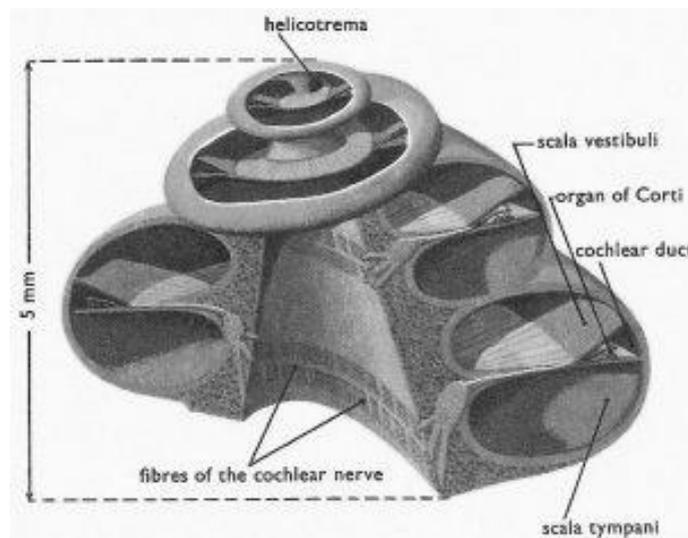
The inner ear consists of two compartments or *labyrinths*, one contained within the other. The **bony labyrinth** is a complex system of interconnected cavities

and canals in the temporal bone. The **membranous labyrinth** lies within the bony labyrinth and consists of a complex system of small sacs and tubules that also form a continuous space enclosed within a wall of epithelium and connective tissue.

The three components of the inner ear are: 1) Semicircular canals, 2) Vestibule, 3) Cochlea.

The **vestibule** is the central space of the bony labyrinth. The **utricle** and **sacculle** of the membranous labyrinth lie in an elliptical and spherical recess, respectively. The **semicircular canals** extend from the vestibule posteriorly, and the **cochlea** extends from the vestibule anteriorly.

The **Semicircular Canals**, three narrow bony-walled tubes, each forming about three-quarters of a circle, lie at approximately right angles to each other in superior, posterior, and horizontal planes. At the lateral end of each semicircular canal, close to the vestibule, is an enlargement called an ampulla. Each inner ear has three ampullae. The three canals open into the vestibule through five openings, with the superior and posterior semicircular canals sharing a common ampulla medially.



**Figure: Structure of Cochlea**

The **Cochlea** is a conically shaped helix connected to the vestibule. The lumen of the cochlea, like that of the semicircular canals, is continuous with that of the vestibule. It connects to the vestibule on the side opposite the semicircular canals. Between its base and the apex, the cochlea makes about 2-3/4 turns around a central bony core called the modiolus. A sensory ganglion, the **spiral ganglion**, lies in the modiolus. One opening of the canal, the cochlear **round window** on its inferior surface near the base, is covered by a thin membrane

(the secondary tympanic membrane) by which it absorbs or damps vibrations reaching it. The organ of Corti that projects into the endolymph of the cochlear duct is the sense organ for hearing.

### **5.5.1. Organ of Corti**

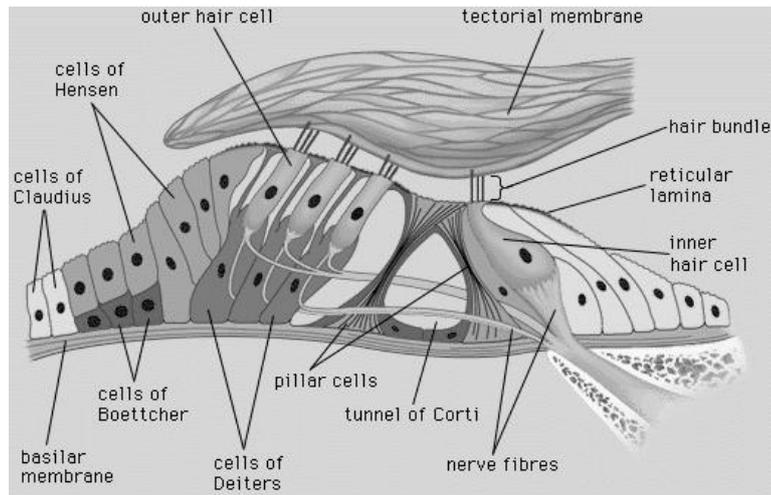
The organ of Corti ("spiral organ") is the receptor organ for hearing and is located in the mammalian cochlea. This organ is described as "a masterpiece of cellular micro-architecture," This highly varied strip of epithelial cells epithelium allows for transduction of auditory signals into nerve impulses action potential. Transduction occurs through vibrations of structures in the inner ear causing displacement of cochlear fluid and movement of hair cells at the organ of corti to produce electrochemical signals.

The organ of Corti is named after one of the first anatomists to give a detailed description of the neuro-sensory cochlea. Italian anatomist Alfonso Giacomo Gaspare Corti (1822–1876) discovered the organ of Corti in 1851. The structure evolved from the Basilar papilla and is now considered one of the most crucial structures for Mechanotransduction in mammals. Seated on the basilar membrane, it is composed of the sensory cells, called hair cells, the neurons, and several types of support cells.

#### **a) Structure**

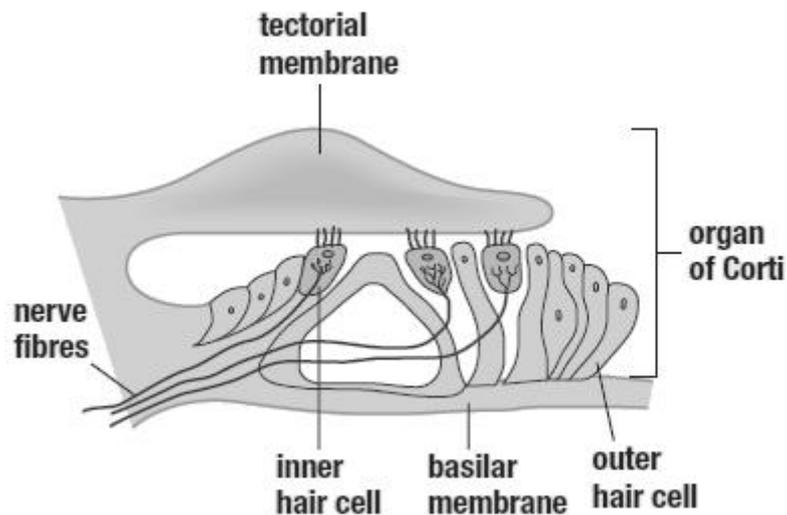
The organ of Corti is located in the cochlea of the inner ear between the vestibular duct and the tympanic duct and is composed of mechanosensory cells, known as hair cells. Strategically positioned on the basilar membrane of the organ of Corti are three rows of outer hair cells (OHCs) and one row of inner hair cells (IHCs). Separating these hair cells are supporting cells: Dieters cells', also called phalangeal cells, which separate and support both the outer and inner hair cells.

Tiny finger like projections are projected from the tops of the hair cells called as stereocilia, which are arranged in a gradated fashion with the shortest stereocilia on the outer rows and the longest in the center. This gradation is thought to be the most important anatomic feature of the organ of Corti because this allows the sensory cells superior tuning capability.



**Figure : Structure of organ of corti**

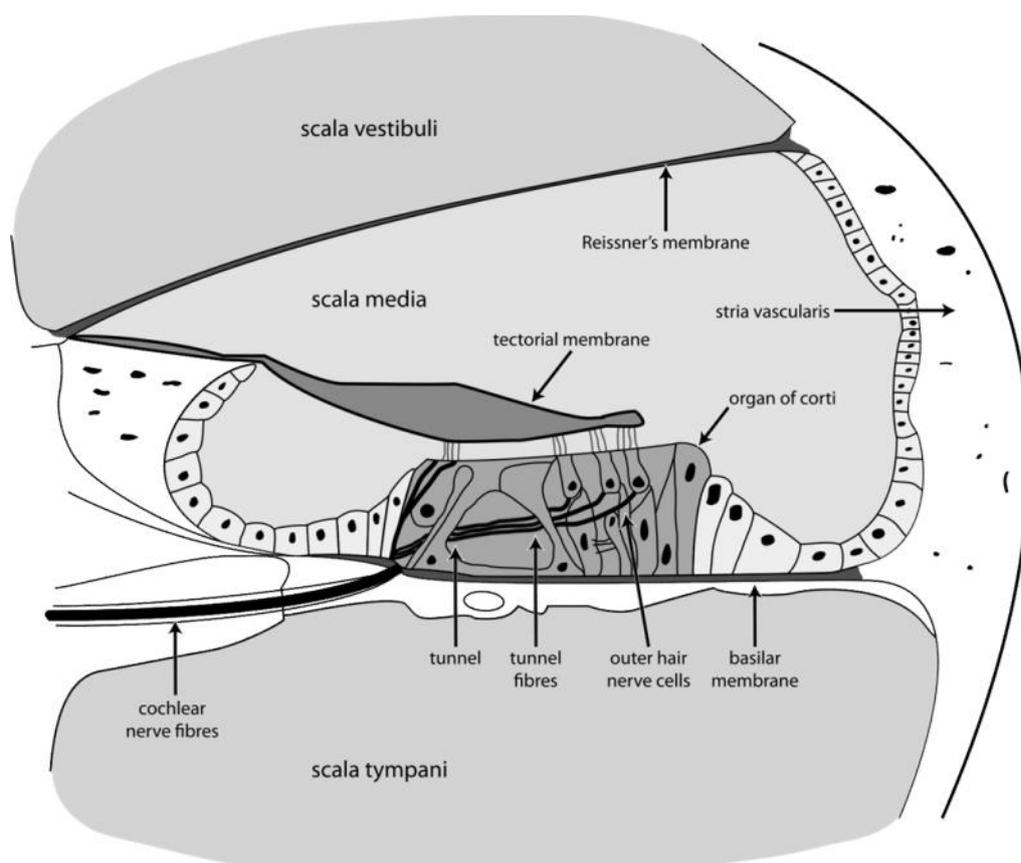
The cochlea is responsible for senses of different frequencies of sound waves interact with different locations on the structure. The base of the cochlea, closest to the outer ear, is the most stiff and narrow and is where the high frequency sounds are transduced. The apex, or top, of the cochlea is wider and much more flexible and loose and functions as the transduction site for low frequency sounds.



**Figure : Structure of organ of corti**

The Organ of Corti is the sensor of sound vibrations. The cochlear duct divides the cochlear canal into three parallel canals or scalae: 1) **Scala media**, the middle compartment in the cochlear canal; 2) **Scala vestibule** or vestibular canal; 3) **Scala tympani** or tympanic canal. The cochlear duct, itself, is the scala media. The scala vestibuli and scala tympani are the spaces above and below, respectively. The scala media is an endolymph-containing space that is continuous with the lumen of the saccule and contains the Organ of Corti,

which rests on its lower wall. The scala vestibule and the scala tympani are perilymph containing spaces and communicate with each other at the apex of the cochlea through a small channel called the **helicotrema**. The scala vestibule is described as beginning at the oval window, and the scala tympani is described as ending at the round window. The upper wall of the scala media, which separates it from the scala vestibuli, is the vestibular (Reissner's) membrane. The lower wall or floor of the scala media is the **basilar membrane**. The organ of Corti rests on the basilar membrane and is overlain by the tectorial membrane.



**Figure: Structure of Organ of Corti**

**b) Function**

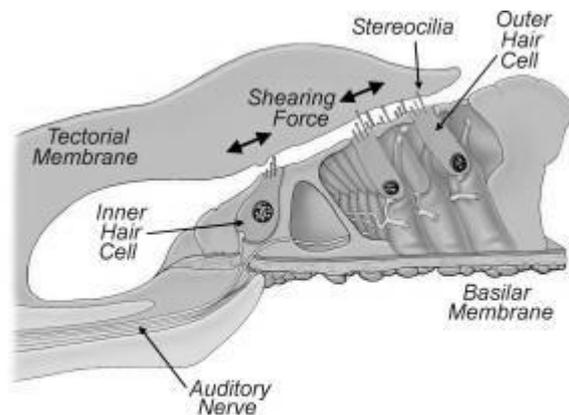
The function of the organ of Corti is to transduce auditory signals and maximize the hair cells' extraction of sound energy. It is the auricle and middle ear that act as mechanical transformers and amplifiers so that the sound waves end up with amplitudes 22 times greater than when they entered the ear.

**c) Auditory transduction**

For auditory signals to reach the organ of Corti in the first place, they must come from the outer ear. Sound waves enter through the auditory canal and

vibrate the tympanic membrane, also known as the eardrum, which vibrates three small bones called the ossicles. As a result, the attached oval window moves and causes movement of the round window, which leads to displacement of the cochlear fluid.

The Organ of Corti is composed of hair cells and supporting cells. Sound waves striking the tympanic membrane are translated into simple mechanical vibrations. The ossicles of the middle ear convey these vibrations to the cochlea. Movement of the stapes in the oval window of the vestibule sets up vibrations or traveling waves in the perilymph of the vestibular canal. The vibrations are transmitted through the vestibular membrane to the scala media (cochlear duct), which contains endolymph, and are also propagated to the perilymph of the tympanic canal. Pressure changes in this closed system are reflected in movements of the membrane that covers the round window in the base of the cochlea.



The Organ of Corti

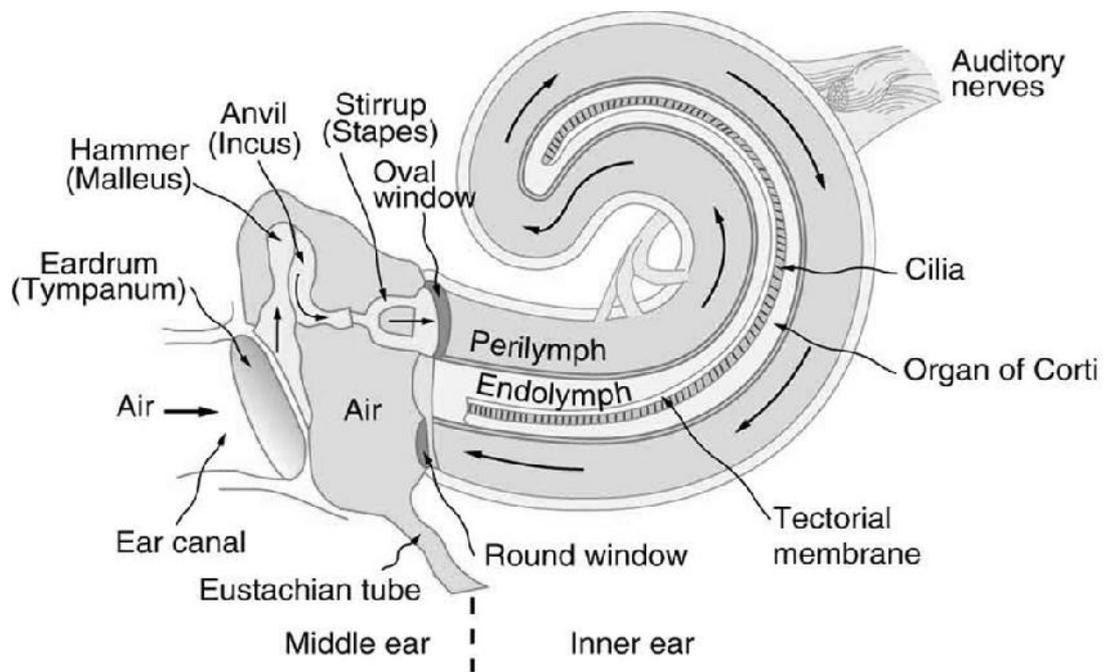
As a result of sound vibrations entering the inner ear, a traveling wave is set up in the basilar membrane. A sound of specified frequency causes displacement of a relatively long segment of the basilar membrane, but the region of maximal displacement is narrow. High-frequency sounds cause maximal vibration of the basilar membrane near the base of the cochlea; low-frequency sounds cause maximal displacement nearer the apex. The point of maximal displacement of the basilar membrane is specified for a given frequency of sound, and this is the basis of frequency discrimination. Perception of sound intensity or loudness depends on the degree of displacement of the basilar membrane at any given frequency range.

Hair cells are attached, through other cells, to the basilar membrane, which vibrates during sound reception. The stereocilia of these hair cells are, in turn,

attached to the tectorial membrane, which also vibrates. The shearing effect between the basilar membrane and the tectorial membrane distorts the stereocilia of the hair cells and this distortion generates membrane potentials that, when conveyed to the brain via the cochlear nerve (cochlear division of the vestibulocochlear nerve, cranial nerve VIII), are perceived as sound.

The basilar membrane on the tympanic duct presses against the hair cells of the organ as perilymphatic pressure waves pass. The organ of Corti lies on the basilar membrane at the base of the scala media and is surrounded by endolymph, a potassium rich fluid. Under the organ of Corti is the scala tympani and above it is the scala vestibuli, both structures exist in a low potassium fluid called perilymph. Because those stereocilia are in the midst of a high concentration of potassium, once their cation channels are pulled open potassium ions as well as calcium ions flow into the top of the hair cell.

With this influx of positive ions the inner hair cells becomes depolarized, opening voltage-gated calcium channels at the basolateral region of the hair cells and triggering the release of the neurotransmitter glutamate. An electrical signal is then sent through the auditory nerve and into the auditory cortex of the brain as a neural message.



**Figure: functioning of cochlea & organ of corti.**

**d) Cochlear amplification**

The organ of Corti is also capable of modulating the auditory signal. The outer hair cells can amplify the signal through a process called electromotility where

they increase movement of the basilar membrane and therefore increase deflection of stereocilia in the inner hair cells. Through its association with the tectorial membrane, motion of the basilar membrane can enhance vibrations in the cochlea.

A crucial piece to this cochlear amplification is the motor protein prestin, which changes shape based on the voltage potential inside of the hair cell. When the cell is depolarized prestin shortens, and because it is located on the membrane of OHCs it then pulls on the basilar membrane and increasing how much the membrane is deflected, creating a more intense effect on the IHCs. When the cell hyperpolarizes prestin lengthens and eases tension on the IHCs, which decreases the neural impulses to the brain. In this way, the hair cell itself is able to modify the auditory signal before it even reaches the brain.

#### e) **Hearing loss**

The organ of Corti can be damaged by excessive sound levels, leading to noise-induced health effects. The most common kind of hearing impairment, sensorineural hearing loss, includes as one major cause the reduction of function in the organ of Corti. Specifically, the active amplification function of the outer hair cells is very sensitive to damage from exposure to trauma from overly-loud sounds or to certain ototoxic drugs. Once outer hair cells are damaged, they do not regenerate, and the result is a loss of sensitivity and an abnormally large growth of loudness in the part of the spectrum that the damaged cells serve.

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## **5.6. Olfactory epithelium**

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### **5.6.1. Odour**

By researchers following some classes of odors (related to shapes of odorant molecules were given as the following:

#### **Basic types of compounds producing various odors.**

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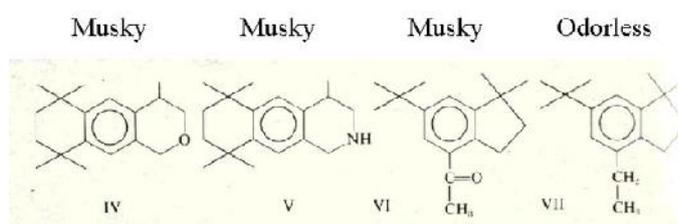
<b>Odor type</b>	<b>Compound</b>
Floral	Alcohols e.g. $\alpha$ -phenylethyl alcohol
Fruity	Esters e.g diethyl adipate
Minty	Esters e.g. methyl salicylate

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Camphorous	1,8-cineole
Musky	Ring ketones, civetone

This is only an attempt to establish classes of basic odors, as for instance both fruity and minty compounds are esters. The more general observation is that higher vertebrates can smell a vast array of different smells that hardly fall into classes at all. Humans can probably distinguish over a thousand different odors.

Functional groups may or may not be important determinants of odor.



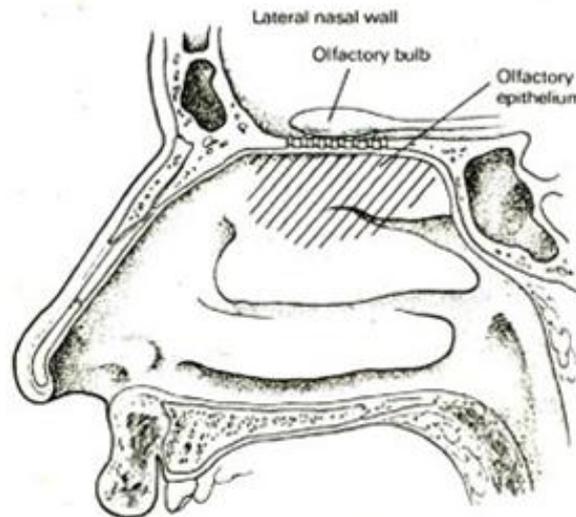
The sense of smell, working at a distance, is almost as sensitive as is theoretically possible: In some insect chemoreceptors a single molecule of odorant per receptor cell is sufficient to be detected. We can estimate the sensitivity in humans by knowing the concentration of the odorant in the inspired air:

The sense of smell has a vividness and evocative quality that are different from any other sense. This may result from the intimate connections of the olfactory system with the paleocortex, or evolutionarily older part of the brain, and with the hypothalamus. It is thus considered to be able to evoke memories which may be processed in the paleocortex (hippocampus and prepyriform cortex), and to have some very primitive functions, in connection with eating and reproduction, that are mediated by the hypothalamic/pituitary system.

Olfaction has been the subject of studies in a wide variety of vertebrates and invertebrates. Much has been written about the role of pheromones which serve as odorous attractants or repellants in insects and higher species. Whether humans consider sweat or other odors attractive is a matter of debate. However, the use of perfumes, which are often floral extracts combined with animal musk, has been practiced for centuries.

### 5.6.2. Anatomy of Olfactory Sensory Cells

We do not smell with our noses, but with our olfactory epithelium. As shown in below, this is where the sensory cells for olfaction in higher vertebrates are located, in the upper and middle conchae of the nasopharynx.



**Figure: Olfactory cells located at olfactory epithelium (in upper and middle of conchae of nasal pharynx)**

#### 5.6.2.1. Olfactory epithelium

The olfactory epithelium is an area inside the nose which is responsible for intercepting different odours and passing them on to the brain. The mechanics of the olfactory epithelium are not fully understood; this structure contains a huge number of neurons, but the exact way in which they interact with and distinguish between smells is a bit of a mystery. The larger the area covered by the olfactory epithelium, the more neurons, and the better the sense of smell.

Like other layers of epithelial tissue in the body, the olfactory epithelium contains a number of layers of cells. These cells include specialized neurons which communicate with the olfactory bulb via long axons, and olfactory hair cells which have highly sensitive receptors which pick up odours. The olfactory epithelium is also quite delicate, and it can be damaged by exposure to chemicals, strong odours, and head injuries.

The olfactory epithelium is located inside the back of the nose. As people breathe in through the nose, fine hairs and mucus near the opening of the nose trap particles which could be harmful, and the rest of the air passes over the olfactory epithelium. The neurons in the epithelium respond to specific odours

and send a signal to the brain to tell it what the nose knows. Essentially, the olfactory epithelium is like a laboratory: when people are exposed to odours, they don't smell them instantly, but rather wait for them to be processed and for their brains to return the results.

Different animals have varying degrees of sensitivity to smell. Animals rely on their olfactory epithelium to alert them to the presence of predators, potential food sources, or contamination which could make food or water dangerous to consume. Certain odours appear to trigger stronger responses than others; sour milk, for example, is often very easy to detect, because it can be dangerous to drink, while people and animals are less sensitive to more benign odours.

Damage resulting in loss of odour sensitivity isn't just unfortunate because people can't stop and smell the roses any more. Anosmia, as the loss of the sense of smell is known, can actually be quite dangerous, because people miss important clues to danger, such as the smell of a gas leak, when they can't smell.

#### **5.6.2.2. Structure of Olfactory epithelium**

The olfactory epithelium is a specialized epithelial tissue inside the nasal cavity that is involved in smell. In humans, it measures about 3 square centimeters (on each side) and lies on the roof of the nasal cavity about 7 cm above and behind the nostrils. The olfactory epithelium is the part of the olfactory system directly responsible for detecting odors.

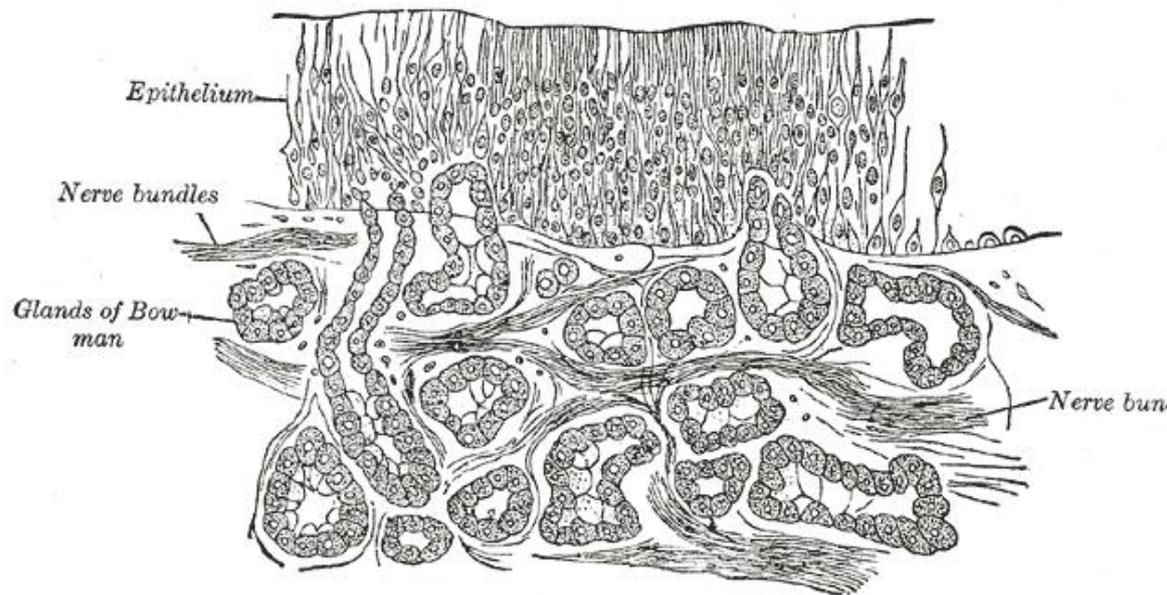
There are basal cells, supporting (sustentacular) cells, and olfactory sensory (receptor) cells. Olfactory cilia project from the sensory cells into a layer of mucus approximately 35  $\mu\text{m}$  thick which covers the sensory epithelium. Molecules of an odorant substance must diffuse across this mucus layer before they can come in contact with the membrane of the olfactory cell, located in the cilia. The main source of the mucus is considered to be the sustentacular cells and the acinar cells of Bowman's glands. The olfactory sensory cells are primary sensory neurons. (In the taste system, by contrast, endodermally-derived sensory cells transduce taste stimuli into secretory activity that excites the connected sensory axons.)

#### **Layers of olfactory epithelium**

Olfactory epithelium consists of four distinct cell types:

1. Olfactory cells
2. Supporting cells

3. Basal cells
4. Brush Cells



**Section of the olfactory mucous membrane.**

### **1. Olfactory cells**

The olfactory cells of the epithelium are bipolar neurons which congregate to form the olfactory nerve. The olfactory nerves go through the cribriform plate and terminate on the dendrites of the mitral cells located in the glomeruli of the olfactory bulb. The apical poles of these neurons are covered with non-motile cilia, with the plasma membrane containing odorant-binding proteins acting as olfactory receptors. The incoming odorants are made soluble by the serous secretion from Bowman's glands, located in the lamina propria of the mucosa.

Olfactory cells are nerve cells, part of the nervous system and classified as part of the peripheral sensory nervous system. They are located in the scent-sensing organs of humans and other animals, have a specific shape that is dependent on their specific location, and vary greatly in their number and sensitivity. Olfactory cells are the structures which helps us to enjoy the scent, warn us about gas leaks, allow drug-sniffing dogs to track down criminals etc.

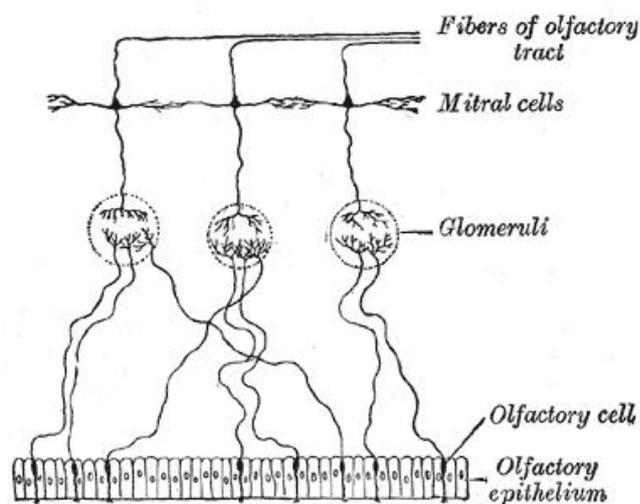
Olfactory cells are neurons, also known as nerve cells. They are part of the nervous system, a body system made of a vast network of nerves and electrochemical connections. The nervous system is responsible for gathering information from the surrounding environment, relaying this

information to the brain, and transmitting the brain's commands to the body. Their primary function is to gather sensory information related to the sense of smell. Because of this, and the fact that they are not located in the brain or spinal cord, they are classified as part of the peripheral sensory nervous system.

These cells are generally located in the nose and in other organs responsible for detecting scent stimuli. In humans, these cells are located in the olfactory epithelium, a densely-packed cluster of cells at the back of the nose. Other mammals such as dogs have a second set of olfactory cells located in the Jacobson's organ. The Jacobson's organ hangs in the back of the throat and helps give dogs their incredible sense of smell.

Olfactory cells have a specific shape dependent upon their location in the body. Cells located in the olfactory epithelium have an elongated shape with a knob on one end. By contrast, cells located in a Jacobson's organ have a more rounded shape.

Finally, scent smells in both humans and animals vary in both their number and in their sensitivity. For example, humans have five million olfactory cells. Dogs, by contrast, have upwards of 220 million olfactory cells. Humans are capable of recognizing over 10,000 distinct scents. While it is not yet known how many scents dogs can distinguish, scientists suspect they can distinguish far more than humans can.



**Figure: Plan of olfactory neurons**

## **2. Supporting cells**

Analogous to neural glial cells, the supporting cells (Sustentacular cells) of the olfactory epithelium function as metabolic and physical support for the olfactory cells. Histologically, the supporting cells are pseudostratified ciliated columnar epithelium. The nuclei of supporting cells are more apically located than those of the other olfactory epithelial cells.

### **3. Basal cells**

Resting on the basal lamina of the olfactory epithelium, basal cells are stem cells capable of division and differentiation into either supporting or olfactory cells. The constant divisions of the basal cells leads to the olfactory epithelium being replaced every 2–4 weeks.

Basal cells can be divided on the basis of cellular anatomy histological markers into two populations: the horizontal basal cells which line the olfactory epithelium and the slightly more superficial globose basal cells. Horizontal basal cells are now thought to be the primary stem cell population supplying new cells in this system, According to some scientists the globose basal cells are the true stem cells.

### **4. Brush cells**

A microvilli-bearing columnar cell with basal surface in contact with afferent nerve endings, specialised for transduction of general sensation. Nerve fibres are terminal branches of trigeminal nerve (cranial nerve V), rather than the olfactory nerve, as afferent olfactory signals are.

### **5. Bowman's (olfactory) glands**

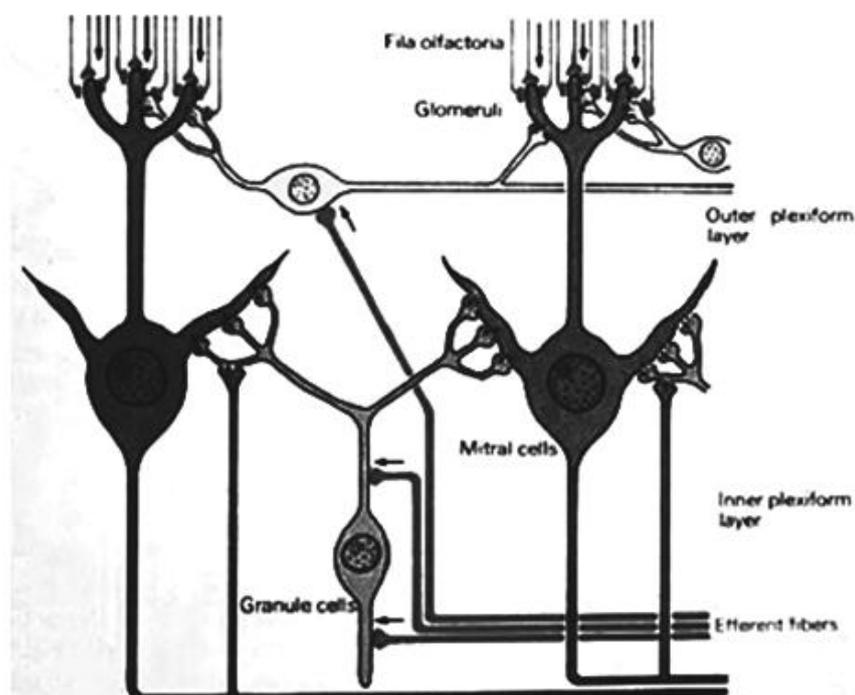
Tubuloalveolar serous secreting glands lying in the lamina propria of the mucosa. These glands deliver a proteinaceous secretion via ducts onto the surface of the mucosa. The role of the secretions are to trap and dissolve odiferous substances for the bipolar neurons. Constant flow from the Bowman's glands allows old odors to be constantly washed away.

There are also endings of the trigeminal system present in the nasal mucosa; these are thought to be the pathway for irritating or "stinging" sensations from the nose. The olfactory epithelium can be damaged by inhalation of toxic fumes, physical injury to the interior of the nose, and possibly by the use of some nasal sprays. Because of its regenerative capacity, damage to the olfactory epithelium can be temporary but in extreme cases, injury can be permanent, leading to anosmia.

### 5.6.2.3. Neural Pathways for Olfactory Signals

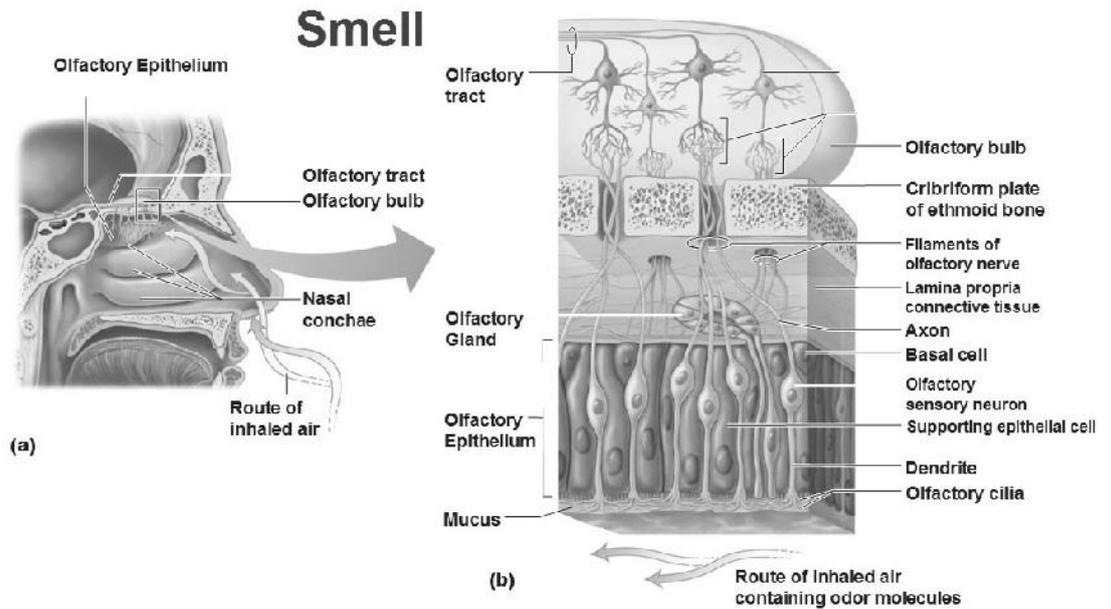
The axons of the sensory cells are called the fila olfactoria, and pass through the cribriform plate of the ethmoid bone to reach the olfactory bulb. The relay points for the sensory axons of the fila olfactoria are glomeruli in the olfactory bulb. The glomeruli contain the dendrites of the large mitral cells, which are the main afferent pathway for olfaction. Axons of the mitral cells turn in the granular layer and become the olfactory tract. Other important cells are granule cells, which connect to the mitral cells, and tufted cells, whose axons also exit in the olfactory nerve.

The fila olfactoria synapse with mitral cells and periglomerular cells, in the lowest, or glomerular layer of the bulb. Periglomerular cell axons then synapse on other mitral cells. Granule cells receive efferent, or centrifugal inputs from the anterior olfactory nucleus on the same side, and synapse on mitral cells. Mitral cell axons then exit the bulb as the olfactory tract.



**Figure: Axons of mitral cells form the olfactory tract**

Recent researches show that about 2000 olfactory sensory cells, coding for a single odorant receptor type, project into a single glomerulus. With granule and periglomerular cells, there are many synaptic interactions and possibilities for modifications of the neural olfactory code at the level of the olfactory bulb.

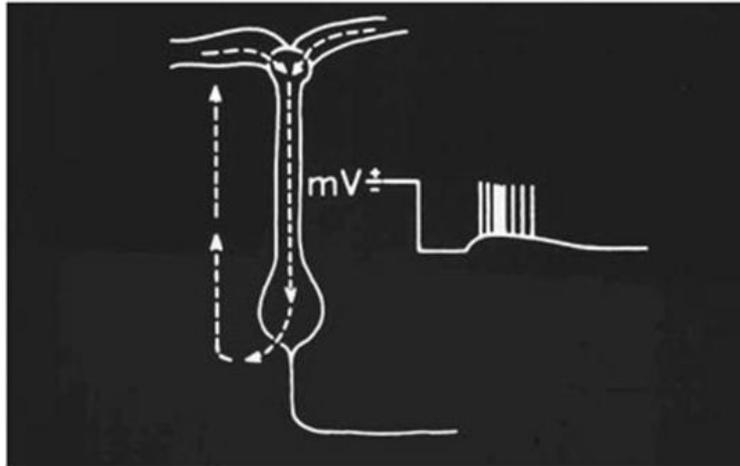


**Figure : Neural Structure of Olfactory receptor**

The olfactory tract eventually leads to the olfactory bulb on the opposite side, to the prepyriform area and the pyriform lobe, the hippocampus, and, via the amygdaloid complex to the autonomic nuclei of the hypothalamus, where signals may influence the release of hormones involved in reproduction.

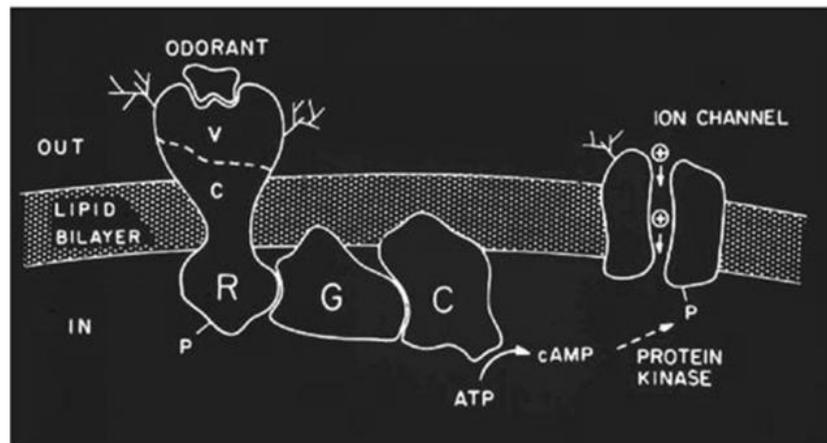
#### **5.6.2.4. Electrophysiology of Olfactory Sensory Cells**

Analysis of current flows in olfactory epithelium when electrodes are placed at various depths has suggested that the action potentials arise in an area of the sensory neuron that is relatively remote from the ciliary odorant receptors. The transduction current produced by the formation of odorant-receptor complex in the cilia flows centripetally down the sensory cell body. Action potentials are initiated in the cell-body region of the sensory neuron, near the point where the cell tapers in an axon hillock before the axon.



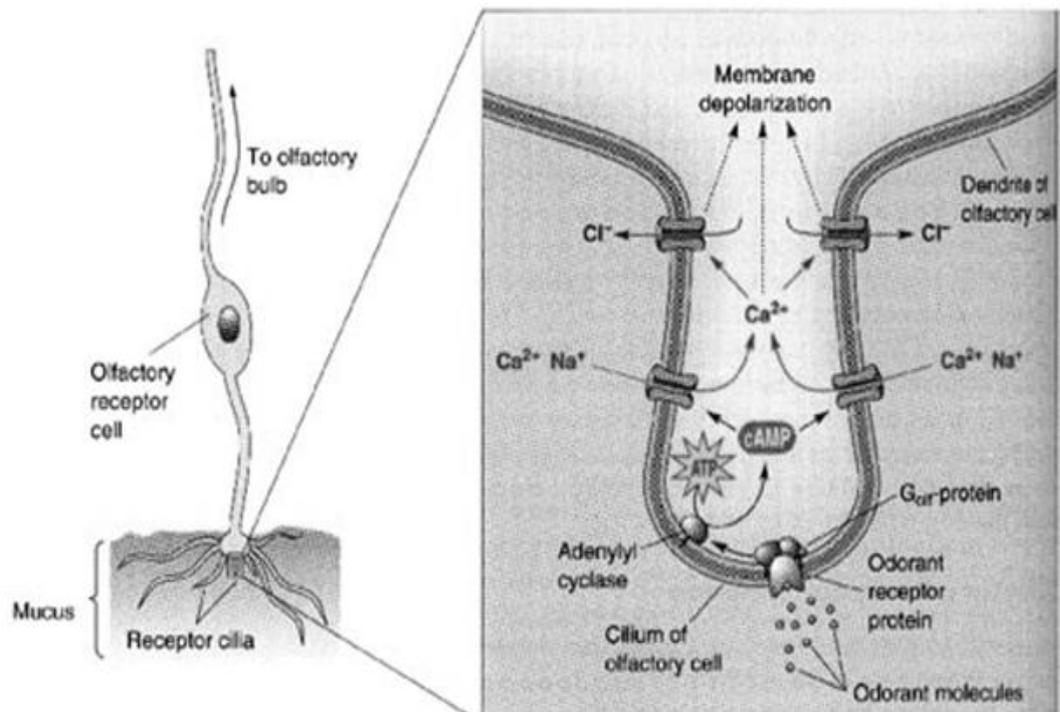
**Figure: Action potentials in olfactory neurons arise in a ciliary receptors**

More recently, it has been revealed that the intimate membrane currents produced in the active zones of olfactory cells, when various odorants are applied to them. One interesting result is that the nucleotide cyclic adenosine monophosphate (cAMP) has a direct gating effect on ionic conductances in the membrane. Cyclic AMP may serve as an intracellular second messenger between olfactory stimulants coming in contact with the sensory cell membrane and the resulting changes in ionic conductances.



**Figure: The receptor molecule (R) has a so-called constant (c) and variable (v) region for different odorants. When an odorant occupies the receptor, a GTP-binding protein (G) is activated, which modulates the activity of the adenylate cyclase (C) catalyzing the production of cAMP. This intracellular messenger activates a protein kinase to cause phosphorylation**

of the ion channel polypeptides, and opening or closing the associated ion channels.



**Figure: Transduction mechanism of Olfactory cells**

It is possible to make suspensions of homogenized olfactory epithelium or detached olfactory cilia that contain both olfactory receptors and some associated ionic channels. These suspensions may then be added to artificial lipid bilayers membranes, and the behavior of the receptor/channel complexes studied under controlled conditions. In the case of epithelial homogenates, both ATP and GTP must be present in the bathing solutions for the olfactory response to occur.

#### **5.6.2.5. Possible Mechanisms of Odour Discrimination**

Current studies have explain the detection of specific odorants by the olfactory system, the existence of perhaps 1000 different genes coding for specific receptors. Each odorant receptor gene is expressed by only ~0.1% of the olfactory sensory neuron population, suggesting that each sensory neuron may express only a single receptor type. The sense of smell may involve many receptors that are able to bind with one or a small number of odorants; since there are so many structurally diverse odorous ligands, it is likely that there would be a large number of different receptor types.

Thus, the olfactory sense may detect up to a thousand different odors by using individual receptors. Subfamilies of receptors may detect structurally similar compounds. Receptors of the same type project to only a few glomeruli in the olfactory bulb. Each glomerulus that receives input from a given receptor type is specific for a given epitope on an odorant. Since odorants contain more than one epitope, a particular odorant is recognized by the pattern of glomeruli that it excites.

#### **5.6.2.6. Reflex Olfactory Pathways**

The axons of mitral cells pass from the olfactory bulb centrally toward the brain as the olfactory tract. The tract then divides to form separate medial and lateral olfactory tracts. The lateral olfactory tract ultimately terminates in the periamygdaloid cortex of the temporal lobe. This pathway probably represents the conscious smell pathway. The medial olfactory tract may terminate in the septal nuclei, the contralateral amygdala, or the anterior continuation of the hippocampus.

The body reflexly responds to both pleasant and unpleasant odors. The reflex responses are classified as viscerosomatic or viscerovisceral, depending on the nature of the response. Viscerosomatic reflexes include the reflex movements of the eyes, facial muscles, neck and the rest of the body in response to both pleasant and unpleasant odors. Viscerovisceral reflexes include salivary and gastric secretions in response to certain pleasant odors and vomiting in response to very obnoxious odors. Both the medial and lateral olfactory tracts contribute to the reflex pathways.

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### **5.7 Summary**

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The **nervous system** and the important **sensory organs** play a key role in the day-to-day functioning of the animal. The nervous system integrates and controls the various functions of the body, while the sensory organs detect the various stimuli in the animal's environment that it reacts to.

Sense organs allow us to interact with the world. Our eyes allow us to see. Identify threats, find food, recognize other people, animals, things, allow us to read and recognize signs. Taste organs in our tongue allow us to taste food and protect us from toxic substances by making them taste bad. Our ears allow us to communicate with people and alert us to dangers etc. Similarly sense organs in our skins allow us to sense touch and feel the Temperature etc. In summary sense organs are essential for our survival.

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## 5.8 Self Assessment Questions

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1. Define Synapse
2. What is Reflex arc?
3. Describe various receptors of human body.
4. Describe the general mechanism of stimulus transduction of receptors.
5. What are the photoreceptors? Describe the retina in detail.
6. Describe the functional architecture of the retina and its function.
7. Explain the structure and auditory transduction of the organ of corti in detail.
8. Write a short note on olfactory epithelium.
9. Describe the functioning of olfactory epithelium
10. Give classification of human body receptors.
11. Define Transduction
12. Describe the Transduction in the Gustatory System

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## 5.9 Reference Books

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- Animal Physiology: Adaptation And Environment – KNET SCHEMDT NELSON – Cambridge University Press
- Animal Physiology : Mechanism And Adaptation- R ECKERT RANDALL- WH Freeman And Co
- Principles Of Animal Physiology ( PB) – CHRISTOPHER MOYES- Pearson Education
- Text Book Of Animal Physiology - SHERWOOD – Cengage Learning India
- Introduction To Animal Physiology – I KAY- Garland Publishing
- Animal Physiology- MARGARET BROWN- Apple Academic
- Text Book Of Animal Physiology – R. NAGABHUSHNAM, KODARKAR & SAROJINI- Oxford IBH Co

## Unit-6

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# Physiology of Respiration: - Regulation of breathing and transport of O<sub>2</sub> and CO<sub>2</sub> & Mechanism of Respiration

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### Structure of the unit

- 6.0 Objectives
- 6.1 Introduction
- 6.2 Breathing Mechanism
  - 6.2.1 Inspiration
  - 6.2.2 Expiration
  - 6.2.3 Lungs Volume
  - 6.2.4 Lungs capacities
  - 6.2.5 Regulation of Breathing
- 6.3 Exchange of Gases
  - 6.3.1 Partial Pressure
  - 6.3.2 Gaseous exchange in Lungs
  - 6.3.3 Transportation of O<sub>2</sub>
  - 6.3.4 Oxygen – dissociation curve
  - 6.3.5 Transportation of CO<sub>2</sub>
  - 6.3.6 Respiratory Quotient (RQ)
  - 6.3.7 Respiratory Substrate
- 6.4 Types of Respiration
- 6.5 Respiratory Organ of Human
  - 6.5.1 Accessory Respiratory Organ/Conducting Respiratory Organ
  - 6.5.2 Essential or Main Respiratory Organ
- 6.6 Mechanism of Respiration
  - 6.6.1 Glycolysis
  - 6.6.2 Anaerobic respiration

- 6.6.3 Aerobic respiration
- 6.6.4 Kreb's Cycle
- 6.6.5 Terminal oxidation
- 6.6.6 Significance of Citric Acid Cycle
- 6.6.7 Substrate level Phosphorylation

- 6.8 Glossary
- 6.9 Self Learning exercise
- 6.10 References

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## 6.1 Objectives

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After going through this unit you will be able to understand:

- What is respiration?
- Breathing and breathing mechanism.
- Respiratory organs and substrates.
- Oxygen-association curve and Bohr Effect.
- Terminal oxidation and substrate Phosphorylation.
- Lungs Volume and Capacities.
- Significance of critic acid cycle.
- Regulation of respiration by factors.

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## 6.1 Introduction

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This chapter is very important for a student to know about how energy produced in body and role of oxygen during respiration.

All living organisms require a continuous supply of energy for carrying out different function such as growth, development, movement and uptake of materials. The energy needed by the cells is obtained through cellular respiration. Cellular respiration is enzymatically controlled catabolic process in which energy released and involves a stepwise oxidative breakdown of organic substances inside living cells. We can compare respiration and combustion (burning of coal, oil and wood etc.) in these two process involves (i) Breakdown of complex organic substances (ii) Utilization of oxygen (iii)

Production of CO<sub>2</sub> and (iv) release of energy, but there are some basic differences between the respiration and combustion, combustion release a large amount of energy in single step, and most of it released as heat and sometimes partly as light. Cellular respiration releases energy in steps and each step is coupled with the synthesis of ATP. Only small amount of energy is spending wastefully as a heat.

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## 6.2 Breathing Mechanism

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The physical movements associated with the gaseous exchange are called breathing. In this process oxygen is carried out to respiratory surface and CO<sub>2</sub> remove from body. During breathing volume of thoracic cavity increases and decreases alternately and due to this lungs also swollen and flattened by alternate manner.

Breathing is involuntary process in which 75% role performed by diaphragm and 25% by movement of ribs.

Thoracic part of body is like a cage in most of mammals which is known as a thoracic cage or respiratory cage. Ventral side of respiratory cage having sternum and dose side having vertebral column posterior side of respiratory cage is covered by diaphragm and lateral side ribs.

There are two types of muscles present between ribs.

- (i) Internal Intercostals muscles (IIM)
- (ii) External intercostals muscles (EIM)

Due to contraction in external intercostals muscles ribs and sternum move upward and outward while ribs and sternum move downward and inward by relaxation of external intercostals muscles.

Diaphragm is present between abdomen cavity and thoracic cavity. Central part of diaphragm is like a semilunar tendon where radial muscles attached to diaphragm. Diaphragm is flattened and volume of thoracic cavity increases due to contraction in radial muscles.

Breathing process completed in two steps.

1. Inspiration
2. Expiration

Inspiration and expiration occurred by alternate manner.

### 6.2.1 Inspiration

This is active phase of breathing in mammals.

Ribs and sternum move upward and outward due to contraction in external intercostals muscles during inspiration width of chest increases from side to side from front to body and from top to bottom in this process.

Diaphragm flattened by contraction of radial muscles (a) Depth of Chest increases (b) capacity of thorax is increased (c) pressure between pleural surface is reduced.

Elastic tissue of lungs is stretched.

(a) Air pressure within alveoli is now less atmospheric pressure.

(b) Air is sucked into alveoli from atmosphere.

### **6.2.2 Expiration**

This is passive phase of breathing in mammals.

Sternum and ribs move downward and inward (a) width of chest decreases.

Diaphragm become dome shaped due to relaxation in radial muscles

(a) Diaphragm ascends (b) Depth of chest decreases (c) capacity of thorax is decreased (d) pressure between pleural surface increased.

Elastic tissue of lungs relaxed

(a) Air Pressure within alveoli is now greater than atmospheric pressure (b) Air is forced out of alveoli to atmosphere.

### **6.2.3 Lung Volume**

(a) Tidal Volume (TV) - It is the volume of air normally inspired or expired in one breath without any effort. It is about 500ml for an average adult human male. Only about 550 ml of air enters the lung alveoli for the exchange of gases the remaining 150ml fills the respiratory passage.

(b) Inspiratory Reserve Volume (IRV) – It is air that can be breathed in by maximum inspiratory effort after an ordinary respiration. Volume of air is 2 to 3.2 litres.

(c) Expiratory Reserve Volume (ERV) – The volume of air that can be breathed out by maximum expiratory effort after an ordinary respiration. It is about 1 to 1.5 litres.

(d) Residual volume (RV) – It is amount of air which remains in the lungs after maximal expiration. It is about 1.5 litres.

#### 6.2.4 Lung capacities

- (a) Inspiratory Capacity (IC) – Maximum volume of air than can be inspired from the end expiratory position.

$$TV+IRV = 2.5 \text{ to } 3.0 \text{ Litres}$$

- (b) Functional Residual capacity (FRC)- Volume of air remaining in the lungs after a quit expiration.

$$RV+ERV = 2.5 \text{ Litres}$$

- (c) Total Lung Capacity (TLC) – Volume of air that the lung can hold after a maximum possible inspiration.

$$IC+FRC = 5.0-6. \text{ Litres}$$

- (d) Vital capacity (VC)- It is the volume of air that can be breathed out by maximal expiratory effort after a maximum inspiration

$$IC+ERY = 4.5 \text{ Litres}$$

The vital capacity is depend upon age, sex and size of the individual. There is correlation between height of individual in cm. and vital capacity of lungs.

#### 6.2.5 Regulation on Breathing

The control of Breathing is nervous.

**Nervous control** – The intercostals muscles and the muscular diaphragm are innervates by phrenic and thoracic motor nerve from the central nervous system. The nerves are influenced by the respiratory centres situated in the medulla oblongata. These are inspiratory centre and expiratory centre. Both these centres do not work together. When inspiratory centre is active expiratory centre is inactive and vice versa. This makes the respiration a periodic process. There is another centre called pneumotaxic centre located in pons of the brain. This centre acts on the inspiratory centre and produces periodic inhibition. Nerve impulses originated from the inspiratory centre stimulate the muscles of the diaphragm to flatten the diaphragm and due to intercostals muscles to raise the ribs.

These process completed inspiration. The inspiratory centre also sends impulse to the pneumotaxic centre during inspiration which in turn transmits impulses to the expiratory centre. The expiratory centre is stimulated and sends impulses to the intercoastal muscles that lower the ribs and cause expiration.

The expiratory centre is connected to the vagus nerve which innervates the alveoli of the lungs. There is stretch receptor present in lungs. During inspiration when lungs expand, these receptors are stimulated and send impulses to the expiratory centre through vagus. Expiratory centre send inhibitory impulses to the inspiratory centre to stop inspiration. The reflex from the stretch receptors serves as a second feedback mechanism for the regulation of the respiratory cycle or breathing mechanism. This is called Hering - Breuer reflex.

### 6.3 Exchange of Gases

All cells have the essential structural organization and enzymes to carry out the oxidative process in small steps. In this process organic compound mainly carbohydrates are oxidized and produce carbon dioxide (CO<sub>2</sub>). In most organisms, oxidation needs the participation of molecular oxygen obtained from outside. Oxygen is ultimately reduced to form water. Thus oxidation of carbohydrates, besides releasing energy leads to the formation of carbon dioxide and water.

The carbon dioxide produced in respiration must be eliminated from the cell. Therefore, respiration involves exchange of gases between the cell and its surrounding and also between the organism and its environment.

#### 6.3.1 Partial Pressure

Exchange of gases between the cell and its surrounding and also between the organism and its environment depend upon the partial pressure of gases. Exchange of gases takes place by diffusion.

(a) Partial pressure of oxygen in Environment

$$PO_2 = 21/100 \times 760 = 159.60 \text{ mm Hg.}$$

(b) Partial pressure of CO<sub>2</sub> in environment

$$PCO_2 = 0.04/100 \times 760 = 0.30 \text{ mm Hg}$$

Atmosphere		Alveolar Air		Blood
PO <sub>2</sub> = 160 mm Hg	→	PO <sub>2</sub> = 100 mm Hg	→	PO <sub>2</sub> = 40 mm Hg

$\text{PCO}_2 = 0.3 \text{ mm Hg}$	$\leftarrow \text{PCO}_2 = 100 \text{ mm Hg}$	$\leftarrow \text{PCO}_2 = 45 \text{ mm Hg}$
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Table-1

It is clear from above table that gases having tendency to move higher partial pressure to lower partial pressure during diffusion.

### 6.3.2 Gaseous Exchange in Lungs

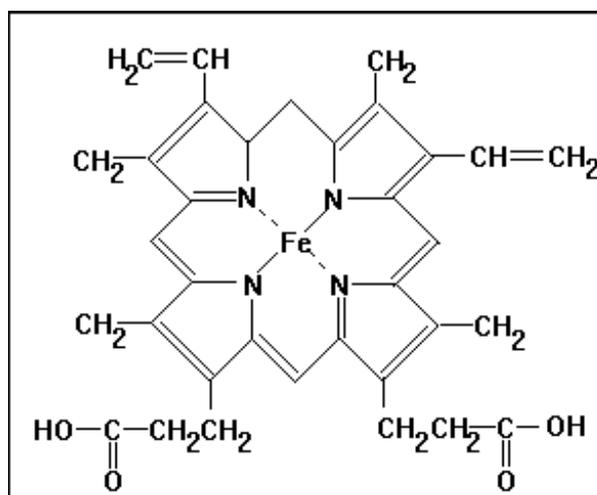
Higher graded animals having lungs, which are essential respiratory organs. Alveoli is smallest unit of the lungs. Alveoli possesses respiratory surface where exchange of gases takes place. Alveoli are covered by very thin squamous epithelium. Epithelium of alveoli connected with wall of blood capillaries and form respiratory surface.

### 6.3.3 Transportation of oxygen

Transportation of oxygen completed by two forms which are as following:

- (a) In form of dissolved stage with water present in blood (2-4%)
- (b) In form of binding stage with Haemoglobin (96-98%)

Large amount (96-98%) of oxygen is transported by haemoglobin, haemoglobin is chromo protein which having prosthetic group and globin protein. The haemoglobin is found in the blood within erythrocytes (Red blood corpuscles).

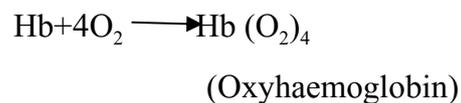


Structure of haemoglobin

The three dimensional structure of haemoglobin was solved using X-Ray crystallography in 1959 by max Perutz. The major type of haemoglobin found

in adults (HbA) is made up of two different polypeptide chains the  $\alpha$ -chain that consists of 141 amino acid residue and  $\beta$ -Chain of 146 residues ( $2 \times 2$ ). Each chain consists of eight  $\alpha$ -helices and a haem prosthetic group. Therefore, haemoglobin can bind four molecules of  $O_2$ . The haem prosthetic group is made up of a protoporphyrin IX ring structure with an iron atom in the ferrous ( $Fe^{2+}$ ) oxidation state. This  $Fe^{2+}$  bonds with four nitrogen atoms in the center of the the protoporphyrin ring and forms two additional bonds on either side of the plane the protoporphyrin ring.

haemoglobin increases carrier capacity of oxygen. Molecules of oxygen do not oxidise atom present in haem porphyrin ring due to this reason oxidation stage of ferrous ( $Fe^{2+}$ ) not changed and this process is known as oxygenation binding of oxygen ( $O_2$ ) with haemoglobin form oxyhaemoglobin.



Colour of haemoglobin is violet while oxyhaemoglobin having red colour. 1gram haemoglobin can carry 1.34ml of  $O_2$  present in 100 ml blood and can be calculated as following:

$$15 \times 1.34 = 20 \text{ ml (100ml blood having 14-15 gram Hb)}$$

#### 6.3.4 Oxygen dissociation curve

Graphical presentation of saturated percentage haemoglobin and partial pressure of oxygen is known as oxygen dissociation curve.

The oxygen haemoglobin dissociation curve plot the proportion of haemoglobin in its saturated form on the vertical axis against the prevailing oxygen tension on the horizontal axis.

Approximately 75% haemoglobin saturated when partial pressure of  $O_2$  ( $PO_2$ ) is 40mm Hg. When partial pressure of  $O_2$  ( $PO_2$ ) is 100 mm Hg in this stage saturated percentage of haemoglobin is 97.5%.

Oxygen dissociation curve is sigmoid shaped.

The partial pressure of oxygen in the blood at which the haemoglobin is 50% saturated, typically about 26.6 mm Hg for healthy person is known as  $P_{50}$ .

PO <sub>2</sub>	% saturation of Hb
10	13.5
20	35
30	57
40	75
50	83.5
60	89
70	92.7
80	94.5
90	96.5
100	97.5

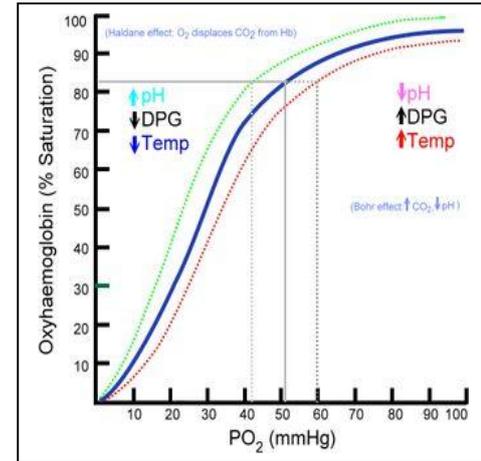


Table-2  
dissociation curve

Diagram-1 Oxygen

The PO<sub>2</sub> is a conventional measure of haemoglobin affinity for oxygen. In the presence of disease or other condition that change the haemoglobin's oxygen affinity and consequently shift the curve to the right or left and P<sub>50</sub> change accordingly.(Diagram-1)

Partial pressure of oxygen (PO<sub>2</sub>) is less toward tissues, Due to this phenomenon dissociation of oxyhaemoglobin take place and oxygen released.

**Bohar effect:** Shifting of oxygen dissociation curve toward right side in presence of CO<sub>2</sub> and hydrogen ion concentration (H<sup>+</sup>) is known as Bohr effect.(Diagram-1)

Bohar effect is helpful for transportation of oxygen. Shifting of oxygen-dissociation curve toward right side induce more release of oxygen. High temperature also shift oxygen dissociation curve toward right side.

### Foetal haemoglobin and oxygen dissociation curve:

foetal haemoglobin (HbF) is structurally different from normal adult hemoglobin (HbA), giving HbF a higher affinity for oxygen than HbA. HbF is composed of two alpha ( ) and two gamma chains whereas HbA is composed of two alpha ( ) and two beta ( ) Chains.

The foetal oxygen dissociation curve is shifted to the left relative to the curve for the normal adult because of structural differences(Diagram-2).

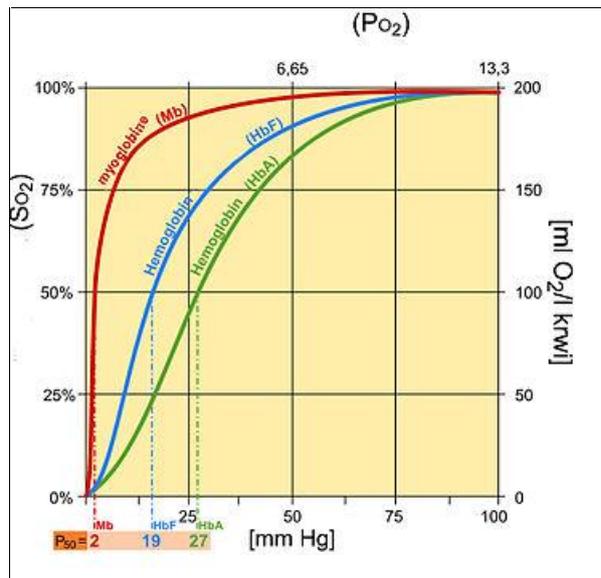


Diagram-2

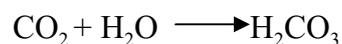
Typically, foetal arterial oxygen pressures are lower than adult arterial oxygen pressures. Hence higher affinity to bind oxygen is required at lower levels of partial pressure in the foetus to allow diffusion of oxygen across the placenta.

### 6.3.5 Transportation of CO<sub>2</sub>

Transportation of CO<sub>2</sub> takes place by three forms (Diagram 3 & 4).

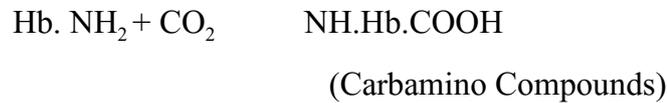
(a) As carbonic Acid

7% of transported in form of carbonic acid.



(b) As carbamino compounds

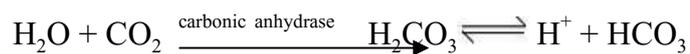
23% of total CO<sub>2</sub> transported in form of carbamino compounds. CO<sub>2</sub> bind with protein present in haemoglobin and produce compounds of carbomino known as carbamino haemoglobin.



(c) As Bicarbonates

70% of total CO<sub>2</sub> transported in form of bicarbonate. CO<sub>2</sub> bind with water inside RBC and produce carbonic Acid (CH<sub>2</sub>CO<sub>3</sub>). Due to presence of carbonic anhydrase enzyme in RBC this reaction increase more than 5000 times.

Carbonic acid dissociate in form of H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> ions.



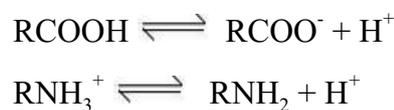
This reaction also occurs outside then red blood cells, in the plasma, but it is much slower due to the lack of carbonic anhydrase.

The hydrogen ions formed from the dissociated carbonic acid combine with the haemoglobin in the red blood cell.



Hydrogen ions produced in the red blood cell from the dissociation of carbonic acid are buffered primarily by haemoglobin.

Plasma proteins are effective buffers (but to a lesser extent than haemoglobin) due to their free carboxyl and free amino group that dissociate.



Haemoglobin is a powerful acid-base buffer as in addition to the free carboxyl and free amino groups, haemoglobin also contains 38 histidine residue that also dissociate. Haemoglobin has six time the buffering capacity of plasma protein due to the presence of the histidine residues.

Haemoglobin is therefore, important in maintaining the acid-base balance of the blood as well as transporting oxygen.

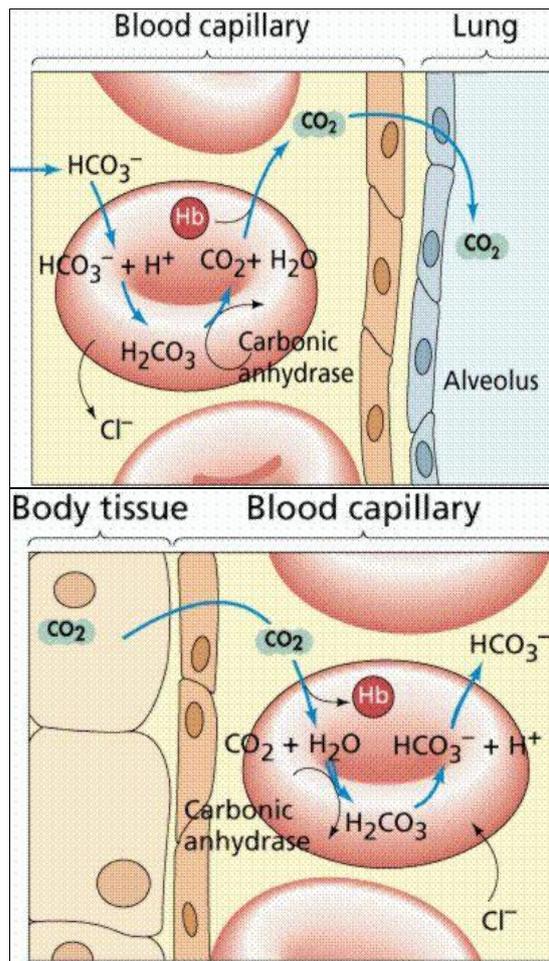


Diagram 3 & 4: Showing Transport of  $\text{CO}_2$

Bicarbonate ions diffuse out of the red blood cell into the plasma while chloride ions diffuse into the RBC this is known as the **chloride shift**.

Bicarbonate and chloride ions are transported across the RBC cell membrane in opposite directions by the bicarbonate- chloride carrier protein.

The chloride shift is extremely rapid, occurring within 1 second.

The chloride shift results in the chloride contents of venous blood being greater than that of arterial blood.

#### **Effect of carbon monoxide:**

The problem with carbon monoxide ( $\text{CO}$ ) is that it inhibits ability of  $\text{O}_2$  to bind with haemoglobin. Haemoglobin binds 230 to 250 time rapidly with carbon monoxide ( $\text{CO}$ ) as compare to oxygen ( $\text{O}_2$ ). Haemoglobin has a very high affinity for  $\text{CO}$ . The usual function of haemoglobin is to bind oxygen ( $\text{O}_2$ ) and take it to a place in the body that need  $\text{O}_2$  and then release the oxygen. When hemoglobin binds  $\text{CO}$ , it binds so tightly that will not let go.

Therefore, the haemoglobin that binds CO becomes 'poisoned' and can no longer bind with oxygen, destroying its function. Then parts of body do not receive the essential oxygen and effectively suffering (Asphyxia).



(Reversible)



(Irreversible)

### 6.3.6 Respiratory Quotient (RQ)

The ratio of the volume of carbon dioxide produced to the volume of oxygen consumed in respiration over a period of time is called respiratory quotient (RQ)

$$\text{RQ} = \frac{\text{Volume of CO}_2 \text{ evolved/released}}{\text{Volume of O}_2 \text{ absorbed/utilized}}$$

RQ is determined with the help of apparatus called respirometer. The value of RQ varies with different substrate utilized in respiration. Its value can be 1, 0, more than 1 or less than 1

- (i) For carbohydrate (RQ) = 1
- (ii) For protein (RQ) = > 1
- (iii) For Fat (RQ) = < 1

### 6.3.7 Respiratory substrate

The organic substances which can be catabolised in the living cells to release energy are called respiratory substrate. The most common respiratory substrate is glucose. It is formed from storage carbohydrate like starch in most plants and glycogen in animals. Fats may be used as respiratory substrate in some cases. Proteins are used in respiration rarely as during germination of protein rich seeds and spores. In higher graded animals, proteins are employed as

respiratory substrate only when carbohydrate and fat reserves have been used up, as during long fasting Respiration involving protein as respiratory substrate is called protoplasmic respiration whereas that uses carbohydrates or fat is termed as floating respiration. Protoplasmic respiration cannot be carried on for long as it liberate toxic ammonia.

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## 6.4 Types of Respiration

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- (a) **External respiration:** In this type respiration, respiratory substrate approached to respiratory surface and exchange of  $O_2$  and  $CO_2$  take place at respiratory surface.
  - (b) **Internal respiration:** In this type respiration, transportation or exchange of  $O_2$  and  $CO_2$  take place between blood and tissues.
  - (c) **Cellular respiration:** In this type respiration, oxidation of organic substances takes place and energy released.
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## 6.5 Respiratory organs of Human

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There are two types of respiratory organs present in human.

### 1.5.1 Accessory respiratory organ/conducting respiratory organ

The different organs of the respiratory system such as nose, larynx, trachea, bronchi carry out the oxygen from external environment to respiratory surface (Lungs) are called Accessory/conducting respiratory organs (Diagram-5)

### 6.5.2 Essential or Main respiratory organ

The organs of the respiratory system such as lungs in which respiratory surface is present and exchange of  $O_2$  and  $CO_2$  take place known as essential or main respiratory organs.

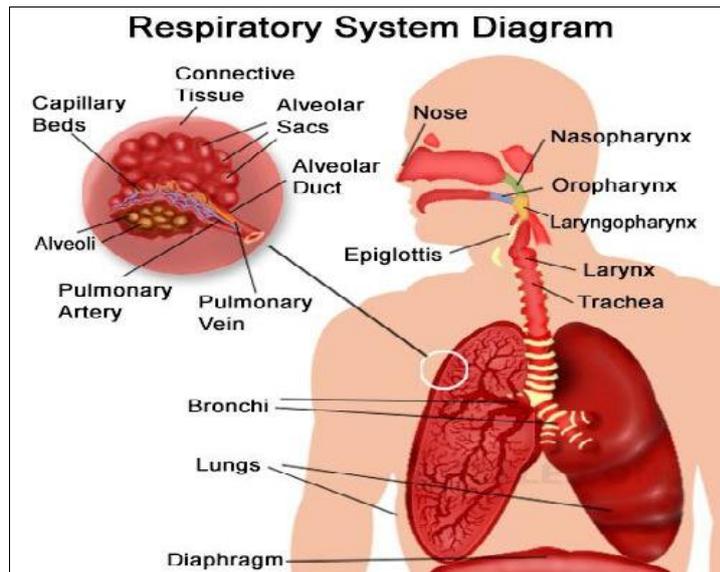


Diagram-5

**Lungs:** Human lungs are conical organ present inside the pleural cavities. They carry out the work of supplying the body with oxygen and removing carbon dioxide. The left lung is divided into 2 lobes (superior and inferior) while the right lung into 3 lobes (superior inferior and middle.)

Alveoli are structural unit of the lungs. The alveoli are sac-shaped bodies present inside the lungs at the tip of alveolar duct. The alveoli function like an interface for the exchange of oxygen and carbon dioxide between lungs and capillaries. Capillaries connect the alveoli with the tissues of the body. The process of gas exchange in alveoli is characterized by inhalation of oxygen and exhalation of  $\text{CO}_2$ . Oxygen enters the blood cells by means of alveoli and network of capillaries. Oxygen is carried to the tissues of different parts of the body by means of blood.  $\text{CO}_2$  is collected by blood and carried to lungs.

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## 6.6 Mechanism of respiration

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Both aerobic and anaerobic types of respiration have a common series of initial steps termed glycolysis. During glycolysis carbohydrates are converted into pyruvic acid through a series of enzymatic reactions. The pyruvic acid thus formed enters mitochondria where  $\text{O}_2$  and necessary enzymes are available. Pyruvic acid finally converted into  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . This series of reaction is known as Krebs cycle or tricarboxylic cycle (TCA cycle) or citric acid cycle.

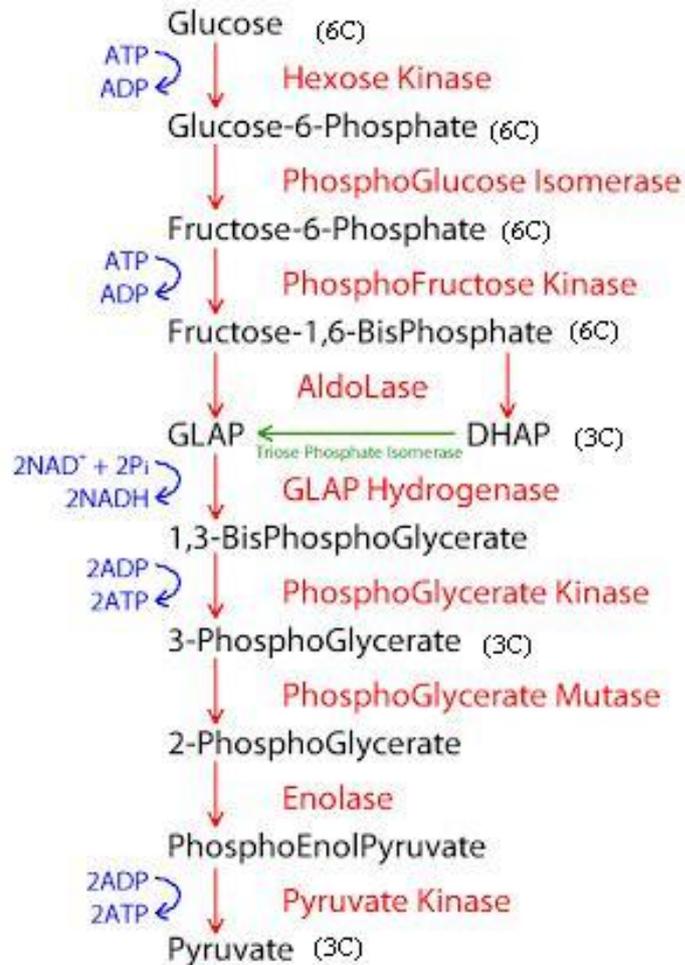
### 6.6.1 Glycolysis

It is also known as EMP pathways, because it was discovered by three German scientists Gustav Embden, Otto Myerhof and J. Parnas. Glycolysis is common to both aerobic as well as anaerobic modes of respiration and is therefore called common pathway. Glycolysis is the first step in the breakdown of glucose and is common to all organisms. It occurs in the cytosol and breakdown of glucose into two molecules of a three carbon compound pyruvic acid, releasing some energy (as ATP) and reducing power (as NADH<sub>2</sub>).

The steps of glycolysis are as following:

- (1) *Phosphorylation*: Glucose phosphorylated to glucose 6-phosphate by ATP in the presence of enzyme hexokinase and Mg<sup>2+</sup>.
- (2) *Isomerisation*: Glucose -6- phosphate is changed into its isomer, fructose – 6 – phosphate with the help of enzyme phosphohexose isomerase (phosphogluco isomerase).
- (3) *Phosphorylation*: Fructose -6- phosphate is phosphorylated by ATP to form fructose 1,6 diphosphate in presence of enzyme phosphofructokinase and Mg<sup>2+</sup> (This is rate limiting step).
- (4) *Splitting*: Fructose 1, 6 diphosphate is then broken down into two molecules of triose phosphate (glyceraldehyde-3 phosphate). Dihydroxy acetone phosphate is further converted into glyceraldehydes 3-phosphate with the help of enzyme phaspho-triose isomerase.
- (5) *Dehydrogenation and phosphorylation*: Each glyceraldehyde 3 phosphate molecule loses hydrogen to NAD<sup>+</sup> to form NADH+ H<sup>+</sup> and accept inorganic phosphate from phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) to form 1,3 diphosphoglucerate in the presence of enzyme triose phosphate dehydrogenase.
- (6) *Dephosphorylation (ATP formation)*: One of the two phosphates of 1, 3 diphosphoglycerate is linked by high energy bond. In the presence of enzyme phosphoglycerate kinase, 1, 3 diphosphoglycerate is converted to 3 phosphoglycerate. One molecules of ADP is phosphorylated to ATP in the reaction.
- (7) *Isomerisation*: 3 phosphoglycerate is changed to its isomer 2-phosphoglycerate by the enzyme phosphoglyceromutase.
- (8) *Dehydration*: 2 phosphoglycerate loses a molecule of water in the presence of enzyme Mg<sup>2+</sup> and changes into phosphoenol pyruvate.

(9) *Dephosphorylation*: High energy phosphate group of phosphoenol pyruvate is transferred to a molecule of ADP with the help of the enzyme pyruvate kinase in the presence of  $Mg^{2+}$  and  $K^+$ .



**Diagram-6: Glycolysis overview**

**Net product of glycolysis:** In glycolysis process two molecules of ATP are consumed during double phosphorylation of glucose to form fructose 1,6 – diphosphate. In return four molecules of ATP are produced by substrate level phosphorylation and two molecules of  $NADH_2$  are produced at the time of oxidation of glyceraldehyde 3- phosphate to 1, 3 – diphosphoglycerate. The whole process may be explained as follow.



Two molecules of  $\text{NADH} + \text{H}^+$  on oxidation process during E.T.S produces 6 molecules of ATP. Therefore, a net gain of 8ATP molecules occurs during glycolysis.

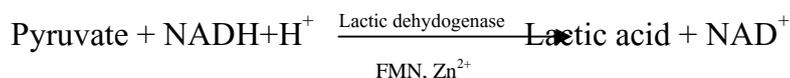
### 6.6.2 Anaerobic respiration

Anaerobic respiration is an enzyme controlled partial breakdown of organic compounds without using oxygen and releasing only a fraction of energy. The term anaerobic respiration is often used in connection with higher organism. In micro organisms, anaerobic respiration often called fermentation. In anaerobic respiration electrons are removed from the substrate during oxidation but are not finally transferred to molecular oxygen.

The final electron acceptors are compounds such as pyruvic acid or acetaldehyde, which form a part of anaerobic respiratory pathway. The end products are lactic acid or ethyl alcohol and not water.

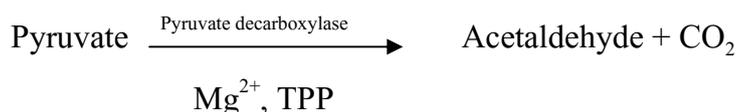
Pyruvate formed at the end of glycolysis is anaerobically broken down to yield various products depending upon the organism and the type of tissue. The two common products are ethyl alcohol and lactic acid.

#### (a) Lactic acid formation in skeletal muscle cells

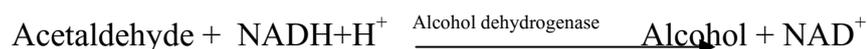


- Skeletal muscles usually derive their energy by anaerobic respiration.
- Anaerobic respiration produces much less energy than aerobic respiration. The main reasons are (I) There is incomplete breakdown of respiratory substrate (II)  $\text{NADH}_2$  produced during glycolysis is often reutilized, (III) Regeneration of  $\text{NAD}^+$  from  $\text{NADH}_2$  does not produce ATP (IV) Electron transport chain is absent (V) oxygen is not used for accepting electrons and protons.

#### (b) Alcoholic Fermentation



- Acetaldehyde then accepts hydrogen from  $\text{NADH}_2$  and is reduced to ethyl alcohol (ethanol) producing oxidized  $\text{NAD}^+$ . The process is catalyzed by the enzyme alcohol dehydrogenase.



### (c) Significance of Anaerobic Respiration

- (i) Carbon dioxide released by yeast cells in alcoholic fermentation is used in baking industry for making the bread light and spongy.
- (ii) Dairy industry depends upon the action of lactic acid bacteria which convert milk sugar lactose into lactic acid.
- (iii) Anaerobic respiration is important during periods of oxygen deficiency.
- (iv) Various types of wines, beers and whisky are prepared through alcoholic fermentation of sugary solution with yeasts.

### 6.6.3 Aerobic respiration

In aerobic respiration molecular oxygen acts as the ultimate acceptor of electrons and protons removed from the substrate. Aerobic respiration is carried out by the enzymes of the matrix and the inner membrane of the mitochondria. CO<sub>2</sub> produced in the process diffuse out of the cell.

In presence of oxygen, pyruvic acid generated in the cytosol is transported to mitochondria and thus initiate the second phase of respiration. Before pyruvic acid enters Krebs's cycle operative in the mitochondria, one of the three carbon atoms of pyruvic acid is oxidized to carbon dioxide in a reaction oxidative decarboxylation.



The pyruvate is first decarboxylated and then oxidized by the enzyme pyruvate dehydrogenase, The remaining 2 carbon acetate unit is accepted by sulphur containing compound coenzyme A (CoA) to form acetyl CoA.

Acetyl coenzyme A (CoA) is connecting link between glycolysis and kreb's cycle. During this process NAD<sup>+</sup> is reduced to NADH.

Aerobic respiration consists of two distinct but mutually dependent processes.

- (i) kreb's cycle or tricarboxylic acid cycle (TCA) in which reduced coenzyme are formed and CO<sub>2</sub> is evolved
- (ii) Terminal oxidation in which the reduced coenzymes are oxidized and oxygen is reduced to water.

#### 6.6.4 Krebs's cycle or TCA cycle

Tricarboxylic acid (TCA) cycle was discovered by British biochemist Sir Hans Krebs. The cycle is also named as citric acid cycle or Tricarboxylic acid (TCA) cycle.

Krebs's cycle occurs in mitochondrial matrix and serves as a common oxidative pathway for carbohydrate, fats and protein.

The Krebs's cycle begins with entrance of acetyl CoA into a reaction to form citric acid.

The various steps of Krebs's cycle are as follows:

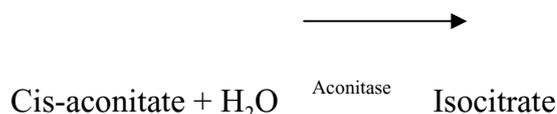
1. *Condensation*: In the first reaction, 2 carbon compound acetyl CoA combine with 4 carbon compound oxaloacetate (OAA) in the presence of condensing enzyme citrate synthetase to form a tricarboxylic 6-carbon compound called citric acid and CoA is liberated. Citric acid is the first product of the Krebs's cycle.



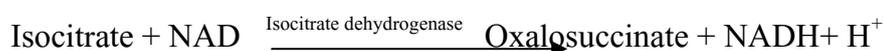
2. *Dehydration*: Citrate undergoes reorganization in the presence of iron-containing enzyme aconitase forming cis-aconitate.



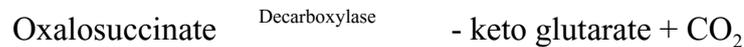
3. *Hydration*: Cis-aconitate is further reorganized into 6 carbon isocitrate with the addition of water in the presence of enzyme aconitase.



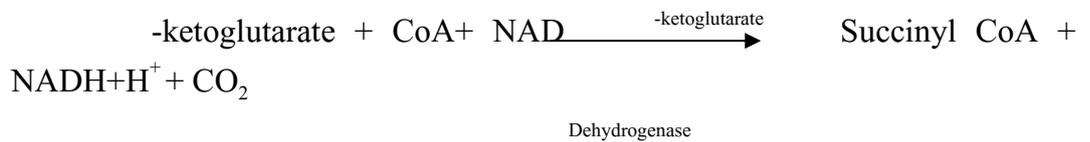
4. *Dehydrogenation*: Isocitrate is dehydrogenated to oxalosuccinate in the presence of enzyme isocitrate dehydrogenase and  $\text{Mn}^{2+}$ . A pair of hydrogen atoms is released which is accepted by  $\text{NAD}^+$  to form  $\text{NADH} + \text{H}^+$ .



5. *Decarboxylation*: Oxalosuccinate is decarboxylated to form a 5- carbon - keto glutarate in the presence of enzyme decarboxylase, one molecule of CO<sub>2</sub> is released in reaction.



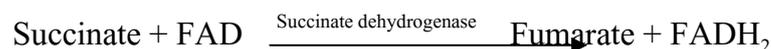
6. *Dehydrogenation and decarboxylation*: -Keto glutarate is both dehydrogenated (By NAD<sup>+</sup>) and decarboxylated by an enzyme complex - ketoglutarate dehydrogenase.



7. *Formation of GTP*: Succinyl CoA splits into succinate and CoA in the presence of enzyme succinyl thiokinase. The reaction releases sufficient energy to form GTP (in animal cell) and ATP (in plants).



8. *Dehydrogenation*: Succinate undergoes dehydrogenation to form 4- carbon fumarate with the help of enzyme succinate dehydrogenase and liberates a pair of hydrogen atom. The later pass to FAD to form FADH<sub>2</sub>.

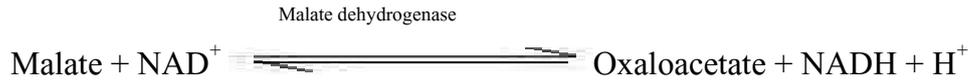


9. *Hydration*: Fumarate is changed into 4-carbon malate with the addition of water in the presence of enzyme fumarase.

Fumarase



10. *Dehydrogenation*: Malate is dehydrogenated in the presence of enzyme malate dehydrogenase to produce 4-carbon oxaloacetate. Hydrogen is accepted by  $\text{NAD}^+/\text{NADP}^+$ .



Oxaloacetate picks up another molecule of activated acetate to repeat the cycle.

The overall equation for this phase of respiration may be written as follows:

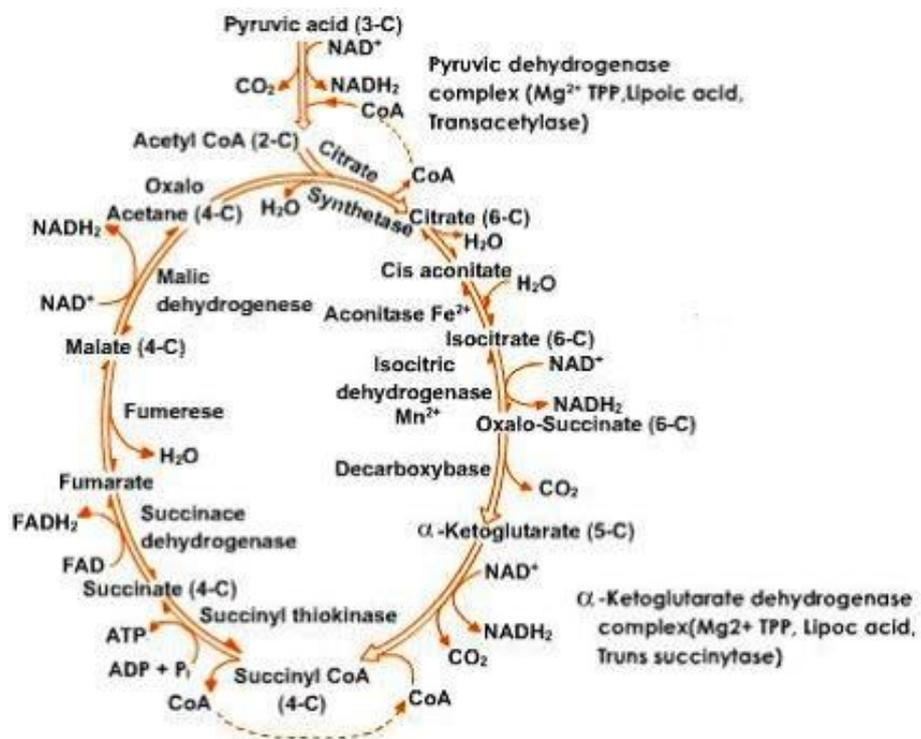
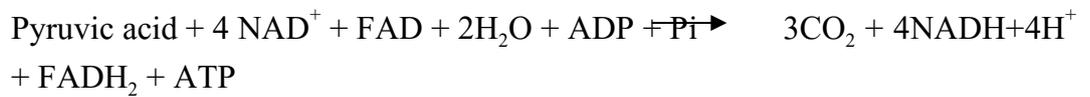
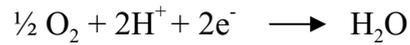


Diagram-8: Krebs's Cycle

### 6.6.5 Terminal oxidation

It is the last step of aerobic respiration which involves the passages of both electrons and protons of reduced coenzymes to oxygen.





Terminal oxidation consists of two processes: (a) Electron transport (b) Phosphorylation

a) **Electron transport Chain:** The inner mitochondrial membrane contains some proteins which act as  $\text{H}^+$  ion and electron transporting enzymes. The enzymes are arranged in ordered manner in a specific series called electron transport chain (ETC) or mitochondrial chain.

An electron transport chain is a series of enzymes and cytochromes in the inner mitochondrial membrane that take part in the passage of electrons from a substance to its ultimate acceptor. The electron carriers include flavins, iron-sulphur complexes, quanonones and cytochrome. Most of them are prosthetic group of proteins.

The glucose molecule is completely oxidized by the end of the TCA cycle. But the energy is not released unless NADH and  $\text{FADH}_2$  are oxidized by the electron transport chain. Electrons from NADH produced in the mitochondrial matrix during citric acid cycle are oxidized by an NADH dehydrogenase (complex-I) and electrons are then transferred to ubiquinone also receives reducing equivalents via  $\text{FADH}_2$  that is produced during oxidation of succinate, through the activity of enzyme succinate dehydrogenase (complex-II) in the citric acid cycle.

The reduced ubiquinone (ubiquinol) is then oxidized with the transfer of electrons to cytochrome (via cytochrome  $\text{bc}_1$  complex (complex-III) is small protein attached to the outer surface of inner membrane and acts as a mobile carrier for transfer of electrons between complex III and IV complex. Complex IV refers to cytochrome C oxidase complex containing cytochrome a and  $\text{a}_3$  and two copper centres.

When the electrons pass from one carrier to another via complex I to IV in the electron transport chain they are coupled to ATP synthase (complex-V) for the production of ATP from ADP and inorganic phosphate. The number of ATP molecules synthesized depends on nature of the electron donor. Oxidation of one molecule of NADH produces 3 molecules of ATP while that of one molecule of  $\text{FADH}_2$  produces 2 molecules of ATP.

At each step of electron transport the electron acceptor has a higher electron affinity than the electron donor. The energy from such electron transport is utilized in transporting protons ( $\text{H}^+$ ) from the matrix across the inner membrane to its outer side (outer membrane). The difference in proton

concentration on the outer side and inner sides of the inner mitochondrial membrane is known as proton gradient.

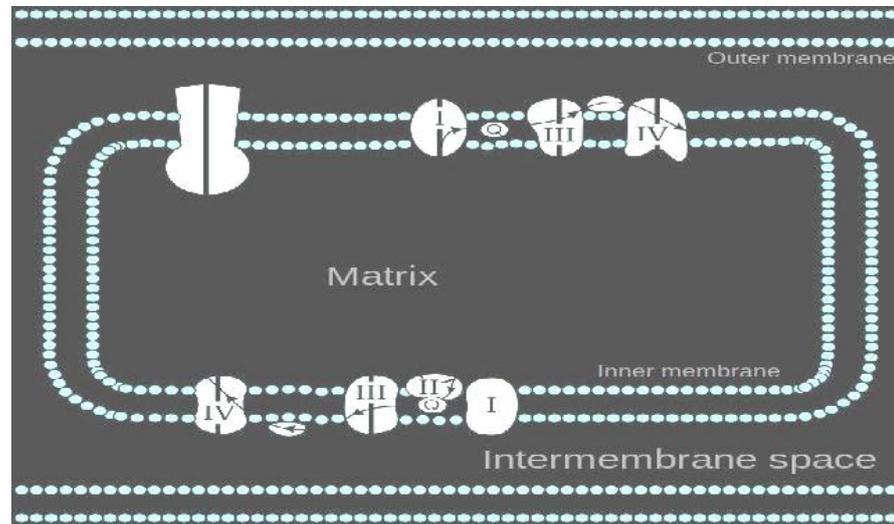


Diagram-7: Electron transport chain in mitochondrial membrane

b) **Oxidative phosphorylation:** oxidative phosphorylation is the synthesis of energy rich ATP molecules with the help of energy liberated by oxidation of reduced coenzymes ( $\text{NADH}_2$  and  $\text{FADH}_2$ ) produced during respiration.

The enzyme required for their synthesis is known as ATP synthatase. This enzyme is present in  $F_1$  or head piece of  $F_0$ - $F_1$  or elementary particle. The particles are located in inner mitochondrial membrane.

The enzyme ATP synthatase becomes active in ATP formation only when there is proton gradient, having higher concentration of protons on the  $F_0$  side (outer side) as compared to  $F_1$  side (inner side). Due to higher proton concentration outside the inner membrane protons return to the matrix down the proton gradient. Just like a flow of water from a higher to a lower level can be utilized to turn a water wheel or a hydroelectric turbine, the energy released by the flow of proton down the gradient is utilized in synthesizing ATP. The enzyme ATP synthetase, synthesizes ATP from ADP and inorganic phosphate using the energy from the proton gradient (Chemiosmotic hypothesis of Peter Mitchek, 1961).

Transport of two electrons from  $\text{NADH}+\text{H}^+$  by the electron transport chain simultaneously transfers three pairs of proton to the outer compartment. One high energy ATP bond is produced per pair of protons returning to the matrix through the inner membrane particles. Therefore, oxidation of one molecule

of NADH<sub>2</sub> produces 3 ATP molecules while that of FADH<sub>2</sub> forms only 2 ATP molecules as the FADH<sub>2</sub> donates its electron further down the chain.

#### 6.6.6 Significance of TCA\Citric acid cycle

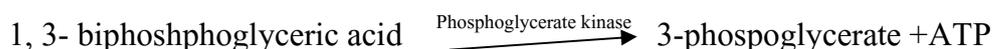
- a. It is responsible for controlled release of energy during aerobic respiration.
- b. It is common pathway of oxidative breakdown of carbohydrates, fatty acids and amino acid.
- c. Citric acid cycle is the major pathway for the synthesis of reduced coenzymes.
- d. Krebs cycle is a catabolic pathway it also provides a number of intermediates for anaerobic pathways. Therefore, Krebs's cycle is often called an amphibolic pathway.

#### 6.6.7 Substrate level phosphorylation

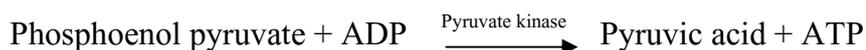
Formation of ATP directly from metabolism is known as substrate level phosphorylation.

There are two steps in the glycolysis process where substrate level phosphorylation occurred.

1. One of the two phosphate groups of 1,3-bisphosphoglycerate is linked by a high energy bond. In the presence of the enzyme phosphoglycerate kinase, 1,3-bisphosphoglycerate is converted to 3-phosphoglycerate. In this reaction, one molecule of ADP is phosphorylated to ATP in the reaction.



2. High energy phosphate group of phosphoenolpyruvate is transferred to a molecule of ADP with the help of the enzyme pyruvate kinase in the presence of Mg<sup>2+</sup> and K<sup>+</sup>.



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### 6.7 Factor affecting respiration

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Certain chemical factors like CO<sub>2</sub>, O<sub>2</sub> and acidity etc. stimulate the respiratory centres in the medulla oblongata.

- a) Effect of low oxygen supply: If the oxygen does not reach the blood, asphyxia occurs. Reduction in the supply of  $O_2$  to the tissues causes hypoxia where as if the tissues are completely deprived oxygen the condition is known as anoxia.
- b) Effect of  $CO_2$ : The inspiratory centre is sensitive to the  $CO_2$  in the blood stimulates the respiratory centres directly and increase the rate and depth of respiration. During exercise or emotional stress the impulses are rapid due to the higher production of  $CO_2$ . In contrary, decrease in  $CO_2$  depresses the rate and depth of respiration. This is called acapnia.
- c) Effect of acidity: when there is slight fall of blood pH; it stimulates respiratory centre. Increased  $CO_2$  lowers the pH resulting acidosis that enhances ventilation. During exercise acidity increases the rate and depth of respiration. Low level of  $CO_2$  increase pH and making blood alkaline and decreases respiration rate.

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## 6.8 Glossary

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- **Partial Pressure:** In a mixture of gases, each gas has partial pressure which is the hypothetical pressure of that gas if it alone occupied the volume of the mixture at the same temperature.
- **Exchange of Gas:** It involves exchange of gases between the cell and its surrounding and between organisms and its surrounding.
- **Diffusion:** The movement of atoms or molecules from an area of higher concentration to an area of lower concentration.
- **Haemoglobin A (HbA):** It is type of haemoglobin which present in cell of adult human.
- **Haemoglobin F (HbF):** It is a type of haemoglobin present in foetal cell of human.
- **Oxyhaemoglobin:** It is formed by combination of oxygen with haemoglobin pigments.
- **Respiratory Quotient (RQ):** The ratio of the volume of carbon dioxide produced to the volume of oxygen consumed in respiration over a period of time is called Respiratory quotient.

- **Floating Respiration:** In this, respiratory substrates are carbohydrate and fat.
- **Photoplasmic respiration:** If respiratory substances are protein then this type of respiration is called as protoplasmic respiration.
- **Breathing:** To inhale and Exhale air using lungs.
- **Inspiration:** This is active phase of breathing in mammals.
- **Expiration:** This is passive phase of breathing in mammals.
- **Glycolysis:** It is common pathway of aerobic as well as anaerobic respiration.
- **Electron transport Chain (ETC):** It is a series of enzymes and cytochromes in the inner mitochondrial membrane that take part in the passage of electron from a substance to its ultimate acceptor.
- **Oxidative Phosphorylation:** The synthesis of energy rich ATP molecules with the help of energy liberated by oxidation of reduced coenzymes (NADH<sub>2</sub> and FADH<sub>2</sub>).
- **Adenosine triphosphate (ATP):** This is known as energy currency due to presence of two high energy phosphate bonds.
- **Flavin adenine dinucleotide (FAD):** It is coenzyme which can reduce by hydrogen.
- **Nicotinamide adenine dinucleotide (NAD):** It is reduced form of coenzyme formed during respiration.

## 6.9 Self learning Exercise

### Section- A (very short answer type)

1. Oxygen dissociation curve is .....
2. Maximum carbon dioxide transported in form of .....in human.
3. The main principle on which exchange of gases is based is.....
4. The main aim of chloride shift is .....

5. After taking a long deep breath we do not respire for some second due to.....
6. Which part of human brain controls the breathing movement?
7. A muscular transverse partition in mammals that separate thorax from abdomen is called.....
8. Lungs ventilation movement is due to.....

**Section- B (Short answer type)**

1. What do you know about bohr's effect?
2. Explain glycolysis briefly?
3. Differentiate between substrate level phosphorylation and oxidative phosphorylation.
4. Explain inspiration?
5. How oxygen is transported in human body?
6. Where is the pneumotaxic centre is located in human? What is its significance in breathing?
7. Significance of TCA cycle?
8. Write short note on respiratory quotient (RQ)?
9. Write down nervous control of respiration?
10. What are the effects of carbon monoxide in respiration?

**Section-C (long answer type)**

1. What is breathing? Write mechanism of breathing in detail.
2. Explain aerobic respiration in detail.
3. Describe electron transport system in detail.
4. How are respiratory gases transported by blood in man?
5. Explain lung volumes and capacities in detail.
6. Describe oxygen dissociation curve in detail?

**Answer key of Section-A**

1. Sigmoid
2. Bicarbonate ion
3. Diffusion
4. Acid-base equilibrium

5. Less CO<sub>2</sub> in blood
  6. Medulla oblongata
  7. Diaphragm
  8. Coastal muscle and diaphragm
- 

## **6.10 References**

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- Animal Physiology by Marshall
- Mammals Physiology by Richard Hill
- Cell and Molecular Biology by Karp
- Animal Physiology by Berry

# Unit -7

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## Stress Physiology

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### Structure of the Unit

#### 7.1 What is stress?

Abiotic stress/ Environmental stress

Biological stress and strain

Stress v/s strain

Elastic strain

Plastic strain

#### 7.2 Resistance

Stress resistance

Elastic resistance

Plastic resistance

Zero Stress

Avoidance

Tolerance

Adaptations

#### 7.3 Signs and symptoms of stress overload in human being

Cognitive symptoms

Emotional symptoms

Physical symptoms

Behavioral symptoms

#### 7.4 The Body's Stress Response

Coping with stress

Emotion-focused

Problem-focused

Avoidance

Eustress

- Distress
- Acute stressors
- Chronic stressors
- Alarm
- Shock phase
- Antishock phase
- Resistance
- Recovery
- Exhaustion
- 7.5 Glands associated with stress
- 7.6 Hormones associated with stress
- 7.7 Hypoxia
- 7.8 Homeostasis
- 7.9 Negative feedback
- 7.10 Allostasis
- 7.11 Allostasis v/s homeostasis
- 7.12 Acclimatization
- 7.13 Self Learning Exercise
- 7.14 References

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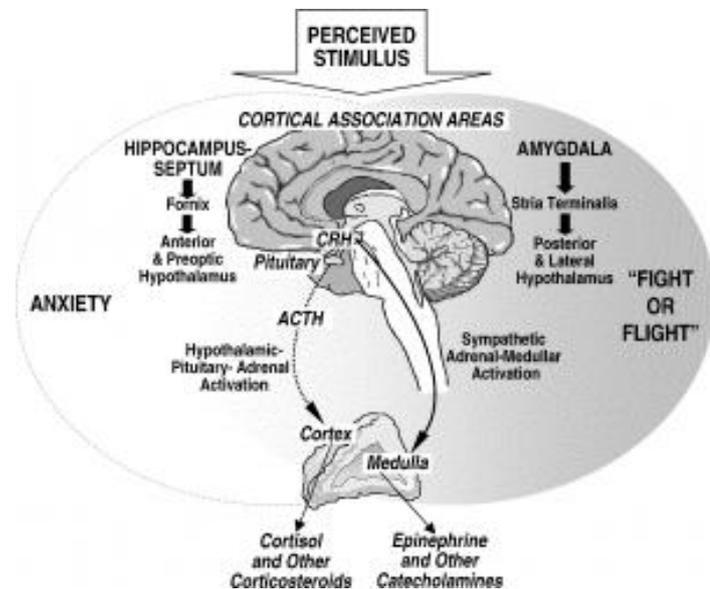
## **7.1 What is stress?**

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The term "stress" derived from the Latin stringere, "to draw tight." Stress in physics is any force applied to an object. Stress in biology is any change in environmental conditions that might bring about a change in organism's behaviour. These conditions include freeze, chill, heat, drought, flood, salty, pest and air pollution etc. Physiologists define stress as how the body reacts to a stressor.

The stress response is the body's way of protecting you and is an automatic process known as the "fight-or-flight-or-freeze" reaction, or the stress response. Upon immediate disruption of either psychological or physical equilibrium the body responds by stimulating the nervous, endocrine and immune systems. The

reaction of these systems causes a number of physical changes that have both short and long term effects on the body.



### A. Abiotic stress/ Environmental stress

The occurrence of unfavorable environmental factors such as moisture deficit / excess, high radiation, low and high temperature, salinity of water and soil, nutrient deficiency or toxicity and pollution of atmosphere, soil and water are likely to affect the crop growth in terms of morphology (plant size, architecture, malformation of plant organs, growth (height, volume, weight), physiological and metabolic processes and yield of crop plants.

Environmental stress can be grouped into the following categories:

1. Physical stress refers to brief but intense exposures to altered temperature, rainfall, light, depth, radiations etc.

#### Example

Thermoregulatory mechanisms and adaptations to maintain an optimum temperature in vertebrates include:

- Thermal migration: During noon, the desert animals move to shady places, burrowing animals escape excessive heat or cold by going deeper into the soil, many birds migrate seasonally to compensate environmental temperature.

- Basking: Various poikilotherms or ectothermic animals like lizards make use of solar energy and some others make use of metabolic heat to raise their body temperature.
  - Aestivation or summer sleep: Aestivation is a condition in which the cold blooded animals maintain their body temperature by reducing metabolic activities and protect them selves from scorching heat/high temperature during summer, so it's also known as summer sleep.
  - Hibernation or winter sleep: This is a state of dormancy occurring during winter to escape from excessive cold and is also called winter sleep.
  - Formation of antifreeze substances: To survive in very cold climates, the antarctic ice fish contains a glycoprotein which prevents ice formation in body fluid.
  - Sub-cutaneous fat: Deposition of fat is particularly advantageous for a homeotherm in a cold climate.
  - Increased metabolism, acceleration of thyroid activity, vaso-constriction, vaso-dilation, sweating, panting etc. are some other important physiological responses to combat temperature stress.
  - Diapause is the stage of development in which larvae stop their development in unfavourable condition (like very high/low temp., and also scarcity of food) and when they get favourable condition they again start development where they stop. It's only found in those animals which developed by process of metamorphosis.
2. Change in salinity, oxygen concentration etc. result in what is termed as chemical stress.

### **Example**

Osmoregulation is a process which enables the animals to maintain a suitable internal medium by regulating the movement of water and salts between the body fluids and the external medium. The mechanism includes:

- Removal of excess salts: When the marine forms drink sea water to compensate their water loss, the salt increases and the excess salt is to be removed. The marine fishes have chloride secretory cells in

their gills which secrete out the excess of salts present in the body fluid. Marine turtles have salt glands for the same purpose.

- Removal of excess water: In case of fresh water forms, endosmosis takes place and animals like *Amoeba* removes the excess water by contractile vacuole, fishes by glomerular kidney etc.

## **B. Biological stress and strain**

Biological stresses differ from mechanical stresses. Biological stresses are associated with the diverse interactions that occur among organisms of the same or different species. Biological stresses can result from competition, herbivory, predation, parasitism, and disease. The organisms are able to erect barriers between the body and environmental stress by expending energy. Biological stresses always cause certain amount of injury which is irreversible and is a plastic strain. Hence, a biological stress is defined as any environmental factor capable of inducing a potentially injurious strain in living organisms. The living organism may show physical strain e.g. cessation of cytoplasmic streaming or a chemical strain e.g. a shift in metabolism. If the strain is severe, the organism may suffer a permanent set i.e. injury or death.

### **Stress v/s strain**

Any environmental factor potentially unfavorable to an organism is termed as stress. The effect of stress on organism's condition is called strain. According to Newton's law of motion, a force is always accompanied by a counterforce, for an action there is always equal and opposite reaction. Stress is the action and whereas strain is the reaction. A body of an organism subjected to stress is in a state of strain.

Strain may be elastic or plastic strain:

### **Elastic strain**

Up to a point, a strain may be completely reversible and when the stress is relieved, the organism becomes normal.

### **Plastic strain**

Beyond the point of elastic strain, the strain may be partially reversible or partially irreversible, which is called plastic strain or permanent set.

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## **7.2 Resistance**

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Resistance is the ability to adapt or to tolerate stress.

### **Stress resistance**

Ability of the organisms to survive under adverse environmental condition is termed as stress resistance. The stress resistance of biological organisms is of two main types.

#### **Elastic resistance**

Ability of the organism to prevent reversible or elastic strain (physical or chemical change) when exposed to a specific stress.

#### **Plastic resistance**

Plastic resistance is the ability of an organism to prevent irreversible or plastic strain. The term resistance to environmental stress has been used for plastic resistance and elastic resistance is not clearly recognized.

#### **Zero Stress**

This is the level of exposure of organism to environmental factors that leads to neither injury nor reduction in growth and yield of crops.

The animals usually respond in two major ways:

**Avoidance:** For eg., movement, migration, hibernation, aestivation etc.

**Tolerance:** For eg., adapting to the prevailing conditions including desert adaptations, cold adaptations etc.

#### **Example**

Lethal freezing occurs when insects are exposed to temperatures below the melting point (MP) of their body fluids; therefore, insects that do not migrate from regions with the onset of colder temperatures must devise strategies to either tolerate or avoid freezing of intracellular and extracellular body fluids. Surviving colder temperatures, in insects, generally falls under two categories: Freeze-tolerant insects can tolerate the formation of internal ice and freeze-avoidant insects avoid freezing by keeping the bodily fluids liquid. The general strategy adopted by insects also differs between the northern hemisphere and the southern hemisphere. In temperate regions of the northern hemisphere where cold temperatures are expected seasonally and are usually for long periods of time, the main strategy is freeze avoidance. In temperate regions of the southern hemisphere, where seasonal cold temperatures are not as extreme or long lasting, the main strategy is freeze tolerance. However, in the Arctic, where freezing occurs seasonally, and for extended periods (>9 months), freeze tolerance also predominates.

## Freeze avoidance

Freeze avoidance involves both physiological and biochemical mechanisms. One method of freeze avoidance is the selection of a dry hibernation site in which no ice nucleation from an external source can occur. Insects may also have a physical barrier such as a wax-coated cuticle that provides protection against external ice across the cuticle. The stage of development at which an insect over-winters varies across species, but can occur at any point of the life cycle (i.e., egg, pupa, larva, and adult).

Freeze-avoidant insects that cannot tolerate the formation of ice within their bodily fluids need to implement strategies to depress the temperature at which their bodily fluids will freeze. Super cooling is the process by which water cools below its freezing point without changing phase into a solid, due to the lack of a nucleation source. Water requires a particle such as dust in order to crystallize and if no source of nucleation is introduced, water can cool down to  $-42^{\circ}\text{C}$  without freezing. In the initial phase of seasonal cold hardening, ice-nucleating agents (INAs) such as food particles, dust particles and bacteria, in the gut or intracellular compartments of freeze avoidant insects have to be removed or inactivated. Removal of ice-nucleating material from the gut can be achieved by cessation in feeding, clearing the gut and removing lipoprotein ice nucleators from the haemolymph. Examples include the over wintering in lesser stag beetle larva, and in some species, by the shedding of the mid-gut during moulting.

In addition to physical preparations for winter, many insects also alter their biochemistry and metabolism. For example, some insects synthesize cryoprotectants such as polyols and sugars, which reduce the lethal freezing temperature of the body. Although polyols such as sorbitol, mannitol, and ethylene glycol can also be found, glycerol is by far the most common cryoprotectant and can be equivalent to  $\sim 20\%$  of the total body mass. Glycerol is distributed uniformly throughout the head, the thorax, and the abdomen of insects, and is in equal concentration in intracellular and extracellular compartments. The depressive effect of glycerol on the super cooling point (SCP) is thought to be due to the high viscosity of glycerol solutions at low temperatures. This would inhibit INA activity and SCPs would drop far below the environmental temperature. At colder temperatures (below  $0^{\circ}\text{C}$ ), glycogen production is inhibited, and the breakdown of glycogen into glycerol is enhanced, resulting in the glycerol levels in freeze avoidant insects reaching

levels five times higher than those in freeze tolerant insects which do not need to cope with extended periods of cold temperatures.

Though not all freeze avoidant insects produce polyols, all hibernating insects produce thermal hysteresis factors (THFs). A seasonal photoperiodic timing mechanism is responsible for increasing the antifreeze protein levels with concentrations reaching their highest in the winter. In the pyrochroid beetle, *Dendroides canadensis*, a short photoperiod of 8 hours light and 16 hours of darkness, results in the highest levels of THFs, which corresponds with the shortening of daylight hours associated with winter. These antifreeze proteins are thought to stabilize SCPs by binding directly to the surface structures of the ice crystals themselves, diminishing crystal size and growth. Therefore, instead of acting to change the biochemistry of the bodily fluids as seen with cryoprotectants, THFs act directly with the ice crystals by adsorbing to the developing crystals to inhibit their growth and reduce the chance of lethal freezing occurring.

### **Freeze tolerance**

Freeze tolerance in insects refers to the ability of some insect species to survive ice formation within their tissues. All insects are ectothermic, which can make them vulnerable to freezing. In most animals, intra- and extracellular freezing causes severe tissue damage, resulting in death. Insects that have evolved freeze-tolerance strategies manage to avoid tissue damage by controlling where, when, and to what extent ice forms. In contrast to freeze avoiding insects that are able to exist in cold conditions by supercooling, freeze tolerant organisms limit supercooling and initiate the freezing of their body fluids at relatively high temperatures. Physiologically, this is accomplished through inoculative freezing, the production of ice nucleating proteins, crystalloid compounds, and/or microbes.

Although freeze-avoidance strategies predominate in the insects, freeze tolerance has evolved at least six times within this group (in the Lepidoptera, Blattodea, Diptera, Orthoptera, Coleoptera and Hymenoptera). Freeze tolerance is also more prevalent in insects from the Southern Hemisphere (reported in 85% of species studied) than it is in insects from the Northern Hemisphere (reported in 29% of species studied). It has been suggested that this may be due to the Southern Hemisphere's greater climate variability, where insects must be able to survive sudden cold snaps yet take advantage of unseasonably warm weather as well. This is in contrast to the Northern Hemisphere, where

predictable weather makes it more advantageous to over winter after extensive seasonal cold hardening. Examples of freeze tolerant insects include: the woolly bear, *Pyrrharctia isabella*; the flightless midge, *Belgica antarctica*; and the alpine cockroach, *Celatoblatta quinquemaculata*.

**Adaptation** is permanent resistance to stress in morphology and structure, physiology and biochemistry under long-term stress condition. The deep sea adaptations, the desert adaptations, the cave adaptations all fall into this category.

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### **7.3 Signs and symptoms of stress overload in human beings (Behavioural Stress)**

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The following table lists some of the common warning signs and symptoms of stress. Signs of stress may be cognitive, emotional, physical, or behavioral:

#### **Cognitive symptoms**

Memory problems  
Inability to concentrate  
Poor judgment  
Pessimistic approach or thoughts  
Anxious or racing thoughts  
Constant worrying

#### **Emotional symptoms**

Moodiness  
Irritability or short temper  
Agitation, inability to relax  
Feeling overwhelmed  
Sense of loneliness and isolation  
Depression or general unhappiness

#### **Physical symptoms**

Aches and pains  
Diarrhea or constipation  
Increased frequency of urination  
Indigestion



Changes in blood glucose

Nausea, dizziness

Chest pain, rapid heartbeat

Loss of sex drive

Frequent colds

Irregular periods

### **Behavioral symptoms**

Eating more or less

Sleeping too much or too little

Isolating oneself from others

Procrastinating or neglecting responsibilities

Using alcohol, cigarettes, or drugs to relax

Nervous habits (e.g. nail biting, pacing)

Stress can also result in physical symptoms such as increased adrenaline, muscular tension, stomach pain, and exhaustion. Stress over a long period of time has also been correlated with increased risk for heart disease and cancer.

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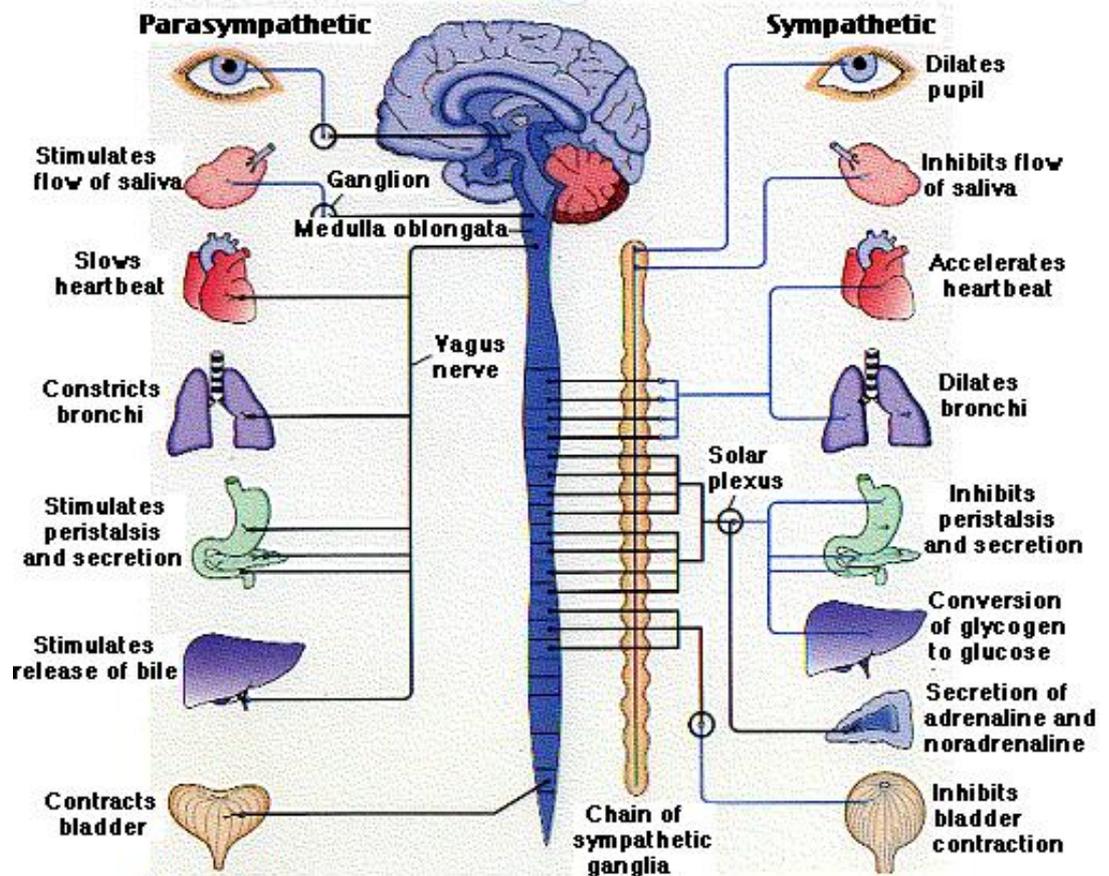
## **7.4 The Body's Stress Response**

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Stress affects the mind, body, and behavior in many ways, and everyone experiences stress differently.

When a threat is perceived, the nervous system responds by releasing a flood of stress hormones, including adrenaline and cortisol. These hormones rouse the body for emergency action.

The heart pounds faster, muscles tighten, blood pressure rises, breath quickens, and the senses become sharper. These physical changes increase the organism's strength and stamina, speed up reaction time, and enhance their focus—preparing them to either fight or flee from the danger at hand.



## Coping with stress

There are three types of coping with stress:

**Emotion-focused:** is a coping style in which the individual tries to diminish his or her negative emotions resulting from stress.

**Problem-focused:** is a coping style in which the individual faces the problem head on, i.e. the person actively takes action to fix or resolve the problem.

**Avoidance:** is avoiding emotions and solutions to problems in hopes that they will both disappear on their own.

Selye coined the word "stress" to refer to inappropriate physical reactions to any demand. He further divided this term into 2 types of stress, eustress and distress.

**Eustress** is good stress, i.e. stress that motivates and improves function.

**Distress** is overwhelming stress that results in anxiety, depression, and the other symptoms mentioned above.

**Acute stressors** affect an organism in the short term while, **chronic stressors** over the longer term.

How organisms respond to a stress is characterized by three phases:

a **nonspecific mobilization phase**, which promotes sympathetic nervous system activity;

a **resistance phase**, during which the organism makes efforts to cope with the threat; and

an **exhaustion phase**, which occurs if the organism fails to overcome the threat and depletes its physiological resources.

**Alarm** is the first stage, which is divided into two phases: the shock phase and the antishock phase.

**Shock phase:** During this phase, the body can endure changes such as hyposmolarity, hypoglycemia, hyponatremia—the stressor effect. This phase resembles Addison's disease. The organism's resistance to the stressor drops temporarily below the normal range and some level of shock (e.g. circulatory shock) may be experienced.

**Antishock phase:** When the threat or stressor is identified or realized, the body starts to respond and is in a state of alarm. During this stage, the locus coeruleus/sympathetic nervous system is activated and catecholamines such as adrenaline are being produced, hence the fight-or-flight response. The result is: increased muscular tonus, increased blood pressure due to peripheral vasoconstriction and tachycardia, and increased glucose in blood. There is also some activation of the hypothalamic-pituitary-adrenal (HPA) axis, producing glucocorticoids (stress-hormone).

**Resistance** is the second stage and increased secretion of glucocorticoids play a major role, intensifying the systemic response—they have lypolytic, catabolic and antianabolic effects: increased glucose, fat and aminoacid/protein concentration in blood. Moreover, they cause lymphocytopenia, eosinopenia, neutrophilia and polycythemia. In high doses, cortisol begins to act as a mineralocorticoid (aldosteron) and brings the body to a state similar to hyperaldosteronism. If the stressor persists, it becomes necessary to attempt some means of coping with the stress. Although the body begins to try to adapt to the strains or demands of the environment, the body cannot keep this up indefinitely, so its resources are gradually depleted.

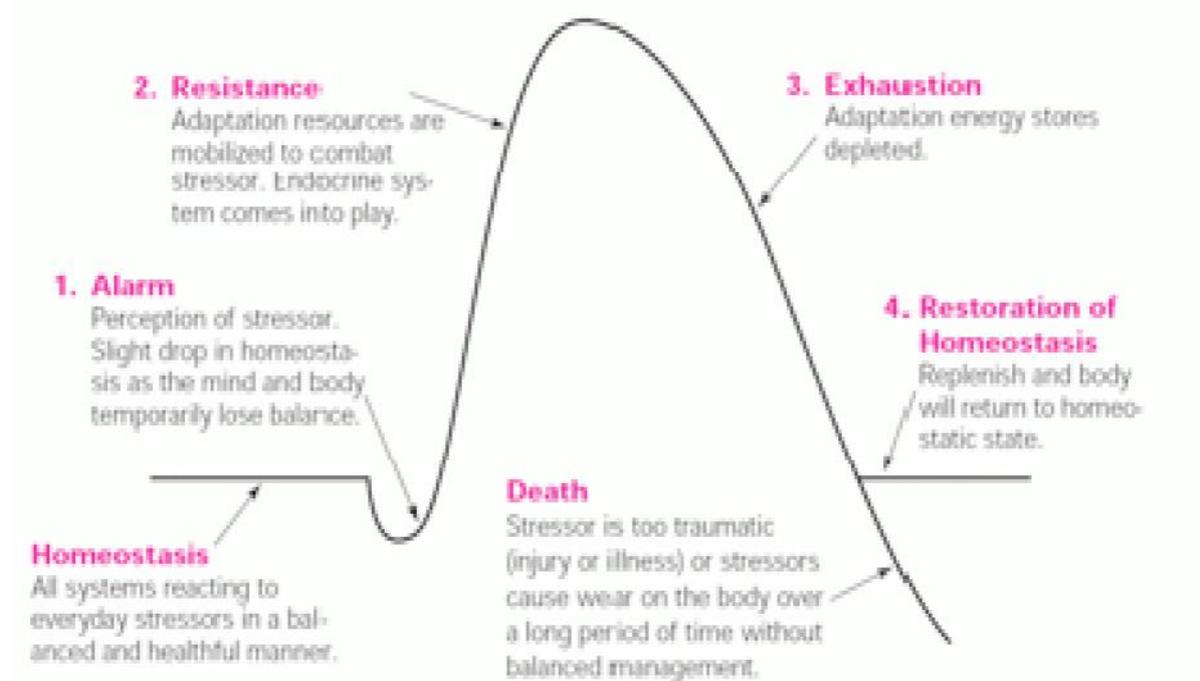
The third stage could be either exhaustion or recovery:

**Recovery** stage follows when the system's compensation mechanisms have successfully overcome the stressor effect (or have completely eliminated the

factor which caused the stress). The high glucose, fat and amino acid levels in blood prove useful for anabolic reactions, restoration of homeostasis and regeneration of cells.

**Exhaustion** is the alternative third stage in the GAS model. At this point, all of the body's resources are eventually depleted and the body is unable to maintain normal function. The initial ANS symptoms may reappear (sweating, raised heart rate, etc.). If stage three is extended, long-term damage may result (prolonged vasoconstriction results in ischemia which in turn leads to cell necrosis), as the body's immune system becomes exhausted, and bodily functions become impaired, resulting in decompensation.

The result can manifest itself in obvious illnesses, such as peptic ulcer and general trouble with the digestive system, diabetes, or even cardiovascular problems, along with clinical depression and other mental illnesses.



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## 7.5 Glands associated with stress

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### Hypothalamus

Often called the seat of emotions is involved with emotional processing. When a thought is perceived as a threat, it secretes a substance called corticotrophin-releasing factor to the Pituitary gland to activate the fight-or-flight response.

### **Pituitary gland**

The pituitary gland is a small organ that is located at the base of the brain just under the hypothalamus. This gland releases various hormones that play significant roles in regulating homeostasis. During a stress response, the pituitary gland releases hormones into the blood stream, namely adrenocorticotrophic hormone, which modulates a heavily regulated stress response system.

### **Adrenal gland**

The adrenal gland is a major organ of the endocrine system that is located directly on top of the kidneys and is chiefly responsible for the synthesis of stress hormones that are released into the blood stream during a stress response. Cortisol is the major stress hormone released by the adrenal gland.

In addition to the locus coeruleus existing as a source of the neurotransmitter norepinephrine within the central nervous system, the adrenal gland can also release norepinephrine during a stress response into the body's blood stream, at which point norepinephrine acts as a hormone in the endocrine system.

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## **7.6 Hormones associated with stress**

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### **Corticotropin-releasing hormone**

Corticotropin-releasing hormone is the neurohormone secreted by the hypothalamus during a stress response that stimulates the anterior lobe of the pituitary gland by binding to its corticotropin-releasing hormone-receptors, causing the anterior pituitary to release adrenocorticotrophic hormone.

### **Adrenocorticotrophic hormone**

Adrenocorticotrophic hormone is the hormone secreted by the anterior lobe of the pituitary gland into the body's blood stream that stimulates the cortex of the adrenal gland by binding to its adrenocorticotrophic hormone-receptors, thus causing the adrenal gland to release cortisol.

### **Cortisol**

Cortisol is a steroid hormone, belonging to a broader class of steroids called glucocorticoids, produced by the adrenal gland and secreted during a stress response. Its primary function is to redistribute energy (glucose) to regions of the body that need it most (i.e., the brain and major muscles during a fight-or-flight situation). As a part of the body's fight-or-flight response, cortisol also acts to suppress the body's immune system.

Cortisol is synthesized from cholesterol in the adrenal cortex. Its primary function is to increase blood sugar through gluconeogenesis, suppress the immune system and aid in fat and protein metabolism.

### **Norepinephrine**

Norepinephrine is a neurotransmitter released from locus coeruleus when stimulated by the hypothalamus during a stress response. Norepinephrine serves as the primary chemical messenger of the central nervous system's sympathetic branch that prepares the body for fight-or-flight response.

### **Serotonin**

Serotonin is a neurotransmitter synthesized in the raphe nucleus of the pons of the brainstem and projects to most brain areas. Serotonin is thought to play an important role in mood regulation. Stress-induced serotonin dysfunctions have been associated with anxiety, fear and depression-like symptoms.

### **Neuropeptide Y**

Neuropeptide Y is a protein that is synthesized in the hypothalamus and acts as a chemical messenger in the brain. Traditionally, it has been thought to play an important role in appetite, feeding behaviour, and satiety, but more recent findings have implicated Neuropeptide Y in anxiety and stress, specifically, stress resiliency.

### **Endocrine system**

When a stressor acts upon the body, the endocrine system is triggered by the release of the neurotransmitter noradrenaline by the ANS. Noradrenaline stimulates the hypothalamic-pituitary-adrenal axis (HPA) which processes the information about the stressor in the hypothalamus. This quickly signals the pituitary gland and finally triggers the adrenal cortex. The adrenal cortex responds by signaling the release of the corticosteroids cortisol and corticotropin releasing hormone (CRH) directly into the bloodstream.

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## **7.7 Hypoxia**

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Hypoxia (also known as Hypoxiation or Anoxemia) is a condition in which the body or a region of the body is deprived of adequate oxygen supply. Hypoxia may be classified as either generalized, affecting the whole body, or local, affecting a region of the body. Although hypoxia is often a pathological condition, variations in arterial oxygen concentrations can be part of the normal

physiology, for example, during hypoventilation training or strenuous physical exercise.

Hypoxia differs from hypoxemia in that hypoxia refers to a state in which oxygen supply is insufficient, whereas hypoxemia refers specifically to states that have low arterial oxygen supply. Hypoxia in which there is complete deprivation of oxygen supply is referred to as "anoxia".

Generalized hypoxia occurs in healthy people when they ascend to high altitude, where it causes altitude sickness leading to potentially fatal complications. Hypoxia also occurs in healthy individuals when breathing mixtures of gasses with low oxygen content, e.g. while diving underwater.

Hypoxia is also a serious consequence of preterm birth in the neonate. The main cause for this is that the lungs of the human fetus are among the last organs to develop during pregnancy.

Hypoxia can result from a failure at any stage in the delivery of oxygen to cells. This can include decreased partial pressures of oxygen, problems with diffusion of oxygen in the lungs, insufficient available hemoglobin, problems with blood flow to the end tissue, and problems with breathing rhythm.

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## **7.8 Homeostasis**

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Homeostasis is a concept central to the idea of stress. In biology, most biochemical processes strive to maintain equilibrium (homeostasis), a steady state that exists more as an ideal and less as an achievable condition. Environmental factors, internal or external stimuli, continually disrupt homeostasis; an organism's present condition is a state of constant flux moving about a homeostatic point that is that organism's optimal condition for living. Factors causing an organism's condition to diverge too far from homeostasis can be experienced as stress. A life-threatening situation such as a major physical trauma or prolonged starvation can greatly disrupt homeostasis. On the other hand, an organism's attempt at restoring conditions back to or near homeostasis, often consuming energy and natural resources, can also be interpreted as stress. In such instances, an organism's fight-or-flight response recruits the body's energy stores and focuses attention to overcome the challenge at hand.

Examples of homeostasis include the regulation of temperature and the balance between acidity and alkalinity (pH). It is a process that maintains the stability of

the human body's internal environment in response to changes in external conditions.

The concept was described by Claude Bernard in 1865 and the word was coined by Walter Bradford Cannon in 1926. Homeostasis requires a sensor to detect changes in the condition to be regulated, an effector mechanism that can vary that condition; and a negative feedback connection between the two.

All living organisms depend on maintaining a complex set of interacting metabolic chemical reactions. From the simplest unicellular organisms to the most complex plants and animals, internal processes operate to keep the conditions within tight limits to allow these reactions to proceed. Homeostatic processes act at the level of the cell, the tissue, and the organ, as well as for the organism as a whole.

Principal Homeostatic processes include the following:

"Warm-blooded" animals (endothermic mammals and birds) maintain a constant body temperature, whereas ectothermic animals (almost all other animals) exhibit wide body temperature variation. An advantage of temperature regulation is that it allows an organism to function effectively in a broad range of environmental conditions. For example, ectotherms tend to become sluggish at low temperatures, whereas a co-located endotherm may be fully active. That thermal stability comes at a price, since an automatic regulation system requires additional energy. If the temperature rises, the body loses heat by sweating or panting, via the latent heat of evaporation. If it falls, this is counteracted by increased metabolic action, by shivering, and—in fur- or feather-coated creatures—by thickening the coat.

- Regulation of the pH of the blood at 7.365 (a measure of alkalinity and acidity).
- All animals also regulate their blood glucose concentration. Mammals regulate their blood glucose with insulin and glucagon.
- The kidneys are used to remove excess water and ions from the blood. These are then expelled as urine. The kidneys perform a vital role in homeostatic regulation in mammals, removing excess water, salt, and urea from the blood.
- If the water content of the blood and lymph fluid falls, it is restored in the first instance by extracting water from the cells. The throat and

mouth become dry, so that the symptoms of thirst motivate the animal to drink.

- If the oxygen content of the blood falls, or the carbon-dioxide concentration increases, blood flow is increased by more vigorous heart action and the speed and depth of breathing increases.
- Sleep timing depends upon a balance between homeostatic sleep propensity, the need for sleep as a function of the amount of time elapsed since the last adequate sleep episode, and circadian rhythms that determine the ideal timing of a correctly structured and restorative sleep episode.
- Personality traits are often conceptualized as a person specific set point level around which mood states fluctuate in time.

### **Control mechanisms**

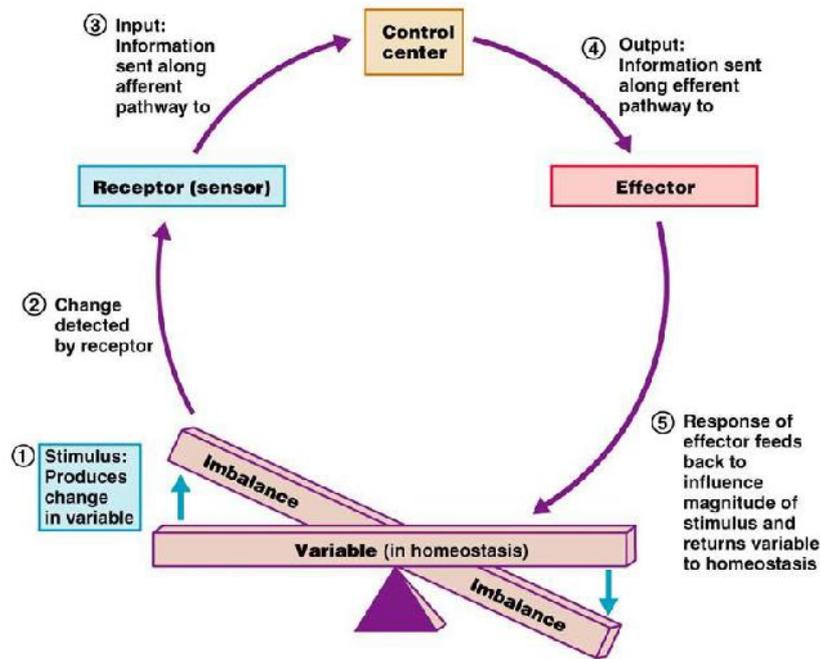
All homeostatic control mechanisms have at least three interdependent components for the variable being regulated: The receptor is the sensing component that monitors and responds to changes in the environment. When the receptor senses a stimulus, it sends information to a "control center", the component that sets the range at which a variable is maintained. The control center determines an appropriate response to the stimulus. The control center then sends signals to an effector, which can be muscles, organs, or other structures that receive signals from the control center. After receiving the signal, a change occurs to correct the deviation by depressing it with negative feedback.

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## **7.9 Negative feedback**

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Negative feedback mechanisms consist of reducing the output or activity of any organ or system back to its normal range of functioning. A good example of this is regulating blood pressure. Blood vessels can sense resistance of blood flow against the walls when blood pressure increases. The blood vessels act as the receptors and they relay this message to the brain. The brain then sends a message to the heart and blood vessels, both of which are the effectors. The heart rate would decrease as the blood vessels increase in diameter (known as vasodilation). This change would cause the blood pressure to fall back to its normal range. The opposite would happen when blood pressure decreases, and would cause vasoconstriction.




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## 7.10 Allostasis

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**Allostasis** is the process of achieving stability, or homeostasis, through physiological or behavioral change. It means "variable;" thus, "remaining stable by being variable". This can be carried out by means of alteration in HPA axis hormones, the autonomic nervous system, cytokines, or a number of other systems, and is generally adaptive in the short term. Allostasis is essential in order to maintain internal viability amid changing conditions.

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## 7.11 Allostasis v/s homeostasis

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Homeostasis is the regulation of the body to a balance, by single point tuning such as blood oxygen level, blood glucose or blood pH. For example, if a person walking in the desert is hot, the body will sweat and they will quickly become dehydrated. Allostasis is adaptation but in regard to a more dynamic balance. In dehydration, sweat occurs as only a small part of the process with many other systems also adapting their functioning, both to reduce water use and to support the variety of other systems that are changing to aid this. In this case, kidneys may reduce urine output, mucous membrane in the mouth, nose and eyes may dry out; urine and sweat output will decrease; the release of arginine vasopressin (AVP) will increase; and veins and arteries will constrict to maintain blood pressure with a smaller blood volume.

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## 7.12 Acclimatization

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Acclimatization is the process in which an individual organism adjusts to a gradual change in its environment (such as a change in temperature, humidity, photoperiod, or pH), allowing it to maintain performance across a range of environmental conditions. Acclimation occurs in a short period of time (days to weeks), and within the organism's lifetime (compare to adaptation). This may be a discrete occurrence or may instead represent part of a periodic cycle, such as a mammal shedding heavy winter fur in favor of a lighter summer coat. Organisms can adjust their morphological, behavioral, physical, and/or biochemical traits in response to changes in their environment. While the capacity to acclimate to novel environments has been well documented in thousands of species, researchers still know very little about how and why organisms acclimate the way that they do. When used as a technical term (such as in the study of physiology), acclimatization refers to a natural process (e.g., shedding heavy winter fur with natural seasonal change), whereas the term acclimation is reserved for changes occurring in response to an artificial or controlled situation, such as changes in temperature imposed in an experimental manipulation.

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## 7.13 Self Learning Exercise

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### Section A (Very Short Answer Type)

1. What do you understand by the term “stress”?
2. What is biological strain.

### Section B ( Short Answer Type)

1. Describe abiotic stress/ environmental stress.
2. Differentiate stress and strain.
3. Define elastic and plastic strains.
4. Give an account of stress resistance.
5. What do you understand by the term “Zero Stress”?
6. Differentiate eustress and distress.
7. Discuss stressors.
8. Describe hypoxia.
9. How is allostasis different from homeostasis?

### Section C (Long Answer Type)

1. Correlate the terms avoidance, tolerance and adaptation and discuss in details the phenomenon.
2. Describe the signs and symptoms of stress overload in human being.
3. How does the body cope up with stress? Discuss.
4. Describe the phases associated with how organisms respond to a stress.
5. Give an account of glands associated with stress.
6. Discuss the hormones associated with stress.
7. Explain homeostasis and the regulation mechanism associated with it.
8. Explain the phenomenon of acclimatization.

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## Unit-8

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# **Innate and acquired Immunity; phylogeny and ontogeny of immune system, organization and structure of lymphoid organs, cells of the immune system and their differentiation**

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### **Structure of Unit**

- 8.0 Objectives
  - 8.1 Introduction
  - 8.2 Types of immunity
  - 8.3 Phylogeny and ontogeny of immune system
  - 8.4 Organization and structure of lymphoid organs
  - 8.5 Cells of the immune system and their differentiation
  - 8.6 Summary
  - 8.7 Glossary
  - 8.8 Self Learning Exercise
  - 8.9 References
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### **8.0 Objectives**

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After studying this unit, you should be able to understand:

- Innate and acquired immunity and barriers against infection.
- Phagocytosis and inflammation
- Active and passive immunity.
- Phylogeny and ontogeny of immune system.
- Various lymphoid organs of body.
- Different types of immune cells.

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## 8.1 Introduction

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The life of every organism is constantly threatened by other organism, which is the nature of the living world. To defend itself, each species has evolved a variety of protective mechanisms. Immunity is the resistance exhibited by the host towards the possible attack of an infectious agent or a disease producing organism. For their continual battle with microorganisms, vertebrates have developed a very elaborate set of protective measure, called the immune system. The resistance acquired by an individual through this system is called immunity.

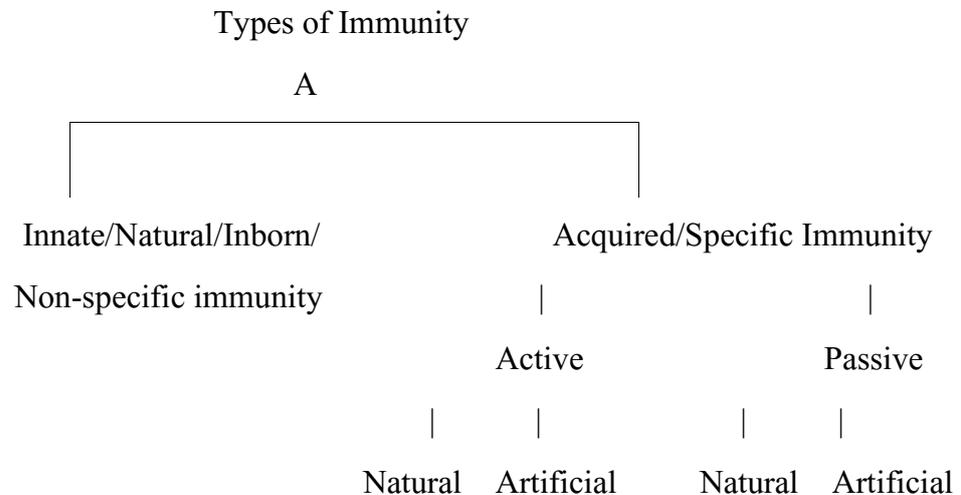
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## 8.2 Types of immunity

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The human body provides two levels of protection from infectious diseases –

- (a) Non-specific resistance or innate immunity
- (b) Specific resistance or adaptive immunity.



### 8.2.1 Innate immunity

Innate immunity of an animal is also known as natural immunity, native immunity or inherited immunity (Latin: innasci- to be born in). It is a general or non-specific defence mechanism of resistance, which prevents the body by different types of pathogens. This type of immunity is developed in an animal by virtue of its genetic and constitutional make up. This type of resistance is

active right from the time a child is born (hence innate). It is totally independent of any previous contact with micro-organism. The specificity of innate immunity is relatively low as it lacks ability to differentiate one microbe from another.

The innate immune responses do not involve antibodies and do not improve or increase with repeated infection with same pathogen. Innate immunity provides the early line of defence while adaptive immunity occurs after 5 to 6 days of antigen exposure.

The extent of innate immunity may be different in different organisms. For example, man can easily get mumps while cats and dogs are immune to this disease. This level of natural immunity may vary between species, races or individual. Species immunity refers to that immune power which is shown by all members of a species. For instance, all human beings are totally insusceptible to plant pathogen and to many pathogens of animals such as rinderpest etc. Such immunities are believed to be based on physiological and anatomical differences. Racial immunity is those that exist among various races and people of the world. The classic example of this is the high resistance of Algerian sheep to anthrax. These racial differences are genetic in origin. The difference in innate immunity exhibited by different individuals in a race is known as individual immunity. The genetic basis of this type of immunity is evident from studies of twins. The homozygous twins have similar type of response to lepromatous leprosy and tuberculosis, which is not seen in heterozygous twins. The individual immunity may also be influenced by age, nutrition, hormones and many other factors.

The important aspects of innate immunity would include:

- a. Mechanical barriers
- b. Chemical barriers
- c. Phagocytosis
- d. Fever
- e. Inflammation

**Mechanical barriers:**

The mechanical or physical barriers such as skin and mucous membrane act as a first line of defence against infection. (Fig.8.1) These physical hindrances blocking the entry of pathogens into the host body.

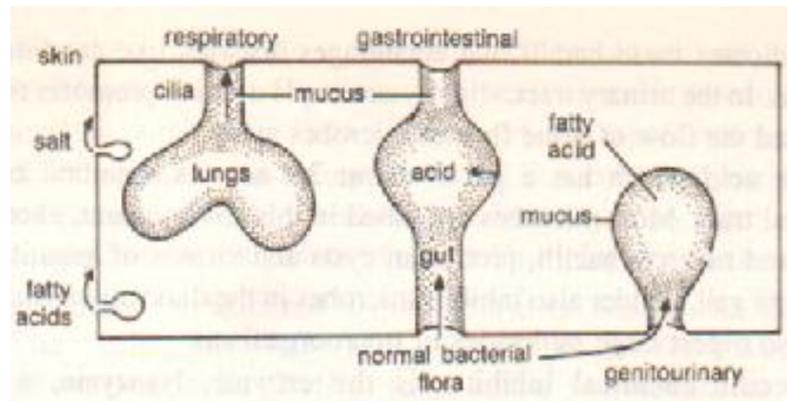


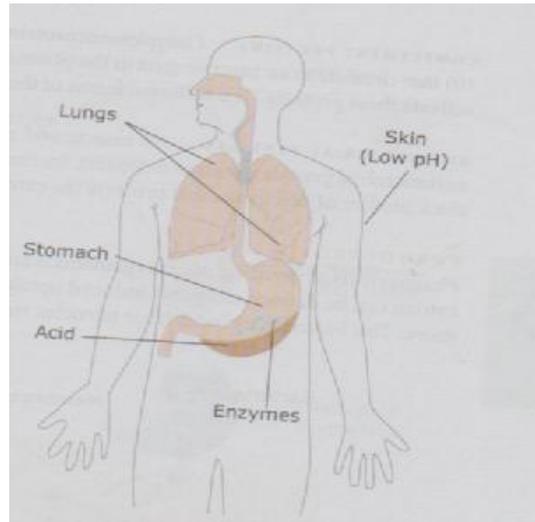
Fig 8.1 – Mechanical barriers ( first line of defence )

The intact skin is impermeable to most infectious agents. The outer keratinous covering of skin does not support viral replication or bacterial penetration. Any break in the integrity of skin facilitates the entry of pathogens. Moreover, the epidermis of the skin is constantly shed off, resulting in the continual removal of any developing pathogens

Various regions of the body which are not protected by intact skin generally lined by mucous-membrane. Several features of mucous membrane provide resistance to parasites. In the respiratory tract, goblet cells secrete mucus which traps heavy particles and microbes in the air. Ciliated epithelial cells move the particles alongwith mucous upto the throat, thereby cleaning foreign material from the respiratory tract. The mucous membrane of the gastrointestinal tract, the urinogenital tract and the conjunctiva also offers the same protection. Some important mechanical factors are also involved in protecting the mucous membrane such as the physiological fluids like tears and saliva, the trapping action of hairs in nasal chamber, the expulsive effects of coughing and sneezing, the cool temperature of the upper respiratory tract etc.

### **Chemical Barriers :**

In addition to mechanical barriers the host body also have several chemical barriers that contribute to innate immunity.(Fig.8.2)



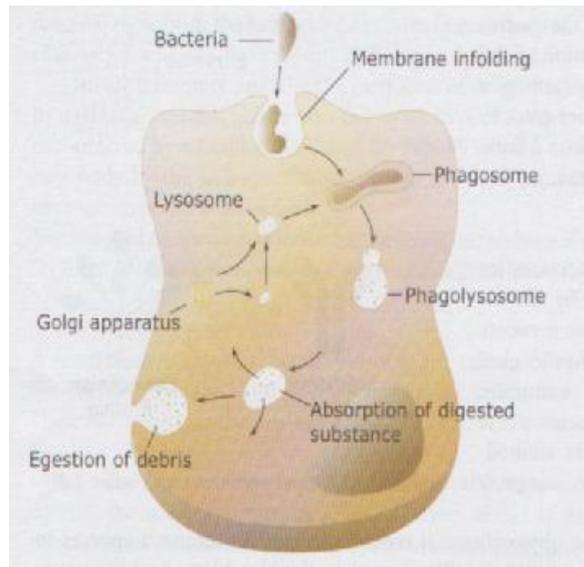
**Fig 8.2- Chemical barriers of body**

Acidic gastric secretions, low pH of the skin, presence of lysozyme in all mucous secretion, a large array of gastric and duodenal enzymes, antibodies and inhibitors, interferons, complement proteins and anti microbial peptide collectively involved in remarkable defence mechanisms to pathogenic invasion.

**Phagocytosis :**

Phagocytosis is a non-specific immune response that destroys invading micro organisms. Phagocytic cells, originally discovered by Metchnikoff (1833) were classified by him into microphages and macrophages. Both macrophages and microphages (also called neutrophils) are very rich in degenerative proteolytic enzymes. Both the types of phagocytic cells are generated by common hematopoietic myeloid stem cells like other blood component. These cells play effective role in Phagocytosis.

When a phagocyte comes into contact with a micro-organism, it engulfs the organism to form a membrane-bound structure called the phagosome. This fuses with lysosomes to form a phagolysosome. The release of lysosomal enzymes degrades the bacteria. The useful products are absorbed back into the cells while the waste is egested out from the cell. (Fig 8.3)



**Fig 8.3- Phagocytosis**

**Fever :**

Fever is a natural defence mechanism and helps not only to stimulate physiological processes but sometimes, actually destroy the infecting pathogens. It has been believed that pathogens affect hypothalamus thermo regulatory centre and stimulate it to raise the body temperature. Due to this, cell metabolism increases. Substances, that act upon the hypothalamus, to increase the body temperature are known as pyrogens. Fever is beneficial to the host because it inhibits the growth of temperature sensitive pathogens. Increased cell metabolism encourages rapid tissue repair and phagocytosis .

**Inflammation:**

Inflammation is a non-specific defence response by the body to either an injury or an infection in the tissue. It may be characterized by heat (calor), redness (rubor), swelling (tumor) and pain (dolor). The injury or infection could be caused by mechanical agents like cut or pin prick, chemical agents as an acid or a bee venom, physical agents as heat or UV rays and/or infectious agent such as parasites.

The process of inflammation is stimulated by a variety of tissue products such as histamine, bradykinin etc. The mediators released by damaged cells, chemicals released by invading micro-organism, products of the complement system and reaction products of the blood clotting system also trigger the process of inflammation. The injury on infection initiates a process which limits the extent of the injury. The arterioles at the site constrict initially and then

dilate leading to an increased blood flow. Dilation of blood vessels leads to increased capillary permeability, a flow of plasma into the tissue and fluid accumulation at the site of irritation. Microphages attach to the vessels close to the injury and migrate through the wall to begin phagocytosis. After some time macrophages replaces the microphages.

A product of phagocytosis is a mixture of plasma, dead tissue cells, leukocytes and bacteria collectively called pus. When this material becomes enclosed in a wall of fibrin, through clotting mechanism, a sac may form. This sac is the abscess or boils. When several abscesses accumulates, and enlarged structure is formed which is known as carbuncle. Thus, inflammation and phagocytosis are inter related processes. The main aim of these processes is to confine the injury or infection to the site of entry and to repair or replace the injured tissue.

### 8.2.2 Acquired Immunity

The resistance that an individual acquires during the life time against a specific pathogen is known as “acquired immunity”. It is also called adaptive immunity and/or specific immunity. It is more developed and specific defence mechanism. It can differentiate between a variety of different antigens, invading pathogens and self-antigens and induce different types of immune response. Adaptive and innate immunity cooperate to produce more effective defence mechanism against infection. Both the types of immunity is totally independent to each other. Some important character of the innate and acquired immunity are listed in table 1.

**TABLE - 1 : Innate and Adaptive Immunity**

<b>Characteristics</b>	<b>Innate Immunity</b>	<b>Adaptive Immunity</b>
Specificity	Broad	Highly specific
Diversity	Limited	Large
Immunogenic memory	None	Present
Recognition	Recognition of conserved molecular patterns	Recognition of specific antigenic determinants
Self-Foreign discrimination	Present	Present

Response	Rapid (minutes)	Delayed (usually days)
Components	Mechanical and chemical barriers, phagocytes, natural killer cells, complement, acute-phase proteins,	Antibodies, T and B lymphocytes, antigen-presenting cells

The most efficient acquired immunity is generally based on antibodies. It may be of two types –

- (1) Active acquired immunity
- (2) Passive acquired immunity

**(1) Active acquired immunity:**

Active immunity is that type of resistance which is developed by an individual as a result of antigenic stimulus. It involves active functioning of host's immune system to produce antibodies and/or immunologically active cells. This type of immunity have a latent period, which is required for activation of host's immune system. Once developed, the active immunity is long lasting. If an individual actively immunised against an antigen, experiences the same antigen subsequently, the immune response occurs more quickly and effectively. This is known as secondary response. It is possible due to memory cells, which are the cells, that is generated after initial contact with antigen and has a relatively longer life as compared to other immune cells. Upon stimulation (with same antigen), these cells can give rise to a rapid immune response.

Active immunity may be natural or artificial. Natural active immunity develops due to an infection from which a person recovers. For example, a person, who has recovered from an attack of small pox, develops natural active immunity. Such immunity is usually long-lasting, but the duration varies with the types of pathogen.

Artificial active immunity is the resistance induced by vaccines. Vaccines are preparations of live or killed micro-organisms or their products. It is the most common method of immunization or vaccination. Some examples of vaccines are as follows:

1. Bacterial vaccine like BCG for tuberculosis
2. Viral vaccine of small pox etc.

Vaccines initiate an infection without causing any disease. The immunity lasts for several years but booster doses may be necessary. Now a days vaccine are also developed by variety of other methods such as recombinant DNA technology.

**TABLE 2**  
**Comparison of active and passive immunity**

	<b>Active Immunity</b>	<b>Passive Immunity</b>
1	Produced actively by the host's immune system	Received passively by the host, No participation of host's immune system.
2	Induced by infection or by contact with immunogens (vaccines, allergens etc.)	Conferred by introduction of readymade antibodies
3	Effective protection	Less effective
4	Immunity effective only after a lag period	Immunity effective immediately.
5	Immunological memory present	No immunological memory
6	Not applicable in immunodeficient hosts	Applicable in immunodeficient hosts.
7	Long lasting	Transient

## **(2) Passive acquired immunity**

The resistance which is transferred to a recipient in a 'ready-made' form is known as passive immunity. Here the host's immune system has no active role. There is no antigenic stimulus, no latent period, no secondary response in passive immunity. (Table 2). In this type of immunity preformed antibodies are administered into host body. It is less effective than active immunity. It is of short life span, lasting usually for days or weeks, only till the passively transferred antibodies are metabolised and eliminated. The main advantage of

this immunisation is that it is immediate in action and, therefore, very useful when instant immunity is desired.

Natural passive immunity is the resistance passively transferred from mother to the baby. In human infants, it is possible through the placenta while in animals such as pigs, it occurs orally through the colostrums. Human infants acquire a satisfactory level of immunological independence at the age of three months, till then maternal antibodies give protection against infection to them.

Artificial passive immunity is the resistance passively transferred to a recipient by administration of antibodies. The agents, used for this purpose are hyper immune sera of animal or human origin. The oldest and most common method is to use hyper immune horse sera, which is prepared by active hyper immunisation in horses using the appropriate antigen. For example antitetanus serum.

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## **8.3 Phylogeny and ontogeny of immune system**

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### **8.3.1 Phylogeny**

Presence of the most specific immune system is not the dynamic features of vertebrates only but a rich variety of immune responses are found in invertebrates also. Although the occurrence of antibodies, B cells and T cells are limited to vertebrates alone, but it has been proved that a non-adaptive immunity is always occurs in invertebrates. They have various mechanisms that can differentiate between self or non self and provide a non-specific barrier to the entry of pathogen. Some of the elements of immunity are detectable in almost all living beings (example phagocytosis).

These similarities are important because they suggest that the invertebrate mechanisms are precursors of the vertebrate ones. These links may be the most compelling evidence that the immune systems of human and other mammals evolved from more ancient creatures over hundreds of millions of years.

The host defence systems arise when the life did, with the protozoans, the simplest form of life. In its defensive function, protozoan phagocytosis is not very different from the function of phagocytic cells of human. In animals ranging from starfish to humans, phagocytic cells travel through a circulatory system or through the coelom. Another fundamental aspect of immunity – the ability to distinguish self from non-self also dates back to early in life's history. Some protozoans live in colonies must be able to recognize one another. The

oldest and simplest metazoan (the sponges) also have the property to differentiate self from non self, its cells attack graft from other sponges. This rejection response is not identical to vertebrate's rejection response. In vertebrates, because of immunological memory if one graft from a donor is rejected, a second graft from the same donor will be rejected more quickly. But in sponges and jellyfish, speed of both the rejection is same. These results suggest that the memory component of the immune response is missing here. Star fish and other higher invertebrates also lack immunological memory.

Presence of various non-specific barriers in invertebrates shows their specific power of innate immunity. It includes mucous that surrounds the body of coelenterates and annelids. The presence of a tough exoskeleton, such as shells, forms a mechanical barrier in the case of arthropods, echinoderms and molluscs. The body fluids of invertebrates contain many factors that have strong antibacterial and anti microbial activity. These include agglutinins, lysozyme, non-lysozyme bactericidins and anti microbial proteins.

One such factor, cecropin A, found in silk moths shows approx 40 percent homology with immunoglobulin domains and could represent a primitive form of immunoglobulin. Surprisingly, plants, which diverged from vertebrates at least a billion years ago, also respond to invading pathogens by producing a variety of antimicrobial molecules that kill the pathogens. Two other features of the vertebrate immune system the complement and lymphocytes are also not found in invertebrates. However, in place of complement, several phyla of invertebrates, exhibit a similar response, called the prophenoloxidase (proPo) system. Like the complement system, proPo is activated by a series of enzymes. This system plays a role in encapsulating foreign objects.

Invertebrates lack lymphocytes and antibody, but they have some mechanisms that seem to be precursors of vertebrate immunity. For example, lymphocytes like cells have been found in earthworms. Lectins isolated from earthworms, snails, clams and other invertebrate animals participate in the coating of foreign particles and enhancing phagocytosis. Similarly, lectins isolated from the flesh fly and from the sea urchin are related to a family of collectins (a vertebrate protein), which play an important role in immune cells of human beings.

Some molecules which are structurally and even functionally similar to antibodies are also present in invertebrates. Vertebrate antibodies (or immunoglobulins) have a specific structure called the Ig fold. The Ig fold

probably emerged during the evolution of metazoa when it became necessary for specialized cells to recognize one another. Hemolin, a protein isolated from the blood of moths, is a member of immunoglobulin super family. Some other molecules of this super family are also found in several invertebrates like grasshoppers and flies. These observations suggest that antibody-based immunity have its roots in invertebrate defense mechanism. Cytokines (including interferons, interleukins and tumor necrosis factor) are the regulators of every aspect of vertebrate immunity. It has been also suggested that invertebrates have some cytokines which are similar to vertebrate cytokine IL-1, IL-6 and TNF. These invertebrate cytokines believed to perform same function as in vertebrates. The most widespread antibacterial protein the lysozyme is produced by insects when infection sets. Lysozyme is also a part of human innate immunity.

It has been believed that the multi-component, adaptive immune systems began with the first vertebrates. In some ways, sharks and skate immunity is similar to that of human. These fishes have spleen as a rich source of B cells like human beings. Similarly they also have a thymus, where T cell matures. Graft rejection is also found in sharks, but the activity takes long time. Sharks also have four types of antibodies with similar type of proteins that of humans.

### 8.3.2 Ontogeny

The immune system is a part of the hematopoietic system, which comprises all the cells of the blood. This system, like the skin, is constantly renewed throughout life. Thus, the development of the immune system, which starts in the embryo, is continued throughout the individual's life span, but the rate decreasing with age.

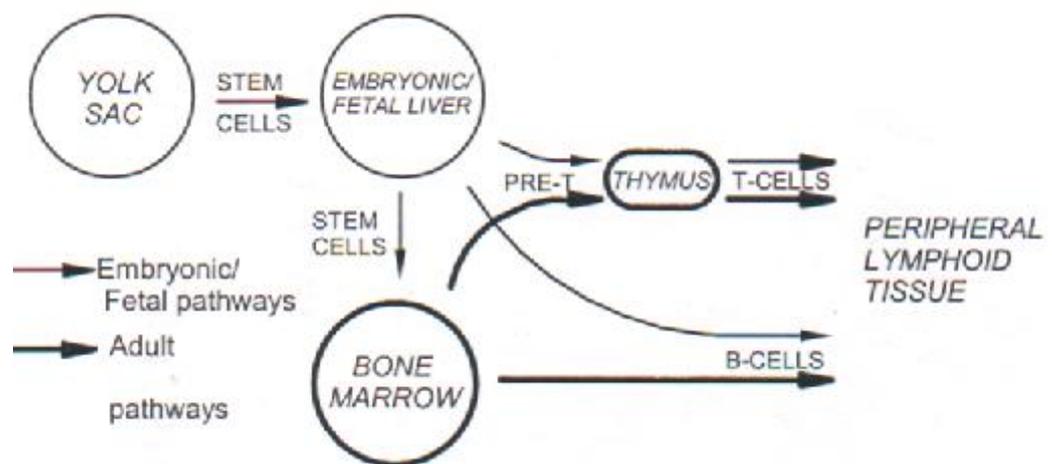
Hematopoietic stem cells (HSC) originate in the yolk sac of developing embryo. They are arising from certain endothelial cells in the aorta. From yolk sac, HSC migrate early into the fetal liver and later into the bone marrow. The generation of immunocompetent cells from hematopoietic precursors in the bone marrow, is a process which continues throughout the life. Human infants are born with a functioning immune system and are additionally protected by transplacentally acquired maternal IgG for the first few months of life.

#### **Origin and development of Hematopoietic Stem Cells**

Various cells of the hematopoietic system are continuously generated from a single kind of precursor cells known as the hematopoietic stem cell (HSC). These stem cells are undifferentiated cells which are capable of asymmetric

division, resulting in two classes of products. One class includes new stem cells identical to the parent cells. The stem cells are therefore said to exhibit the property of self-renewal. The second class of products includes cells in various stages of differentiation, finally developing each of the mature cells of the blood and immune system, including B and T cells, granulocytes, monocytes, RBCs and platelets. The two differentiated daughters of the HSC are the common lymphocyte progenitor, CLP and the common myeloid progenitor, CMP. The CLP gives rise to B and T Cell progenitors while the CMP gives rise to the progenitors of erythrocytes, megakaryocytic, eosinophils, mast cells/basophiles and granulocyte/monocytes.

The first stem cells appear early in human embryonic development (at about two weeks of gestation) in the blood islands of the yolk sac (Fig.8.4). As the developing blood vessels being making connections with the embryo, stem cells move into the developing fetal liver and finally into the spleen. By the time of birth neither the liver nor the spleen remains a site of haematopoiesis in human; the stem cells have migrated into the bone marrow.



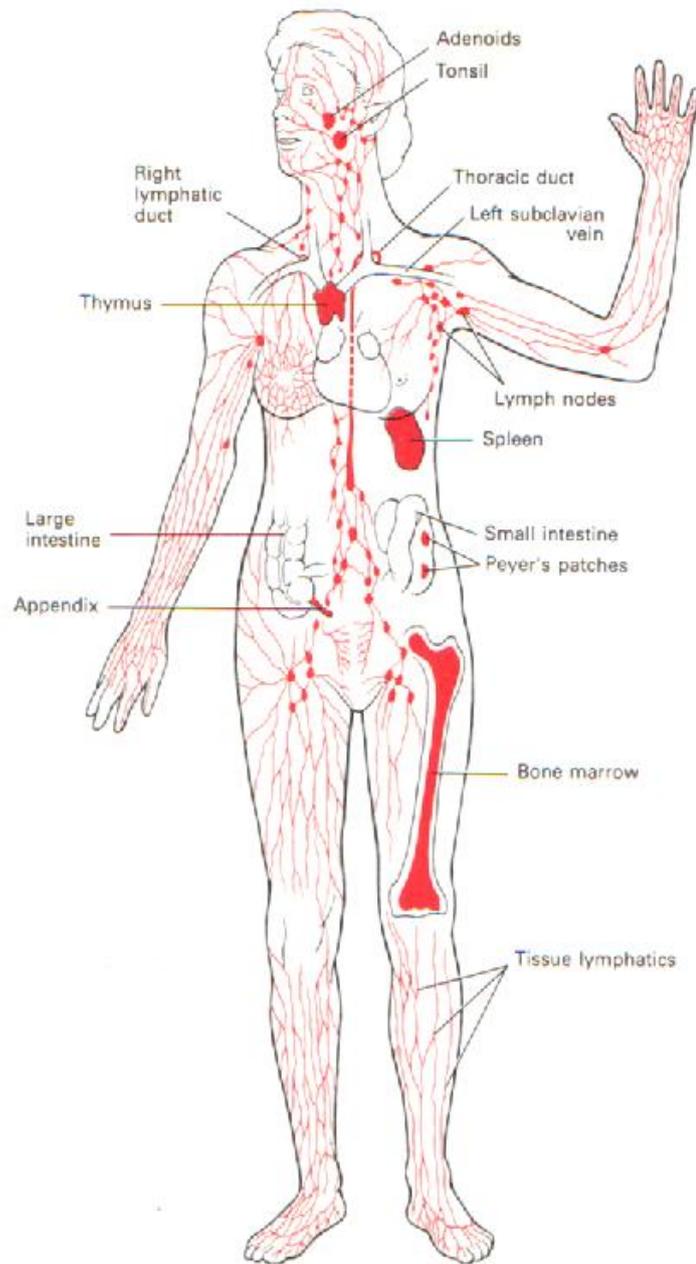
**Fig 8.4 – Cell migration in fetus and adult**

In normal human adults, the development of various cells of hematopoietic system is restricted to the bone marrow, exceptionally the T cells, which are produced exclusively within the thymus, from the precursors (pre T cells) which are bone marrow derived.

## 8.4 Organization and structure of lymphoid organs

Entry of antigens always induces various immune responses in our body. These immune responses kill the antigen bearing pathogen. The cells involved in these responses are the basic components of our defence system - the lymphoreticular

system. This system is a complex organization of cells of diverse morphology. It consists of lymphoid and reticuloendothelial components. In which the lymphoid cell (lymphocytes and plasma cells) are primary component of specific immune response. These lymphoid cells along with lymphoid organs makes lymphoid system.(fig.-8.5)



**Fig. 8.5 – Different lymphoid organs of human body**

Based upon the functions the lymphoid organs can be classified mainly into two types :-

1. Central (Primary) Lymphoid organs

- Bone Marrow
- Bursa of Fabricius (in birds)
- Thymus

2. Periferal (Secondary) Lymphoid organs

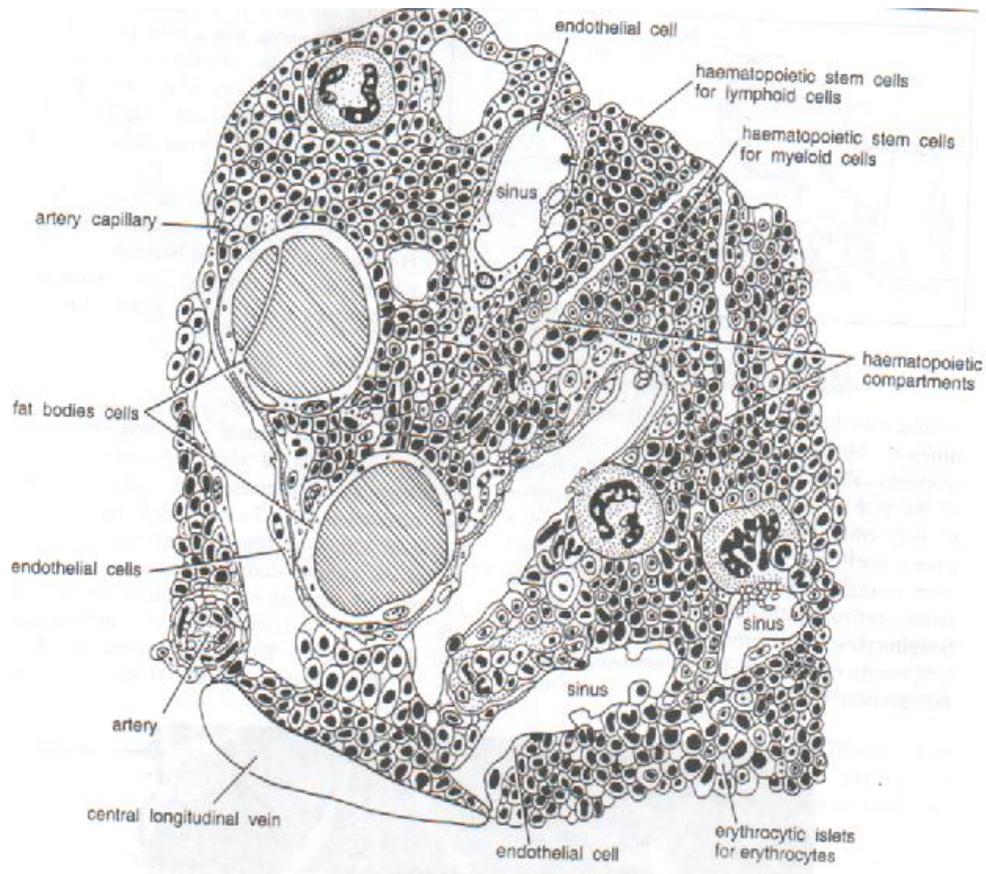
- Spleen
- Lymph nodes
- Mucosal associated Lymphoid tissue (MALT)

#### 8.4.1 Primary lymphoid organs

The central or primary lymphoid organs are the organs in which precursor lymphocytes proliferate, develop and acquire immunological capability. Different types of lymphocytes formed in the bone marrow. From bone marrow these cells migrate to various organs for maturation. T Cells mature in the thymus where as B cells in the foetal liver and bone marrow.

(i) **Bone Marrow** - Bone marrow is a connective tissue present in bones. This is a major source of stem cells for pre B and T cells and for serum immunoglobulin. This also produces different blood components, cytokines and entire leukocytes. During early foetal life, production of blood cells takes place inside the mesenchyme of the yolk sac. After some time blood cells are produced in the spleen and liver. The bone marrow becomes a major haematopoietic tissue from the fifth month of foetal life. Bone marrow is of two type – red marrow, which is red, because of red blood cells and yellow marrow, which is yellow because of fat cells. Both the types of marrow are inter changeable.

In human beings and other mammals red bone marrow serves as the main source of lymphoid cells and blood cells. It is found in the cavities of most bones such as humerus, radius-ulna, femur, tibia-fibula, ribs etc. The bone marrow is composed of haematopoietic compartments, lymphoid compartment, fat bodies, sinuses and blood capillaries (Fig. 8.6).



**Fig. 8.6- T. S of mammalian bone marrow**

Each haematopoietic compartment is made up of basement membrane, endothelial lining cells, erythrocytic islets, myeloid cells giving rise to leukocytes such as monocytes, neutrophils, basophils, mast cells, eosinophils and macrophages. The lymphoid compartment gives rise to immune components such as B cells, T cells and NK cells. The maturation as well as differentiation of different types of cells are stimulated by a number of cytokines. Cytokines are produced by macrophages and stroma cells of bone marrow. T cells differentiation is started in the marrow, then they migrate to the thymus where differentiation is completed. B cells originate and mature in the bone marrow. From there, they enter into the blood stream through vascular sinuses.

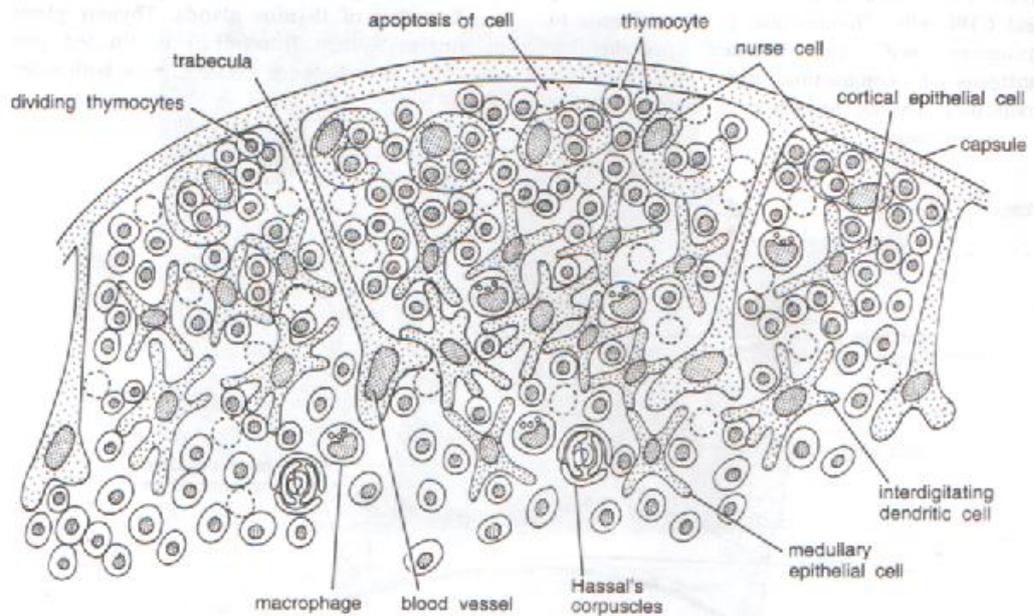
**(ii) Bursa of Fabricius (BOF)** –This is a specific lymphoid organ found in birds. BOF arises as a pouch from the dorsal part of the cloaca. Its development, structure and functions are comparable to the thymus. BOF,

becomes a lymphoid organ at about the 15<sup>th</sup> day of embryonation, develops full functional ability near hatching and starts involuting by 7-13 weeks of age (the age of puberty). Stem cells from the yolk sac, fetal liver and bone marrow enter into the bursa and develop into immune competent 'bursal' (B) lymphocytes. At the time of antigenic stimulation these cells transform into plasma cells and secrete antibodies.

BOF is a blind oval sac like structure of approx. 1 cm diameter. It is connected by cloaca by a duct. It is made up of two regions (1) capsule and (2) sub-capsule. Just below the capsule, a muscle layer is also present, which consists of outer circular muscle fibre, middle longitudinal fibre and inner circular muscle fibre. Sub capsule is made up of mucosa. Vilus like processes, called as 'plicae', occupies the lumen of BOF. Plicae is composed of many polyhedral follicles. The follicles are made up by outer cortex and inner medulla. Both cortex and medulla have reticuloepithelial cells which gives rise to lymphocytes.

**(iii) Thymus-** Thymus is one of the most important primary lymphoid organ which plays very significant role in natural, humoral and cell mediated immunity. Thymus develops from the epithelium or III<sup>rd</sup> and IV<sup>th</sup> pharyngeal pouches at about the VI<sup>th</sup> week of gestation. The thymus became fully mature by the III<sup>rd</sup> month of gestation. In man, it reaches its maximum size just prior to birth.

Thymus is located behind the upper part of the sternum, just above the heart between breast bone on both sides of the lungs. It is a two lobed structure. It is made up of capsule, para cortex, cortex and medulla.



**Fig. 8.7- T.S of thymus of man**

Capsule is the outer covering of the thymus. Capsule penetrates deeply into the cortex and medulla as trabeculae (Fig. 8.7). The cortex is filled with actively proliferating small lymphocytes. The medulla contains the Hassall's corpuscles. Hassall's corpuscles or thymic corpuscles are composed of tightly packed epithelial cells that may be remnants of degenerating cells, as well as, lymphocytes, eosinophils and macrophages.

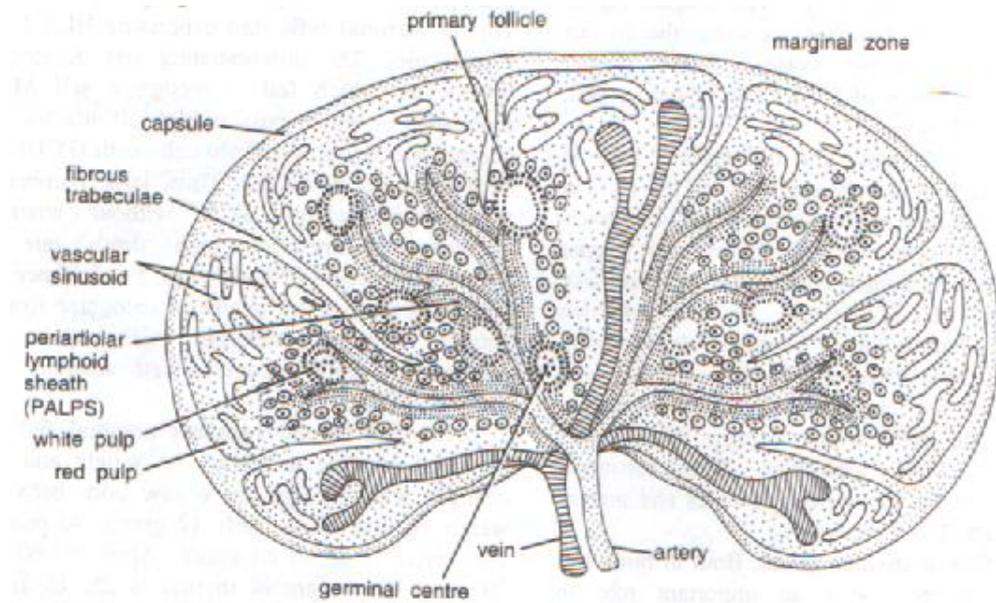
Till recently, the thymus was an organ without any recognised function. The work done by Good (1954) and Miller (1961) paved the way for the understanding of the role of thymus in the cell mediated immunity. The primary function of the thymus is the production of T lymphocytes. Mesenchyme stem cells from yolk sac, fetal liver and bone marrow reach the thymus and differentiate into the T cells. This is an interesting fact that, from the total amount of produced T cells, only about one percent leave the thymus. The rest are destroyed locally. The reason of this wasteful process is not known. In addition to T cells maturation, thymus also plays a key role in 'education' of T cells. In the thymus, T cells are 'educated' so that they become capable to recognize 'self' and 'non-self' proteins or antigens. T cells, also become competent to help other immune components for neutralizing antigens, in the thymus. Maturation of T cells, in the thymus also make them capable to migrate into other lymphoid organs.

#### 8.4.2 Secondary lymphoid organs

After maturation and proliferation, the lymphocytes migrate into lymph and blood streams, accumulate in the peripheral or secondary lymphoid organs and activate the appropriate immune response. The secondary lymphoid organs consists of well organized encapsulated organs, like spleen and lymph nodes and non-encapsulated lymphoid tissues. These organs are the major sites of production of effective T and B cells. The non-encapsulated lymphoid tissues include Peyer's patches of small intestine, tonsils in the pharynx and submucosal lymphoid follicles of appendix, gut, liver etc. These lymphoid tissues which are found in association with mucosal surfaces are collectively called mucosa-associate lymphoid tissue (MALT).

(1) **Spleen** – The spleen is the largest lymphoid organ. It is an encapsulated, bean-shaped, dark brownish organ filled with spongy pulp. It is situated on the left side of the body below the diaphragm. The spleen is richly supplied with blood vessels, but it is not connected with lymphatic vessels. Spleen is very efficient in trapping lymphocytes, monocytes, macrophages, immune complexes, antigens from the blood and helps these cells to produce antibody. It is major site of immune response to blood-borne antigens. Spleen also have several non-immunological functions. It is the important site for storage of platelets of blood.

Spleen of man is covered by a capsule of white connective tissue, which extends into the spleen at various places as tubular structure called trabeculae. Trabeculae divide the spleen into several interconnected two types of compartments – red pulp and white pulp (Fig. 8.8). Red pulp contains RBC (normal and defective) degraded haemoglobin, iron pigment etc. This is the site where old or damaged blood cells are destroyed. White pulp is composed of peri arterial lymphoid sheath (PALS). PALS are composed of B and T cell rich areas. It also have primary lymphoid follicles. During an immune response, these primary follicles become secondary follicles and develops into germinal centres. The region, between the red and white pulp is known as marginal zone. It contains B cells, T cells and macrophages.



**Fig. 8.8- L.S of spleen of man**

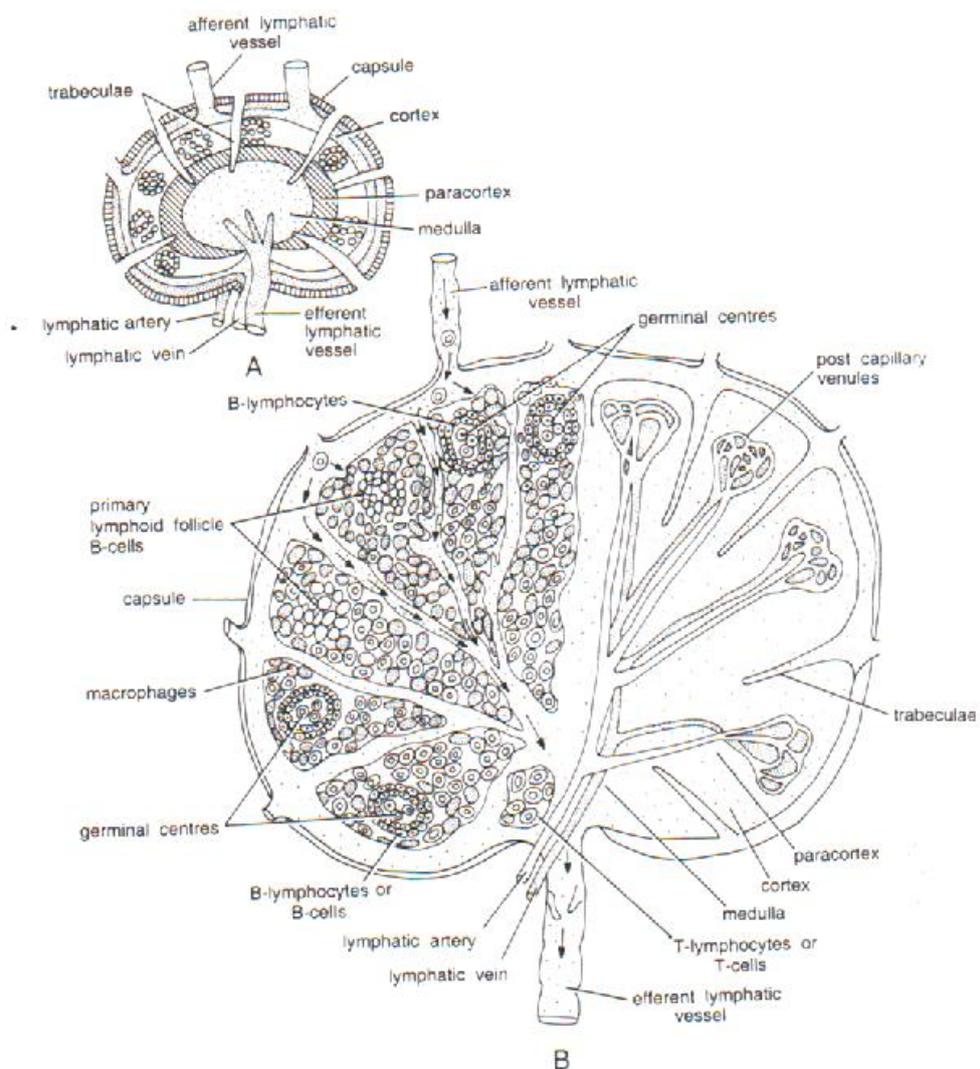
The marginal zone is the major receiving depot of the spleen, receiving large quantities of blood. Here, antigen is trapped by macrophages and dendritic cells, which carries it to the PALPS. These cells present the antigen on T cells. T cells activated the B cells. After activation, B cells and T cells migrate to primary follicles in the marginal zone. Due to antigenic exposure primary follicles convert into secondary follicles containing germinal centres. Here B cells turn into plasmocytes and secretes appropriate antibodies.

**(2) Lymph Nodes** - Lymph nodes are the first organized secondary lymphoid organ to interact with antigens. They are small, encapsulated, bean shaped bodies which contains lymphocytes, macrophages and dendrite cells. Lymph nodes are made up of net like tissue of reticular cells. The interstitial fluid - lymph flows into lymphatic capillaries which communicate into larger lymph vessels through primary and secondary lymphoid follicles and lymph nodes . Lymph nodes act as a filter for the lymph, each group of nodes draining a specific part of the body. They phagocytose foreign materials including micro organism. They are also important for B and T cells proliferation and circulation.

Lymph node is made up of capsule, cortex, para cortex and medulla. The fibrous capsule is formed the outer most layer of lymph node, from which it penetrate deeply into the node as trabeculae. Its outer surface is convex but contains an indentation, the hilus, through which lymphatic vessels leave the node and blood vessels enter. The node can be divided into two parts, outer

cortex and inner medulla. The cortex contains primary lymphoid follicles, B cells and germinal centres. Below the cortex, between the cortex and medulla, one more part paracortex is present. It contains T cells and dendritic cells (fig. 8.9). Below the para cortex the medulla is present which contains medullary cortex, B cells, CD 4 cells, plasma cells, macrophages and reticular cells.

Antigens trapped in lymph are carried by lymphatic vessel into lymph node. Here, they are processed and presented by dendritic cells into the para cortex. In para cortex, due to antigenic stimulation B cells activates and transformed into plasmocytes and secretes antibodies. One important feature of lymph nodes is that T cells and B cells are kept in different compartment which are known as B cell and T cells areas. B cell area lies in outer cortex whereas T cells are confined to paracortical area.



**Fig. 8.9- L.S of mammalian lymph node A. showing different parts  
B. showing different cells**

Lymph nodes appear in the human foetus in the third month, survive for 60 years or more with slight atrophy. The function of the Lymph nodes is similar to that of spleen, the main difference is that the spleen is for blood-borne antigens while lymph nodes are for antigens of lymph.

**(3) Mucosa – associated lymphoid Tissue (MALT)** -The various mucous membranes of body such as digestive, respiratory and urinogenital system's mucosal linings represent the main sites for the entry of microbes into the body. Hence, almost 50 percent of the lymphoid tissue in the human body is located within the lining of the major tracts, defending these surfaces against microbes. The lymphoid tissues are collectively called mucosa-associated lymphoid tissue (MALT) and include nasal associated lymphoid tissue, gut associated lymphoid tissue, bronchus associated lymphoid tissues and genitourinary associated lymphoid tissues. MALT plays important role in trapping, filtering and transport of antigens to peyer's patches (present in small intestine) where the antigens are destroyed.

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## **8.5 Cells of the immune system and their differentiation**

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The immune system is composed of different types of cells that collectively protect the body from pathogens. These cells can destroy bacteria, parasites, tumour cells and viral-infected cells. All the cells of immune system arise from haematopoietic stem cells in the bone marrow by the process called haematopoiesis. These haematopoietic stem cells are always at stable level in an adult life because of the self-renewal capacity. These stem cells converts into two type of progenitor cells : myeloid progenitor, which gives rise to erythrocytes, granulocytes, macrophages and mast cells etc. and lymphoid progenitor gives rise to B lymphocytes and T lymphocytes. Moreover, some of these cells can transform from one to another depending on the functional need.

### **8.5.1 Lymphocytes**

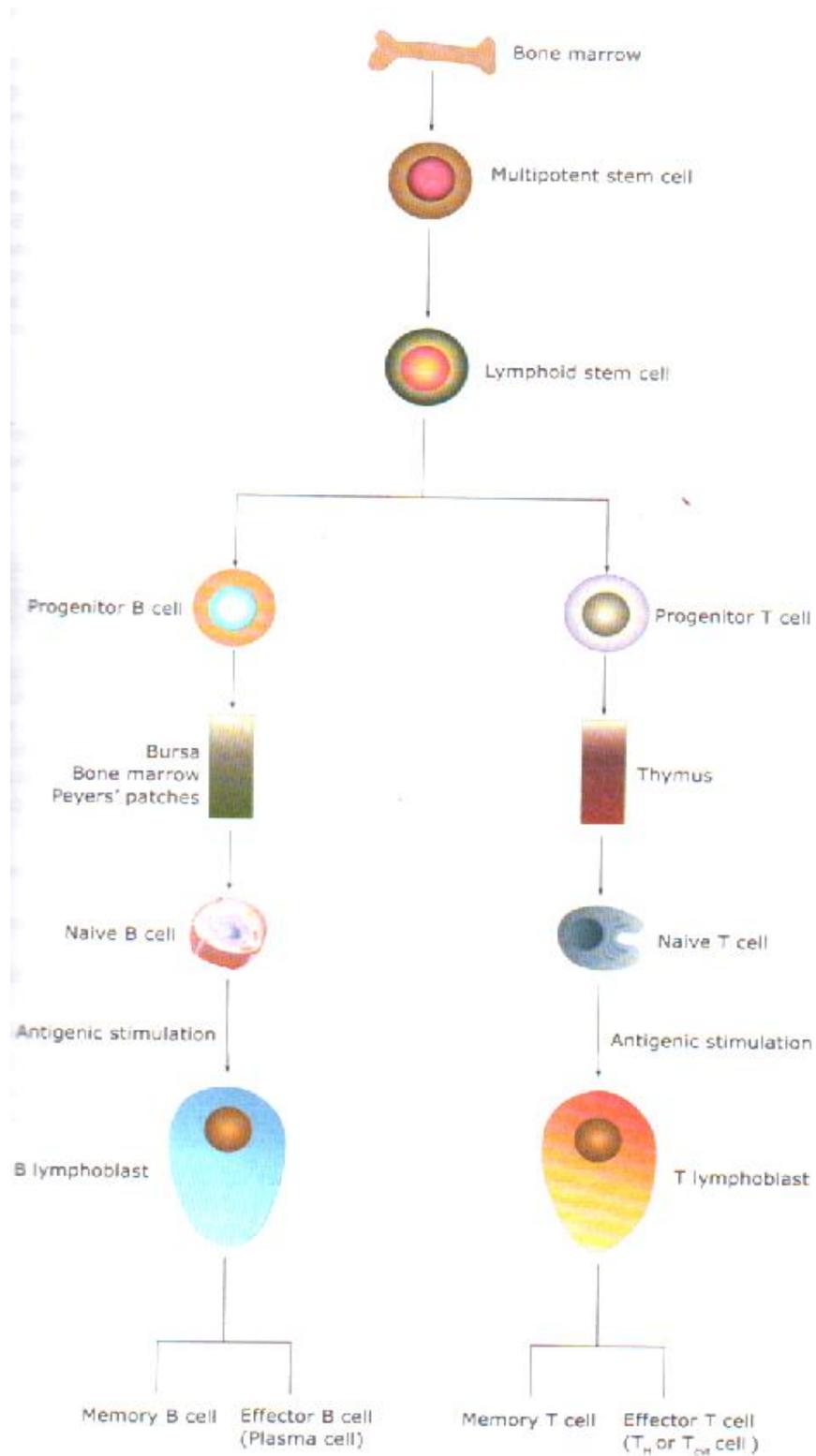
Lymphocytes are the major cellular component of the immune system. They are small, rounded cells, found in peripheral blood, lymph, lymphoid organs and many other tissues. They constitute 20-40 percent of white blood cells population, while in lymph and lymphoid organs they form the predominant

cell type. According to the size, lymphocytes can be classified into small, medium and large lymphocytes. Small lymphocytes are most numerous. Depending on their life span, they can be classified as short lived and long lived lymphocytes. The short lived cells act as effective cells in immune response, whereas long lived cells are the store house for immunological memory. The short lived cells have a life span of about 2 weeks whereas the long lived cells may last for 3 years or more, or even for life in man.

Lymphocytes are produced at a very high rate of about  $10^9$  per day. Like all other blood cells lymphocytes originate in the lymphoid organ. In the initial stages of development the lymphocytes cannot be distinguished morphologically. Such small, non-motile lymphocytes are referred to as naive cells or small lymphocytes. These cells are in the  $G_0$  state of the cell cycle. Due to antigenic stimulation some of the naive cells enter the  $G_1$  Stage of cell cycle, whereas the rest of the naive cells die after a short life span. The cells which enter the  $G_1$  stage become larger and are called lymphoblasts. These cells then enter the S phase and finally the activated lymphocytes divide. All these events collectively known as blast transformation. The mature lymphoblasts divide and differentiate into antigen responsive effector cells and memory cells. (Fig. 8.10)

Mainly three types of sub population of lymphocytes – B cells, T cells and NK cells are present in body. They are functionally different but appear morphologically similar. These populations of lymphocytes do not remain distinct, but mix together in the process known as ‘lymphocyte recirculation’. There is a constant flow of lymphocytes through the blood, lymph, lymphoid organs and other body tissues. Thus, the lymphocytes of appropriate specificity would reach at the required site and provide the immunity.

Lymphocytes can be identified or marked in cell population by a large number of membrane bound molecules expressed on their surface. The reaction of a mature lymphocyte to its specific antigen may be induction of either ‘tolerance’ or induced an immune response. The nature of immune response is lymphocyte dependent. Stimulated T cells produce certain activation products like lymphokines and induce cell mediated immunity (CMI), while activated B cells divide and transform into plasma cells which produce antibodies and induce humoral immunity or antibody mediated immunity (AMI).



**Fig. 8.10- Blast transformation in B and T cells**

## T Lymphocytes

T lymphocytes constitute major part (about 80%) of human blood lymphocytes. This type of lymphocytes develop from their precursor in the thymus. Although these cells also produce in bone marrow but in an immature state, known as prothymocytes, they leave the bone marrow and migrate into the thymus. A maturation process called thymic education converts prothymocytes into mature T cells. These mature T cells released into the blood stream. T cells also have the surface receptor for antigen binding but they are not antibody molecules like B cells. T cell receptors recognize antigen only when they are presented with self MHC molecules.

T cells can be divided into different sub populations based on their functions and on their surface antigens. According to their functions T cells may be classified into regulator cells and effector cells. Regulator T cells may be helper (inducer) cells ( $T_H$ ) or suppressor cells ( $T_S$ ). Helper T cells activate B cell response whereas suppressor T cells inhibit antibody production by B cells and the reaction of effector T cells. Therefore, balanced activity of helper T cells and suppressor T cells produces optimal immune response. Over activity of  $T_H$  cells or lesser activity of  $T_S$  cells causes abnormal immune response as seen in autoimmunity. Similarly over activity of  $T_S$  cells or decreased activity of  $T_H$  cells leads to immunodeficiency.

The  $T_H$  cells are responsible for immune response to protein antigens in general, and for helping B cells to make different types of antibodies. Depending on the cytokine secretion these cells can be divided into two groups. The  $T_{H1}$  subset secretes pro-inflammatory cytokines important for the killing of intracellular microbes and generation of Tcyt cell.  $T_{H2}$  cells secrete anti-inflammatory cytokines, which are important for B cell proliferation.  $T_{H2}$  cytokines are also important in parasitic infection.

Effector T Cells may be cytotoxic T Cells (CTL), cells responsible for delayed type hyper sensitivity (DTH) and those which undergo rapid proliferation in mixed lymphocyte activity (MLR). Tcyt cells (CTL) are activated when they interact with antigen – MHC complex on the surface of an altered self cell. Activated Tcyt cells bind, interact and eliminate these altered self-cells (virus-infected cells or tumour cells).

### **8.5.2 B Lymphocytes**

B Cells were first shown in an organ of birds called bursa of fabricus, so they are named as B Cells. In man, B cells mature in the bone marrow and again the tissue starting with 'b' is just a coincidence. Human beings do not have any organ anatomically similar to that of bursa. From bone marrow, B cells migrate to secondary lymphoid organs and tissues for maturation.

B cells are the only cells, capable of producing antibody and establish the antibody mediated immunity (AMI) or humoral immunity. They contribute about 10 percent of lymphocyte population . Each B cells express surface immunoglobulin molecule, which act as specific antigen receptor.

B cells can be divided into two subsets – B1 and B2. B1 cells arise early in ontogeny and express mainly IgM antibodies. They mature independently of the bone marrow and their response against antigens is also independent of T cells. B2 Cells are primarily responsible for humoral immunity. They are involved in the synthesis of IgG ,IgA and IgE molecules,which are T cell dependent . Activation of B cells always require T cells.

When the mature naive B cells are appropriately stimulated with proper antigen, they divide and differentiate into two types of subpopulation , the plasma cells and memory cells. Plasma cells are infrequently present in blood and are normally restricted to the secondary lymphoid organs. Plasma cells synthesize and secrete only one type of specific antibody of one immunoglobulin class. These cells are short lived cells (one or two weeks). Memory cells are long lived cells that retain “antigenic-memory”. These cells when restimulated (again activated with same antigen) can again transform themselves into antibody secreting plasma cells and memory cells within a short span of time.

### **8.5.3 Null Cells (NK Cells)**

The small amount (10%) of blood lymphocytes which do not express the antigen receptors are called null cells. They are also produced in bone marrow and found throughout the body, but mainly in blood. They are not antigen-specific and don't exhibit immunological memory. Although these cells lack antigen-binding receptors, yet they have various surface antigens. The functions and nature of these cells are not fully understood.

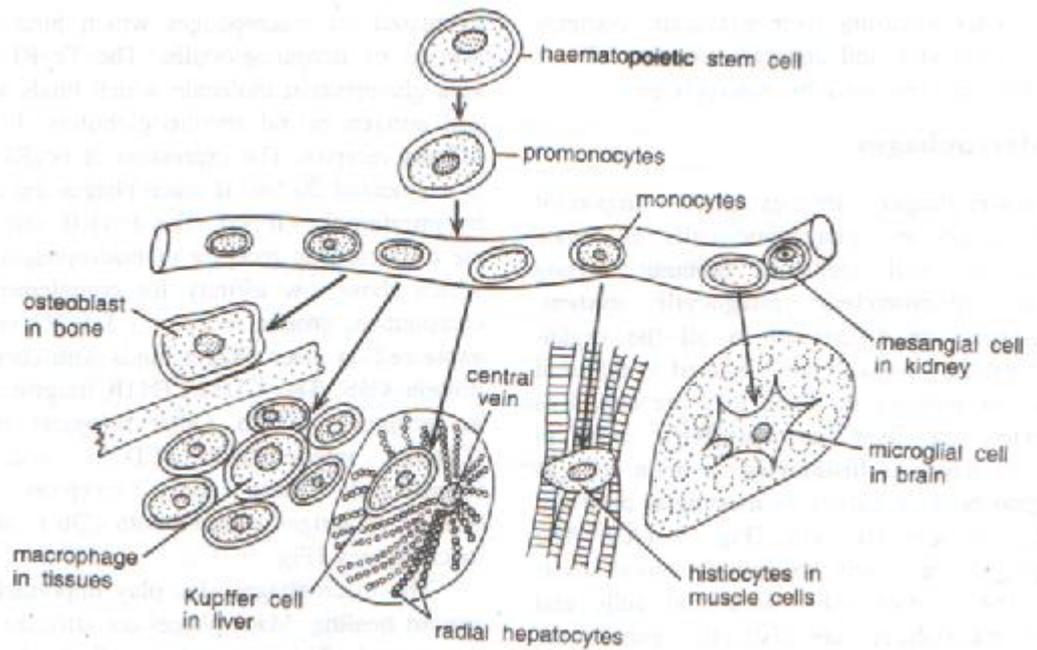
A specific sub population of null cells has been identified which have the surface receptors for IgG . They are capable of destroying IgG sensitised target cells. They are known as killer (K) cells. Another subpopulation of null cells is the natural killer (NK) cells. They are capable of killing the virally transformed

cells and involved in allograft and tumour rejection. Interferon increases NK cells activity. These cells present in the spleen and peripheral blood.

#### **8.5.4 Phagocytic cells (Macrophages)**

The mononuclear phagocytes are the second major immune cells population. They contribute to an important function called phagocytosis. As described earlier phagocytosis is phylogenetically the oldest defence mechanism in animals. Protozoans shows a good capacity of phagocytosis. The importance of this mechanism in defence has been discovered by Metchnikoff (1882) in starfish. The mononuclear phagocytic system includes macrophages and microphages. Both the types of cells originate in the bone-marrow. Myeloid progenitor cells differentiate into promonocytes and finally into blood monocytes. The human blood monocytes (macrophages) are the largest lymphoid cells with bean shaped nucleus and granular cytoplasm. Cells from this circulating pool of monocytes migrate into various organs and tissues and after maturation they become tissue specific macrophages. During this differentiation these cells swell to much larger size, synthesize more hydrolytic enzymes and acquire increased phagocytic ability.

Tissue macrophages can live for months or even for years unless destroyed by phagocytic activity. According to their different organs and tissue they have been given special names. For instance, they are known as Kupffer cells in liver, mesangial cells in kidney, alveolar macrophages in the lungs, sinus macrophages in spleen and lymph nodes, microglial cells in the brain, serosal macrophages in the peritoneal cavity and osteoblasts in the bones(Fig.8.11).



**Fig. 8.11- Macrophages in different tissues**

The primary function of macrophages is phagocytosis. Macrophages move slowly to the target, their cytoplasm projected out pseudopodia and engulf the abnormal cell and foreign particles. They accumulate in areas of inflammation or tissue damage. Thus, the macrophage serves as “scavenger cells” of the body. They phagocytose invading microbes, antigens and even injured or dead self tissues or cells. This activity can be enhanced by cytokines secreted by  $T_H$  cells.

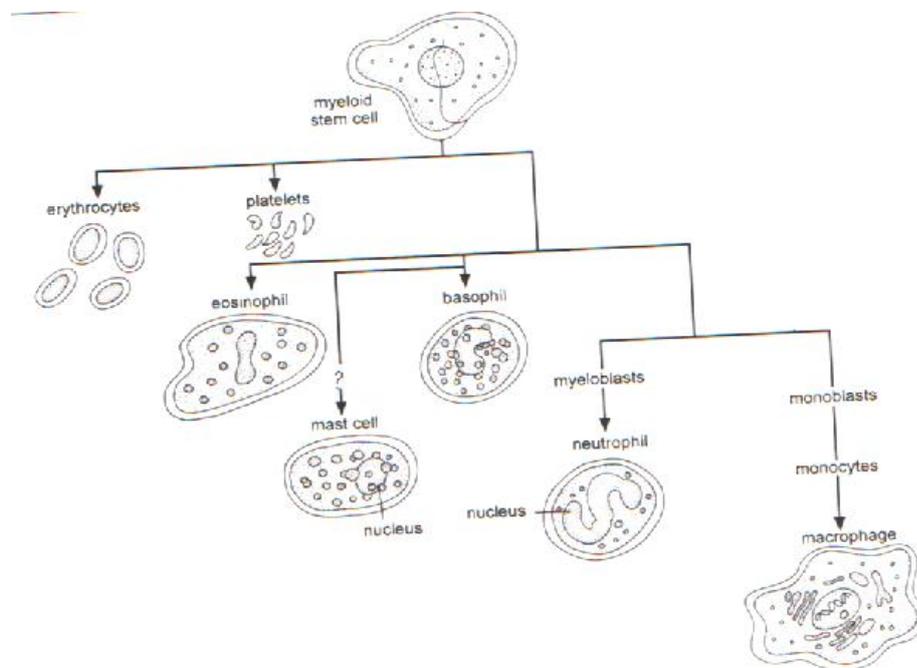
These cells serve as an important link between innate immunity and specific immune response. They phagocytose the foreign particles (innate immunity) as well as act as antigen presenting cells which can be recognized by T cells (Humoral immunity). It has been also recorded that activated macrophages are more efficient than antigen presenting B cells, but less efficient than dendritic cells. When stimulated by cytotoxic antibodies and certain lymphokines, macrophages become ‘armed’ and capable of antigen specific cytotoxicity, important in anti tumour activity and graft rejection.

### **8.5.5 Granulocytes**

Granulocytes are the subpopulation of white blood cells developed by myeloid progenitor (fig.8.12). These type of cells contains granular cytoplasm (hence the name). According to the staining properties of these granules these cells are

of three types eosinophils, basophils and neutrophils. These cells act as an effector cells at the time of inflammation. They are also participate in innate immunity and are stimulated by cytokines.

**Neutrophils-** Neutrophils are the cells which have the property that their cytoplasmic granules stain at natural pH (hence the name). They are also known as polymorphonuclear leukocyte due to presence of multilobed nucleus (3 or 4 lobes connected by thread like chromatin). Neutrophils originate in the bone marrow. The development from myeloid stem cells to neutrophils takes about 2 weeks. In first week myeloid stem cells proliferate and converts into progenitor neutrophils and in second week they develop into mature neutrophils with the development of granules. Now, they are called polymorphonuclear granulocytes. These cells migrate to the blood and various tissues of body.



**Fig. 8.12- Different myeloid progenitor cells**

Neutrophils are actively phagocytic in nature. Neutrophils have a large array of lytic enzymes and antibiotic protein in their granules. They are predominant cell type in acute inflammation. They contribute 95% of circulating granulocytes. They do not have any role in specific immune response.

**Eosinophils-** Eosinophils contribute about 5% of the total leukocyte population. They have bilobed nuclei and their cytoplasmic granules stain red with acid dye. They are motile phagocytes migrate from the blood to the tissues. They produced in bone marrow and mature in spleen. The phagocytic activity of eosinophils is less important than the neutrophils. They are often produced in very large number in allergic inflammation, parasitic infection and around antigen-antibody complex.

The granules of eosinophils contain toxins known as “major basic protein” and “eosinophils cationic proteins”. These are toxic for helminths, which are relatively resistant to the enzymes of neutrophils and macrophages.  $T_H$  derived cytokines activate the growth and differentiation of eosinophils, which contribute to the accumulation of eosinophils at the site of allergy and parasitic infection.

**Basophils and Mast cells-** Basophils are non-phagocytic, circulating granulocytes. Their nucleus is not definitely lobed. The granules of these cells stain bluish-black with basic dye. They contribute only 0.5% of the total leukocyte population. Basophiles originate from haematopoietic cells in the bone marrow. From bone marrow, they migrate to the blood and tissues (mast cells). They are important in allergic reaction. Their cytoplasm has large number of basophilic granules containing heparin, histamine, serotonin and other hydrolytic enzymes. Degranulation of these cells resulting in the release of pharmacologically active agents constitute the effective mechanism in anaphylactic and atopic allergy. They shows high affinity to IgE antibodies.

**Dendritic cells (DC)-**These are the antigen presenting cells of skin and tissues of innate and humoral immunity. They are so named, because they are star shaped and their surface has many folds or spike like projections, resembles with dendrites of nervous system. Like many other immune cells they also originate from the bone marrow and located in the blood, lymph and most of other tissues. From various tissues the dendritic cells loaded with antigen bearing pathogen and carry them to nearest lymph node. They are act as professional antigen presenting cells due to the presence of B7 molecule at their surfaces.

These are several types of dendritic cells with different properties, functions and locations.

- Langerhans cells are present in the skin and mucous membrane.

- Interstitial dendritic cells are present in most of the organs (for example, liver, lungs heart etc.)
- Dermal dendritic cells are found in dermis of the skin.
- Circulating dendritic cells are found in blood and lymph.
- Interdigitating dendritic cells are present in thymic medulla, splenic white pulp and lymph nodes.

An infection of the tissue by bacteria or virus or even simple inflammation causes maturation of immature dendritic cells. After become infected the immature cells process the antigen, become activated, circulate and carry the antigen to lymphoid organs. Now, these cells appear as interdigitating dendritic cells and stimulate T cells to produce antigen specific response.

**Platelets-** Blood platelets are also involved in immune responses. They are produced in the bone marrow. They contain granules. At the site of injury, platelets attach to the damaged surface and degranulate. The released substances activate the complement system, increase vascular permeability and attract WBC to the site.

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## 8.6 Summary

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- The defence mechanism of the body towards disease causing pathogen is of two types – non specific (innate) immunity and specific (adaptive) immunity.
- Innate immunity includes various defensive mechanisms like (a) mechanical and chemical barriers, (b) Phagocytosis, (c) Fever, (d) Inflammation and (e) acute phase proteins.
- Invertebrate also have the innate immunity.
- All the cells of immunity derived from haematopoietic stem cells.
- Two types of cells (plasma cells and memory cells) are the speciality of acquired immunity.
- When a B cell activated with an antigen they differentiate into plasma cells and memory cells.
- Plasma cells secrete antibodies that destroy pathogens.

- T cells includes two group of cells – helper T (TH) cells and Cytotoxic T (Tcyt) cells.
- T<sub>H</sub> Cells secrete cytokines which helps in B cells activation.
- T cyt cells are important for destruction of tumour cells/virus infected cells.
- All the blood cells (erythrocytes, leukocytes and platelets) are originate from haematopoietic stem cells (HSC).
- HSC gives rise to the lymphoid progenitor, which is the precursor of B, T and NK cells and myeloid progenitor which is the precursor of neutrophils, basophils, eosinophils.
- The organs of immune system called as primary lymphoid organ (bone marrow and thymus) and secondary lymphoid organ (lymph nodes, spleen etc.)
- Primary lymphoid organs are that organs where all the lymphocytes mature where as in secondary lymphoid organs they are interact with antigens.
- Bone marrow is the major site for the synthesis and maturation of lymphocytes (except T cells which nature in thymus).
- B lymphocytes are responsible for antibody production where T cells involved in cell mediated immunity.
- Neutrophils, eosinophils, dendritic cells and macrophages are phagocytic in nature while basophils and mast cells are non-phagocytic cells.

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## 8.7 Glossary

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- **Acquired Immunity or Adaptive Immunity** – The power of resistance acquired during the lifetime of an individual.
- **Active Immunity** – The immunity developed by an individual as a result of active functioning of their immune apparatus.
- **Antibody** – Specific globulin whose formation is induced by the introduction of an antigen in the body.
- **Antigen** – Any foreign substance which when introduced into the body induces an immune response.

- **B Cells** – A subset of lymphocytes which mature in bone marrow or in the bursa and produce antibody.
- **Basophil** - Circulating non-phagocytic granulocytes, developed in bone marrow. Stained with basic dyes.
- **Blast Cell** – Cell which have large amount of cytoplasm, immediately prior to division.
- **Bone marrow** – Living connective tissue of bones which contain haematopoietic stem cells for producing various immune cells.
- **Bursa of Fabricius** – Primary lymphoid organ of birds, site for B cell maturation in birds.
- **Cell mediated Immunity** – Immunity which involves T Cells and macrophages for destruction of pathogens.
- **Complement** – A group of serum proteins which participate in enzyme cascade and provides extracellular immunity.
- **Cortex** – The outer region of an organ such as thymus or lymphnodes.
- **Cytokines** \_ A protein that mediates cellular interactions and regulate various aspects of health including immune responses.
- **Cytotoxic T Cells** – An effector T cell that can kill infected host cells.
- **Degranulation** – Release of contents stored in granules of mast cells and basophils.
- **Dendritic cells** – Highly efficient antigen presenting cells of secondary lymphoid organs.
- **Effector cells** – Cells which are fully capable to set an immune response.
- **Eosinophil** - Cytoplasmic granulocyte, stained with acidic dye, effective against helminths.
- **Granulocyte** – A myeloid cell with prominent cytoplasmic granules.
- **Helper T cell** – A subpopulation of T cells that promotes immune responses by releasing cytokines.

- **Haematopoiesis** – Formation and differentiation of blood cells.
- **Haematopoietic stem cells** – Cells which produce progenitor cells of different haematopoietic lineages.
- **Histamine** – Basic content of mast cells and basophils, active in allergies.
- **Humoral Immunity** – Defence system which involves production of antibodies.
- **Immune Response** – Specific defence response by a host to eliminate or reject foreign particle.
- **Immunity** – Resistance power of body against to an infection.
- **Immunocompetent** – Immunologically capable cells or tissues.
- **Immunoglobulin** – A glycoprotein with antibody activity.
- **Inflammation** – A nonspecific defence response to a tissue injury or infection which induces reddened swelling and pain.
- **Innate Immunity** – Non specific, inborn defence mechanism.
- **Interferon** – Anti viral cytokines produced by virus infected cells.
- **Interleukins** – Group of proteins that act as a growth and differentiation factor for immune cells.
- **Kupffer cells** – Macrophages found in the sinusoids of liver.
- **Langerhans cells** – Dendritic cells found in the skin.
- **Leukocyte** – White blood cells.
- **Lymph** – Extracellular Tissue fluid that flows through lymphatic vessels.
- **Lymph Node** – Secondary lymphoid organ that containing lymphocyte rich tissues.
- **Lymphocyte** – Small mononuclear leukocytes which express antigen specific receptors; includes B and T cells.
- **Lymphokine** – Cytokines produced by lymphoid cells.

- **Macrophage** – Large mononuclear phagocyte.
- **Major histocompatibility complex (MHC)** – A cluster of genes, divided into 3 classes; Class I, II and III. Class I and II involved in antigen presentation, Class III helps in complement fixation.
- **Mast cells** – Bone marrow derived granulocytes plays an important role in inflammation and hyper sensitivity.
- **Memory cells** – A type of lymphocyte generated at the time of first exposure to any particular pathogen but not in active form. Upon II<sup>nd</sup> stimulation these cells gives rise more rapid and effective immune response.
- **Myeloid lineage** –Lineage of blood cells that gives rise to granulocytes.
- **Naive lymphocyte** – Lymphocytes that have not yet encountered antigen.
- **Natural Killer Cell (NK Cells)** – Lymphocytes that is neither B nor a T cell. They are natural killer of pathogens.
- **Neutrophil** – Circulating phagocytic granulocytes, stained with neutral dyes.
- **Null Cells** - See N K cells.
- **Ontogeny** – The embryonic development of an individual.
- **Paracortex** – T cell rich area of the lymph node between cortex and medulla.
- **Passive Immunization** – Administration of preformed antibody in an individual.
- **Pathogen** – Infection causing organism.
- **Peyer's patches** – Secondary lymphoid organ of small intestine.
- **Phagocytosis** – Process of engulfment of pathogens by phagocytes.
- **Phylogeny** – Evolutionary history of a plant or animal species.
- **Primary Immune Response** – Immune response towards the first exposure to antigen.

- **Primary lymphoid organs** – Organs in which B and/or T cells mature like bone marrow and thymus.
- **PUS** – A mixture of dead bacteria, leukocyte and fluid, formed as a result of inflammatory response.
- **Secondary immune response** – A more rapid immune response occurs after a second exposure to same antigen.
- **Secondary lymphoid organ** – Organs where T cells and B cells proliferate and get activated such as lymph nodes.
- **Serum** – Yellow residual fluid derived from clotted blood.
- **Spleen** – Largest secondary lymphoid organ.
- **Stem cells** – Cells that can give rise to many differentiated cell lines.
- **T cells** – Thymus derived lymphocytes involved in cell mediated immune response.
- **Vaccine** – Preparation of inactivated pathogen used to induce immunity in the host.
- **Vaccination** – Administration of vaccine into host body.

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## 8.8 Self Learning Exercise

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### Section A (Very short Answer type)

1. The raised body temperature prevents:
  - (a) Entry of virus in host
  - (b) Replication of virus in the host
  - (c) Proliferation of virus in the host.
  - (d) Establishment of virus in the host.
2. Which of the following is not a chemical barrier ?
 

(a) Stomach acid	(b) Interferon
(b) Lysozyme	(d) Mucous
3. Which of the following is predominant leukocyte ?
 

(a) Eosinophil cell	(b) Basophil cell
(b) Neutrophil cell	(d) CD 3 cell

4. Bursa of Fabricius in chicks is found:  
(a) Above kidney                      (b) Near liver lobes  
(b) Near Cloaca                      (d) Around spleen
5. Which of the following is antigen presenting cell?  
(a) CD 8 cell                      (b) CD 4 Cell  
(b) B Cell                      (d) NK Cell
6. The dendritic cells are found in:  
(a) Epidermal cells                      (b) Endodermal cells  
(b) Liver cells                      (d) Osteocytes
7. Scientist who discovered the phagocytosis –  
(a) William Ibrahim                      (b) Benjamin  
(c) Elie Metschnikoff                      (d) Leuckart
8. Kupffer cells are found in:  
(a) Hepatocytes                      (b) Ostiocytes  
(c) Chondreocytes                      (d) Plasmocytes
9. Which of the following are active in innate immunity:  
(a) CD 4 cells                      (b) CD 8 Cells  
(b) B cells                      (d) N K Cells

#### **Section B (Short Answer Type)**

1. Differentiate between innate and acquired immunity.
2. Discuss the role of NK cells in immunity.
3. Write a short note on Bursa of fabricious.
4. Describe the structure and function of thymus.
5. Write a note on Payer's Patches.
6. Give a short note on mechanical barriers.
7. Elaborate haematopoietic stem cells.
8. Describe the significant of lymphoid organs.

#### **Section C (Long Answer Type)**

1. Describe the mechanism of innate immune responses.
2. Write a detail note on ontogeny of immune system.

3. What do you understand by lymphoid organs. Give a detail account of primary lymphoid organs.
4. Describe the different cells of immunity.
5. Write a note on – B cell, T cells, macrophages, NK Cells.

Answer key of section A

1(b) 2(b) 3(c) 4(c) 5(c) 6(a) 7(c) 8(a) 9(d)

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## **8.9 References**

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- Text book of Microbiology by R. Ananthanarayan and Jayaram Paniker
- The Elements of Immunology by Fahim Halim Khan
- Immunology by S S Lal and S Kumar
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## Unit - 9

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# Nature of immune responses; Nature of antigens and super antigens; Factors influencing immunogenicity; Epitopes and haptens

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### Structure of unit:

- 9.0 Objectives
- 9.1 Introduction
- 9.2 Innate and Acquired Immunity
- 9.3 Modes of Immunization
- 9.4 Characteristics of the Immune Response
- 9.5 Cells Involved In the Acquired Immune Response
- 9.6 Clonal Selection Theory
- 9.7 Humoral and Cell-Mediated Immunity
- 9.8 Elements of Innate Immunity
- 9.9 Elements of Acquired Immunity
- 9.10 Antigen
- 9.11 Requirements for Immunogenicity:
- 9.12 Primary and Secondary Immune Responses:
- 9.13 Antigenicity and Antigen-Binding Site:
- 9.14 Epitopes Recognized By B And T Cells:
- 9.15 Major Classes of Antigens:
- 9.16 Adjuvants:
- 9.17 Benefits of Immunology
- 9.18 Damaging Effects of the Immune Response
- 9.19 Regulation of the Immune Response
- 9.20 The Future of Immunology

- 9.21 Summary
  - 9.22 Glossary
  - 9.23 Self-Learning Exercise
  - 9.24 References
- 

## 10.0 Objectives

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After going through this unit you will be able to understand:

- Innate and acquired immunity
  - Mode of immunization
  - Characteristics of immune responses
  - Clonal selection theory
  - Cells and organs involved in innate and acquired immunity
  - Antigen, epitopes and haptens.
- 

### 9.1 Introduction

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The Latin *immunis*, meaning *free from burden*, has provided the English term immunity. In biology, the burden is disease – caused by a variety of viruses, fungi, bacteria, protozoa, worms and toxins – and physiological role of the immune system is to keep it at bay.

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### 9.2 Innate and Acquired Immunity

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The term immunity refers to all the mechanisms used by the body as protection against environmental agents that are foreign to the body. These agents may be microorganisms or their products, foods, chemicals, drugs, pollen, or animal hair and dander. Immunity may be innate or acquired.

**9.2.1 Innate Immunity-** Innate immunity is conferred by all those elements with which an individual is born and which are always present and available at very short notice to protect the individual from challenges by “foreign” invaders. These elements include body surfaces and internal components, such as the skin, the mucous membranes, and the cough reflex, which present effective barriers against invasion by many microorganisms.

Numerous internal components are also features of innate immunity: fever, interferons and other substances released by leukocytes as well as a variety of

serum proteins such as -lysin, the enzyme lysozyme, polyamines and the kinins, among others. All of these elements either affect pathogenic invaders directly or enhance the effectiveness of host reactions to them. Other internal elements of innate immunity include phagocytic cells such as granulocytes, macrophages and microglial cells of the central nervous system, which participate in the destruction and elimination of foreign material that has penetrated the physical and chemical barriers.

**9.2.2 Acquired Immunity-** Acquired immunity is more specialized than innate immunity and it supplements the protection provided by innate immunity. Acquired immunity came into play relatively late, in evolutionary terms, and is present only in vertebrates.

Although an individual is born with the capacity to mount an immune response to a foreign invader, immunity is acquired by contact with the invader and is specific to that invader only, known as *acquired immunity*. The initial contact with the foreign agent (immunization) triggers a chain of events that leads to the activation of certain cells (lymphocytes) and the synthesis of proteins, some of which exhibit specific reactivity against the foreign agent. By this process, the individual acquires the immunity to withstand and resist a subsequent attack by, or exposure to, the same offending agent.

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## 9.3 Modes of Immunization

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**9.3.1 Active Immunization** Active immunization, involves the inoculation of immunogenic material to induce an immune response (and therefore immunological memory) in the recipient. Active immunity takes longer to develop than passive, but also lasts much longer, and may often be life-long.

Various forms of antigenic material may serve as a vaccine:

i) Killed organisms. The viruses which cause rabies, influenza and polio can be collected, killed by treatment with heat or chemicals, and used as effective vaccinating agents. The bacteria responsible for cholera, whooping cough (pertussis) and typhoid fever can be used in the same manner.

ii) Attenuated organisms. Live viruses, but in a weakened or "attenuated" form, provide effective vaccination for measles, mumps and polio, and more recently for influenza. Attenuated bacterial vaccines also exist, typified by those for anthrax and for tuberculosis (BCG, "Bacille de Calmette-Guerin", an attenuated form of the organism which causes bovine tuberculosis). The advantage of

attenuated organism over killed ones is that they can set up active infections and provide more effective stimulation of protective immune responses.

iii) Toxoids. In the case of diphtheria and tetanus infections, the real danger in the disease comes not from the presence of the organisms themselves but from the potent toxins which they produce. Effective immunity can be induced by immunization with chemically modified toxins, or toxoids, which are no longer toxic but still highly immunogenic (and, of course, cross-reactive with the native toxins).

iv) Purified antigens ("subunit vaccines"). Vaccines for meningococcus (*Neisseria meningitidis*), pneumococcus (*Streptococcus pneumoniae*) and the Hepatitis B virus each consist of purified antigens from these organisms, polysaccharide for the first two and protein for the last. In those cases where effective antigens can be identified and purified which which can stimulate stronger responses showing class switching and greater memory, and are effective in younger children.

v) "Naked" DNA. Direct inoculation of DNA encoding a protein results in a strong and long-lived immune response to that protein, both humoral and cell mediated. It is thought that the DNA transfect local APCs resulting in expression of the encoded protein in the context of both MHC Class I and Class II.

### **9.3.2 Passive Immunization**

Injection of antibody to a pathogen can provide very rapid, although short-lived, resistance to infection, and is referred to as passive immunization. Passive immunization is generally used when there is no time to wait for the development of active immunity or when no effective active vaccine exists.

### **9.3.3 Adoptive Immunity**

Adoptive immunity illustrated by the transfer of immune reactivity to non-immune (and/or irradiated) recipients using immunocompetent cells, typically spleen cells. Obviously, such adoptive transfer of reactivity cannot be carried out in humans due to histocompatibility barriers, and adoptive immunity essentially does not exist in human medicine.

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## **9.4 Characteristics of The Immune Response**

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The acquired immune response has several generalized features that characterize it and serve to distinguish it from other physiologic systems such as circulation, respiration, or reproduction. These features are:

**Specificity:** The ability to discriminate among different molecular entities presented to it and responds only to those uniquely required, rather than making a random, undifferentiated response.

**Adaptiveness:** The ability to respond to previously unseen molecules that may in fact never have existed before on earth.

**Discrimination between “Self” and “Nonself”:** A cardinal feature of the specificity of the immune response is its ability to recognize and respond to molecules that are foreign or “nonself” and avoid making a response to those molecules that are “self”. This distinction and the recognition of foreign antigen are conferred by specialized cells, namely, lymphocytes, which bear on their surface receptors specific for foreign antigen.

**Memory:** A property shared with the nervous system is the ability to recall previous contact with a foreign molecule and respond to it in a “learned” manner, i.e., a more rapid and larger response.

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## 9.5 Cells Involved In the Acquired Immune Response

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A convenient way to define the cell types involved in acquired immunity is to divide the host defence mechanisms into two categories: B-cell responses and T-cell responses. B and T cells are derived from a common lymphoid precursor cell but differentiate along different developmental lines, respectively. In short, B cells develop and mature in the bone marrow, and T cells develop in the bone marrow but undergo critical maturation steps in the thymus.

Antigen-presenting cells (APC), such as macrophages and dendritic cells, constitute the third cell type participating in the acquired immune response. Although these cells do not have antigen-specific receptors themselves, they process and present antigen to the antigen-specific receptors expressed by T cells. The APC express a variety of cell-surface molecules that facilitate their interaction with T cells. Among these, the major histocompatibility complex (MHC) molecules are encoded by a set of polymorphic genes expressed within a population. In clinical settings, MHC molecules determine the success or failure of organ and tissue transplantation.

Other cell types, such as neutrophils and mast cells, also participate in acquired immune responses. In fact, they participate in both innate and acquired immunity. While these cells have no specific antigen recognition properties and can be activated by a variety of substances, collectively termed cytokine, they

are an integral part of the network of cells that participate in host defences and often display potent immune regulatory properties.

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## 9.6 Clonal Selection Theory

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Clonal selection theory proposed and developed by Jerne and Burnet (both Nobel Prize winners) and by Talmage. The essential postulates of this theory are summarized below.

The specificity of the immune response is based on the ability of its components (namely, antigen-specific T and B lymphocytes) to recognize particular foreign molecules (antigens) and respond to them in order to eliminate them. The immune response is capable of recognizing literally thousands of foreign antigens, how is the response to any one accomplished? The theory proposed that:-

1. T and B lymphocytes of myriad specificities exist before there is any contact with the foreign antigen.
2. The lymphocytes participating in the immune response have antigen-specific receptors on their surface membranes. As a consequence of antigen binding to the lymphocyte, the cell is activated and releases various products. In the case of B lymphocytes, the receptors are molecules (antibodies) bearing the same specificity as the antibody that the cell will subsequently produce and secrete (Fig. 1). T cells have complex receptors denoted as T-cell receptors (TcRs). Unlike the B cell, the T-cell products are not the same as their surface receptors, but are other protein molecules that participate in elimination of the antigen.
3. Each lymphocyte carries on its surface receptor molecules of only a single specificity as demonstrated in B cells, and holds true also for T cells.

These three postulates describe the existence of a large repertoire of possible specificities formed by cellular multiplication and differentiation before there is any contact with the foreign substance to which the response is to be made.

The introduction of the foreign antigen then selects from among all the available specificities those with specificity for the antigen enabling binding to occur. Again, the scheme shown for B cells also applies to T cells. However, T cells have receptors that are not antibodies and secrete molecules other than antibodies.

The remaining postulates of the clonal selection theory account for this process of selection by the antigen from among all the available cells in the repertoire.

4. Immunocompetent lymphocytes combine with the foreign antigen, or a portion of it, termed epitope, by virtue of their surface receptors. They are stimulated under appropriate conditions to proliferate and differentiate into clones of cells with the corresponding identical receptors to the particular portion of the antigen, termed antigenic determinant or epitope. With B-cell clones this will lead to the synthesis of monoclonal antibodies having precisely the same specificity. T cells will be similarly "selected" by appropriate antigens or portions thereof. Each selected T cell will be activated to divide and produce clones of the same specificity. Thus, the clonal response to the antigen will be amplified; the cells will release various cytokines, and subsequent exposure to the same antigen would now result in the activation of many cells or clones of that specificity. Instead of synthesizing and releasing antibodies as the B cells do, the T cells synthesize and release cytokines. These cytokines, which are soluble mediators, exert their effect on other cells to grow or become activated and eventually eliminate the antigen. It should be noted that several distinct regions of an antigen (epitopes) can be recognized, several different clones of cells will be stimulated, in the case of B cells, to produce antibody, the sum total of which would represent an antiserum specific for that antigen but made up of antibodies of differing specificity, and in the case of T cells, all the T-cell clones recognizing various epitopes on the same antigen will be activated to perform their function.

A final postulate was added to account for the ability to recognize "self" antigens without making a response:

5. Circulating "self" antigens that reach the developing lymphoid system prior to some undesignated maturational step will serve to shut off those cells that recognize it specifically, and no subsequent immune response will be induced.

This formulation of the immune response had a truly revolutionary effect on the field and changed forever our way of looking at and studying immunology.

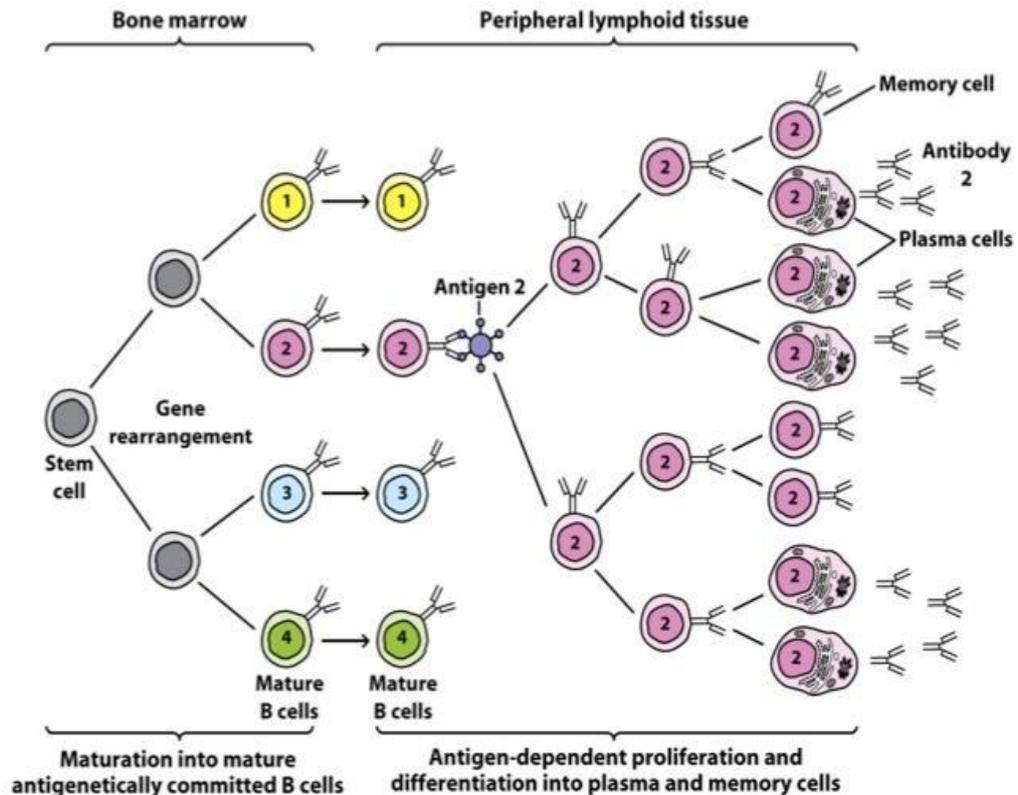


Figure 1:- Clonal selection theory of B cells leading to antibody production.

## 9.7 Humoral and Cell-Mediated Immunity

There are two branches of immune responses that have different sets of participants and different sets of purposes but with one common aim: to eliminate the antigen. These two branched interact with each other and collaborate to achieve the final goal of eliminating antigen. One of these two arms is mediated mainly by B cells and circulating antibodies, hence it is termed **humoral immunity**. The other is mediated by T cells that, do not synthesize antibodies but instead synthesize and release various cytokines that affect other cells. Hence, this kind of immune response is termed **cellular** or **cell-mediated immunity**.

**9.7.1 HUMORAL IMMUNITY-** Humoral immunity is mediated by serum antibodies which are the proteins secreted by the B-cell compartment of the immune response. Antibodies are a heterogenous mixture of serum globulins, all of which share the ability to bind individually to specific antigens. All serum globulins with antibody activity are referred to as **immunoglobulins (Ig)**. All immunoglobulin molecules have common structural features, which enable them to do two things: (1) recognise and bind specifically to a unique structural entity on an antigen, namely, the epitope, and (2) perform a common biologic

function after combining with the antigen. The binding between antigen and antibody is not covalent but depends on many relatively weak forces, such as hydrogen bonds, van der Waals forces, and hydrophobic interactions. Since these forces are weak, successful binding between antigen and antibody depends on a very close fit over a sizable area, much like the contacts between a lock and a key.

Besides, the help provided by T cells in the generation of antibody responses, noncellular components of the innate immune system, collectively termed the **complement system**, play a key role in the functional activity of antibodies when they interact with antigen. The reaction between antigen and antibody serves to activate the system, which consists of a series of serum enzymes. The end result is lysis of the target in the case of microbes such as bacteria or enhanced **phagocytosis** by phagocytic cells. The activation of complement also results in the recruitment of highly **phagocytic polymorphonuclear (PMN)** cells or neutrophils, which are active in innate immunity.

**9.7.2 CELL-MEDIATED IMMUNITY-** In contrast to humoral immune responses that are mediated by antibodies, cell-mediated responses are T-cell mediated. However, this is an oversimplified definition. The effector cell responsible for the elimination of a foreign antigen such as a pathogenic microbe can be an activated T cell expressing a pathogen-specific TCR or a phagocytic cell that gets activated by innate receptors which they express and the cytokines produced by activated T cells. Each T cell, bearing approximately  $10^5$  identical antigen receptors (TCRs), circulates directly to the site of antigen expressed on APC and interacts with these cells in cognate (cell-to-cell) fashion.

There are several major subsets of T cells exist, including helper T cells ( $T_H$  cells), which express molecules called CD4, and cytotoxic T cells ( $T_C$  cells), which express CD8 molecules on their surface. Another population of T cells possessing suppressor activity is the T-regulatory cell ( $T_{reg}$  cells). The functions ascribed to these T cells include the following:

**B-cell help-**  $T_H$  cells cooperate with B cells to enhance the production of antibodies.  $T_H$  cells function by releasing cytokines, which provide various activation signals for the B cells. **Inflammatory effects-** On activation, certain  $T_H$  cells releases cytokines that include the migration and activation of monocytes and macrophages, leading to inflammatory reactions.

**Cytotoxic effects-** Certain T cells, called T-cytotoxic ( $T_C$ ) cells, are able to deliver a lethal hit on contact with their target cells, leading to their death.

**Regulatory effects-** Subsets of Helper T cells ( $T_H1$ ,  $T_H2$ ) have distinct regulatory properties that are mediated by the cytokines they release.

**Cytokine effects-** Cytokines produced by each of the T-cell subsets exert, directly or indirectly, numerous effects on many cells, lymphoid and nonlymphoid.

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## **9.8 Elements of Innate Immunity**

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Innate immunity is carried out by nonspecific physical and chemical barriers, cellular barriers and molecular pattern-based reactions. Here we will describe the major components of innate immunity.

### **9.8.1 Physical and Chemical Barriers of Innate Immunity:**

Most organisms and foreign substances cannot penetrate intact skin but can enter the body if the skin is damaged. Some microorganisms can enter through sebaceous glands and hair follicles. However, the acid pH of sweat and sebaceous secretions and the presence of various fatty acids and hydrolytic enzymes (e.g., lysozymes) all have some antimicrobial effects, which minimize this route of infection. In addition, soluble proteins, including the interferons and certain members of the complement system found in the serum, contribute to nonspecific immunity. Interferons are a group of proteins made by cells in response to virus infection which essentially induce a generalized antiviral state in surrounding cells. Activation of the complement system in response to certain microorganisms results in a controlled enzymatic cascade which targets the membrane of pathogenic organisms and leads to their destruction. An important innate immune mechanism involved in the protection of many areas of the body, including the respiratory and gastrointestinal tracts, is the coverage of surfaces in these areas with mucous. In these areas, the mucous membrane barrier traps microorganisms, which are then swept toward the external openings by ciliated epithelial cells. The hairs in the nostrils and the cough reflex are also helpful in preventing organisms from infecting the respiratory tract. Alcohol consumption, cigarette smoking, and narcotics suppress this entire defense system.

### **9.8.2 Intracellular and Extracellular Killing of Microorganisms:**

Once an invading microorganism has penetrated the various physical and chemical barriers that constitute the first line of defense, it encounters the next

line of defense, which consists of various specialized cells whose purpose is to destroy the invader. These include the polymorphonuclear leukocytes, monocytes, and macrophages each of which is derived from hematopoietic precursor cells. It is important to understand two fundamental cellular activities associated with many members of this group of cells: endocytosis and phagocytosis.

**9.8.2.1 Endocytosis.** Endocytosis is the ingestion by cells of macromolecules present in extracellular fluid. This can occur either by pinocytosis, which involves nonspecific membrane invagination, or by receptor-mediated endocytosis, a process involving the selective binding of macromolecules to specific membrane receptors. In both cases, ingestion of the foreign macromolecules generates endocytic vesicles filled with the foreign material, which then fuse with acidic compartments called endosomes. Endosomes then fuse with lysosomes containing degradative enzymes (e.g., nucleases, lipases, proteases) to reduce the ingested macromolecules to small breakdown products, including nucleotides, sugars, and peptides.

**9.8.2.2 Phagocytosis.** phagocytosis, the ingestion by individual cells of invading foreign particles such as bacteria, is a critical protective mechanism of the immune system. Many microorganisms release substances that attract phagocytic cells. Phagocytosis may be enhanced by a variety of factors that make the foreign particle an easier target. These factors, collectively referred to as opsonins (Greek word meaning "prepare food for"), consist of antibodies and various serum components of complement. After ingestion, the foreign particle is entrapped in a phagocytic vacuole (phagosome), which fuses with lysosomes to form the phagolysosome. The phagolysosome release its powerful enzymes, which digest the particle.

Phagocytes can also damage invading pathogens through the generation of toxic products in a process known as the respiratory burst. Production of these toxic metabolites is induced during phagocytosis of pathogens such as bacteria and is catalyzed by a set of interrelated enzyme pathways. The most important toxic products produced by the respiratory burst are nitric oxide (catalyzed by inducible nitric oxidase synthase), hydrogen peroxide and superoxide anion (catalyzed by phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase], and hypochlorous acid (catalyzed by myeloperoxidase). In addition to being toxic to bacteria, each of these microbicidal products can also damage host cells. Fortunately, a series of protective enzymes produced by phagocytes primarily limits their microbicidal activity to the phagolysosome,

thereby focusing their toxicity on ingested pathogens. These protective enzymes include catalase, which degrades hydrogen peroxide, and superoxide dismutase, which converts the superoxide anion into hydrogen peroxide and oxygen. The absence of or an abnormality in any one of the respiratory burst components results in a form of immunodeficiency that predisposes individuals to repeated infections.

### **9.8.3 Cells Involved in the Innate Immune System:**

Several cell types participate in innate host defense mechanisms. Upon activation (contact with microorganisms) these cells produce and often release biologically active soluble substances, including potent antimicrobial products (e.g., peroxide) and cytokines, which have different effects on the various host cells. They are also involved in the induction of acquired immune responses mediated by B and T cells. Thus, it is important to know cells involved in innate immune system and recognize their important role in acquired immune responses.

**9.8.3.1 Polymorphonuclear (PMN) Leukocytes.** PMN leukocytes are a population of cells also referred to as granulocytes. These include the basophils, mast cells, eosinophils, and neutrophils. Granulocytes are short-lived phagocytic cells that contain enzyme-rich lysosomes, which can facilitate destruction of infectious microorganisms. They also produce peroxide and superoxide radicals, which are toxic to many microorganisms. Some lysosomes also contain bactericidal proteins such as lactoferrin. PMN leukocytes play a major role in protection against infection. Defects in PMN cell function are accompanied by chronic or recurrent infection.

**9.8.3.2 Macrophages.** macrophages are phagocytes derived from blood monocytes. The monocyte is a small, spherical cell with few projections, abundant cytoplasm, little endoplasmic reticulum, and many granules. Following migration of monocytes from the blood to various tissues, they undergo further differentiation into a variety of histologic forms, all of which play a role in phagocytosis, including the following:

- **Kupffer cells**, in the liver; large cells with many cytoplasmic projections.
- **Alveolar macrophages**, in the lung.
- **Splenic macrophages**, in the white pulp of the spleen.

- **Peritoneal macrophages**, free floating in peritoneal fluid.
- **Microglial cells**, in the central nervous tissue.

In general, macrophages have two major functions. One, as their name ("large eater") implies, is to engulf and break down trapped materials into simple amino acids, sugars, and other substances for excretion or reuse, with the aid of the degradative enzymes in their lysosomal granules. Thus these cells play a key role in the removal of bacteria and parasites from the body. The second major function of macrophages is to take up antigens, process them by denaturation or partial digestion, and present the fragments to antigen-specific T cells (the process of antigen presentation).

**9.8.3.3 Dendritic Cells.** Dendritic cells are long-lived and reside in an immature state in most tissues where they recognize and phagocytize pathogens and other antigens. They are found in the skin are called Langerhans cells. These cells are derived from the same hematopoietic precursor cells as monocytes. Direct contact of all dendritic cells with pathogens leads to their maturation and allows them to significantly increase in their antigen presentation capacity. Moreover, mature dendritic cells have the ability to activate naive antigen-specific T cells. These cells are important players in both innate immunity and the initiation of acquired immune responses.

**9.8.3.4 Natural Killer Cells.** Altered features of the membranes of abnormal cells, such as those found on virus-infected or cancer cells, are recognized by natural killer (NK) cells, which are cytotoxic. NK cells probably play a role in the early stages of viral infection or tumorigenesis, before the large numbers of activated cytotoxic T lymphocytes are generated. Histologically, NK cells are large granular lymphocytes. The intracellular granules contain preformed biologically potent molecules that are released when NK cells make contact with target cells. Some of these molecules cause the formation of pores in the membrane of the target cell, leading to its lysis. Other molecules enter the target cell and cause apoptosis (programmed cell death) of the target cell by enhanced fragmentation of its nuclear DNA. Hence, they are able to lyse certain virus-infected cells and tumor cells without prior stimulation.

From these brief descriptions, you can see that each of the cellular components of the innate immune system has diverse roles in the achievement of two common goals: (1) eliminating foreign substances and pathogens from the host and (2) generating antigen-specific acquired immune responses that ultimately give rise to long-term immunity.

#### **9.8.4 Inflammation**

An important function of phagocytic cells is their participation in inflammatory reactions. Inflammation, a major component of the body's defense mechanisms, is a physiologic process typically initiated by tissue damage from endogenous factors such as tissue necrosis or bone fracture as well as exogenous factors. Exogenous factors include mechanical injury (cuts), physical injury (burns), chemical injury (exposure to corrosive chemicals), immunologic injury (hypersensitivity reaction), and biologic injury (infections caused by pathogenic microorganisms). The clinical signs of inflammation are pain, redness, and heat. These can be explained by increased blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids that move from the blood vessels to surrounding tissue, and cellular influx.

#### **9.8.5 Fever**

Although fever, an elevation in body temperature, is one of the most common manifestations of infection and inflammation, there is still limited information about the significance of fever in the course of infection in mammals. Fever is caused by many bacterial products, most notably the endotoxins of Gram-negative bacteria. Fever results when cytokines called endogenous pyrogens are produced by innate immune cells (monocytes and macrophages) in response to the presence of endotoxins.

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### **9.9 Elements of Acquired Immunity**

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In contrast to innate immunity, which is an attribute of every living organism, acquired immunity is a more specialized form of immunity. The various elements that participate in innate immunity do not exhibit specificity against the foreign agents they encounter whereas acquired immunity always exhibits such specificity. The first encounter with a foreign substance that has penetrated the body triggers a chain of events that induces an immune response with specificity against that foreign substance. Acquired immunity develops only after exposure to, or immunization with, a given substance.

#### **9.9.1 Cells and organs involved in acquired immunity**

There are two major types of cells that participate in acquired immunity: B lymphocytes (so named because they originate in the bone marrow), and T lymphocytes (named for their differentiation in the thymus). B lymphocytes and T lymphocytes are responsible for the specificity exhibited by the acquired immune response. B lymphocytes synthesize and secrete into the bloodstream

antibodies with specificity against the foreign substance. The T lymphocytes, which also exhibit specificity against the foreign substance by virtue of their receptors, do not make antibodies, but they themselves seek out the invader to produce their effects. T lymphocytes also interact with B cells and "help" the later make antibodies; they activate macrophages, and they have a central role in the development and regulation of acquired immunity.

### **9.9.2 Lymphatic Organs:**

The lymphatic organs are those organs in which lymphocyte maturation, differentiation, and proliferation take place. Lymphocytes are derived from the pluripotential hematopoietic bone marrow stem cells, which give rise to all blood cells. The erythroid and myeloid cells, which differentiate into erythrocytes and granulocytes, are derived from these stem cell progenitors. Lymphoid progenitor cells differentiate into lymphocytes.

The lymphoid organs are generally divided into two categories. The primary or central lymphoid organs are those in which the maturation of T and B lymphocytes into antigen-recognizing lymphocytes occurs. Developing T and B cells acquire their antigen-specific receptors in primary lymphoid organs. The secondary lymphoid organs are those organs in which antigen-driven proliferation and differentiation take place.

**9.9.2.1 PRIMARY LYMPHOID ORGANS.** There are two major primary lymphoid organs, one in which the T cells develop and the other in which the B cells develop.

**The Thymus Gland.** Progenitor cells from the bone marrow migrate to the primary lymphoid organ, the thymus gland, where they differentiate into T lymphocytes. The thymus gland is a bilobed structure, derived from the endoderm of the third and fourth pharyngeal pouches. During fetal development, the size of the thymus increase. The growth continues until puberty. Thereafter, the thymus undergoes atrophy with aging.

The thymus is a lymphoepithelial organ and consists of epithelial cells, organized into cortical and medullary areas that are infiltrated with lymphoid cells (thymocytes). The cortex is densely populated with lymphocytes of various sizes, most of which are immature. T lymphocytes mature in the cortex and migrate to the medulla, which they then leave to enter the peripheral blood circulation, through which they are transported to the secondary lymphoid organs. It is in these secondary lymphoid organs that the T cells encounter and respond to foreign antigens.

Maturation of the T lymphocyte involves the commitment of a given T cells to recognize and respond to a given determinant or epitope of a foreign antigen. This recognition is achieved by a specific receptor on the T cell, which is acquired during differentiation in the thymus. Mature T lymphocytes in the medulla are capable of responding to foreign antigens in the same way that they would respond in the secondary lymphoid organs. However, the thymus is considered to be a primary lymphoid organ, where antigen-driven proliferation and differentiation do not take place. It is of interest that only 5-10% of maturing lymphocytes survive and eventually leave the thymus; 90-95% of all thymocytes die in the thymus. It is clear that the lymphocytes that die have developed specificity to "self" structures or have failed to make functional receptors and therefore, eliminated. The lymphocytes that survive develop specificity against foreign antigens.

**Bursa of Fabricius and the Bone Marrow.** A primary lymphoid organ was first discovered in birds. In birds, B cells undergo maturation in the bursa of Fabricius. This organ, situated near the cloaca, consists of lymphoid centers that contain epithelial cells and lymphocytes. Unlike the lymphocytes in the thymus, these lymphocytes consist solely of antibody-producing B cells. Mammals do not have a bursa of Fabricius.

**9.9.2.2 Secondary Lymphoid Organs.** The secondary lymphoid organs consist of certain structures in which mature, antigen-committed lymphocytes are stimulated by antigen to undergo further division and differentiation. The major secondary lymphoid organs are the spleen and the lymph nodes. In addition, tonsils, appendix, clusters of lymphocytes distributed in the lining of the small intestine (peyer's patches), as well as lymphoid aggregates spread throughout mucosal tissue are considered secondary lymphoid organs. In these secondary lymphoid organs, mature lymphocytes interact with antigen and differentiate to synthesize specific antibodies. The secondary lymphoid organs have two major functions: they are highly efficient in trapping and concentrating foreign substances, and they are the main sites of production of antibodies and the generation of antigen-specific T lymphocytes.

**The Spleen.** The spleen is the largest of the secondary lymphoid organs. It is highly efficient in trapping and concentrating foreign substances carried in the blood. It is the major organ in the body in which antibodies are synthesized and from which they are released into the circulation. The spleen is composed of white pulp, rich in lymphoid cells, and red pulp, which contains many sinuses

as well as large quantities of erythrocytes and macrophages, some lymphocytes, and a few other cells.

The areas of white pulp are located mainly around small arterioles, the peripheral regions of which are rich in T cells, with B cells present mainly in germinal centers. Approximately 50% of spleen cells are B lymphocytes; 30-40% are T lymphocytes. Following antigenic stimulation, the germinal centres contain large numbers of B cells and plasma cells. These cells synthesize and release antibodies.

**Lymph Nodes.** Lymph nodes are small ovoid structure (normally less than one cm in diameter) found in various regions throughout the body. They are close to major junctions of the lymphatic channels, which are connected to the thoracic duct. The thoracic duct transports lymph and lymphocytes to the vena cava, the vessel that carries blood to the right side of the heart from where it is redistributed throughout the body.

Lymph nodes are composed of a medulla with many sinuses and a cortex, which is surrounded by a capsule of connective tissue. The cortical region contains primary lymphocytic follicles. On antigenic stimulation, these structures form germinal centers that contain dense populations of lymphocytes-mostly B cells-that are undergoing mitosis. The deep cortical area of paracortical region contains T cells and macrophages. The macrophages trap, process, and present antigen to the T cells that have specificity against that antigen, events that result in activation of the T cells. The medullary area of the lymph node contains antibody-secreting plasma cells that have travelled from the cortex to the medulla via lymphatic vessels.

Lymph nodes are highly efficient in trapping antigen that enters through the afferent lymphatic vessels. In the node, the antigen interacts with macrophages, T cells, and B cells, and that interaction brings about an immune response, manifested by the generation of antibodies and antigen-specific T cells. Lymph, antibodies, and cells leave the lymph node through the efferent lymphatic vessel, which is just below the medullary region.

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## 9.10 Antigen

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Immune responses arise as a result of exposure to foreign stimuli. The compound that evokes the response is referred to as either antigen or immunogen. The distinction between these terms is functional. An antigen is any agent capable of binding specifically to components of the immune system, such as the BCR on B lymphocytes and soluble antibodies. By contrast, an

immunogen is any agent capable of inducing an immune response and is therefore immunogenic. There are many compounds that are incapable of inducing an immune response but are capable of binding with components of the immune system. Thus all immunogens are antigens, but not all antigens are immunogens. This difference becomes obvious in the case of low-molecular-weight compounds, a group of substances that includes many antibiotics and drugs. By themselves, each of these compounds is incapable of inducing an immune response, but when coupled with a much larger entity, such as a protein, the resultant conjugate induces an immune response that is directed against various parts of the conjugate, including the low-molecular-weight compound. This low-molecular-weight compound is referred to as a hapten whereas the high-molecular compound to which the hapten is conjugated is referred to as a carrier.

Thus a **hapten** is a compound that, by itself, is incapable of inducing an immune response; however, an immune response can be induced against the hapten when it is conjugated to a carrier.

Immune responses have been demonstrated against all the known biochemical families of compounds, including carbohydrates, lipids, proteins, and nucleic acids. Similarly, immune responses to drugs, antibiotics, food additives, cosmetics, and small synthetic peptides can also be induced, but only when these are coupled to a carrier.

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## 9.11 Requirements for Immunogenicity

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A substance must possess the following characteristics to be immunogenic: (1) foreignness, (2) high molecular weight, (3) chemical complexity, and in most cases (4) degradability and interaction with the host's MHC.

**Foreignness:-** Animals normally do not respond immunologically to self. Thus, for example, if a rabbit is injected with its own serum albumin, it will not mount an immune response; it recognizes the albumin as self. By contrast, if rabbit serum albumin is injected into a guinea pig, the guinea pig recognizes the rabbit serum albumin as foreign and mounts an immune response because it recognizes the substance as foreign. Thus, the first requirement for a compound to be immunogenic is foreignness. The more foreign the substance, the more immunogenic it is.

**High Molecular Weight:-** The second requirement for immunogenicity is a certain minimum molecular weight. In general, small compounds that have a

molecular weight less than 1000 Da (e.g., penicillin, progesterone, aspirin) are not immunogenic; those of molecular weight between 1000 and 6000 Da (e.g., insulin, ACTH) may or may not be immunogenic; and those of molecular weight greater than 6000 Da (e.g., albumin, tetanus toxin) are generally immunogenic.

**Chemical Complexity:-** The third characteristic of immunogenicity is a certain degree of physicochemical complexity. For example, simple molecules such as homopolymers of amino acids (e.g., a polymer of lysine with a molecular weight of 30,000 Da) are seldom good immunogens. Similarly, even though it has a molecular weight of 50,000 Da, the homopolymer of poly- $\gamma$ -D-glutamic acid (the capsular material of *Bacillus anthracis*) is not immunogenic. These compounds, although of high molecular weight, are not sufficiently chemically complex to be immunogenic. However, if complexity is increased by attaching various moieties—such as dinitrophenol or other low-molecular-weight compounds—that, by themselves, are not immunogenic, the entire macromolecule becomes immunogenic.

**Degradability:-** For antigens that activate T cells to stimulate immune responses, interactions with MHC molecules expressed on APC must occur. Before they can express antigenic epitopes (small fragments of the immunogen) on their surface, APC must first degrade the antigen through a process known as antigen processing (enzymatic degradation of antigen). Once degraded and noncovalently bound to MHC, epitopes stimulate the activation and clonal expansion of antigen-specific effector T cells.

In general, a substance must have all four of the characteristics described in order to be immunogenic: it must be foreign to the individual in whom it is administered, have a relatively high molecular weight, possess a certain degree of chemical complexity, and be degradable.

**Haptens:** Substances called haptens fail to induce immune responses in their native form because of their low molecular weight and their chemical simplicity. These compounds are not immunogenic unless they are conjugated to high-molecular-weight, physiochemically complex carriers. Thus an immune response can be evoked to thousands of chemical compounds, those of high molecular weight and those of low molecular weight, provided the latter is conjugated to high-molecular-weight complex carriers.

**Further Requirements for Immunogenicity:**

Several other factors play roles in determining whether a substance is immunogenic. The genetic makeup (genotype) of the individual plays an important role in determining whether a given substance will stimulate an immune response. Genetic control of immune responsiveness is largely controlled by genes mapping within the MHC. Another factor that plays a crucial role in immunogenicity relates to the individual's B- and T-cell repertoires. Acquired immune responses are triggered following the binding of antigenic epitopes to antigen-specific receptors on B and T lymphocytes. If an individual lacks a particular clone of lymphocytes bearing the identical antigen-specific receptor needed to respond to the stimulus, an immune response to that antigenic epitopes will not take place.

Insufficient doses of antigen may not stimulate an immune response for one of two reasons: (1) The amount administered fails to activate enough lymphocytes or (2) such a dose renders the responding cells unresponsive. The number of doses administered also affects the outcome of the immune response generated.

Finally, the route of administration can affect the outcome of the immunization strategy because it determines which organs and cell populations will be involved in the response.

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## **9.12 Primary and Secondary Immune Responses**

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The first exposure of an individual to an immunogen is referred to as the primary immunization, which generates a primary response. Many events take place during this primary immunization. Cells process antigen, triggering antigen-specific lymphocytes to proliferate and differentiate. T-lymphocyte subsets interact with other subsets and induce the latter to differentiate into T lymphocytes with specialized functions. T lymphocytes also interact with B lymphocytes, inducing them to synthesize and secrete antibodies.

A second exposure to the same immunogen results in a secondary response. This may occur after the response to the first immune event has leveled off or has totally subsided. The secondary response differs from the primary response in many respects. Most notably and biologically relevant is the much quicker onset and the much higher magnitude of the response. The secondary response is also called the memory or anamnestic response, and the B and T lymphocytes that participate in the memory response are termed memory cells.

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## **9.13 Antigenicity and Antigen-Binding Site**

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An immune response induced by an antigen generates antibodies or lymphocytes that react specifically with the antigen. The antigen-binding site of an antibody or a receptor on a lymphocyte has a unique structure that allows a complementary fit to some structural aspect of the specific antigen. The portion of the immunoglobulin that specifically binds to the antigenic determinant or epitope is concentrated in several hypervariable regions of the molecule, which form the complementarity-determining region (CDR).

Various studies indicate that the size of an epitope that combines with the CDR on a given antibody is approximately equivalent to five to seven amino acids. These dimensions were calculated from experiments that involved the binding of antibodies to polysaccharides and to peptide epitopes. Such dimensions would also be expected to correspond roughly to the size of the complementary antibody-combining site, termed the paratope, and this expectation has been confirmed by X-ray crystallography. The small size of an epitope (peptide) that binds to a specific TCR (peptides with 8-12 amino acids) is made functionally larger, since it is noncovalently associated with MHC proteins of the APC. This bimolecular epitope-MHC complex then binds to the TCR, forming a trimolecular complex (TCR-epitope-MHC).

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### **9.14 Epitopes Recognized By B and T Cells**

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According to a large body of evidence, the properties of many epitopes recognized by B cells differ from those recognized by T cells. In general, membrane-bound antibody present on B cells recognizes and binds free antigen in solution. Thus, these epitopes are typically on the outside of the molecule, accessible for interaction with the B-cell receptor. Terminal side chains of polysaccharides and hydrophilic portions of protein molecules generally constitute B-cell epitopes. B-cell epitopes may also form as a result of the folded conformation of molecules. In such epitopes, called conformational or discontinuous epitopes, noncontiguous residues along a polypeptide chain are brought together by the folded conformation of the protein.

In contrast to B cells, T cells are unable to bind soluble antigen. The interaction of an epitope with the TCR requires APC to process the antigen; after enzymatic degradation takes place, the resulting small peptides associate with MHC. Thus, T-cell epitopes can only be continuous of linear since they are composed of a single segment of a polypeptide chain. Illustrate the structural organization of a class I MHC bound to an antigenic peptide. Generally such processed epitopes are internal denatured linear hydrophobic areas of proteins.

Polysaccharides, on the other hand, are not processed by APC and are not known to bind or activate T cells. Thus polysaccharides contain epitopes recognized solely by B cells, but protein epitopes can be recognized by both B and T cells.

Thus, they may consist of a single epitope (haptens) or have varying numbers of the same epitope on the same molecule (e.g., polysaccharides). The most common antigens (proteins) have varying numbers of different epitopes on the same molecule.

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## 9.15 Major Classes of Antigens

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The following major chemical families may be antigenic:

- 1. Carbohydrates (polysaccharides).** Polysaccharides can induce antibody responses in the absence of T cell help. Polysaccharides that form part of more complex molecules (glycoproteins) will elicit T-cell dependent immune responses, part of which is directed specifically against the polysaccharide moiety of the molecule. An immune response, consisting primarily of antibodies, can be induced against many kinds of polysaccharide molecules, such as components of microorganisms (e.g., teichoic acid of Gram-negative bacteria).
- 2. Lipids.** Lipids are rarely immunogenic, but an immune response to lipids may be induced if the lipids are conjugated to protein carriers. Thus, in a sense, lipids may be regarded as haptens. Immune responses to glycolipids and to sphingolipids have also been demonstrated.
- 3. Nucleic acids.** Nucleic acids are poor immunogens by themselves, but they become immunogenic when they are conjugated to protein carriers. DNA, in its native helical state, is usually nonimmunogenic in normal animals. However, immune responses to nucleic acids have been reported in many instances.
- 4. Proteins.** Because virtually all proteins are immunogenic, the most common immune responses are those to proteins. The greater the degree of complexity of the protein, the more vigorous the immune response to that protein will be. Because of their size and complexity, proteins contain multiple epitopes.

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## 9.16 Adjuvants

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To enhance the immune response to a given immunogen, various additives or vehicles are often used. An adjuvant is a substance that, when mixed with an immunogen, enhances the immune response against the immunogen. It is important to distinguish between a carrier for a hapten and an adjuvant. A hapten will become immunogenic when conjugated covalently to a carrier; it will not become immunogenic if mixed with an adjuvant. Thus, an adjuvant enhances the immune response to immunogens but does not confer immunogenicity on haptens. Interest in the identification of adjuvants for use with vaccines is growing because many new vaccine candidates lack sufficient immunogenicity. This is particularly true of peptide-based vaccines. Adjuvant mechanisms include (1) increasing the biologic or immunologic half-life of vaccine antigens, (2) increasing the production of local inflammatory cytokines, and (3) improving antigen delivery and antigen processing and presentation by APC, especially the dendritic cells. Empirically, it has been found that adjuvants containing microbial components (e.g., mycobacterial extracts) are the best adjuvants. Pathogen components induce macrophages and dendritic cells to express costimulatory molecules and to secrete cytokines.

While many adjuvants have been developed in animal models and tested experimentally in humans, only one has been accepted for routine vaccination. Currently, aluminum hydroxide and aluminum phosphate (alum) are the only adjuvants approved for human vaccines administered to normal individuals in the United States. Many adjuvants have been used in experimental animals. One commonly used adjuvant, Freund's complete adjuvant, consists of killed *Mycobacterium tuberculosis* or *Mycobacterium butyricum* suspended in oil, which is then emulsified with an aqueous antigen solution. The oil-emulsified state of the adjuvant-antigen mixture allows the antigen to be released slowly and continuously, helping sustain the recipient's exposure to the immunogen. Other microorganisms used as adjuvants are bacille Calmette-Guerin (BCG) (an attenuated mycobacterium), *Corynebacterium parvum*, and *Bordetella pertussis*.

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## **9.17 Benefits of Immunology**

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So far we have discussed the theoretical aspects of immunology; its practical applications are of important for survival and must be part of the education of students. The field of immunology has been in the public limelight since the late 1960s, when successful transplantation of the human kidney was achieved. More recently, the spectacular transplantation of the human heart and other major organs, such as the liver, has been the focus of much publicity. Public

interest in immunology was intensified by the potential application of the immune response to the detection and management of cancer, and in the 1980s the general public became familiar with some aspects of immunology because of the alarming spread of acquired immune deficiency syndrome (AIDS).

Of great impact to humanity is the success of immunology in the prevention and virtual elimination of many infectious diseases. Vaccination against infectious diseases has been an effective form of prophylaxis. Immunoprophylaxis against the virus that causes poliomyelitis has reduced this dreadful disease to insignificant importance in many parts of the world and, for the first time, a previously widespread disease, smallpox, has been eliminated from the face of the earth. Recent developments in immunology hold the promise of immunoprophylaxis against malaria and several other parasitic diseases that plague many parts of the world and affect billions of people. Vaccination against diseases of domestic animals promises to increase the production of meat in developing countries, while vaccination against various substances that play roles in the reproductive processes in mammals offers the possibility of long-term contraception in humans and companion animals such as cats and dogs.

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## **9.18 Damaging Effects of The Immune Response**

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Acquired immunity directed against a foreign material has as its ultimate goal the elimination of the invading substance. In the process some tissue damage may occur as the result of the accumulation of components with nonspecific effects. This damage is generally temporary. As soon as the invader is eliminated, the situation at that site reverts to normal.

There are instances in which the power of the immune response, although directed against innocuous foreign substances such as some medications, inhaled pollen particles, or substance deposited by insect bites, produces a response that known collectively as hypersensitivity reactions or allergic reactions.

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## **9.19 Regulation of the Immune Response**

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Complexity of the immune response and its potential for inducing damage must operate under carefully regulated conditions, as does any other physiologic system. These controls are multiple and include feedback inhibition by soluble products as well as cell-cell interactions of many types that may either heighten or reduce the response. The net result is to maintain a state of homeostasis such

that when the system is perturbed by a foreign invader, enough response is generated to control the invader and then the system returns to equilibrium; in other words, the immune response is shut down. However, its memory of that particular invader is retained so that a more rapid and heightened response will occur should the invader return.

An important form of regulation concerns the prevention of immune responses against "self" antigens. For various reasons, this regulation may be defective and an immune response against "self" is mounted. This type of immune response is termed autoimmunity, and is the cause of diseases such as some forms of arthritis, thyroiditis, and diabetes that are very difficult to treat.

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## **9.20 The Future of Immunology**

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A peek into the world of the future for the student of immunology suggests many exciting areas in which the application of molecular biologic technique promises significant dividends. As examples, we may take vaccine development and control of the immune response. In the past, rather than the laborious, empirical search for an attenuated virus or bacterium for use in immunization, it is now possible to obtain the nucleotide sequence of the DNA that encodes the component of the invading organism that accounts for the protective immune response. These sequences about the segment of the encoded protein most likely to be responsible for inducing immunity. Such segments can be readily synthesized and tested for use as a vaccine.

Another area of great promise is the characterization and synthesis of various cytokines- substances that enhance and control the activation of various cells associated with the immune response as well as with other functions of the body. Again, the techniques of gene isolation, clonal reproduction, and biosynthesis have contributed to rapid progress. Powerful and important modulators have been synthesized by the methods of recombinant DNA technology and are being tested for their therapeutic efficacy in a variety of diseases, including many different cancers.

Finally, and probably one of the most exciting areas, is the technology to genetically engineer various cells and even whole animals such as mice that lack one or more specific trait ("gene knockout") or carry a specific trait (transgenic). This allows the immunologist to study the effect of these traits on the immune system and on the body as a whole with the aim of understanding the intricate regulation, expression, and function of the immune response and with the ultimate aim of controlling the trait to the benefit of the individual.

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## 9.21 Summary

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1. Immunity refers to all the mechanisms used by the body as protection against environmental agents that are foreign to the body. There are two types of immunity: (1) Innate, and (2) acquired.
2. Innate immunity is present from birth and its role is to provide a first line of defense against pathogen. Whereas, when an infectious organism is not eliminated by innate immune system, acquired immunity can prevent reinfection with the same organisms with the generation of antigen-specific lymphocytes and memory cells.
3. Specificity, adaptiveness, Discrimination between “Self” and “Nonself”: and memory are the generalized features of The acquired immune responses.
4. There are two branches of immune responses with different participants and different purposes but with one aim: to eliminate the antigen. One is mediated mainly by B cells and circulating antibodies, is termed humoral immunity. The other is mediated by T cells, do not synthesize antibodies but instead synthesize and release various cytokines that affect other cells, is termed cell-mediated immunity.
5. Many non-cellular and cellular elements participate in innate immunity; these include various physical barriers, chemical barriers, pattern recognition receptors, phagocytes, Nk cells, etc.
6. Two major types of cells participate in acquired immunity: (1) B-lymphocytes and (2) T-lymphocytes.
7. Precursor cells of the B and T lineages are found in the bone marrow- a primary lymphoid organ. B-lymphocytes fully differentiate in bone marrow to become mature B-cells. Whereas, T cells differentiate in the thymus to become functional cells.
8. B lymphocytes synthesize and secrete antibodies. T lymphocytes participate in cell mediated immunity and help B cells make antibodies by providing them soluble growth and differentiation factors needed for B cell activation.
9. The compound that evokes the response is referred to as either antigen or immunogen.

Immunogenicity is the capacity of a compound to induce an immune response. Immunogenicity requires that a substance must possess the following characteristics: (1) foreignness, (2) high molecular weight, (3) chemical complexity, and in most cases (4) degradability and interaction with the host's MHC.

10. Hapten is a compound that, by itself, is incapable of inducing an immune response but an immune response can be induced when it is conjugated to a carrier.
11. Major classes of antigens are carbohydrates, lipids, nucleic acids and proteins. The smallest unit of antigen that is capable of binding with antibodies is called epitope. Adjuvants are substances that can accelerate, prolong and enhance the quality of specific immune responses.

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## 9.22 Glossary

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- **Adjuvant:** A substance, given with antigen, that enhances the response to the injected antigen.
- **Carrier:** A large immunogenic molecule or particle to which a hapten or other nonimmunogenic, epitope-bearing molecules are attached, allowing them to become immunogenic.
- **Cell-mediated immunity:** Immune reaction mediated by T cells.
- **Clonal selection theory:** The concept that specificity and diversity of an immune responses are the result of selection by antigen of specifically reactive clones from a large repertoire of performed lymphocytes, each with individual specificities.
- **Complement:** A series of serum proteins involved in the mediation of immune reactions. The complement cascade is triggered classically by the interaction of antibody with specific antigen.
- **Cytokines:** soluble substances secreted by cells, which have a variety of effects on other cells.
- **Epitope:** An alternative term for antigenic determinant.
- **Hapten:** A compound usually of low molecular weight, that is not itself immunogenic but that. After conjugation to a carrier proteins or cells,

becomes immunogenic and induces antibody, which can bind the hapten alone in the absence of carrier.

- **Humoral immunity:** Any immune reaction that can be transferred with immune serum.
- **Immunogen:** A substance capable of inducing an immune response.
- **Immunoglobulin:** A general term for all antibody molecules.
- **Interferon:** A group of proteins having antiviral activity and capable of enhancing and modify the immune response.
- **Macrophage:** A large phagocytic cell of mononuclear series.
- **Major histocompatibility complex:** A cluster of genes encoding polymorphic cell-surface molecules that are involved in interactions with T cells. These molecules also play major role in transplantation rejection.
- **Phagocytosis:** The engulfment of a particle or a microorganism by leukocytes.
- **Pinocytosis:** Ingestion of liquid or very small particles by vesicle formation in a cell.

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## 9.23 Self-Learning Exercises

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### Section -A (Very Short Answer Type):

1. Acquired immunity present only in.....  
B and T cells are derived from a common.....
3. B cells develop and mature in the.....
4. Clonal selection theory proposed and developed by .....
5. Humoral immunity is mediated by .....
6. All serum globulins with antibody activity are referred to as interferons and certain members of the complement system found in the.....
7. macrophages are phagocytes derived from.....
8. The clinical signs of inflammation are.....
9. In birds, B cells undergo maturation in the.....
10. The major secondary lymphoid organs are the.....

11. The compound that evokes the response is referred to as .....
12. The initial contact with the foreign agent is called.....
13. Substances called.....fail to induce immune responses in their native form because of their low molecular weight and their chemical simplicity.
14. The route of administration can affect the.....

**Section -B (Short Answer Type):**

1. Write general features of immune responses.
2. Which cells involved in the immune response?
3. There are several major subsets of T cells, Write their name.
4. What is interferon?
5. Define endocytosis and phagocytosis.
6. What is respiratory burst?
7. Which cells involved in the innate immune system ?
8. Write examples of granulocytes.
9. Define Inflammation.
10. Which are the primary lymphoid organs?
11. Where bursa of Fabricius situated?
12. Write all secondary lymphoid organs.
13. What is haptens and epitope?
14. What is CDR?
15. Give a definition of adjuvants.

**Section -C (Long Answer Type):**

1. Describe innate & acquired immunity.
2. Explain modes of immunization.
3. Describe Clonal selection theory
4. Explain Humoral and cell mediated immunity.
5. Describe Natural killer cells.
6. Write an essay on Lymphatic Organs.

7. What are the requirements for immunogenicity?
8. Explain major classes of antigen.

**Answer Key of Section-A**

1. Vertebrates
2. lymphoid precursor cell
3. bone marrow
4. Jerne, Burnet and Talmage
5. B cells and circulating antibodies
6. Immunoglobulins
7. serum
8. monocytes
9. pain, redness, and heat
10. bursa of Fabricius
11. spleen and the lymph nodes
12. antigen
13. immunization
14. haptens
15. outcome of the immunization

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## **9.24 References**

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## Unit - 10

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# Structure and functions of antibodies, antigen-antibody interaction in vitro and in vivo, complement system

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### Structure of the Unit

- 10.0 Objectives
- 10.1 Introduction
- 10.2 Body Organization of Antibody
  - 10.2.1 Basic Structure
  - 10.2.2 V- Region
  - 10.2.3 Tissue Hinge Region
  - 10.2.4 Constant-Region Domains
- 10.3 Types of Antibody
  - 10.3.1 Immunoglobulin G (IgG)
  - 10.3.2 Immunoglobulin M (IgM)
  - 10.3.3 Immunoglobulin A (IgA)
  - 10.3.4 Immunoglobulin E (IgE)
  - 10.3.5 Immunoglobulin D (IgD)
- 10.4 Antigen Antibody Interaction
  - 10.4.1 Precipitation Reactions
  - 10.4.2 Agglutination Reactions
  - 10.4.3 Radioimmunoassay
  - 10.4.4 Enzyme-Linked Immunosorbent Assay
  - 10.4.5 Western Blotting
  - 10.4.6 Immunoprecipitation
  - 10.4.7 Immunofluorescence
- 10.5 Complement System
- 10.6 Summary

- 10.7 Glossary
- 10.8 Self-Learning Exercise
- 10.9 References

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## 10.0 Objectives

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After going through this unit you will be able to understand

- How Antibodies are used as humoral resistance.
- Level of basic organization.
- Different types of Antibody
- How antigens are interact with antibodies
- Tools and techniques for bonding
- Complement system in human

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## 10.1 Introduction

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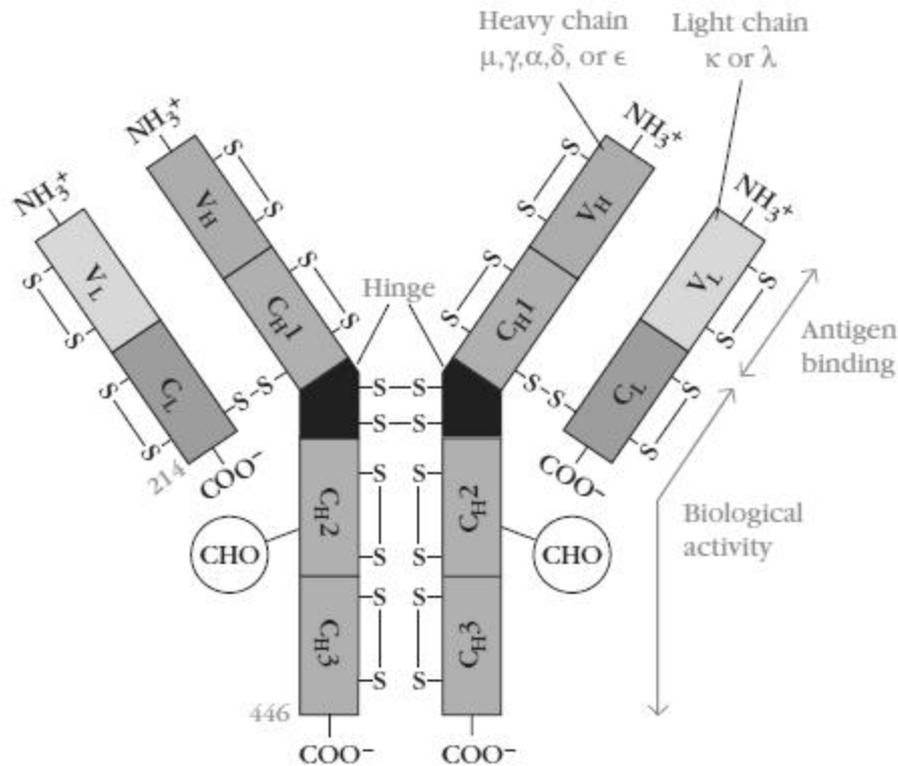
Antibodies are agents of humoral resistance by probing out and neutralizing antigens or marking them for elimination. All antibodies share heterogeneous structural features, bind to antigen. Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen.

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## 10.2 Body Organization of Antibody

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Blood can be separated in a centrifuge into a fluid and a cellular fraction. The fractions of blood have plasma and cellular fraction contains red blood cells, leukocytes, and platelets. If the plasma is clot, the fluid phase is converted into serum. Antibodies reside in the serum. The first antibodies were discovered by A. Tiselius and E. A.Kabat, in 1939. The  $\gamma$ -globulin fraction was identified as containing serum antibodies called immunoglobulins. Antibody molecules have a common structure of four peptide chains. This structure consists of two identical light (L) chains, polypeptides of about 25,000molecular weight, and two identical heavy (H) chains, larger polypeptides of molecular weight 50,000 or more. Each light chain is bound to a heavy chain by a disulfide bond, and by such noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar noncovalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain (H-L).



**Figure-1:** Body Organization of Antibody.

**V- Region:**

The amino acid of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different specificity. These segments of highly variable sequence are called *V regions*: *VL* in light chains and *VH* in heavy.

**Hinge Region:**

The light and heavy chains enclose an extended peptide chain between the CH1 and CH2 domains that have no homology with the other domains. This region, called the hinge region. The region is rich in proline residues and flexible, giving IgG, IgD, and IgA segmental flexibility. As a result, the two Fab arms can assume various angles to each other when antigen is bound.

**Other Constant-Region Domains**

The heavy chains in IgA, IgD, and IgG contain three constant-region domains and a hinge region, whereas the heavy chains in IgE and IgM contain four constant- region domains and no hinge region. The corresponding domains of the two groups are as follows:

<b>IgA, IgD, IgG</b>	<b>IgE, IgM</b>
CH1/CH1	CH1/CH1

Hinge region	CH2/CH2
CH2/CH2	CH3/CH3
CH3/CH3	CH4/CH4

The CH2/CH2 domains in IgE and IgM occupy the same position in the polypeptide chains as the hinge region in the other classes of immunoglobulin.

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## 10.3 Types of Antibody

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### Immunoglobulin G (IgG)

IgG, the most abundant in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule has two heavy chains and two light chains. There are four human IgG subclasses according declining average of serum concentrations: IgG1, IgG2, IgG3, and IgG4. The structural characteristics that differentiate to subclasses from one another are the size of the hinge region and the number and position of the interchain disulfide bonds between the heavy chains. IgG1, IgG3, and IgG4 can cross the placenta and play an important role in protecting the developing fetus.

### Immunoglobulin M (IgM)

IgM consists 5%–10% of the total serum immunoglobulin; with an average serum concentration of 1.5 mg/ml. IgM is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds having ten antigen-binding sites on the periphery of the molecule. Each pentamer contains an additional Fc-linked polypeptide called the J (joining) chain. It appears for polymerization of the monomers to form pentameric IgM; it is added just before secretion of the pentamer. IgM is the first immunoglobulin class produced in a primary response to an antigen. It is also the first immunoglobulin to be synthesized by the neonate.

### Immunoglobulin A (IgA)

IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. In serum, IgA survive as a monomer containing a J-chain polypeptide. IgA serves an important effector function at mucous membrane surfaces, which are the main entry sites for most pathogenic organisms. IgA can cross-link large antigens with multiple epitopes. Binding of secretory IgA to bacterial and viral surface antigens prevents attachment of the pathogens to the mucosal cells, thus

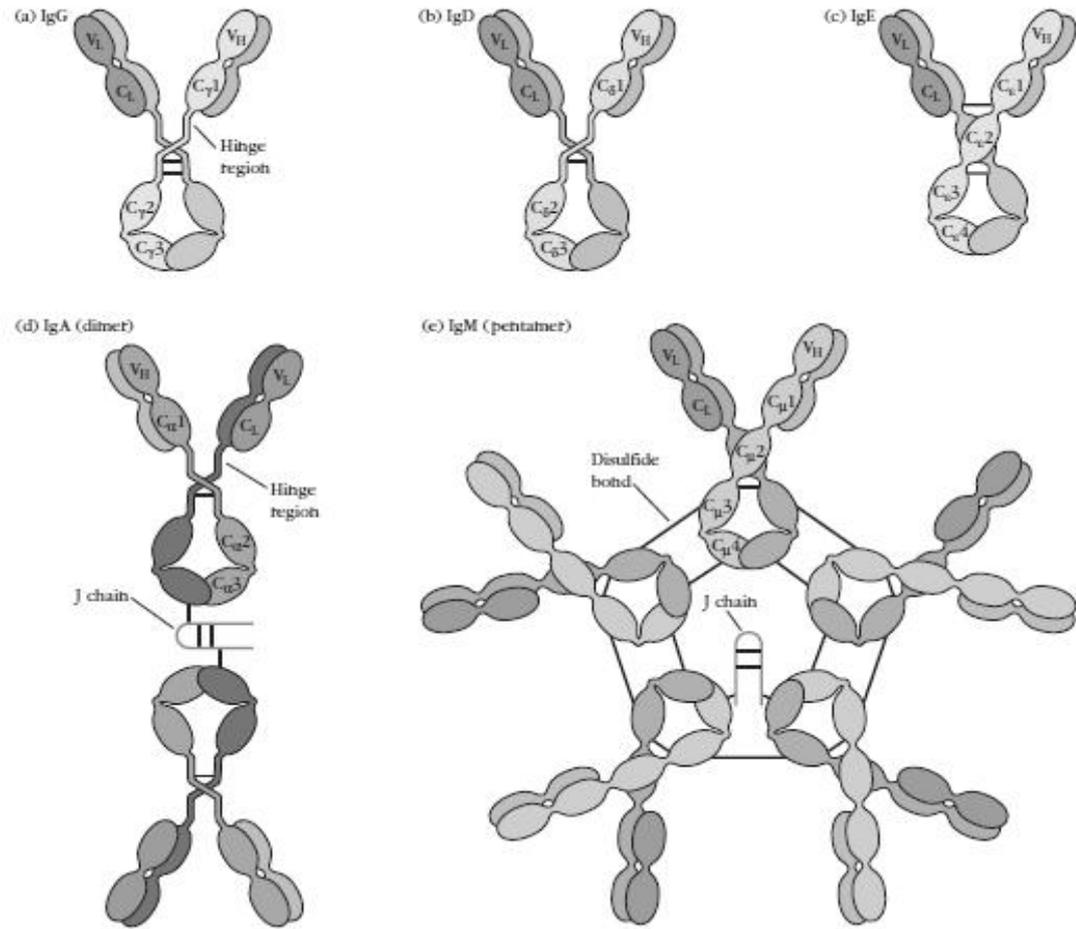
inhibiting viral infection and bacterial colonization. Breast milk contains secretory IgA and many other molecules that help protect the newborn against infection during the first month of life. Because the immune system of infants is not fully functional, breast-feeding plays an important role in maintaining the health of newborns.

### **Immunoglobulin E (IgE)**

The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3g/ml). IgE antibodies mediate the immediate hypersensitivity reactions which are liable for the symptoms of hay fever, asthma, hives, and anaphylactic shock. The presence of a serum component responsible for allergic reactions was first demonstrated in 1921 by K. Prausnitz and H. Kustner, who injected serum from an allergic person intra-dermally into a nonallergic individual. Localized mast-cell degranulation induced by IgE also may release mediators that facilitate a buildup of various cells necessary for antiparasitic defense.

### **Immunoglobulin D (IgD)**

IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with antiisotype antisera against the then-known isotypes: IgA, IgM, and IgG. The new class, called IgD, has a serum concentration of 30 g/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membrane bound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation.



**Figure-2: Types of Antibodies.**

## 10.4 Antigen Antibody Interaction

The antigen antibody interaction is bimolecular association. The association between an antibody and an antigen involves various noncovalent interactions including hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions between the antigenic determinant, or epitope of the antigen and the variable-region (V<sub>H</sub>/V<sub>L</sub>) domain of the antibody molecule, mainly the hypervariable regions, complementarity-determining regions (CDRs). The specificity of antigen-antibody interactions has led to the development of a variety of immunologic assays, which can be used to detect the presence of either antibody or antigen. The Immunoassays have vital roles in diagnosing diseases, monitoring the level of the humoral immune response, and identifying molecules of biological or medical interest.

### Precipitation Reactions

Antibody and soluble antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate. Antibodies that aggregate soluble antigens are called precipitins. Although formation of the soluble Ag-Ab complex occurs within minutes, formation of the visible precipitate occurs more slowly and often takes a day or two to reach completion. Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen: The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments.

In **immuno-electrophoresis**, the antigen mixture is first electrophoresed to separate its components by charge and antiserum is added to the troughs. Antibody and antigen then diffuse toward each other and produce lines of precipitation. Immuno-electrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum. A related quantitative technique, rocket electrophoresis, does permit measurement of antigen levels. In rocket electrophoresis, a negatively charged antigen is electrophoresed in a gel containing antibody. The precipitate formed between antigen and antibody has the shape of a rocket, the height of which is proportional to the concentration of antigen in the well.

### **Agglutination Reactions**

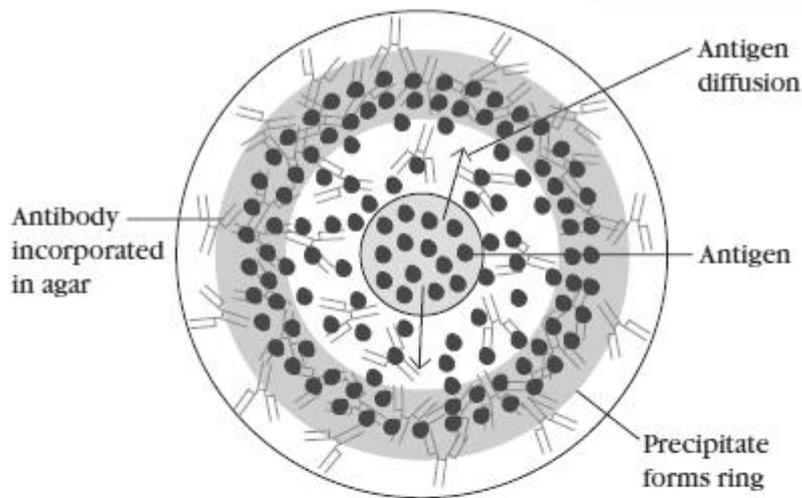
The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Agglutination reactions are parallel to precipitation reactions which depend on the crosslinking of polyvalent antigens. Antibodies that produce such reactions are called agglutinins.

### **Radioimmunoassay**

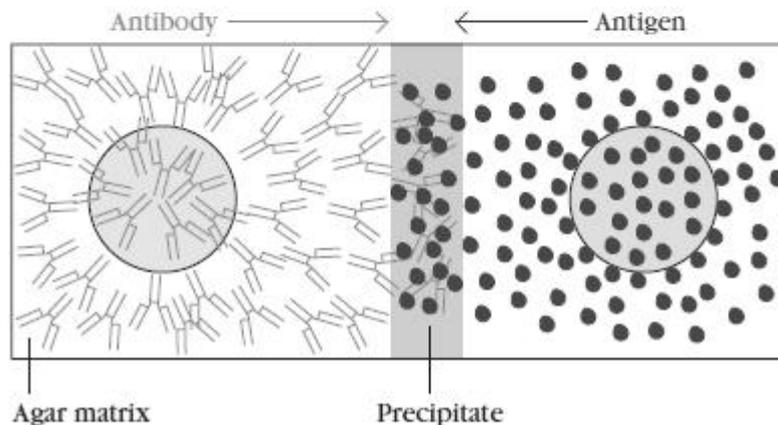
One of the most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA) which was first developed in 1960 by S. A. Berson and Rosalyn Yalow. The technique has assessment for measuring hormones, serum proteins, drugs, and vitamins at concentrations of 0.001 micrograms per milliliter or less. The principle of RIA involves competitive binding of radio labeled antigen and unlabeled antigen to a high-affinity antibody. The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts. The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labeled antigen will be

displaced from the binding sites. The decrease in the amount of radio labeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample.

### RADIAL IMMUNODIFFUSION



### DOUBLE IMMUNODIFFUSION



**Figure-3:** Antigen Antibody Interaction by immunodiffusion.

### Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay is similar to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called a chromogenic substrate. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and  $\beta$ -galactosidase.

### **Indirect ELISA**

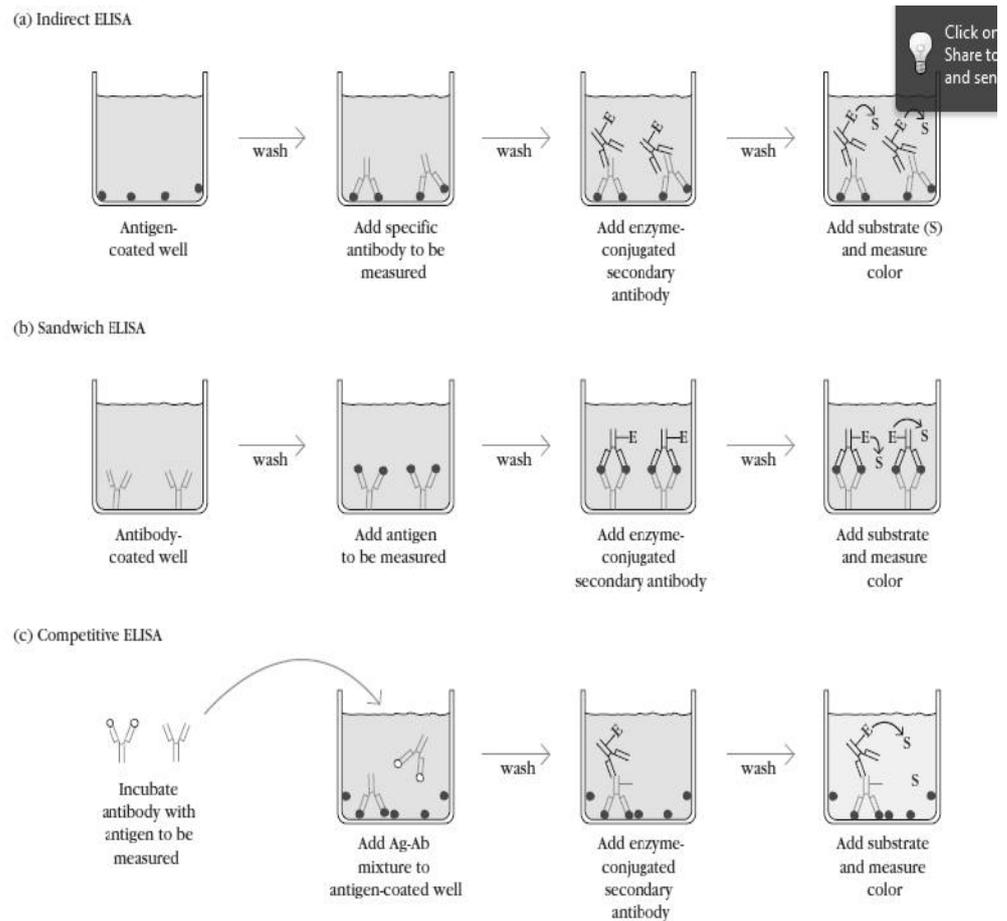
Antibody can be detected or quantitatively determined with an indirect ELISA. Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds.

### **Sandwich ELISA**

Antigen can be detected or measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added to bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

### **Competitive ELISA**

Another variation for measuring amounts of antigen is competitive ELISA. In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen-coated microtiter well. The more antigens present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.

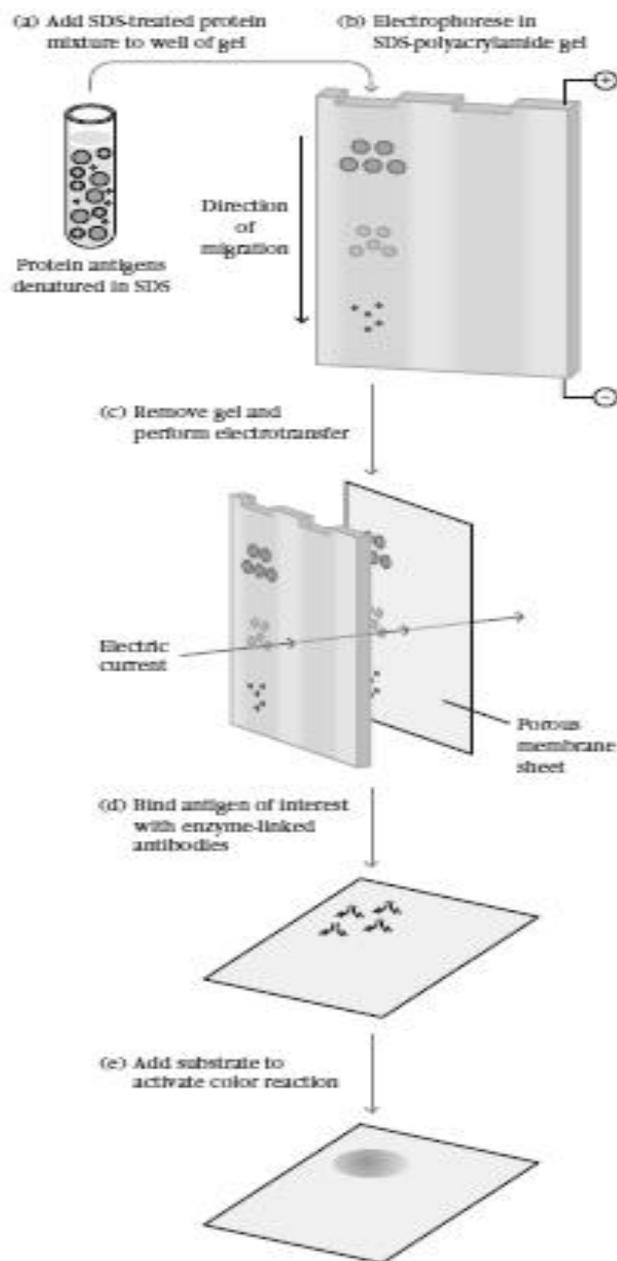


**Figure-4: Antigen Antibody Interaction by ELISA Method.**

### Western Blotting

Identification of a specific protein in a complex mixture of proteins can be accomplished by a technique known as Western blotting. In Western blotting, a protein mixture is electrophoretically separated on an SDS-polyacrylamide gel (SDS-PAGE). The protein bands are transferred to a nylon membrane by electrophoresis and the individual protein bands are identified by flooding the nitrocellulose membrane with radio labeled or enzyme linked polyclonal or monoclonal antibody specific for the protein of interest. The Ag-Ab complexes that form on the band containing the protein recognized by the antibody can be visualized in a variety of ways. If the protein of interest was bound by a radioactive antibody, its position on the blot can be determined by exposing the membrane to a sheet of x-ray film, a procedure called autoradiography. After binding of the enzyme antibody conjugate, addition of a chromogenic substrate that produces a highly colored and insoluble product causes the appearance of a colored band at the site of the target antigen. Western blotting is used to

determine whether the patient has antibodies that react with one or more viral proteins.



**Figure-5: Antigen Antibody Interaction by Western Blotting Method.**

### **Immunoprecipitation**

The immunoprecipitation technique used to isolate the antigen of interest for further analysis. It also provides a sensitive assay for the presence of a particular antigen in a given cell or tissue type. An extract produced by disruption of cells is mixed with an antibody against the antigen of interest in order to form an antigen-antibody complex as precipitate. The antigen

concentration is low, antigen-antibody complexes into precipitates can take hours, even days, and it is difficult to isolate the small amount of immunoprecipitate that forms. For it add a secondary antibody specific for the primary antibody to bind the antigen-antibody complexes. If the secondary antibody is attached to a bead, the immune complexes can be collected by centrifugation. After the secondary antibody binds to the primary antibody, immune precipitates are collected by placing a magnet against the side of the tube. The Ag-Ab complex is collected by immunoprecipitation, washed free of unincorporated radio labeled amino acid and other impurities, and then analyzed.

### **Immunofluorescence**

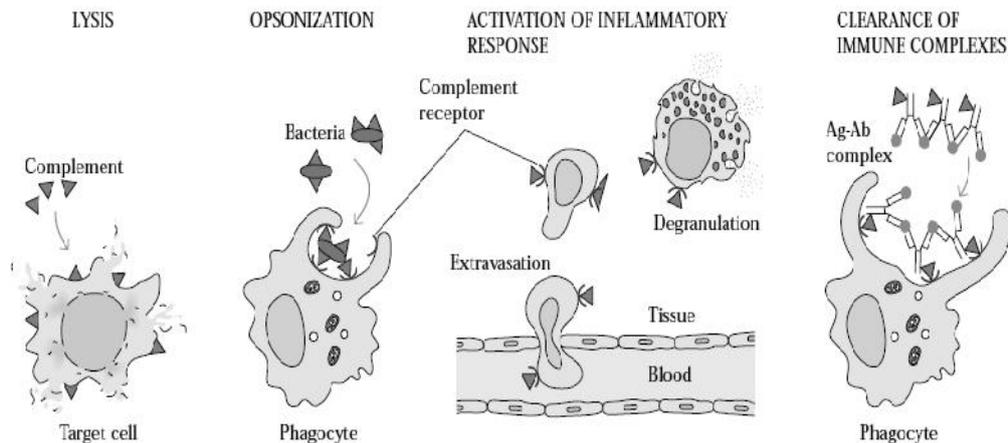
In 1944, Albert Coons discovered that antibodies could be labeled with molecules having the property of fluorescence known as immunofluorescence. If antibody is labeled with a fluorescent dye, labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wavelength. Fluorescent compounds such as fluorescein and rhodamine are commonly use. Fluorescein, an organic dye is absorbing blue light (490 nm) and emits an intense yellow-green fluorescence (517 nm). Rhodamine, another organic dye, absorbs in the yellow-green range (515 nm) and emits a deep red fluorescence (546 nm). Because it emits fluorescence at a longer wavelength than fluorescein, it can be used in two-color immunofluorescence assays.

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## **10.5 Complement System**

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The complement system is major effector of the humoral division of the immune system in the 1890s, when Jules Bordet at the Institut Pasteur in Paris explained bacteriolytic activity requires two different substances: first, the specific antibacterial antibodies, which survive the heating process, and a second, heat-sensitive component responsible for the lytic activity. Bordet developed a simple test for the lytic activity, the easily detected lysis of antibody-coated red blood cells, called hemolysis. Paul Ehrlich in Berlin independently carried out similar experiments and coined the term *complement*, defining it as “the activity of blood serum that completes the action of antibody.”



**Figure -6: The multiple activities of the complement system.**

The biological activities of this system affect both innate and acquired immunity and reach far beyond the original observations of antibody mediated lysis of bacteria and red blood cells. After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of basic functions including: Lysis of cells, bacteria, and viruses Opsonization, which promotes phagocytosis of particulate antigens Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules, Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and Liver .

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## 10.6 Summary

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An antibody molecule has two identical light chains and two identical heavy chains, which are linked by disulfide bonds. Each heavy chain has an amino-terminal variable region followed by a constant region. The five antibody classes have different functions, average serum concentrations, and half-lives. Antigen-antibody interactions depend on types of noncovalent interactions. The complement system comprises a group of serum proteins, many of which exist in inactive forms. Complement activation occurs by the classical, alternative, lectin pathways which are initiated differently.

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## 10.7 Glossary

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- **Agglutination** is the clumping together of particles, usually by antibody molecules binding to antigens on the surfaces of adjacent particles.

- **Allotypes** are allelic polymorphisms that can be detected by antibodies specific for the polymorphic gene products; in immunology, allotypic differences in immunoglobulin molecules were important in deciphering the genetics of antibodies.
- **Antigen:antibody complexes** are noncovalently associated groups of antigen and antibody molecules that can vary in size from small soluble complexes to large insoluble complexes that precipitate out of solution; they are also known as immune complexes.
- **C domains** -The constant regions of the polypeptide chains of immunoglobulin molecules are made up of one or more constant domains or C domains of similar structure; each immunoglobulin chain also has a single variable or V domain.
- **The complement system** is a set of plasma proteins that act together to attack extracellular forms of pathogens. Complement activation can occur spontaneously on certain pathogens or by antibody binding to the pathogen. The pathogen becomes coated with complement proteins that facilitate pathogen removal by phagocytes and can also kill certain pathogens directly.
- **The enzyme-linked immunosorbent assay (ELISA)** is a serological assay in which bound antigen or antibody is detected by a linked enzyme that converts a colorless substrate into a colored product.
- **Fc receptors** are receptors for the Fc portion of immunoglobulin isotopes. They include the Fcγ and Fcε receptors.
- **Immunodiffusion** is the detection of antigen or antibody by the formation of an antigen:antibody precipitate in a clear agar gel.
- **Immuno-electrophoresis** is a technique in which antigens are first separated by their electrophoretic mobility and are then detected and identified by immunodiffusion.
- **Plasma** is the fluid component of blood containing water, electrolytes, and the plasma proteins

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## 10.8 Self-Learning Exercise

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### Section -A (Very Short Answer Type):

1. Serum is separated from-----
2. Heavy (H) chains are formed of----- Polypeptides in antibody.

3. J- chain is found in -----
4. Define ELISA Word
5. Immunodiffusion is -----
6. Allotypes are .....

**Section -B (Short Answer Type):**

1. Name the antibodies types.
2. What is Immunoelectrophoresis?
3. A Short note on Agglutination. Give an example of natural Agglutination.
4. Explain Antigen: antibody complexes.
5. What is Precipitation Reactions?
6. A Short note on complement system.
7. Differentiate to Precipitation and Immunoprecipitation.
8. What is Western Blotting?

**Section -C (Long Answer Type)**

1. Explain the Body Organization of Antibody.
2. Write about ELISA with their types.
3. Describe Types of Antibody, draw suitable diagrams.
4. What is antibody? Write on Antigen Antibody Interaction in *invitro*.

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## 10.9 References

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- Berzofsky,1991. Fundamental Immunology,
- Kuby– Immunology
- janeway-2001, Immunobiology

## Unit - 11

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# Major Histocompatibility complex in mouse and HLA system in humans

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### Structure of the Unit

- 11.0 Objectives
- 11.1 Introduction
- 11.2 Body Organization of MHC
  - 11.2.1 Basic Structure
  - 11.2.2 Class I
  - 11.2.3 Class II
- 11.3 MHC in mouse
- 11.4 HLA system in Humans
- 11.5 Significance of HLA & MHC System
- 11.6 Summary
- 11.7 Glossary
- 11.8 Self-Learning Exercise
- 11.9 References

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### 11.0 Objectives

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After going through this unit you will be able to understand

- What is MHC
- How MHC is function as humoral resistance.
- Level of basic organization.
- MHC in mice
- HLA system in human

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### 11.1 Introduction

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The major histocompatibility complex is group of genes on a single chromosome which acts central role to develop both humoral and cells mediated immune responses. T and B cells use surface molecules to recognize

antigen. In contrast to antibodies or B-cell receptors can identify an antigen alone while T-cell receptors recognize pieces of antigen that are located on the surface of other cells with binding groove of a cell surface protein known major histocompatibility complex (MHC). These encoded proteins establish tissue transplantation between two genetically different individuals will be accepted or rejected. George Snell, Jean Dausset and Baruj Benacerraf received the Nobel Prize in 1980 for discovery of the MHC in mice and humans.

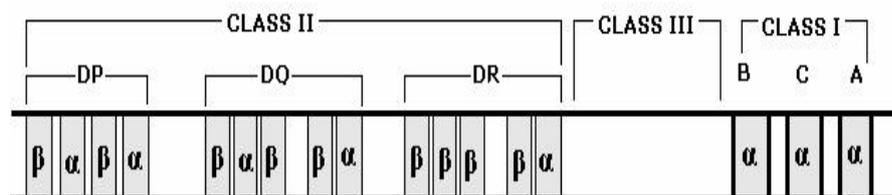
However, the HLA (human leukocyte antigens) are the MHC antigens present as long continuous stretch of DNA on chromosome 6 in humans. They play essential role in the initiation and regulation of the immune response and defence against microorganisms. The physiologic function of MHC molecules is to present peptide antigen to T lymphocytes. These antigens can separate in three classes: class I, class II and class III.

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## 11.2 Body Organization of MHC

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The MHC antigens present as long continuous stretch of DNA on chromosome 6 in humans having overall size of 3.5 million base pairs. MHC molecule's both class I and class II have been isolated and purified, and three-dimensional structures of their extracellular domains have been determined by x-ray crystallography. Class I and class II MHC molecules are membrane-bound glycoproteins with close relation in structure and function. Certain membrane glycoproteins play role as highly specialized antigen-presenting molecules with channels. These channels form extraordinarily stable complexes with peptide ligands with presenting on the cell surface for recognition by T cells receptor (TCR) engagement. The class I gene complex contains three loci A, B and C. Each locus is coded for  $\alpha$  chain polypeptides. The class II gene complex also contains one  $\beta$  and a variable number of  $\alpha$  chain polypeptides at least three loci, DP, DQ and DR. Class III region is not actually a part of the HLA complex but it is located within the HLA region. In contrast, class III MHC is a group of unrelated proteins who does not share structural or function relationship with class I and II molecules. They have no role in graft rejection

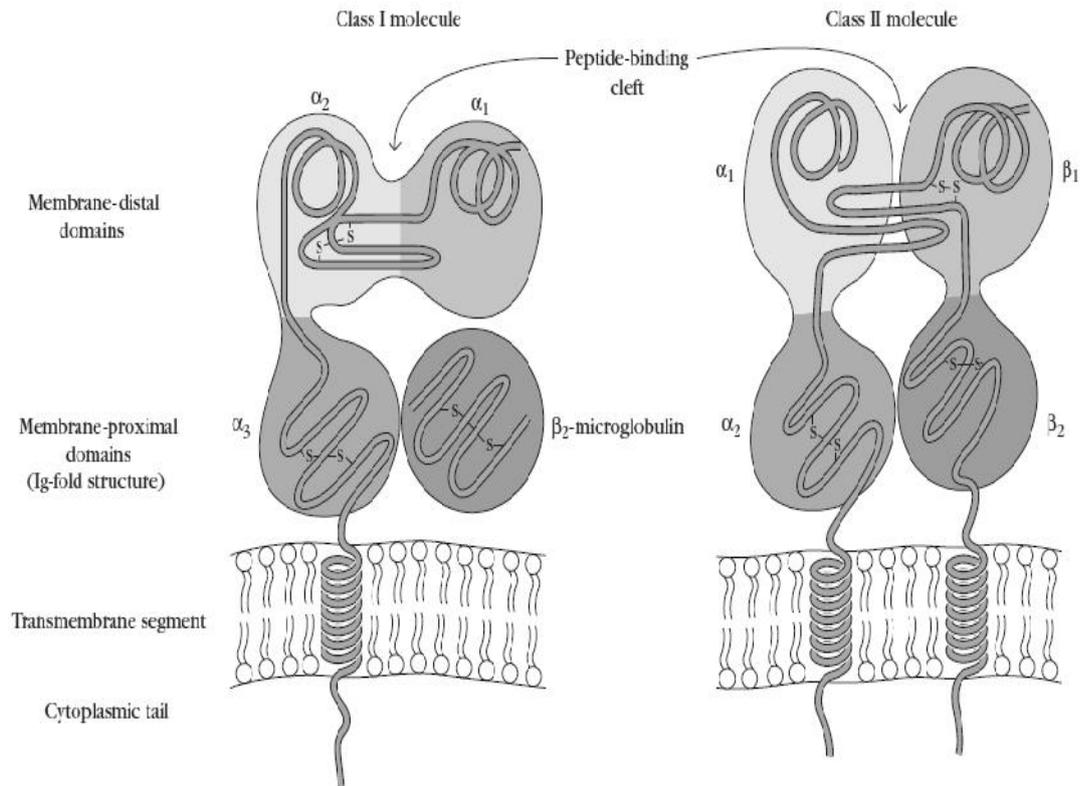


**Figure: 1-** Body organization of MHC Molecules. (Sridhar Rao, 2006)

- **Class I Molecules**

MHC I presented as  $\alpha$ -chain composed of three domains— 1, 2, and 3. The 1 located upon the non-MHC molecule  $\beta_2$  microglobulin. In human, this non-MHC Molecule is existed on chromosome 15. The 3 is trans-membrane that is attached the MHC class I molecule to the cell membrane. Class I MHC molecules contain two separate polypeptide chains, the heavier (44-47 KDa)  $\alpha$ -chain and the lighter (12 KDa)  $\beta$ -chain. The 3 domain and  $\beta_2$ -microglobulin are arranged on two  $\beta$ -pleated sheets formed by antiparallel  $\beta$  strands of amino acids. The 3 domain conserved in class I MHC molecules for strongly interaction with the CD8 cell surface molecule establish on TC cells. A peptide-binding groove is produced amid 1 and 2 helices with  $\beta$ -pleated sheet as its base. It is large enough to bind a peptide of 8 to 10 amino acids. The specific binding of a peptide molecule in the peptide-binding groove of MHC need peptide at a fixed position. CD8 T lymphocytes recognizes peptide antigen only in MHC I molecules.

During the x-ray crystallographic analysis of class I molecules, small noncovalently associated peptides that had co-crystallized with the protein were found in the groove. Class I molecules present peptide fragments in the cytosol to the CD8 lymphocytes.



**Figure: 2-** Schematic diagrams of a class I and a class II MHC molecules.(Kuby,2003)

- **Class II Molecules**

Class II MHC molecules are also classified in the immunoglobulin superfamily. Class II MHC molecules contain two different polypeptide chains, a 33-KDa chain and a 28 KDa chain. Both chains are associated by non-covalent bonds containing 2 domains  $\alpha_1$  and  $\alpha_2$  and  $\beta_1$  and  $\beta_2$  with  $\alpha_2$  and  $\beta_2$  being the membrane proximal domains. Like class I  $\alpha$ -chains, class II MHC molecules are membrane bound glycoproteins. These glycoproteins contain a transmembrane Segment and a cytoplasmic tail segment. The  $\alpha_1$  and  $\beta_1$  domains form the Antigen binding cleft that can hold a peptide of 13-18 Da. Subsequently of MHC class I, A peptide-binding groove of class II molecule is composed of by floor of eight antiparallel strands and sides of antiparallel helices. The class II molecule lacks the conserved residues in the class I molecule that bind to the terminal amino acids of short peptides to forms an open pocket. Class II possesses an open-ended groove in compare to socket-like opening of class I.

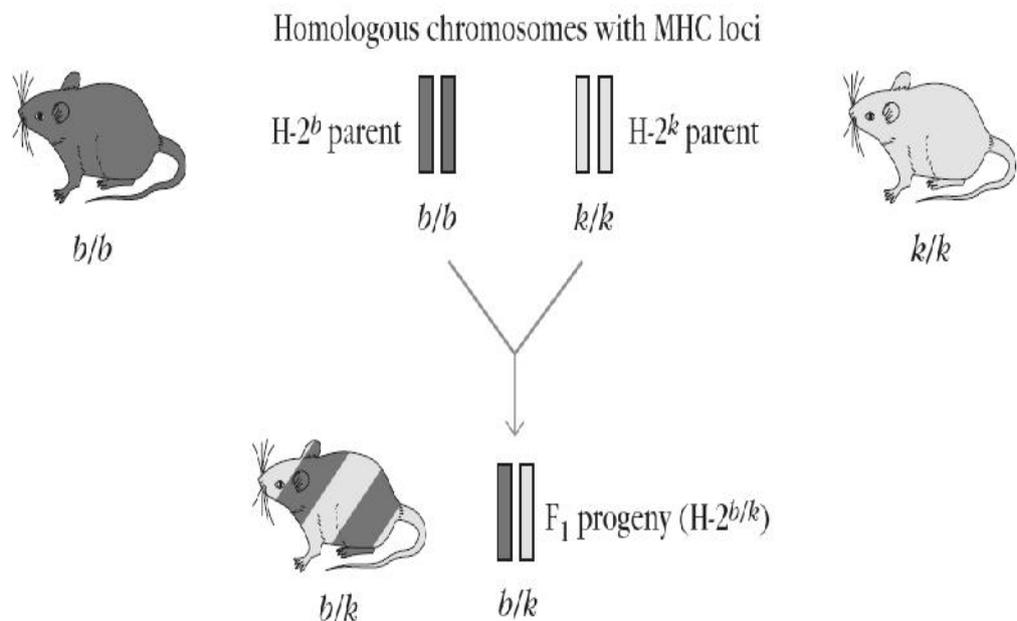
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### 11.3 MHC in mouse

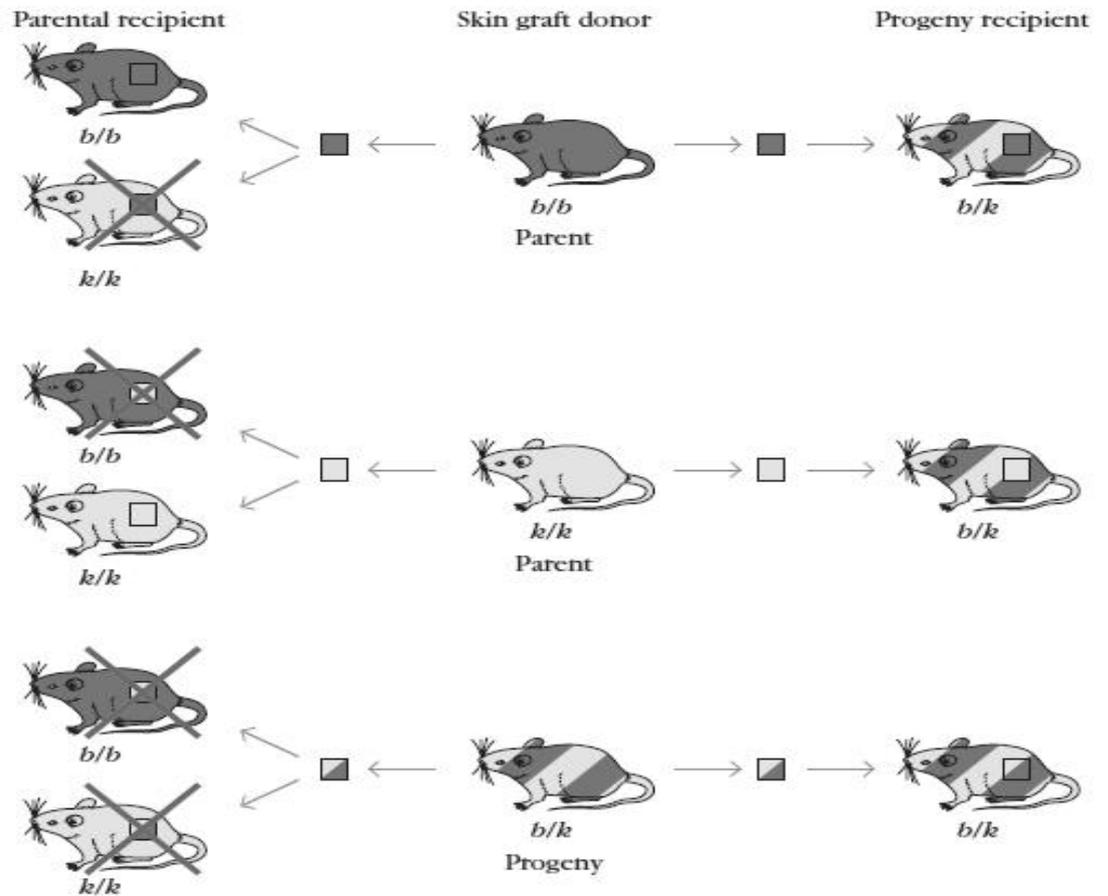
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Congenic mouse strains are produced to study the MHC Complex (H-2 complex) in mice. Inbred mouse strains are identical at all genetic loci. Two strains are congenic. The phenotypic variations between congenic strains are associated to the genetic regions that differentiate the strains.

The MHC in Congenic strains can identify by producing a series of crosses, backcrosses, and selections. In Figure, the H-2 complex of homozygous strain B is crossed into background genes of homozygous strain A to produce a congenic strain, represented A.B. In newly formed congenic strain, first & second letter refer to the strain providing genetic background and genetically different MHC region. Thus, new strain A.B will be identified for the MHC locus genetically to strain A accepting contribution of strain B.



**Figure:-3** Mating of inbred mouse strains with different MHC Haplotypes. (Kuby, 2003)



**Figure:- 4** Acceptance or rejection of skin grafts is controlled by the MHC type of the inbred mice. (Kuby, 2003)

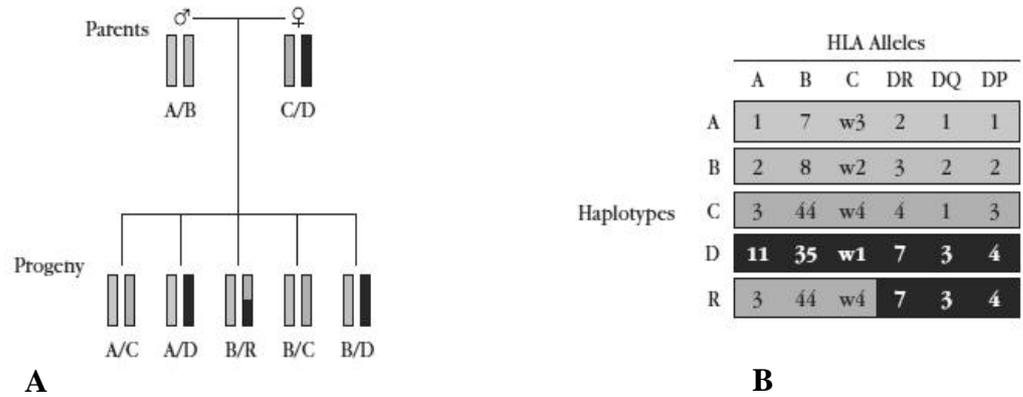
Throughout production of congenic strains, an incident cross occurs with H-2 complex yielding a recombinant strain. This recombinant strains differ from the parental strains or the congenic strain within the H-2 complex. These recombinant strains may be useful to analyze the MHC with assessment of functional divergence between strains.

The functional divergence of strains can be different in a few genes contained by the MHC. The generation of new H-2 haplotypes generates an illustration of means by which MHC maintains to heterogeneity.

## 11.4 HLA system in Humans

The human leukocyte antigen (HLA) system is the human adaptation of the major histocompatibility complex (MHC) genes that found in most vertebrates. The HLA Genes is located on chromosome 6 where encodes cell-surface antigen-presenting proteins. The encoded proteins are regulated the immune system in humans. The class I MHC region is about 2000 kb long containing

approximately 20 genes in humans. In HLA system, class I region are characterized in HLA-A, HLA-B, and HLA-C. In humans, the non-classical class I genes also present included the *HLA-E*, *HLA-F*, *HLA-G*, *HFE*, *HLA-J*, and *HLA-X* loci as well as *MIC*. The *MIC* gene products are expressed at low levels in epithelial cells influencing heat shock proteins.



**Figure:-5** (A) Inheritance of HLA haplotypes in a human family.

(B) The genes that make up each parental haplotype in family. (Kuby, 2003)

The class II MHC region contains the classical class as HLA-DR, DP, and DQ in humans. In the human DR region contains three or four functional  $\alpha$ -chains which's products can be expressed together with the  $\beta$ -chain gene product in a cell. The human DP and DQ regions contain two  $\beta$ -chain gene in compare to DR region. In the human class II region, non-classical genes identified as DM and DO. The DM genes encode a class II-like molecule for loading of antigenic peptides into the class II MHC molecules. Class II DO molecules are expressed only in the thymus and mature B cells.

The class III region of the MHC in humans contains a heterogeneous collection of gene. These genes encode several complement components, two steroid 21-hydroxylases, heat-shock proteins and cytokines.

## 11.5 Significance of HLA & MHC System

- **Anthropology:** The HLA types show a discrepancy widely among different ethnic populations. This discrepancy allows establishing relationship among populations and migration pattern.
- **Transplantation:** The HLA & MHC take part a dominant role in transplant immunity. Pre-transplant histocompatibility testing is extremely imperative for organ transplantation. From Pre-transplant

histocompatibility testing, the outcome relates to living donors matched with the recipient for superiority in one or more haplotypes than unrelated cadaveric donors.

- **In cancer:** Some HLA-mediated diseases are also support to cause of cancer. Gluten-sensitive enteropathy is associated with T-cell lymphoma. The HLA systems is participated a protective role to recognize increasing antigens those cannot be tolerated due to low levels in normal position. Abnormal cells might be targeted for apoptosis, which can be considered as referee for many cancers before diagnosis.
- **Transfusion**
- **Forensic science**
- **Disease Correlation**

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## 11.6 Summary

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The major histocompatibility complex (MHC) & HLA are groups of closely associated genes that encode proteins involved in intercellular identification and antigen appearance on T lymphocytes in vertebrates and Human. The feature of MHC molecules is polymorphism that recognized the antigens as a peptide bound by a specific allelic variant. At least three properties of MHC molecules are affected the MHC polymorphism: the range of peptides bound; the conformation of the bound peptide; and the direct interaction of the MHC molecule with the T-cell receptor. Class I MHC molecules consist of a large glycoprotein chain with 3 extracellular domains and a transmembrane segment, and 2-microglobulin. Class II MHC molecules are composed of two noncovalently associated glycoproteins and chain. The class III region of the MHC encodes molecules that include a diverse group of proteins with playing no role in antigen presentation.

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## 11.7 Glossary

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- **Allelic:** Relating to one of a series of two or more alternate forms of a gene that occupy the same position or locus on a specific chromosome.
- **Allograft:** A tissue transplant (graft) between two genetically non-identical members of a species.
- **Allotypes:** Antigenic determinants those are present in allelic (alternate) forms.

- **Autograft:** A tissue transplant from one area to another on a single individual.
- **B lymphocyte (B cell):** The precursors of antibody-forming plasma cells; these cells carry immunoglobulin and class II MHC (major histocompatibility complex) antigens on their surfaces.
- **Class I, II and III MHC molecules:** Proteins encoded by genes in the major histocompatibility complex (q.v.). Class I molecules are designated HLA-A, B, or C. Class II molecules are designated DP, DQ or DR.
- **Major histocompatibility complex (MHC)** the chromosomal region containing genes that control the histocompatibility antigens; in humans it controls the HLA antigens.
- **Myeloma:** A tumor of plasma cells, generally secreting a single species of immunoglobulin.
- **T cell:** A lymphocyte which undergoes a developmental stage in the thymus.
- **Tolerance:** Diminished or absent capacity to make a specific response to an antigen, usually produced as a result of contact with that antigen under non-immunizing conditions.

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## 11.8 Self-Learning Exercise

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### Section -A (Very Short Answer Type):

1. MHC is located on-----in Mouse.
2. Class II MHC molecules are composed of-----
3. HLA is-----
4. Congenic Mouse are-----
5. In Human, MHC contain -----Long Region and -----genes
6. In humans, the non-classical class I genes are -----

### -- Section -B (Short Answer Type):

1. Define the MHC.
2. What is Anthropology?
3. A Short note on Significance of HLA & MHC System

4. A Note on MHC Class II domain structure.
5. Differentiate to MHC System And HLA System.
6. What is Transfusion?

### **Section -C (Long Answer Type)**

1. Explain the Body Organization of MHC.
2. How determine the identical MHC haplotypes in different inbred mouse strains?
3. What is HLA system? Write on structure of HLA system in Humans.

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## **11.9 References**

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- Shridhar Rao P.N., 2006, MHC (Major Histocompatibility Complex)
- Kuby–2003, Immunology
- Roitts & Delves-2001, Essential Immunobiology
- Steven A. Frank, 2002, Immunology and Evolution of Infectious Disease

## Unit - 12

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# Organization and expression of Ig genes; T-cell and B-cell generation, activation and differentiation; Cytokine

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### Structure of Unit

- 12.0 Objectives
- 12.1 Introduction
- 12.2 Genetic Models Of Ig Structure
  - 12.2.1 Germ-Line and Somatic-Variation Models
  - 12.2.2 Dreyer and Bennett Proposed the Two-Gene Model
  - 12.2.3 Tonegawa's Bombshell—Immunoglobulin Genes Rearrange
- 12.3 Multigene Organization Of Ig Genes
  - 12.3.1  $\kappa$ -chain multigene family
  - 12.3.2  $\lambda$ -chain multigene family
  - 12.3.3 heavy-chain multigene family
- 12.4 Gene Rearrangements In Variable-Region
  - 12.4.1 Light-Chain DNA Undergoes V-J Rearrangements
  - 12.4.2 Heavy-Chain DNA Undergoes V-D-J Rearrangements
- 12.5 Generation Of Antibody Diversity
- 12.6 Class Switching Among Constant-Region Genes
- 12.7 Expression of Ig Genes
  - 12.7.1 Expression of membrane or secreted immunoglobulin
  - 12.7.2 Simultaneous expression of IgM And IgD
  - 12.7.3 Synthesis, Assembly, and Secretion of Immunoglobulins
- 12.8 Regulation of Ig-Gene Transcription
- 12.9 Generation, Activation And Differentiation Of T-Lymphocytes
  - 12.9.1 Maturation of T-Cell

12.9.2	T_Cell activation
12.9.3	T-cell differentiation
12.10	Generation, Activation and Differentiation Of B-Lymphocytes
12.10.1	B-Cell Maturation
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## **12.0 Objectives**

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After going through this unit you will be able to understand:

- Genetic Models of Ig Structure
- Gene Rearrangements in Variable-Region
- Multigene Organization of Ig Genes
- Generation of Antibody Diversity
- Class Switching among Constant-Region Genes
- Expression of Ig Genes
- Synthesis, Assembly, and Secretion of Immunoglobulins
- Regulation of Ig-Gene Transcription
- Generation, Activation and Differentiation of T-lymphocytes
- Generation, Activation and Differentiation of B-lymphocytes
- Cytokine

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## 12.1 Introduction

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One of the most remarkable features of the vertebrate immune system is its ability to respond to an apparently limitless array of foreign antigens. As immunoglobulin (Ig) sequence data accumulated, virtually every antibody molecule studied was found to contain a unique amino acid sequence in its variable region but only one of a limited number of invariant sequences in its constant region. The genetic basis for this combination of constancy and tremendous variation in a single protein molecule lies in the organization of the immunoglobulin genes.

In germ-line DNA, multiple gene segments encode portions of a single immunoglobulin heavy or light chain. These gene segments are carried in the germ cells but cannot be transcribed and translated into complete chains until they are rearranged into functional genes. During B-cell maturation in the bone marrow, certain of these gene segments are randomly shuffled by a dynamic genetic system capable of generating more than  $10^6$  combinations. Subsequent processes increase the diversity of the repertoire of antibody binding sites to a very large number that exceeds  $10^6$  by at least two or three orders of magnitude. The processes of B cell development are carefully regulated: the maturation of a progenitor B cell progresses through an ordered sequence of Ig-gene rearrangements, coupled with modifications to the gene that contribute to the diversity of the final product. By the end of this process, a mature, immune competent B cell will contain coding sequences for one functional heavy chain variable-region and one light-chain variable-region. The individual B cell is thus antigenically committed to a specific epitope. After antigenic stimulation of a mature B cell in peripheral lymphoid organs, further rearrangement of constant-region gene segments can generate changes in the isotype expressed, which produce changes in the biological effector functions of the immunoglobulin molecule without changing its specificity. Thus, mature B cells contain chromosomal DNA that is no longer identical to germ-line DNA. While we think of genomic DNA as a stable genetic blueprint, the lymphocyte cell lineage does not retain an intact copy of this blueprint. Genomic rearrangement is an essential feature of lymphocyte differentiation, and no other vertebrate cell type has been shown to undergo this process.

This chapter first describes the detailed organization of the immunoglobulin genes, the process of Ig-gene rearrangement. Then it describes the class switching, the role of differential RNA processing in the expression of

immunoglobulin genes, and the regulation of Ig-gene transcription. Besides, it describes generation, activation and differentiation of T-cell and B-cell and cytokines.

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## **12.2 Genetic Models Of Ig Structure**

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The results of the immunoglobulin-sequencing revealed a number of features of immunoglobulin structure that were difficult to reconcile with classic genetic models. Any viable model of the immunoglobulin genes had to account for the following properties of antibodies:

- The vast diversity of antibody specificities
- The presence in Ig heavy and light chains of a variable region at the amino-terminal end and a constant region at the carboxyl-terminal end
- The existence of isotypes with the same antigenic specificity, which result from the association of a given variable region with different heavy-chain constant regions

### **12.2.1 Germ-Line and Somatic-Variation Models**

For several decades, immunologists sought to imagine a genetic mechanism that could explain the tremendous diversity of antibody structure. Two different sets of theories emerged. The germ-line theories maintained that the genome contributed by the germ cells, egg and sperm, contains a large repertoire of immunoglobulin genes; that are sufficient to produce more than 10<sup>8</sup> different specificities. According to this model no special genetic mechanisms to account for antibody diversity. They argued that the immense survival value of the immune system justified the dedication of a significant fraction of the genome to the coding of antibodies. In contrast, the somatic-variation theories maintained that the genome contains a relatively small number of immunoglobulin genes, from which a large number of antibody specificities are generated in the somatic cells by mutation or recombination.

Whether diversity was generated by germ-line or by somatic mechanisms, a question remained: How could stability be maintained in the constant (C) region while some kind of diversifying mechanism generated the variable (V) region? Neither the germ-line nor the somatic-variation proponents could offer a reasonable explanation for this central feature of immunoglobulin structure.

### **12.2.2 Dreyer and Bennett Proposed the Two-Gene Model**

In 1965, W. Dreyer and J. Bennett suggested that two separate genes encode a single immunoglobulin heavy or light chain, one gene for the V region and the other for the C region. They suggested that these two genes must somehow come together at the DNA level to form a continuous message that can be transcribed and translated into a single Ig heavy or light chain. Moreover, they proposed that hundreds or thousands of V-region genes were carried in the germ line, whereas only single copies of C-region class and subclass genes need exist. The strength of this type of recombinational model (which combined elements of the germ-line and somatic variation theories) was that it could account for those immunoglobulins in which a single V region was combined with various C regions. By postulating a single constant region gene for each immunoglobulin class and subclass, the model also could account for the conservation of necessary biological effect or functions while allowing for evolutionary diversification of variable-region genes.

Studies of DNA hybridization kinetics using a radioactive constant-region DNA probe indicated that the probe hybridized with only one or two genes, confirming the model's prediction that only one or two copies of each constant-region class and subclass gene existed. However, this evidence was not enough to overcome resistance in the scientific community to the hypothesis of Dreyer and Bennet. The suggestion that two genes encoded a single polypeptide contradicted the existing one gene—one polypeptide principle and was without precedent in any known biological system.

### **12.2.3 Tonegawa's Bombshell—Immunoglobulin Genes Rearrange**

In 1976, S. Tonegawa and N. Hozumi found the first direct evidence that separate genes encode the V and C regions of immunoglobulins and that the genes are rearranged in the course of B-cell differentiation. This work changed the field of immunology. In 1987, Tonegawa was awarded the Nobel Prize for this work. They demonstrated that the Dreyer and Bennett two-gene model—one gene encoding the variable region and another encoding the constant region—applied to both heavy and light-chain genes.

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## **12.3 Multigene Organization of Ig Genes**

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The  $\kappa$  and  $\lambda$  light chains and the heavy chains are encoded by separate multigene families situated on different chromosomes. In germ-line DNA, each of these multigene families contains several coding sequences, called gene segments, separated by noncoding regions. During B-cell maturation, these

gene segments are rearranged and brought together to form functional immunoglobulin genes. The  $\kappa$  and  $\lambda$  light-chain families contain V, J, and C gene segments; the rearranged VJ segments encode the variable region of the light chains. The heavy-chain family contains V, D, J, and C gene segments; the rearranged VDJ gene segments encode the variable region of the heavy chain. In each gene family, C gene segments encode the constant regions. Each V gene segment is preceded at its 5' end by a small exon that encodes a short signal or leader (L) peptide that guides the heavy or light chain through the endoplasmic reticulum. The signal peptide is cleaved from the nascent light and heavy chains before assembly of the finished immunoglobulin molecule. Thus, amino acids encoded by this leader sequence do not appear in the immunoglobulin molecule.

### **12.3.1 $\kappa$ -chain multigene family**

A functional  $\kappa$  variable-region gene contains two coding segments—a 5' V segment and a 3' J segment, which are separated by a noncoding DNA sequence in unrearranged germ-line DNA. The  $\kappa$  multigene family in the mouse germ line contains three V gene segments, four J gene segments, and four C gene segments. The J<sub>4</sub> is a pseudogene, a defective gene that is incapable of encoding protein. In humans, the lambda locus is more complex. There are 31 functional V gene segments, 4 J segments, and 7 C segments. In addition to the functional gene segments, the human lambda complex contains many V, J, and C pseudogenes.

### **12.3.2 $\lambda$ -chain multigene family**

The  $\lambda$ -chain multigene family in the mouse contains approximately 85 V gene segments, each with an adjacent leader sequence a short distance upstream (i.e., on the 5' side). There are five J gene segments, one of which is a nonfunctional pseudogene and a single C gene segment. As in the  $\kappa$  multigene family, the V and J gene segments encode the variable region of the light chain, and the C gene segment encodes the constant region. Since there is only one C gene segment, there are no subtypes of light chains. The  $\lambda$ -chain multigene family in humans, which has an organization similar to that of the mouse, contains approximately 40 V gene segments, 5 J segments, and a single C segment.

### **12.3.3 heavy-chain multigene family**

The organization of the immunoglobulin heavy-chain genes is similar to, but more complex than, that of the  $V_L$  and  $J_L$  light-chain genes. An additional gene segment encodes part of the heavy-chain variable region. This encoded gene segment is situated between  $V_H$  and  $J_H$  gene segments to encode the entire variable region of the heavy chain. This gene segment, which encoded amino acids within the third complementarity-determining region (CDR3), was designated D for diversity, because of its contribution to the generation of antibody diversity. The heavy-chain multigene family on human consists of 51  $V_H$  gene segments, 27 functional  $D_H$  gene segments and 6 functional  $J_H$  gene segments. Each  $V_H$  gene segment is preceded by a leader sequence a short distance upstream. Downstream from the  $D_H$  gene segments are six functional  $J_H$  gene segments, followed by a series of  $C_H$  gene segments. A similar heavy chain gene organization is found in the mouse.

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## 12.4 Gene Rearrangements in Variable-Region

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Functional genes that encode immunoglobulin light and heavy chains are assembled by recombinational events at the DNA level. These events and the parallel events involving T-receptor genes are the only known site-specific DNA rearrangements in vertebrates. Variable-region gene rearrangements occur in an ordered sequence during B-cell maturation in the bone marrow. The heavy-chain variable-region genes rearrange first, then the light-chain variable-region genes. At the end of this process, each B cell contains a single functional variable region DNA sequence for its heavy chain and another for its light chain. The process of variable-region gene rearrangement produces mature, immunocompetent B cells; each such cell is committed to produce antibody with a binding site encoded by the particular sequence of its rearranged V genes. Rearrangements of the heavy chain constant-region genes will generate further changes in the immunoglobulin class (isotype) expressed by a B cell, but those changes will not affect the cell's antigenic specificity. The steps in variable-region gene rearrangement occur in an ordered sequence, but they are random events that result in the random determination of B-cell specificity. These steps are described below:-

### 12.4.1 Light-Chain DNA Undergoes V-J Rearrangements

Expression of both  $V_L$  and  $J_L$  light chains require rearrangement of the variable-region V and J gene segments. In humans, any of the functional  $V_L$  genes can combine with any of the four functional J -C combinations. In the mouse,

things are slightly more complicated. DNA rearrangement can join the V 1 gene segment with either the J 1 or the J 3 gene segment, or the V 2 gene segment can be joined with the J 2 gene segment. In human or mouse light-chain DNA, any one of the V gene segments can be joined with any one of the functional J gene segments. Rearranged and genes contain the following regions in order from the 5' to 3' end: a short leader (L) exon, a noncoding sequence (intron), a joined VJ gene segment, a second intron, and the constant region. Upstream from each leader gene segment is a promoter sequence. The rearranged light chain sequence is transcribed by RNA polymerase from the L exon through the C segment to the stop signal, generating a light-chain primary RNA transcript. The introns in the primary transcript are removed by RNA processing enzymes, and the resulting light-chain messenger RNA then exits from the nucleus. The light-chain mRNA binds to ribosomes and is translated into the light-chain protein. The leader sequence at the amino terminus pulls the growing polypeptide chain into the lumen of the rough endoplasmic reticulum and is then cleaved, so it is not present in the finished light-chain protein product.

#### 12.4.2 Heavy-Chain DNA Undergoes V-D-J Rearrangements

Generation of a functional immunoglobulin heavy-chain gene requires two separate rearrangement events within the variable region. A  $D_H$  gene segment first joins to a  $J_H$  segment; the resulting  $DHJH$  segment then moves next to and joins a  $V_H$  segment to generate a  $VHDHJH$  unit that encodes the entire variable region. In heavy-chain DNA, variable-region rearrangement produces a rearranged gene consisting of the following sequences, starting from the 5' end: a short L exon, an intron, a joined VDJ segment, another intron, and a series of C gene segments. As with the light-chain genes, a promoter sequence is located a short distance upstream from each heavy-chain leader sequence. Once heavy-chain gene rearrangement is accomplished, RNA polymerase can bind to the promoter sequence and transcribe the entire heavy-chain gene, including the introns. Initially, both  $C_\mu$  and  $C_\delta$  gene segments are transcribed. Differential polyadenylation and RNA splicing remove the introns and process the primary transcript to generate mRNA including either the  $C_\mu$  or the  $C_\delta$  transcript. These two mRNAs are then translated, and the leader peptide of the resulting nascent polypeptide is cleaved, generating finished  $\mu$  and  $\delta$  chains. The production of two different heavy-chain mRNAs allows a mature, immunocompetent B cell to express both IgM and IgD with identical antigenic specificity on its surface.

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## 12.5 Generation of Antibody Diversity

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As the organization of the immunoglobulin genes was deciphered, the sources of the vast diversity in the variable region began to emerge. To date, seven mechanisms of antibody diversification have been identified in mice and humans: Multiple germ-line gene segments, Combinatorial V-(D)-J joining, Junctional flexibility, P-region nucleotide addition (P-addition), N-region nucleotide addition (N-addition), Somatic hypermutation, Combinatorial association of light and heavy chains.

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## 12.6 Class Switching Among Constant-Region Genes

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After antigenic stimulation of a B cell, the heavy-chain DNA can undergo a further rearrangement in which the  $V_H D_H J_H$  unit can combine with any  $C_H$  gene segment. The exact mechanism of this process, called class switching or isotype switching, is not clear, but it involves DNA flanking sequences (called switch regions) located 2–3 kb upstream from each  $C_H$  segment (except  $C_{\mu}$ ). These switch regions, though rather large (2 to 10 kb), are composed of multiple copies of short repeats (GAGCT and TGGGG). One hypothesis is that a protein or system of proteins that constitute the switch recombinase recognize these repeats and upon binding carry out the DNA recombination that results in class switching. Intercellular regulatory proteins known as cytokines act as “switch factors” and play major roles in determining the particular immunoglobulin class that is expressed as a consequence of switching.

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## 12.7 Expression of Ig Genes

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As in the expression of other genes, post-transcriptional processing of immunoglobulin primary transcripts is required to produce functional mRNAs. The primary transcripts produced from rearranged heavy-chain and light-chain genes contain intervening DNA sequences that include noncoding introns and J gene segments not lost during V-(D)-J rearrangement. In addition, the heavy-chain C-gene segments are organized as a series of coding exons and noncoding introns. Each exon of a  $C_H$  gene segment corresponds to a constant-region domain or a hinge region of the heavy-chain polypeptide. The primary transcript must be processed to remove the intervening DNA sequences, and the remaining exons must be connected by a process called RNA splicing. Short, moderately conserved splice sequences, or splice sites, which are located at the intron-exon boundaries within a primary transcript, signal the positions at which splicing occurs. Processing of the primary transcript in the nucleus removes

each of these intervening sequences to yield the final mRNA product. The mRNA is then exported from the nucleus to be translated by ribosomes into complete H or L chains.

Processing of an immunoglobulin heavy-chain primary transcript can yield several different mRNAs, which explains how a single B cell can produce secreted or membrane bound forms of a particular immunoglobulin and simultaneously express IgM and IgD.

### **12.7.1 Expression of membrane or secreted immunoglobulin**

A particular immunoglobulin can exist in either membrane-bound or secreted form. The two forms differ in the amino acid sequence of the heavy-chain carboxyl-terminal domains (CH3/CH3 in IgA, IgD, and IgG and CH4/CH4 in IgE and IgM). The secreted form has a hydrophilic sequence of about 20 amino acids in the carboxyl terminal domain. This is replaced in the membrane-bound form with a sequence of about 40 amino acids containing a hydrophilic segment that extends outside the cell, a hydrophobic transmembrane segment, and a short hydrophilic segment at the carboxyl terminus that extends into the cytoplasm. Differential processing of a common primary transcript determines whether the secreted or membrane form of an immunoglobulin will be produced. Mature naive B cells produce only membrane-bound antibody, whereas differentiated plasma cells produce secreted antibodies.

### **12.7.2 Simultaneous expression of IgM And IgD**

Differential RNA processing also underlies the simultaneous expression of membrane-bound IgM and IgD by mature B cells. Transcription of rearranged heavy-chain genes in mature B cells produces primary transcripts containing both the  $C_{\mu}$  and  $C_{\delta}$  gene segments. The  $C_{\mu}$  and  $C_{\delta}$  gene segments are close together in the rearranged gene (only about 5 kb apart), and the lack of a switch site between them permits the entire  $VDJC_{\mu}C_{\delta}$  region to be transcribed into a single primary RNA transcript about 15 kb long, which contains four poly-A sites. Sites 1 and 2 are associated with  $C_{\mu}$  and sites 3 and 4 are located at similar places in the  $C_{\delta}$  gene segment. If the heavy-chain transcript is cleaved and polyadenylated at site 2 after the  $C_{\mu}$  exons, then the mRNA will encode the membrane form of the  $\mu$  heavy chain. If polyadenylation is instead further downstream at site 4, after the  $C_{\delta}$  exons, then RNA splicing will remove the intervening  $C_{\mu}$  exons and produce mRNA encoding the membrane form of the  $\delta$  heavy chain. Since the mature B cell expresses both IgM and IgD on its membrane, both processing pathways must occur simultaneously. Likewise,

cleavage and polyadenylation of the primary heavy-chain transcript at poly-A site 1 or 3 in plasma cells and subsequent splicing will yield the secreted form of the  $\mu$  or  $\delta$  heavy chains, respectively.

### 12.7.3 Synthesis, Assembly, and Secretion of Immunoglobulins

Immunoglobulin heavy- and light-chain mRNAs are translated on separate polyribosomes of the rough endoplasmic reticulum (RER). Newly synthesized chains contain an amino-terminal leader sequence, which serves to guide the chains into the lumen of the RER, where the signal sequence is then cleaved. The assembly of light (L) and heavy (H) chains into the disulfide-linked and glycosylated immunoglobulin molecule occurs as the chains pass through the cisternae of the RER. The complete molecules are transported to the Golgi apparatus and then into secretory vesicles, which fuse with the plasma membrane. The order of chain assembly varies among the immunoglobulin classes. In the case of IgM, the H and L chains assemble within the RER to form half-molecules, and then two half-molecules assemble to form the complete molecule. In the case of IgG, two H chains assemble, then an  $H_2L$  intermediate is assembled, and finally the complete  $H_2L_2$  molecule is formed. Inter chain disulfide bonds are formed, and the polypeptides are glycosylated as they move through the Golgi apparatus. If the molecule contains the transmembrane sequence of the membrane form, it becomes anchored in the membrane of a secretory vesicle and is inserted into the plasma membrane as the vesicle fuses with the plasma membrane. If the molecule contains the hydrophilic sequence of secreted immunoglobulins, it is transported as a free molecule in a secretory vesicle and is released from the cell when the vesicle fuses with the plasma membrane.

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## 12.8 Regulation of Ig-Gene Transcription

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In a B cell immunoglobulin genes are expressed at different rates during different developmental stages. The V (D) J recombination reaction, which serves as the basis for generating the vast repertoire of antibody molecules, occurs only in lymphoid cells and like other biological processes is mediated by cis-acting elements and trans-acting factors. Three major classes of cis-regulatory sequences in DNA regulate transcription of immunoglobulin genes.

- Promoters: relatively short nucleotide sequences, extending about 200 bp upstream from the transcription initiation site, that promote initiation of RNA transcription in a specific direction

- Enhancers: nucleotide sequences situated some distance upstream or downstream from a gene that activate transcription from the promoter sequence in an orientation-independent manner
- Silencers: nucleotide sequences that down-regulate transcription, operating in both directions over a distance.

All of these regulatory elements have clusters of sequence motifs that can bind specifically to one or more nuclear proteins. Each  $V_H$  and  $V_L$  gene segment has a promoter located just upstream from the leader sequence. In addition, the  $J$  cluster and each of the  $D_H$  genes of the heavy-chain locus are preceded by promoters. Like other promoters, the immunoglobulin promoters contain a highly conserved AT rich sequence called the TATA box, which serves as a site for the binding of a number of proteins that are necessary for the initiation of RNA transcription. The actual process of transcription is performed by RNA polymerase II, which starts transcribing DNA from the initiation site, located about 25 bp downstream of the TATA box. Ig promoters also contain an essential and conserved octamer that confers B-cell specificity on the promoter. The octamer binds two transcription factors, oct-1, found in many cell types, and oct-2, found only in B cells.

While much remains to be learned about the function of enhancers, they have binding sites for a number of proteins, many of which are transcription factors. A particularly important role is played by two proteins encoded by the E2A gene which can undergo alternate splicing to generate two collaborating proteins, both of which bind to the  $\mu$  and intronic enhancers. These proteins are essential for B-cell development and E2A knockout mice make normal numbers of T cells but show a total absence of B cells. Interestingly, transfection of these enhancer-binding proteins into a T cell line resulted in a dramatic increase in the transcription of  $\mu$  chain mRNA and even induced the T cell to undergo  $D_H + J_H \rightarrow D_HJ_H$  rearrangement.

Silencers may inhibit the activity of Ig enhancers in non-B cells. If so, they could be important contributors to the high levels of Ig gene transcription that are characteristic of B cells but absent in other cell types. One heavy-chain enhancer is located within the intron between the last (3') J gene segment and the first (5') C gene segment ( $C_\mu$ ), which encodes the  $\mu$  heavy chain. Because this heavy-chain enhancer ( $E_\mu$ ) is located 5' of the  $S_\mu$  switch site near  $C_\mu$ , it can continue to function after class switching has occurred. Another heavy-chain

enhancer (3' E) has been detected 3' of the C gene segment. One  $\kappa$ -light-chain enhancer (E<sub>κ</sub>) is located between the J segment and the C segment, and another enhancer (3' E) is located 3' of the C segment. The light-chain enhancers are located 3' of C<sub>4</sub> and 3' of C<sub>1</sub>. Silencers have been identified in heavy-chain and  $\kappa$ -chain DNA, adjacent to enhancers, but not in  $\lambda$ -chain DNA.

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## 12.9 Generation, Activation and Differentiation of T-Lymphocytes

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The generation of mature progenitor T-cells (Maturation) in the thymus and the activation of mature T-cells in the periphery are influenced by the MHC molecules. Those T-cells restricted by Class I MHC develop into CD8<sup>+</sup> T-cells, and those restricted by Class II MHC give rise to CD4<sup>+</sup> T-cells.

### 12.9.1 Maturation of T-Cell

The progenitors of T-cells migrate from the bone marrow into the thymus under the influence of chemotactic factors that are secreted by the thymic epithelial cells. This process starts on about the 11th day of gestation in mice, and in the 8th or nine week of gestation in humans. The generation of a mature T-cell population with a diverse TCR repertoire is divided into an early phase in which the development of thymocytes can proceed in the absence of mature TCR, and a late phase in which further maturation critically depends on the cells expression of a functional  $\beta$  TCR. The early phase covers all the developmental stages prior to the appearance of CD4<sup>+</sup>CD8<sup>+</sup> positive thymocytes. T-cell maturation occurs in the thymus when progenitor T-cells from the bone marrow enter the thymus and rearrange their TCR genes.

On arrival in the thymus, progenitor T-cells do not express surface molecules characteristic of T-cells. They lack detectable CD4 and CD8, and are therefore called Double Negative (DN) cells. The expression of other cell surface molecules particularly C-kit, CD44 and CD25 marks the developmental progression of the DN population in the early phase. The most immature thymocyte lack TCR CD3 expression and reside in the CD4<sup>+</sup> CD8<sup>+</sup> population which are called Triple Negative (TNs). This population is only about 1 to 2 per cent of all thymocytes and passes through a series of differentiation stages. These stages ultimately lead to the coexpression of CD4 and CD8 and the generation of Double Positive (DP) thymocytes.

Pre T-cell receptor (Pre-TCR) is expressed on thymocytes that lack CD4 and CD8 markers on the surface. This receptor consists of a CD3 protein and a disulphide linked heterodimer made of *B* chain of the TCR and a 33 kD type I transmembrane glycoprotein which is called pre-T (pT ) covalently associated with TCR $\beta$ . It belongs to the immunoglobulin superfamily and is encoded by a non-rearranging gene.

In the final stages of maturation two different development pathways are followed, which generate functionally distinct CD4<sup>\*</sup> and CD8<sup>\*</sup> sub-population that exhibit Class II and Class I MHC restriction, respectively. T-cell maturation involves rearrangements of the germ-line T-cell Receptor (TCR) genes and the expression of various membrane markers. In the thymus, developing T-cells, known as thymocytes, proliferate and differentiate along different developmental pathways, which generate distinct sub-populations of mature T-cells. The T-cells diversify by a pair of selection processes.

### **Positive Selection**

Positive selection is an interaction between immature double positive thymocytes with thymic epithelial cells. This interaction is through their TCR which establishes the contact with Class I and Class II MHC expressed on thymic epithelial cells. Those thymocytes which fail to establish such contacts undergo apoptosis. Only those thymocytes whose *BT*CR heterodimer recognizes a self-MHC molecule are selected for survival. Positive selection permits the survival of only those T-cells whose TCRs recognize self-MHC molecules.

### **Negative Selection**

The negative selection eliminates T-cells that react too strongly with self-MHC. The MHC restricted population of positively selected thymocytes shows two types of TCR affinity (high and low) to self-MHC. Those thymocytes which show high affinity with self-MHC molecules undergo negative selection by interaction with bone marrow derived dendritic cells or macrophages in the medulla of the thymus. Tolerance to self antigen is achieved by eliminating self reactive T-cells and only allowing maturation of T-cells that are specific for foreign antigen and altered self molecules. These processes generate a primary T-cell repertoire that is self-tolerant.

The thymocytes that undergo productive TCR gene rearrangement are not put through the selection process which involves positive selection or negative

selection. The positive selection ensures  $\alpha$  TCR expressed on the T-cell of an individual will bind to self-MHC. The cells which fail to undergo positive selection are eliminated within the thymus. Those thymocytes which show high affinity receptors for self-MHC molecules undergo death by apoptosis. The processes are necessary to generate mature T-cells that are MHC restricted and self-tolerant.

About 98 percent of all thymocytes do not mature. They die by apoptosis within the thymus. Most double negative thymocytes progress down a different development pathway. They stop proliferating and begin to rearrange the TCR  $\beta$ -chain genes and express the  $\beta$ -chain. These  $\beta$ -chains combine with a 33 kDa glycoprotein known as pre-T  $\zeta$  chain and associate with the CD3 group to form a complex called pre-T-cell Receptor (pre-TCR). Immature thymocytes in the  $\beta$  pathway express a pre-T-cell receptor.

The pre-TCR recognizes some intra-thymic ligand and transmits the signal through the CD3 complex that activates a protein tyrosine kinase. Once a signal has been transmitted through the pre-TCR, it halts further  $\beta$ -chain gene rearrangement and induces expression of both CD4 and CD8. The thymocytes are now called Double Positive (DP), or CD4<sup>+</sup>CD8<sup>+</sup> cells, which begin to proliferate. Double positive thymocytes that express the  $\alpha$  TCR-CD3 complex and survive thymic selection develop into either single-positive CD4<sup>+</sup> thymocytes or single-positive CD8<sup>+</sup> thymocytes. Thus, in the thymus there is: (1) Positive selection of thymocytes bearing receptors capable of binding self-MHC molecules, which results in MHC-restriction (2) Negative selection by elimination of thymocytes bearing high affinity receptors for self-MHC molecules alone or self-antigen presented by self-MHC, which results in self-tolerance.

Both these processes are necessary to generate mature T-cells that are self-MHC restricted and self-tolerant. Thymic stromal cells, including epithelial cells, macrophages, and dendritic cells play essential roles in positive and negative selection. During positive selection only those cells whose  $\alpha$  TCR heterodimer recognizes a self-MHC molecule are selected for survival.

### **12.9.2 T Cell activation**

The central event in the generation of both humoral and cell mediated immune responses is the activation and the clonal expansion of T-cells. The activated T-cell progresses through the cell cycle, proliferating and differentiating into

memory cells or effector cells. After T-cells interact with antigen, numerous genes are activated. Superantigens are viral or bacterial proteins that bind simultaneously to the *VB* domain of a T-cell receptor and to the  $\beta$  chain of a Class II MHC molecule. Crosslinking of a T-cell receptor and the Class II MHC molecule by a superantigen produces an activating signal that induces T-cell activation and proliferation. Activation of T-cell is initiated by an antigen presenting cell which presents antigenic peptide on its surface in the groove of a Class II MHC molecule.

T-cell activation is initiated by interaction of the TCR-CD3 complex with a peptide-MHC complex on an antigen-presenting cell. The activating signal is transduced by the TCR-CD3 complex and regulated by co-receptors CD4, CD8 and CD5. Signal transduction is accomplished by a series of protein phosphorylation and dephosphorylation events catalyzed by protein kinases and protein phosphatases. In addition to the signals mediated by T-cell receptor and its associated accessory molecules (Signal 1), activation of the T-cell requires a co-stimulatory signal (Signal 2) provided by the antigen-presenting cells.

T-cell recognition of an antigenic peptide-MHC complex on an antigen presenting cell results either in activation and clonal expansion or in a state of non-responsiveness called clonal anergy. The presence or absence of the co-stimulatory signal (Signal 2) determines whether activation results in clonal expansion or clonal anergy. Only dendritic cells, macrophages, and B-cells present antigen together with Class II MHC molecules and deliver the co-stimulatory signal necessary for complete T-cell activation that leads to proliferation and differentiation. The antigen-presenting cells differ in their ability to display antigen and to deliver the co-stimulatory signal.

Class II MHC peptide complex on APC establish the contact with TCR-CD3 complex of T-cell resulting in activating signals. There is a cascade of biochemical events that induces resting T-cell to enter the cell cycle G<sub>0</sub> to G<sub>1</sub>, which results in the expression of high affinity autocrine receptor for IL-2. IL-2 is the T-cell growth factor; it helps in T-cell proliferation, progression and differentiation into effector and memory T-cells. Interaction of T-cell with antigen on Class II MHC-APC will lead to activation of many genes. The gene activation occurs at different intervals. There are immediate genes that are activated within half an hour of antigen recognition, for example, c-Fox, c-Myc, NFAT. Early genes are those which are expressed within one to 2 hours of antigen recognition for example, IL-2, IL-2R, IL-3, IFN- $\gamma$ . Late genes are

expressed more than two days after antigen recognition and are responsible for secretion of various adhesion molecules.

### **12.9.3 T-cell differentiation**

Naive T-cells survive for only about five to seven weeks in the absence of antigen stimulation. Each naive T-cell recirculates from the blood to the lymph nodes and back again every 12 to 24 hours. CD4<sup>\*</sup> and CD8<sup>\*</sup> T-cells leave the thymus and enter the circulation as resting cells in the G<sub>0</sub> stage of the cell cycle as naive T-cells, which have not yet encountered antigen. They continually recirculate between the blood and lymph systems. During recirculation, naive T-cells reside in secondary lymphoid tissues such as lymph nodes. If a naive cell does not encounter antigen in a lymph node, it exits through the efferent lymphatics, and drains into the thoracic duct to rejoin the blood.

On the other hand, if a naive T-cell recognizes an antigen-MHC complex on an appropriate antigen-presenting cell of target cell it gets activated, initiating a primary response. After 48 hours of activation, the naive T-cell enlarges into a blast cell which begins undergoing repeated rounds of cell division. Blast cells divide two to three times per day and keep on doing so for five days thereby generating a large population of clones which differentiate into memory or effector T-cells. Activation depends on the signal induced by engagement of the TCR complex and a co-stimulatory signal induced by the CD28-B7 interaction. These signals trigger entry of the T-cell into the G<sub>1</sub> phase of the cell cycle and induce transcription of the gene for IL-2 and the  $\alpha$  chain of the high affinity IL-2 receptor. It also increases the half-life of IL-2 mRNA. Secretion of IL-2 and its subsequent binding to the high affinity IL-2 receptor induces the activated naive T-cells to proliferate and differentiate. T-cells activated in this way divide 2-3 times per day for 4-5 days, generating a large clone of progeny cells which differentiate into memory or effector T-cell populations. Effector cells are derived from both naive and memory cells after antigen activation. The effector T-cells have a short lifespan ranging from few days to a few weeks. They differ from the naive T-cells in respect to cell surface markers which contribute to their recirculation ability. One subset of CD4 effector cells is called T<sub>h</sub>1 subset which secretes IL-2, IFN- $\gamma$  and TNF- $\beta$  and the cells are responsible for the activation of cytotoxic T-cells and are also involved in the delayed type of hypersensitivity. The other subset T<sub>h</sub>2 secretes IL-4, IL-5, IL-6 and IL-10 and serves as effector helper cell for B-cell activation.

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## 12.10 Generation, Activation And Differentiation Of B-Lymphocytes

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B-cell developmental process can be divided into three broad stages: generation of mature immunocompetent B cells (maturation), activation of mature B cells when they interact with antigen, and differentiation of activated B cells into plasma cells and memory B cells. In many vertebrates, including humans and mice, the bone marrow generates B cells. This process is an orderly sequence of Ig-gene rearrangements, which progresses in the absence of antigen. This is the antigen independent phase of B-cell development. A mature B cell leaves the bone marrow expressing membrane-bound immunoglobulin (mIgM and mIgD) with a single antigenic specificity. These naive B cells, which have not encountered antigen, circulate in the blood and lymph and are carried to the secondary lymphoid organs, most notably the spleen and lymph nodes. If a B cell is activated by the antigen specific to its membrane-bound antibody, the cell proliferates and differentiates to generate a population of antibody-secreting plasma cells and memory B cells. Since B cell activation and differentiation in the periphery require antigen, this stage comprises the antigen dependent phase of B-cell development.

### 12.10.1 B-Cell Maturation

The generation of mature B cells first occurs in the embryo and continues throughout life. Before birth, the yolk sac, fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow.

B-cell development begins as lymphoid stem cells differentiate into the earliest distinctive B-lineage cell—the progenitor B cell (pro-B cell)—which expresses a transmembrane tyrosine phosphatase called CD45R (B220 in mice). Pro-B cells proliferate within the bone marrow, filling the extravascular spaces between large sinusoids in the shaft of a bone. Proliferation and differentiation of pro-B cells into precursor B cells (pre-B cells) requires the microenvironment provided by the bone-marrow stromal cells. If pro-B cells are removed from the bone marrow and cultured *in vitro*, they will not progress to more mature B-cell stages unless stromal cells are present. The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, that support the developmental process.

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At the earliest developmental stage, pro-B cells require direct contact with stromal cells in the bone marrow. This interaction is mediated by several cell-adhesion molecules, including VLA-4 on the pro-B cell and its ligand, VCAM-1, on the stromal cell. After initial contact is made, a receptor on the pro-B cell called c-Kit interacts with a stromal-cell surface molecule known as stem-cell factor (SCF). This interaction activates c-Kit, which is a tyrosine kinase, and the pro-B cell begins to divide and differentiate into a pre-B cell and begins expressing a receptor for IL-7. The IL-7 secreted by the stromal cells drives the maturation process, eventually inducing down-regulation of the adhesion molecules on the pre-B cells, so that the proliferating cells can detach from the stromal cells. At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation.

B-cell maturation depends on rearrangement of the immunoglobulin DNA in the lymphoid stem cells. First to occur in the pro-B cell stage is a heavy-chain  $D_H$ -to- $J_H$  gene rearrangement; this is followed by a  $V_H$ -to- $D_HJ_H$  rearrangement. If the first heavy-chain rearrangement is not productive, then  $V_H$ - $D_HJ_H$  rearrangement continues on the other chromosome. Upon completion of heavy-chain rearrangement, the cell is classified as a pre-B cell. Continued development of a pre-B cell into an immature B cell requires a productive light-chain gene rearrangement. Because of allelic exclusion, only one light-chain isotype is expressed on the membrane of a B cell. Completion of a productive light-chain rearrangement commits the now immature B cell to a particular antigenic specificity determined by the cell's heavy-chain VDJ sequence and light-chain VJ sequence. Immature B cells express mIgM (membrane IgM) on the cell surface. The recombinase enzymes RAG-1 and RAG-2, which are required for both heavy-chain and light-chain gene rearrangements, are expressed during the pro-B and pre-B cell stages. The enzyme terminal deoxyribonucleotidyl transferase (TdT), which catalyzes insertion of N-nucleotides at the  $D_H$ - $J_H$  and  $V_H$ - $D_HJ_H$  coding joints, is active during the pro-B cell stage and ceases to be active early in the pre-B-cell stage. Because TdT expression is turned off during the part of the pre-B-cell stage when light-chain rearrangement occurs, N-nucleotides are not usually found in the  $V_L$ - $J_L$  coding joints. The bone-marrow phase of B-cell development culminates in the production of an IgM-bearing immature B cell. At this stage of development the B cell is not fully functional, and antigen induces death or unresponsiveness rather than division and differentiation. Full maturation is signalled by the co-expression of IgD and IgM on the membrane. This progression involves a

change in RNA processing of the heavy-chain primary transcript to permit production of two mRNAs, one encoding the membrane form of the  $\mu$  chain and the other encoding the membrane form of the  $\lambda$  chain.

A parallel situation occurs during B-cell development. In the pre-B cell, the membrane  $\mu$  chain is associated with the surrogate light chain, a complex consisting of two proteins: a V-like sequence called Vpre-B and a C-like sequence called  $\lambda_5$ , which associate noncovalently to form a light-chain-like structure. The membrane-bound complex of  $\mu$  heavy chain and surrogate light chain appears on the pre-B cell associated with the Ig- $\alpha$ /Ig- $\beta$  heterodimer to form the pre-B-cell receptor. Only pre-B cells that are able to express membranebound  $\mu$  heavy chains in association with surrogate light chains are able to proceed along the maturation pathway.

There is speculation that the pre-B-cell receptor recognizes a not-yet-identified ligand on the stromal-cell membrane, thereby transmitting a signal to the pre-B cell that prevents  $V_H$  to  $D_HJ_H$  rearrangement of the other heavy-chain allele, thus leading to allelic exclusion. Following the establishment of an effective pre-B-cell receptor, each pre-B cell undergoes multiple cell divisions, producing 32 to 64 descendants. Each of these progeny pre-B cells may then rearrange different light-chain gene segments, thereby increasing the overall diversity of the antibody repertoire. The critical role of the pre-B-cell is to generate a signal which is necessary for pre-B cells to proceed to the immature B-cell stage. Four such factors, E2A, early B-cell factor (EBF), B-cell-specific activator protein (BSAP), and Sox-4 are particularly important for B-cell development. These factors play essential roles in the early stages of commitment to the B-cell lineage.

### **12.10.2 B-cell activation and Proliferation**

In G0 stage of cell cycle, Naive B-cells are non-dividing B-cells. Activation of these resting cell drives them into the cell cycle, progressing through G1 into S phase, in which DNA is replicated. Once the cell has reached S phase, it completes the cell cycle, moving through G2 and into mitosis. After export of mature B-cells from the bone marrow, activation, proliferation and differentiation occur in the periphery and requires antigen, Activating signals fall into two categories, namely, the competence and progression signals. Competence signals drive the B-cell from G0 into early G1. Progression signals then drives the cell from G1 into S and further to cell division and differentiation.

Antigen driven activation of B-cells goes through two different methods on the basis of antigen involved. Depending on the nature of the antigen, B-cell activation proceeds by different routes that are either dependent upon  $T_h$ -cells or independent of them; The B-cell response to Thymus Dependent (TD) antigens requires direct contact with  $T_h$ -cells.

Antigen that can activate the B-cells in the absence of direct participation by  $T_h$ -cells are known as Thymus Independent (TI) antigens which are TI-1 and TI-2 types. The TI antigens are multivalent and therefore, induce strong stimulation of B-cells by cross-linking mIg molecules, which act as signal 1. Type 1 antigens are bacterial cell wall components including lipopolysaccharides (LPS). In addition type 1 TI antigens also contain an additional component that provides Signal 2. Most TI-1 antigens are polyclonal B-cell activators and at high concentrations stimulate proliferation and antibody secretion by as many as one third of all B-cells. The prototypic TI-1 antigen is lipopolysaccharide. TI-1 are not B-cell mitogens and therefore, do not act as polyclonal activators. Type 2 TI antigens are bacterial cell wall polysaccharides with repetitive units conjugated to polymeric proteins, for example bacterial flagellins. TI-1 antigens activate both mature and immature B-cells.

Type 2 TI antigens show extensive cross-linking with mIg receptor, generates an extensive competence signal, which itself leads to progression signal that leads to progression of B-cells. TI-2 antigens activate B-cells by extensively cross-linking the mIg receptor. TI-2 antigens activate only the mature cells, and inactivate immature B-cells. Although B-cell response to TI-2 antigens do not require direct involvement of  $T_h$ -cells. Cytokines derived from Th-cells are required for efficient B-cell proliferation and for class-switching isotypes other than IgM. Type 1 TI antigen seems to need an additional signal in the form of cytokine to enter into progression stage.

The response to TI antigens is generally weaker, no memory cells are formed and IgM is the predominant antibody that is secreted. This reflects a low level of class switching. Thus  $T_h$ -cells have an important role in generating memory cells, affinity maturation and class switching to other signalling pathways. The binding of the Ig receptor by antigen activates intracellular signalling pathways. Antigen driven activation and clonal selection of naive B-cells leads to generation of plasma cells and memory B-cells, in absence of antigen induced activation, naive B-cells in the periphery have a short life span, dying within a few weeks by apoptosis.

During B-cell differentiation affinity maturation, somatic hypermutation and class switching occurs. This is followed by the centroblasts differentiating into memory B-cells and plasma cells. A CD40 ligand represents a key negative signal that prevent centroblast from entering into terminal plasma cell differentiation. As long as CD40 ligand is provided centroblasts remain as it is in the presence of IL-2 and IL-10. Withdrawal of this ligand results in the centroblasts rapidly differentiating into plasma cells.

The long lived plasma cells are terminally differentiated B-cells that contribute to immune memory through the continual production of high affinity antibody. Memory B-cells generally express isotype switched and affinity matured membrane Ig and are required to cognate memory  $T_h$ -cell regulation for the response to antigen. Once the antigen binds to mIg receptor on B-cells, the antigen is processed into peptides. Binding of antigen to B-cell receptor mIg results in signal transduction leading to a sequence of events in the cytoplasm and gene action to upregulate the production of certain membrane associated molecules such as Class II MHC, costimulatory molecules B-7 and the receptor for the growth factors and cytokines. Enhanced expression of B-7 and class II MHC molecules on B-cells increases its ability to function as an antigen presenting cell for  $T_h$ -cell activation T-B-Cell conjugated is formed only when B-cell presents the processed antigenic peptide on its class II MHC molecule to  $T_h$ -cell through TCR. This contact is essential for the directional release of cytokines by the  $T_h$ -cells and also for B-cell activation. Three  $T_h$ -cells derived cytokines, IL-2, IL-4 and IL-5 provide progression signal for B-cells to proliferate. Proliferation of B-cells leads to three different events: formation of plasma cells, memory B-cells, class switching and affinity maturation. These events require signals provided by  $T_h$ -cells or follicular dendritic cells. This event in the B-cell differentiation is the result of somatic hypermutation and antigen selection of high affinity clones.

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## 12.11 Cytokine

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A variety of immunocompetent cells such as macrophages and T and B lymphocytes play a central role in the humoral and/or cellular immune response to pathogenic infections and other antigens of various natures. These immune responses are regulated by low-molecular weight soluble factors called cytokines, which are polypeptide hormones with autocrine or paracrine activities secreted by white blood cells and various other cells in the body in response to a number of stimuli.

Cytokines bind to specific receptors on the membrane of target cells, triggering signal-transduction pathways that ultimately alter gene expression in the target cells. The susceptibility of the target cell to a particular cytokine is determined by the presence of specific membrane receptors. In general, the cytokines and their receptors exhibit very high affinity for each other. Because their affinities are so high, cytokines can mediate biological effects at picomolar concentrations.

A particular cytokine may bind to receptors on the membrane of the same cell that secreted it, exerting autocrine action; it may bind to receptors on a target cell in close proximity to the producer cell, exerting paracrine action; in a few cases, it may bind to target cells in distant parts of the body, exerting endocrine action. Cytokines regulate the intensity and duration of the immune response by stimulating or inhibiting the activation, proliferation, and/or differentiation of various cells and by regulating the secretion of antibodies or other cytokines. Binding of a given cytokine to responsive target cells generally stimulates increased expression of cytokine receptors and secretion of other cytokines, which affect other target cells in turn. Thus, the cytokines secreted by even a small number of lymphocytes activated by antigen can influence the activity of numerous cells involved in the immune response. For example, cytokines produced by activated  $T_h$  cells can influence the activity of B cells,  $T_c$  cells, natural killer cells, macrophages, granulocytes, and hematopoietic stem cells, thereby activating an entire network of interacting cells.

Cytokines exhibit the attributes of pleiotropy, redundancy, synergy, antagonism, and cascade induction, which permit them to regulate cellular activity in a coordinated, interactive way. A given cytokine that has different biological effects on different target cells has a pleiotropic action. Two or more cytokines that mediate similar functions are said to be redundant; redundancy makes it difficult to ascribe a particular activity to a single cytokine. Cytokine synergism occurs when the combined effect of two cytokines on cellular activity is greater than the additive effects of the individual cytokines. In some cases, cytokines exhibit antagonism; that is, the effects of one cytokine inhibit or offset the effects of another cytokine. Cascade induction occurs when the action of one cytokine on a target cell induces that cell to produce one or more other cytokines, which in turn may induce other target cells to produce other cytokines.

The term cytokine encompasses those cytokines secreted by lymphocytes, substances formerly known as lymphokines, and those secreted by monocytes

and macrophages, substances formerly known as monokines. Although these other two terms continue to be used, they are misleading because secretion of many lymphokines and monokines is not limited to lymphocytes and monocytes as these terms imply, but extends to a broad spectrum of cells and types. For this reason, the more inclusive term cytokine is preferred.

Many cytokines are referred to as interleukins, a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Some cytokines are known by common names, including the interferons and tumor necrosis factors. Recently gaining prominence is yet another subgroup of cytokines, the chemokines, a group of low-molecular weight cytokines that affect chemotaxis and other aspects of leukocyte behavior. These molecules play an important role in the inflammatory response.

The activity of cytokines was first recognized in the mid-1960s, when supernatants derived from *in vitro* cultures of lymphocytes were found to contain factors that could regulate proliferation, differentiation, and maturation of allogeneic immune-system cells. Soon after, it was discovered that production of these factors by cultured lymphocytes was induced by activation with antigen or with nonspecific mitogens. Biochemical isolation and purification of cytokines was hampered because of their low concentration in culture supernatants and the absence of well-defined assay systems for individual cytokines. A great advance was made with the development of gene-cloning techniques during the 1970s and 1980s, which made it possible to produce pure cytokines by expressing the protein from cloned genes. The discovery of cell lines whose growth depended on the presence of a particular cytokine provided researchers with the first simple assay systems. The derivation of monoclonal antibodies specific for each of the more important cytokines has made it possible to develop rapid quantitative immunoassays for each of them.

Once the genes encoding various cytokines had been cloned, sufficient quantities of purified preparations became available for detailed studies on their structure and function. Cytokines generally have a molecular mass of less than 30 kDa. Structural studies have shown that the cytokines share a similar polypeptide fold, with four  $\alpha$ -helical regions (A–D) in which the first and second helices and the third and fourth helices run roughly parallel to one another and are connected by loops.

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## 12.12 Functions of Cytokines

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Although a variety of cells can secrete cytokines, the two principal producers are the  $T_h$  cell and the macrophage. Cytokines released from these two cell types activate an entire network of interacting cells. Among the numerous physiologic responses that require cytokine involvement are development of cellular and humoral immune responses, induction of the inflammatory response, regulation of hematopoiesis, control of cellular proliferation and differentiation, and the healing of wounds. Although the immune response to a specific antigen may include the production of cytokines, it is important to remember that cytokines act in an antigen-nonspecific manner. That is, they affect whatever cells they encounter that bear appropriate receptors and are in a physiological state that allows them to respond.

Cytokines are involved in a staggeringly broad array of biological activities including innate immunity, adaptive immunity, inflammation, and hematopoiesis. Altogether, the total number of proteins with cytokine activity easily exceeds 100 and research continues to uncover new ones.

The nonspecificity of cytokines seemingly conflicts with the established specificity of the immune system. One way in which specificity is maintained is by careful regulation of the expression of cytokine receptors on cells. Often cytokine receptors are expressed on a cell only after that cell has interacted with antigen. In this way cytokine activation is limited to antigen-activated lymphocytes. Another means of maintaining specificity may be a requirement for direct interaction between the cytokine-producing cell and the target cell to trigger cytokine secretion, thus ensuring that effective concentrations of the cytokine are released only in the vicinity of the intended target. In the case of the  $T_h$  cell, a major producer of cytokines, close cellular interaction occurs when the T-cell receptor recognizes an antigen-MHC complex on an appropriate antigen-presenting cell, such as a macrophage, dendritic cell, or B lymphocyte. Cytokines secreted at the junction of these interacting cells reach high enough local concentrations to affect the target APC but not more distant cells. In addition, the half-life of cytokines in the bloodstream or other extracellular fluids into which they are secreted is usually very short, ensuring that they act for only a limited period of time and thus over a short distance.

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## 12.13 Cytokine Receptors

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To exert their biological effects, cytokines must first bind to specific receptors expressed on the membrane of responsive target cells. Because these receptors are expressed by many types of cells, the cytokines can affect a diverse array of cells. Biochemical characterization of cytokine receptors initially progressed at a very slow pace because their levels on the membrane of responsive cells is quite low. As with the cytokines themselves, cloning of the genes encoding cytokine receptors has led to rapid advances in the identification and characterization of these receptors.

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### **12.14 Therapeutic Uses of Cytokines and Their Receptors**

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The availability of purified cloned cytokines and soluble cytokine receptors offers the prospect of specific clinical therapies to modulate the immune response. A few cytokines—notably, interferons and colony stimulating factors, such as GM-CSF, have proven to be therapeutically useful. However, despite the promise of cytokines as powerful mediators of immune and other biological responses, not many have made their way into clinical practice. A number of factors are likely to raise difficulties in adapting cytokines for safe and effective routine medical use. One of these is the need to maintain effective dose levels over a clinically significant period of time. During an immune response, interacting cells produce sufficiently high concentrations of cytokines in the vicinity of target cells, but achieving such local concentrations when cytokines must be administered systemically for clinical treatment is difficult. In addition, cytokines often have a very short half-life, so that continuous administration may be required. For example, recombinant human IL-2 has a half-life of only 7–10 min when administered intravenously. Finally, cytokines are extremely potent biological response modifiers and they can cause unpredictable and undesirable side effects. The side effects from administration of recombinant IL-2, for instance, range from mild (e.g., fever, chills, diarrhea, and weight gain) to serious, such as anemia, thrombocytopenia, shock, respiratory distress, and coma. Despite these difficulties, the promise of cytokines for clinical medicine is great and efforts to develop safe and effective cytokine-related strategies continue, particularly in areas such as inflammation, cancer therapy, and modification of the immune response during organ transplantation, infectious disease, and allergy.

Some specific examples of various approaches being explored include cytokine receptor blockade and the use of cytokine analogs and cytokine-toxin conjugates. For instance, proliferation of activated  $T_h$  cells and activation of  $T_c$

cells can be blocked by anti-TAC, a monoclonal antibody that binds to the subunit of the high-affinity IL-2 receptor. Administration of anti-TAC has prolonged the survival of heart transplants in rats. Similar results have been obtained with IL-2 analogs that retain their ability to bind the IL-2 receptor but have lost their biological activity. Such analogs have been produced by site-directed mutagenesis of cloned IL-2 genes. Finally, cytokines conjugated to various toxins (e.g., the A chain of diphtheria toxin) have been shown to diminish rejection of kidney and heart transplants in animals. Such conjugates containing IL-2 selectively bind to and kill activated T<sub>h</sub> cells.

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## 12.15 Summary

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1. Immunoglobulin light chains and heavy chains are encoded by three separate multigene families, each containing numerous gene segments and located on different chromosomes.
2. Functional light-chain and heavy-chain genes are generated by random rearrangement of the variable-region gene segments in germ-line DNA.
3. Immunoglobulin gene rearrangements occur in sequential order, heavy-chain rearrangements first, followed by light chain rearrangements. Allelic exclusion is a consequence of the functional rearrangement of the immunoglobulin DNA of only one parental chromosome and is necessary to assure that a mature B cell expresses immunoglobulin with a single antigenic specificity.
4. The major sources of antibody diversity, which can generate  $>10^{10}$  possible antibody combining sites, are: Multiple germ-line gene segments, Combinatorial V-(D)-J joining, junctional flexibility, P-addition, N-addition and somatic mutation.
5. After antigenic stimulation of mature B cells, class switching results in expression of different classes of antibody (IgG, IgA, and IgE) with the same antigenic specificity.
6. Differential RNA processing of the immunoglobulin heavy-chain primary transcript generates membrane bound antibody in mature B cells, secreted antibody in plasma cells, and the simultaneous expression of IgM and IgD by mature B cells.
7. Transcription of immunoglobulin genes is regulated by three types of DNA regulatory sequences: promoters, enhancers and silencers.

8. Progenitor T cells from the bone marrow enter the thymus and rearrange their TCR genes. In most cases these thymocytes rearrange TCR genes and become  $\alpha\beta$  T cells. A small minority rearrange TCR genes and become  $\gamma\delta$  T cells.
9. The earliest thymocytes lack detectable CD4 and CD8 and are referred to as double-negative cells.
10. Positive selection in the thymus eliminates T cells unable to recognize self-MHC and is the basis of MHC restriction. Negative selection eliminates thymocytes bearing high-affinity receptors for self-MHC molecules alone or self-antigen plus self-MHC and produces self-tolerance.
11. Naive T cells are resting cells (G<sub>0</sub>) that have not encountered antigen. Activation of naive cells leads to the generation of effector and memory T cells. Memory T cells, which are more easily activated than naive cells, are responsible for secondary responses. Effector cells are short lived and perform helper, cytotoxic, or delayed-type hypersensitivity functions.
12. The T-cell repertoire is shaped by apoptosis in the thymus and periphery.
13. T cells are not MHC restricted. Most in humans bind free antigen, and most have the same specificity. They may function as part of the innate immune system
14. B cells develop in bone marrow and undergo antigen induced activation and differentiation in the periphery. Activated B cells can give rise to antibody-secreting plasma cells or memory B cells.
15. During B-cell development, sequential Ig-gene rearrangements transform a pro-B cell into an immature B cell expressing mIgM with a single antigenic specificity. Further development yields mature naive B cells expressing both mIgM and mIgD.
16. In the periphery, the antigen-induced activation and differentiation of mature B cells generates an antibody response. The antibody response to proteins and most other antigens requires T<sub>H</sub> cells. These are thymus-dependent or simply T-dependent (T<sub>D</sub>) responses. Responses to some antigens, such as certain bacterial cell-wall products (e.g., LPS) and polymeric molecules with repeating epitopes, do not require T<sub>H</sub> cells and

are independent ( $T_1$ ) antigens. The vast majority of antigens are dependent.

17. B-cell activation is the consequence of signal-transduction process triggered by engagement of the B-cell receptor that ultimately leads to many changes in the cell, including changes in the expression of specific genes.
18. B- and T-cell activation share many parallels, including: compartmentalization of function within receptor subunits; activation by membrane-associated protein tyrosine kinases; assembly of large signaling complexes with protein-tyrosine-kinase activity; and recruitment of several signal-transduction pathways.
19. The properties of the primary and secondary antibody responses differ. The primary response has a long lag period, a logarithmic rise in antibody formation, a short plateau, and then a decline. IgM is the first antibody class produced, followed by a gradual switch to other classes, such as IgG. The secondary response has a shorter lag time, a more rapid logarithmic phase, a longer plateau phase, and a slower decline than the primary response. Mostly IgG and other isotypes are produced in the secondary response rather than IgM, and the average affinity of antibody produced is higher.
20. Cytokines are low-molecular-weight proteins that are produced and secreted by a variety of cell types. They play major roles in the induction and regulation of the cellular interactions involving cells of the immune, inflammatory and hematopoietic systems.
21. The biological activities of cytokines exhibit pleiotropy, redundancy, synergy, antagonism, and, in some instances, cascade induction.
22. There are over 200 different cytokines, most of which fall into one of the following families: hematopoietins, interferons, chemokines, and tumor necrosis factors.
23. Cytokines act by binding to cytokine receptors, most of which can be classified as immunoglobulin superfamily receptors, class I cytokine receptors, class II cytokine receptors, members of the TNF receptor family, and chemokine receptors.

24. A cytokine can only act on a cell that expresses a receptor for it. The activity of particular cytokines is directed to specific cells by regulation of the cell's profile of cytokine receptors.
25. Therapies based on cytokines and cytokine receptors have entered clinical practice.

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## 12.16 Glossary

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- **Affinity:** A measure of the binding constant of a single antigen-combining site with a monovalent antigenic determinant.
- **Allelic exclusion:** The ability of heterozygous lymphoid cells to produce only one allelic form of antigen-specific receptor (Ig or TCR) when they have the genetic endowment to produce both. Genes other than those for the antigen-specific receptors are usually expressed codominantly.
- **Apoptosis:** A form of programmed cell death caused by activation of endogenous molecules leading to the fragmentation of DNA.
- **B-cell receptor (BCR):** The cell surface receptor of B cells for a specific antigen composed of a transmembrane immunoglobulin molecule associated with the invariant Ig  $\alpha$  and Ig  $\beta$  chains in a noncovalent complex.
- **Combinatorial joining:** the joining of V, D and J segments of Ig and TCR genes to generate essentially new genetic information during the development of B and T cells.
- **Complement:** A key effector mechanism in both innate and adaptive immunity for the elimination of microbial pathogens.
- **Complementarity-determining regions (CDRs):** Hypervariable regions of immunoglobulins and T-cell receptors that determine their specificity and make contact with specific ligand. There are three such regions (CDR1, CDR2, and CDR3) in each V domains.
- **Cytokine receptors:** Cellular receptors for cytokines.
- **Cytokines:** Soluble substances secreted by cells which have a variety of effects on other cells.

- **Diversity:** The existence of a large number of lymphocytes with different antigenic specificities in any individual. Diversity is a fundamental property of the adaptive immune system and is the result of variability in the structures of the antigen-binding sites of lymphocytes receptors for antigens (antibodies and TCRs).
- **Domain:** A compact segment of an immunoglobulin or TCr chain, made up of amino acids around an S-S bond.
- **Double –negative thymocyte:** Immature T cells within the thymus that lack expression of both CD4 and CD8.
- **Double –positive thymocyte:** An immediate stage in T-cell development in the thymus characterized by expression of both CD4 and CD8.
- **Germinal centers:** Secondary lymphoid structures that are sites of intense B-cell proliferation, selection, maturation and death during antibody responses.
- **Germline:** Refers to genes in germ cells as opposed to somatic cells. In immunology, it refers to immunoglobulin or TCR genes in their unarranged state.
- **Heavy (H) chain:** The larger of the two types of chains that comprise a normal immunoglobulin or antibody molecule.
- **Helper T cells:** A class of T cells that cooperate with B cells to make antibody in responses to thymus –dependent antigens.
- **Hypervariable regions:** Portions of the light and heavy immunoglobulin chains that are highly variable in amino acid sequence from one immunoglobulin molecule to another and that together constitute the antigen-binding site of an antibody molecule.
- **Immature B cell:** IgM-positive cell in the B-cell lineage easily tolerized by exposure to antigen.
- **Interleukins:** Glycoproteins secreted by a variety of leukocytes that have effects on other leukocytes.
- **Lymphokine:** A cytokine secreted by lymphocytes.

- **Major histocompatibility complex:** A cluster of genes encoding polymorphic cell-surface molecules that are involved in interactions with T cells. These molecules also play major role in transplantation rejection.
- **Mature B cell:** B cells with IgM and IgD on their surfaces.
- **Naïve lymphocytes:** Lymphocytes that have not yet encountered their specific antigen and therefore have never responded to it. All lymphocytes leaving the central lymphoid organs are naïve.
- **Plasma cell:** The antibody-producing end stage of B-cell differentiation.
- **Positive selection:** The process by which developing B and T cells receive signals in the primary lymphoid organ in which they are developing to continue their differentiation.
- **Pre-B cell:** cell in the B-cell lineage which has rearranged heavy but not light chain genes.
- **Pre-T cell:** Cell in T-lymphocyte differentiation in the thymus that has rearranged TCR genes.
- **Pro-B cell:** Earliest stage of B-cell differentiation in which a heavy-chain D segment rearranges to a J gene segment.
- **Promotor:** A DNA sequence immediately 5' to transcription start site of a gene where the proteins that initiate transcription bind.
- **Pro-T cell:** A developing T cell in the thymic cortex that does not express TCR, CD4 or CD8 molecules.
- **Somatic hypermutation:** Change in the variable-region sequence of an antibody produced by a cell following antigenic stimulation, resulting in increased antibody affinity for antigen.
- **Switch region:** Region of B-cell heavy-chain DNA at which recombination occurs in antigen-stimulated cell, allows isotype switch.
- **Thymocytes:** T cells differentiating in the thymus.
- **Variable (V) region:** The N-terminal portion of an Ig or TCR which contains the antigen-binding region of the molecule.

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## 12.17 Self-Learning Exercises

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### Section -A (Very Short Answer Type):

1. The  $\kappa$  and  $\lambda$  light chains and the heavy chains are encoded by separate multigene families situated on.....
2. Intercellular regulatory proteins known as .....act as “switch factors”
3. The immunoglobulin promoters contain a highly conserved AT rich sequence called the .....
4. Pre T-cell receptor is expressed on thymocytes that lack .....on the surface
5. The progenitors of T-cells migrate from the bone marrow into the thymus under the influence of.....
6. Those thymocytes which show high affinity receptors for cells MHC molecules undergo death by.....
7. Naive T-cells survive for only about.....in the absence of antigen stimulation.
8. The generation of mature B cells first occurs in the.....and continues throughout life.
9. Antigen driven activation and clonal selection of naive B-cells leads to generation of.....
10. Humoral and/or cellular immune responses to pathogenic infections and other antigens are regulated by low-molecular weight soluble factors called.....
11. Cytokines secreted by lymphocytes, substances formerly known as ....., and those secreted by monocytes and macrophages, substances formerly known as.....

### Section -B (Short Answer Type):

1. What is Two-Gene Model?
2. Explain Tonegawa’s Immunoglobulin Genes Rearrange model?
3. Write all gene segments of  $\kappa$  and  $\lambda$  light-chain families.
4. Where and when variable-region gene rearrangements occur?
5. Which enzyme removes the introns in the primary transcript?

6. What is class switching?
7. Write the two kind of forms in which, a particular immunoglobulin can exist?
8. Where is the Immunoglobulin heavy- and light-chain mRNAs translated?
9. What is promoter, enhancer and silencer?
10. What is pre-T cell and Pro-T cell?
11. What is positive selection?
12. What is variable region?
13. What is naïve lymphocytes?
14. Write the definition of cytokine.

**Section -C (Long Answer Type):**

1. Describe Genetic models of Ig structure.
2. Explain  $\kappa$ -chain,  $\lambda$ -chain and heavy-chain multigene family.
3. Describe Class switching among constant-region genes.
4. Describe expression of Ig genes.
5. What is the difference between positive and negative selection of T-cell?
6. Explain functions of cytokines.
7. Write an essay on therapeutic uses of cytokines and their receptors.

**Answer Key of Section-A**

16. different chromosomes
17. cytokines
18. TATA box
19. CD4 and CD8 markers
20. chemotactic factors
21. apoptosis
22. five to seven weeks
23. embryo
24. plasma cells and memory B-cells
25. cytokines

26. lymphokines, monokines

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## **12.18References**

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- Immunology by Goldby
- Immunology by C. Vaman Rao
- Immunology by Richard Coico & Geoffrey Sunshine

## Unit - 13

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# Tolerance, Hypersensitivity and Immunity against Infections

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## 13.0 Objectives

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After going through this unit you will be able to understand:

- Central tolerance
- Peripheral tolerance
- Types of hypersensitivity
- Mechanism of hypersensitivity reactions and associated diseases
- Immune response against pathogens, particularly parasites.

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## 13.1 Introduction

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The term immunity is derived from Latin word *immunitas* which refers to the protection from prosecution. Historically, this word meant protection from diseases and infections caused by foreign agents called **immunogens**. Sometimes immune system itself becomes ill and result in damage of self-cells. This is done by two different mechanisms – **autoimmunity and hypersensitivity**.

To have a control over autoimmune situation, immune system keeps itself under a strict surveillance mechanism called **self –tolerance**. This mechanism is executed at two levels *viz* central and peripheral. Both the mechanisms work to eliminate auto- reactive components (B and T cells) of the immune system.

In some situations, the immune system goes wild and shows an exaggerated reaction which causes damage to innocent tissues and cells. This situation is called **hypersensitivity**. Here immune system becomes more detrimental than protective. Here, immune responses are inadequately controlled and inappropriately target the host tissue. Sometime, these responses are triggered by antigen (including otherwise harmless environmental antigens) and sometimes by commensal microorganism. There are multiple types of hypersensitivity reactions that differ in their mechanisms, affected organs and time of manifestation.

Most of the time, immune system develops a complex set of interactions with pathogens. In fact, before setup of an infection, host immune system works to stop the spread and growth of pathogens. In another words body's defense system works to expel out the pathogen and nullify its effects. Body's defense mechanism employs both innate and adaptive immunity for the above said purposes. On the contrary, to combat with the host immune system, pathogen also exhibits different strategies like-anatomical sequestration, surface antigen masking, tegument formation and variation in surface antigen. In some cases, pathogen release toxins which can cause tissue injury and functional deformities in the host tissue.

The present chapter describes the above three important aspects (tolerance, hypersensitivity and interaction between host immune system and pathogens). The first portion of the unit largely deals with the detailed mechanism of tolerance. In the second part- types and mechanism of hypersensitivity along with the associated diseases are discussed. The last part of the chapter describes about the interaction of the host immune system and the invading pathogens (parasites in particular).

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## **13.2 Tolerance**

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Paul Ehrlich, in the beginning of 20<sup>th</sup> century, realized that the immune system could go awry, a situation he termed as horror autotoxicus (later horror autotoxicus was termed as autoimmunity). This could be caused by a sudden inability of host's immune system to distinguish between self and non-self or by a misinterpretation of a self-component as dangerous. This directs humoral and/or T-cell-mediated immune activity against self-components, resulting in an immune attack on host tissues. This condition is called as autoimmunity. Autoimmune reactions, which may cause serious damage to cells and organs, can sometimes become fatal. Autoimmunity, in general, is a rare event and is

under a strict immunological surveillance and control. This control mechanism is collectively termed as self-tolerance (or simply tolerance).

Immunologic tolerance can be defined as a state of immunological unresponsiveness or non-reactivity towards self-antigens. Self-tolerance is a complicated, active and carefully regulated mechanism which involves– (a) elimination of immune cells that can react against self-antigens and (b) active inhibition of immune responses against self-molecules. Self-tolerance is an active antigen dependent process in response to the antigen and is different from immunodeficiency and immunosuppression. Antigens that induce tolerance are called tolerogens rather than immunogens. When self-tolerance processes are working correctly, host tissues remains undisturbed by the immune system and only foreign are attacked. Tolerance is specific response and has memory which exists in either or both B and T lymphocytes. Just as in immunological memory, T cells related to the memory of self-tolerance are quite long lasting.

Immunologic tolerance occurs in two forms: central and peripheral. Central tolerance is concerned with the negative selection of autoreactive lymphocytes in primary lymphoid organs while peripheral tolerance is executed to remove self-reactive lymphocytes from the blood and other peripheral organs.

### **13.2.1 Central tolerance**

The diversity of lymphocytes (generated by a number of random recombination events taking place during their development and maturation) is the main cause of the generation of a large number of lymphocytes bearing self-reactive or autoreactive T-cell surface receptors/ B-cell surface receptors. T cell and B cell receptors require expression of multiple gene segments (the V, D, and J segments). These multiple gene segments undergo recombination to form a functional gene coding for immunoglobulin's (Ig) or T- cell receptors (TCR). Such genetic rearrangements occur through somatic gene arrangements whereby any V (variable)-region gene segment can associate with any D (diversity) or J (joining) gene segment, a process called **V(D)J recombination**. As a result a great variety of TCRs and Igs is generated. Since this is a random process, by chance production of self-reactive immunogenic components is almost inevitable. Without a checking mechanism, such TCR or Ig receptors could produce mature functional T or B cells that can recognize self-antigens, resulting in autoimmune diseases. It is therefore very necessary to delete/ degrade such T or B cell clones possessing receptors that recognize self-

antigens with high affinity. The tolerance thus produced is called **central tolerance**. In other words central tolerance can be defined as a mechanism by which newly developing T cells and B cells present in primary lymphoid organs, are rendered non-reactive to self-molecules. These cells are destroyed or inactivated after they have expressed receptors for self-antigens and before they develop into fully mature immunocompetent lymphocytes.

The idea of central tolerance was suggested by Joshua Lederberg in 1959 in his general theory of immunity and tolerance. He proposed in his theory that lymphocytes learn self-tolerance during their development process. His postulates were experimentally confirmed in 1980's.

Chiefly central tolerance remains associated with the primary lymphoid organs (bone marrow for B cells and thymus for T cells) where developing immune cells are found, prior to export into the peripheral organs. Stimulation of central tolerance depends on encountering self-antigens during maturation phase of lymphocytes and thus central tolerance could only be developed for antigens present in primary lymphoid organs. The tolerance towards antigens depends upon the chemical properties of the antigen, the lymphocytic cell involved and its stage of development. These factors decide whether the lymphocytic cell will become non-responsive (anergic) or undergo directed suicide (negative selection) or alter its antigen receptor (receptor editing) or enter in a regulatory lineage. There is a significant difference in the mechanism of B and T cell tolerance. The details of both the tolerance are described below.

#### **13.2.1.1 B cell central tolerance**

The prerequisite for the action of immunological tolerance is the recognition of self-antigens, present in bone marrow, by immature B cells. These immature B cells are then processed, in one way or another, to reduce their response towards self-antigens or are destroyed. As a result a population of B cells that can recognize antigens derived from pathogens only (non-self) is generated (figure 1). Such B-cells cannot recognize self-antigens.

A defective immature B cells that can recognize self-molecules present in the bone marrow undergoes negative selection, a process called as **clonal deletion** (antigen induced loss of cells of B cell lineage). A developing B cell could encounter two types of antigens, namely multivalent cell surface antigens or low valence soluble antigens:

- (a) An immature B cell expressing surface IgM can recognize multivalent cell surface antigens of self-origin (eg. MHC) is rejected by a process known as

**clonal deletion.** Such cells undergo programmed cell death or apoptosis. It has been seen that the crosslinking of IgM receptors of immature B cells results in death by apoptosis. Immune system, before undergoing apoptosis, tries to rescue the self-reactive B cell by gene rearrangements leading to a process called **receptor editing**. Receptor editing is done to replace the self-reactive receptor (B cell surface receptor, IgM) with a new copy of receptor, which is not auto-reactive. The binding site of B cell surface receptor (BCR) contains elements of both the heavy and the light chain. In receptor editing, one of these is changed to alter the specificity. Light-chain ( $V_L$ ) receptor editing occurs quite frequently and is often sufficient. Rearrangement of heavy chain segment ( $V_H$ ) is less common. During receptor editing, DNA rearrangement machinery is used to replace receptors with autoimmune specificity.

- (b) Few immature B cells, which binds with low valence soluble self-antigens are not eliminated rather their capability to express IgM on their surfaces is sacrificed (It is because of the constant exposure to self-antigen which results in development of receptor tolerance that ultimately leads to down regulation of receptor synthesis). As a result, immature B cells migrate to the peripheral organs with only IgD receptor (generally B cell migrating to peripheral organs contains both IgM and IgD receptors) and hence do not respond to antigen. These B cells are said to be **anergic**. Only those B cells which contain both the receptors are able to recognize antigen.

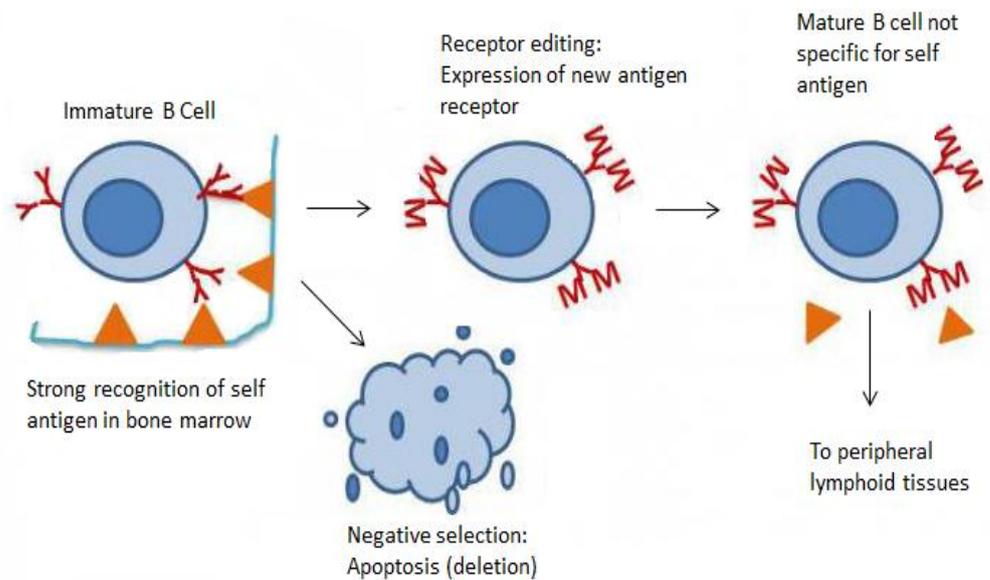


Figure 1: B cell central tolerance

Even if mature self-reacting B cells were to survive intact, they would very rarely be activated. This is because, to proliferate and produce antibodies, an

auto-reactive B cell needs co-stimulatory signals and cytokines from auto-reactive T cells as well as the presence of its recognized antigen. Thus, it is quite possible that most individuals carry substantial numbers of auto-reactive B cells within their mature B-cell stocks that are never activated.

### 13.2.1.2 T cell central tolerance

The ability of the immune system to respond to a foreign antigen depends on the probability that one of the millions of T cells that survive selection process in thymus will bind with one of the many MHC-peptide (antigenic) combinations expressed by an antigen presenting cells (APC). APCs process pathogenic proteins outside the thymus. A perfect T cell should recognize self-major histocompatibility complex (MHC) but should not recognize self-peptides. Thus for survival they have to undergo a very rigorous process of selection. This process of positive and negative selection (figure 2) is carried out in the thymus. The vast majority of DP thymocytes (double positive developing T cells having both  $CD4^+$  and  $CD8^+$  co-receptors) never meets the selection criteria (98%) and die by apoptosis within the thymus. Only 2% to 5% of DP thymocytes actually exit the thymus as mature T cells.

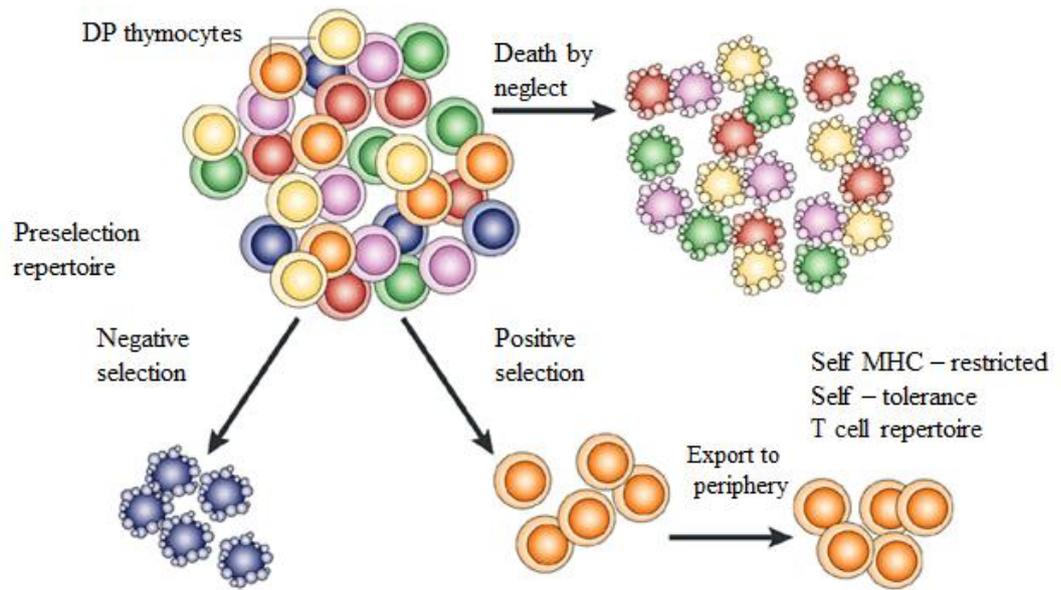
**Positive selection** is made to ensure that the T cells which get matured can recognize antigens in association with MHC. This process takes place in the cortex of thymus and is mediated by thymic epithelial cells having a rich repertoire of MHC molecules. Thymocytes which can bind strongly to surface MHC molecules are saved from apoptosis while all the others have to die (figure 2). The recognition of MHC by T cells is important because in addition to foreign antigens, T cells need to identify and respond to the infected host cells. The selection process is carried out in immature double positive (DP) T cell which bears both co-receptors  $CD4^+$  and  $CD8^+$ . It is only after positive selection that T cell differentiates into  $CD4^+$  cells or  $CD8^+$  cells (i.e. T helper which recognizes MHC II or T cytotoxic which recognizes MHC I). About 95% DP thymocytes do not specifically recognize self-MHC molecules and hence die because of failure in positive selection. These cells do not receive survival signals through their TCRs and die by a process known as **death by neglect**.

Like in B cell development, T cells diversity and development give rise to self-reactive cells. Hence, it is of primary importance to kill them by **negative selection** (any process that clears a repertoire of autoreactive clones) within thymus. About (2%–5%) are eliminated by negative selection. The process takes place at cortico-medullary junction and medulla. Both the places are

dominated by thymic epithelial cells and dendritic cells. The medullary thymic cells exhibit self-antigens on their surface and present them to developing DP-T cells. Those cells which recognize self-molecules are made to die *via* programmed cell death i.e. apoptosis a process known as **clonal deletion (figure 2)**. Clonal deletion process is mediated by the same cells (APCs) and similar interactions (high-affinity TCR engagement coupled with costimulatory signals) which activate mature T cells and this is why TCR signals result in death of immature cells.

Thymus harbours only a small set of cells like thymocytes, stromal cells, macrophages etc. Proteins of only these cells could be shown by MHC molecules present over medullary thymic cells. Hence, under given circumstances, identification of small group of self-reactive cells is only possible. However strikingly, thymus is known to get rid of a huge number of auto-reactive T cells that can react against a number of antigens which are not even present in thymus, for example specific proteins which are exclusively present in tissues and organs like brain, liver, kidney. The answer to the above surprise came in 1990 when researchers came to know that the thymic medullary epithelial cells had an unusual capacity to express and present proteins from all over the body. This capacity was a result of the ability of medullary epithelial cells to express a unique protein called **AIRE (autoimmune regulator)**, which allows cells to express, process and present proteins that are ordinarily found in specific organs only. It works as a classic transcription factor and acts as a part of transcriptional complex which facilitates expression of tissue-specific genes by regulating translation and chromatin packing (AIRE protein binds to chromatin whose histone H3 has no methyl groups attached to its lysine-4). Thus, it allows medullary epithelial cells to express proteins not ordinarily found in the thymus, process them and present them along with MHC molecules to T cells. Thus AIRE can turn on the expression of hundreds of tissue-specific genes. However till now, AIRE's mechanism of action is not completely understood.

Some other mechanisms which do not cause cell death are also used for the thymic negative selection (central tolerance). These include - **clonal arrest** (prevention of maturation of auto-reactive thymocyte receptors), **clonal anergy** (inactivation of auto-reactive cells) and clonal editing (rearrangement of TCR genes for second and third chance); however clonal deletion is perhaps the most common mechanism for thymic negative selection.



**Figure 2: T cell central tolerance**

### 13.2.2 Peripheral Tolerance

**Peripheral tolerance** is another form of immunological tolerance which is developed after mature T and B cells leave the primary lymphoid organs and enter the peripheral organs. Central tolerance is not a leak proof system and at times few defective lymphocytes may leak out from these organs and reach to other primary and secondary organs where they may initiate severe reactions against self-antigens. In fact, a number of lymphocytes with specificity for self-antigens are common in the circulatory system. Their presence can be attributed to two factors *viz* 1) all self-antigens are not expressed in the central lymphoid organs and 2) a minimum threshold affinity of self-tolerance is required for triggering the clonal deletion, as a result some weak self-reactive clones escape the process of selection. Peripheral tolerance mainly controls the level of self-reactive CD4<sup>+</sup> cells which escaped from the thymic selection process. The problem of B-cell tolerance is not very acute because B cells cannot respond to most antigens unless they receive help from T helper cells and thus T cell tolerance is of primary importance.

#### 13.2.2.1 Peripheral T lymphocyte tolerance

Peripheral tolerance occurs when mature T cells starts to recognize self-antigens present in the peripheral tissues. This results in rendering them inactivated or to undergo apoptosis. Mechanisms for peripheral tolerance include anergy, immune suppression by regulatory T cells and activation of induced cell death. If the T cells can bind self-antigens, they may induce autoimmune response. So in situations where central tolerance becomes leaky,

peripheral tolerances becomes necessary to prevent autoimmunity.

**(a) Anergy**

Term **Anergy** can be defined as lack of reaction by the body's defense mechanisms against specific antigens, generally self-antigens. It results in induction of lymphocyte tolerance in peripheral organs. In this process the immune system is unable to mount a normal T cell response against self-antigen. Anergy is thus an important strategy for functional inactivation of auto-reactive T lymphocytes which can recognize self-antigens in the peripheral tissues. It is among one of the three processes that induce tolerance by modifying the immune system so as to prevent self-destruction. Before understanding the process of tolerance, we should first understand the process of T- lymphocyte activation. Activation of naïve T cell requires two stimulations:

- 1) Binding of T cell with the antigen-MHC complex on the membrane of APCs. This is an antigen-specific first signal. MHC can complex with most of the antigens.
- 2) The co-stimulatory signals expressed by antigen-presenting cells bearing pathogens. This co-stimulatory signal comes from B7-CD28 interaction. The B7 (B7-1/B7-2) is a peripheral protein found on the surface of activated APCs as well as on naïve T cells. It interacts with CD28 receptor which is found on the surface of T cells. This interaction produces a signal cascade which leads to the survival and activation of T cells. Blockade of CD28 is effective in stopping T cell activation. T-cells are also known to express another surface protein CTLA-4 (CD152) (cytotoxic T lymphocyte-associated antigen-4), which can also bind to B7 protein. The interaction of T cells and CTLA-4 protein does not allow activation of T cells and acts as a co-inhibitory signal. Thus, the T cell response can be turned on by stimulation of CD28 receptors present on T cells while stimulation of the CTLA-4 receptor turn off the T cell attack. Intracellular CTLA-4 protein receptor is also found in regulatory class of T cells and may be important for their function.

For the process of T cell anergy, either of the above two signals is restricted. Anergy occurs; (a) in the presence of T cells which can recognize self-antigens and (b) absence of adequate level of co-stimulators which are needed for T cell activation. In the absence of a pathogen, APCs of peripheral lymphoid organs (which are in resting state) express low level of co-stimulators like B7 protein.

Though they are in resting phase, APCs constantly present antigens (including self-antigens) to T cells. Binding of antigens to T cell receptors produce initial signals for further activation of T cells. However in case of self-antigens, the B7 protein binds with CTLA-4 receptor present on T cells. The CTLA-4 protein is involved in shutting down T cell responses. The mechanism of CTLA-4 activation is not fully known but it is believed that they may function by masking and eliminating B7-1 and B7-2 proteins of APCs. As a result, the B7 proteins become unavailable to interact with CD28 receptors (which are required for activation of T cells).

CTLA-4 receptors are also known to employ a phosphatase which masks the T cell receptor and thus attenuate the interaction by CD28. As a result of this, the self-reactive T cell does not activate.

### ***(b) Immune Suppression by Regulatory T Cells***

The development of regulatory T cells is another strategy employed by the immune system which reduces the risk of autoimmunity. Some auto-reactive CD4<sup>+</sup> (T<sub>H</sub>) cells get differentiated into regulatory T cells. This subset of T cells is characterized by the presence of a transcription factor called Foxp3. Foxp3 is necessary for the development and functioning of regulatory T cells. The regulator cells express high level of CD25 receptor. CD25 binds with IL-2 (interleukin-2). Interaction of IL-2 and CD25 is very important for the survival and functioning of this set of T cells. In addition to IL-2, another cytokine called transforming growth factor-beta (TGF- $\beta$ ) is very important for the functioning of regulator T cells. TGF- $\beta$  stimulates the generation of regulatory T cells by inducing the expression of Foxp3 receptor. Along with TGF- $\beta$  regulatory T cells are also known to release IL-10. Both of them works for the inhibition of activation of auto reactive T cells by contact dependent mechanism or by the secretion of cytokines that inhibit the T cells (figure 3), however, the detailed mechanism remains unknown. Thus the regulatory T cells inhibit the activation and differentiation of self-reactive T cells into effector T cells.

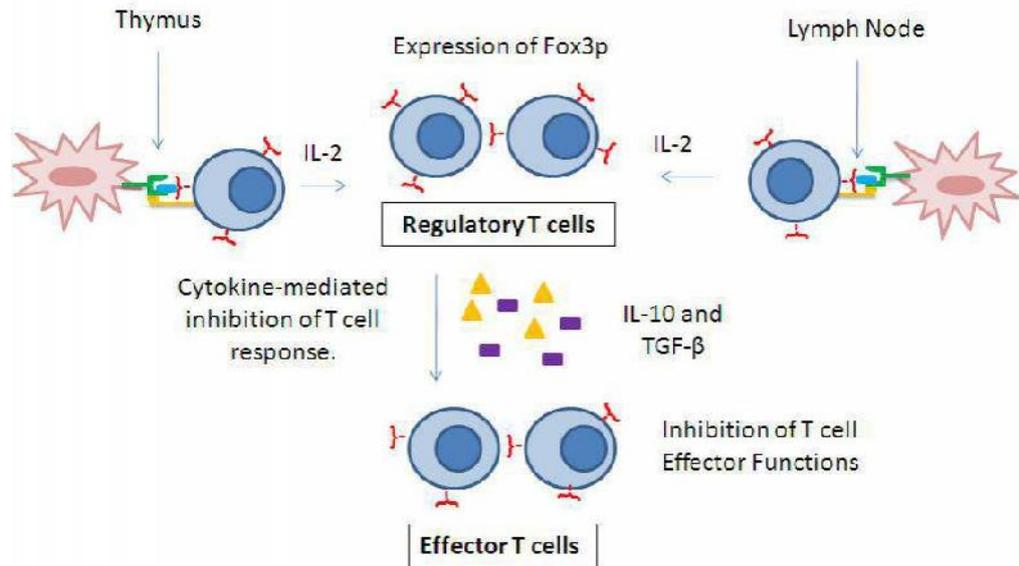


Figure 3: Peripheral Immune tolerance by regulatory T cells

### (c) Activation of Programmed Cell Death

Another mechanism for self-tolerance is induction of apoptosis in auto-reactive T cells. This process is induced after antigen recognition and also known as **activation-induced cell death**. Two pathways are known to induce apoptosis in mature T cells which are activated by self-antigens. In one of the pathways, production of proapoptotic proteins is induced in the T cells which through a signal transduction cascade trigger cell death. Normal T cells (that recognize foreign antigen) are also known to produce apoptotic proteins however their action is counteracted by the production of anti-apoptotic proteins. These antiapoptotic proteins are created by co-stimulatory signals released by B7 (present on APCs)-CD28 (present on T cells) interactions. In the absence of co-stimulatory proteins, T cells cannot synthesis anti-apoptotic proteins and hence die by programmed cell death.

In the second pathway, a self-reactive T cell when interacts with a self-antigen produces death receptors and associated ligands on its surface. Interaction with another similar T cell (expressing death receptors and death ligands), can trigger signal transduction leading to apoptosis of both the cells. Death receptors are known as Fas and the death ligand is known as FasL.

Certain T cell very frequently encounters similar type of antigens. A frequently encountered antigen indicates towards self-antigen, unless chronic infection is occurring i.e. microbial antigen is encountered much less than self-antigen. The T cells that frequently encounter same antigen develop Fas and FasL (death receptor and death ligand). Interaction with similar type of T cells would cause

the death of both the cells.

### **13.2.2.2 Peripheral B cell tolerance**

After maturation, a B cell migrates from the bone marrow. Outside the bone marrow, mature B cell can encounter a range of antigens (including self-antigens) which are not found in the bone marrow. B cells (just like T cell) can become anergic when they encounter self-antigens in peripheral lymphoid tissues and blood. B cells also become anergic when auto reactive T-cells (which are necessary for the activation of B cells) undergo tolerance or are not present. Since, innate immunity do not work for self-antigens, B cells will not encounter any signals that are induced during such responses.

#### ***(a) Anergy and deletion***

At times, some auto-reactive B cells are repeatedly stimulated by self-antigen. Such cells become unresponsive to further activation. These cells, for their survival, require very high level of the growth factors BAFF/BLys. Because of high requirement of growth factors, such cells are not efficient in competing with normal B cells which require low BAFF concentration. Thus auto-reactive B cells have a shortened life span and are eliminated speedily from the peripheral tissues. Some B cells which bind with high avidity to self-antigens in the peripheral organs undergo programmed cell death by the mitochondrial pathway which is independent of growth factors (figure 4).

The self-reactive B cells which are generated by somatic mutation of Ig genes in germinal centers also undergo apoptosis. This B cells elimination is mediated by the interaction of FasL receptor on helper T cells with Fas ligand on the activated B cells. Failure of this pathway of peripheral B cell tolerance may contribute to the autoimmunity.

#### ***(b) Signaling by inhibitory receptors***

Auto-reactive low affinity B cells may be prevented from further activation by appointment of various inhibitory receptors. These inhibitory receptors set a threshold for the activation of B cells. This threshold allow activation of only those B cells which respond to foreign antigens with the help of T cells or innate immunity, but do not allow activation of B cells that respond to self-antigens. SHP-1 tyrosine phosphatase and CD22 are examples of inhibitory receptors. *Immunoreceptor tyrosine-based inhibition motif* (ITIM) (a conserved amino acid sequence generally found in the cytoplasmic tails of some immune response inhibitory receptors) present in the cytoplasmic tail of CD22 are phosphorylated by Lyn Protein. Lyn is a *tyrosine-protein kinase* belonging to

Src family of protein tyrosine kinases. Phosphorylation leads to the recruitment of other enzymes like the phosphotyrosine phosphatases SHP-1. These phosphatases then diminish the activation of molecules involved in cell signaling thus attenuating B cell receptor signaling. As a result activation of auto reactive B cells is inhibited.

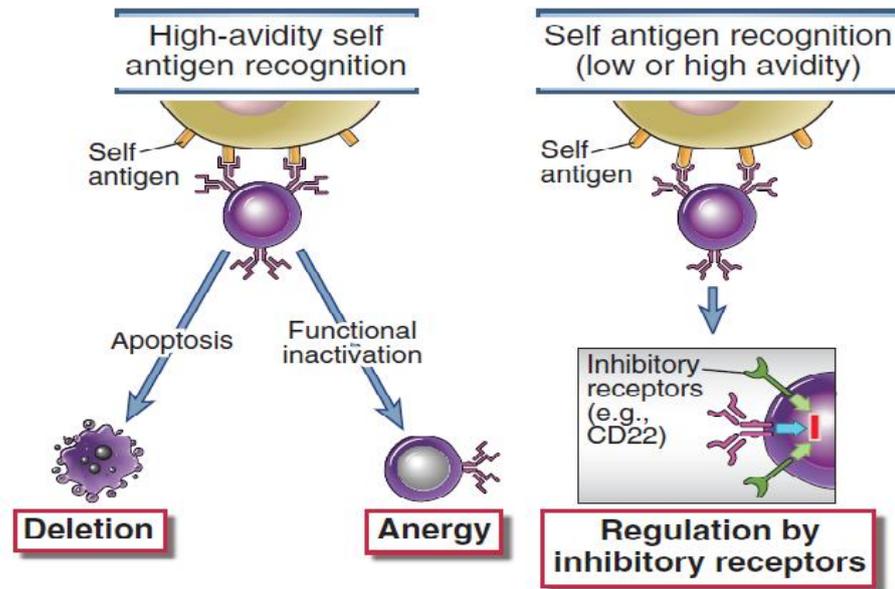


Figure 4: B cell peripheral tolerance

### 13.3 Hypersensitivity

Hypersensitivity is a phenomenon in which immune system goes undesirably wild and cause damage to the individual. It is an exaggerated immune response which may be damaging, uncomfortable and occasionally fatal. Such response is generally mounted against an innocuous antigen, which in affected persons would not be immunogenic normally. Some of these antigens may include environmental allergens like house dust, pollen grains etc. Some drugs, self-cells, plant products, insect venom are also known to cause allergic reactions.

Most of the damage resulting from hypersensitivity reactions is a result of interaction between antigens and antibodies or between antigens and sensitized T-lymphocytes has taken place. Most of the hypersensitivity reactions require an immunologically pre-sensitized state of the host. The nature, severity and symptoms associated with the reaction depend upon the involvement of antibodies or sensitized T-lymphocytes.

#### 13.3.1 Types of hypersensitivity reactions

Hypersensitivity reactions can be classified on the following basis:

**(a) Time taken for the manifestation of reaction:** Based upon the time required

for the execution for hypersensitivity reactions, they can be of two types-

- 1) **Immediate hypersensitivity** reactions which show manifestation of symptoms within a very short time period (minutes or few hours) after antigenic stimulus.
- 2) **Delayed type hypersensitivity (DTH)** reactions which after antigenic stimulus require hours or days to manifest themselves.

Generally immediate hypersensitivity reactions result from antibody-antigen reactions, whereas DTH is caused by T-cell reactions (table 1).

**(b) Body area affected:** Based upon the body area affected by the allergic reactions, they can be classified in two categories-

- 1) **Localized reaction** in which the reaction remains localised to a specific organ or area.
- 2) **Systemic reaction** in which the reaction manifests all over the body or to a larger portion. Systemic reactions are normally of DTH type and are often fatal.

**(c) Mode of action/ mechanism:** Various mechanisms have been reported for different hypersensitivity reactions. Based upon the type of working mechanism, the hypersensitivity reactions are divided into four types. This classification is known as **Gell and Coomb's classification** and is based upon P. G. H. Gell and R. R. A. Coomb's proposal to differentiate between hypersensitivity reactions. Type I, II and III are mediated either by antibodies or antigen-antibody complexes and falls within humoral branch of immunology. Type IV hypersensitivity reactions are mediated by immunological cells like T cell. The details of Gell and Coomb's classification are shown in table 2. The hypersensitivity reactions are studied according to this classification.

### 13.3.2 Type I hypersensitivity reactions

A type I hypersensitive reactions are induced by certain types of antigens referred to as **allergens** (an allergen is an antigen, often a protein, that induces an inappropriate exaggerated immune response in contrast to a classical response produced by most immunogens in the recipient host). An allergen may be the globular proteins present in pollens released from trees, grasses and ragweed, as well as certain food substances, animal remnants and insect venom. Most of these reactions take place in and around mucus membrane surfaces.

Characteristic	Immediate hypersensitivity	Delayed hypersensitivity
Time of reaction after antigenic challenge	Reaction appears and recedes rapidly.	Appears slowly, lasts longer.
Induction	Induced by antigens or haptens.	Antigen or hapten intradermally or by any other route.
Immune response	Circulating antibodies.	It is a 'cell-mediated' reaction.
Transfer of hypersensitivity	Passive transfer possible with serum.	Cannot be transferred with serum; but possible with T cells or transfer factor.
Desensitization	Easy desensitization, but short-lived.	Difficult, but long-lasting.

**Table 1:** Distinguishing features of immediate and delayed type of hypersensitivities.

Type	Name	Mechanism	Example of disease
Type I	Immediate hypersensitivity	Antigen binding and cross linking of IgE followed by mast cell degranulation	Allergic Asthma, Allergic Rhinitis
Type II	Antibody-mediated hypersensitivity	IgM/IgG antibody-antigen interactions on target cell surfaces.	Grave's Disease, Hemolytic Anemia of Newborn
Type III	Immune complex mediated hypersensitivity	Immune complex formation and deposition in tissues, leading to local or systemic inflammatory reactions	Rheumatoid Arthritis, Serum Sickness, Arthus Reaction
Type IV	Delayed type hypersensitivity	Release of cytokines from activated sensitized T <sub>H</sub> 1 cells, resulting in	Contact Dermatitis, Chronic

		accumulation and activation of macrophages and CTL cells.	Transplant Rejection.
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**Table 2: Gell and Coomb's classification of hypersensitivity.**

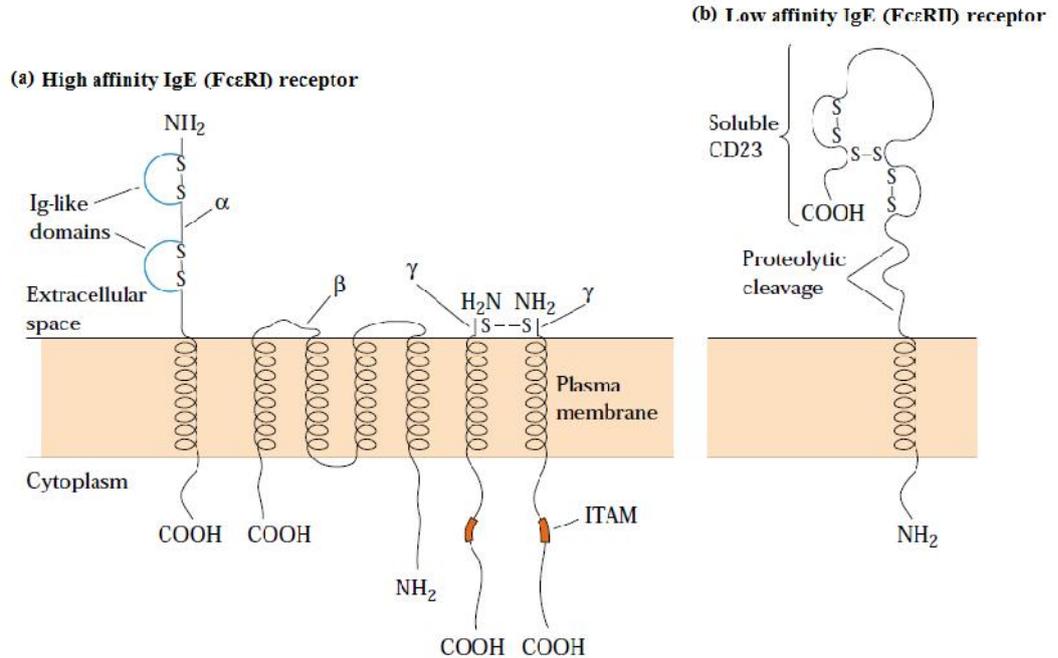
Type I hypersensitivity is an anaphylactic reaction. Anaphylaxis stands for “anti or opposite to protection” and is defined as extravagant reaction of an organism to a foreign substance to which it has previously become sensitized, resulting from the release of histamine, serotonin and other vasoactive substances. It is a type of immediate hypersensitivity and can be localized or systemic.

In contrast to normal immunological reaction in which memory cells produces IgG, a type I reaction is characterized by the release of IgE from the antibody producing plasma cells. The reagenic activity of IgE depends on its ability to bind to a receptor specific for the Fc region of its heavy chain (Fc R). Two classes of Fc R have been identified and are designated as Fc RI and Fc RII (figure 5). They are present on different cell types and basically differ in their affinity towards IgE. Fc RI has more affinity towards IgE and are usually present on mast cells and basophils. They are inducible on Langerhans cells and eosinophils. This receptor is a tetrameric complex and consists of four chains- one Fc RI (alpha chain), one Fc RI (beta chain) and two Fc RI (gamma chains) connected by disulfide bridge. Alpha chain constitutes an antibody binding site, while beta amplifies the downstream signals and gamma chains initiates the downstream signals. and chains contain **immuno receptor tyrosine-based activation motifs (ITAMs)** that are phosphorylated in response to IgE cross-linking. The phosphorylation leads to a cascade of reactions important for manifestation of type I reactions.

Fc RII also known as CD23 is a "low-affinity" IgE receptor. It is found on the surface of mature B cells, activated macrophages, eosinophils, follicular dendritic cells and platelets. This receptor is known to have a significant role in **antibody feedback regulation mechanism** and hence significantly important in IgE regulation.

IgE antibody binds with high affinity to **Fc receptors** on the surface of resting mast cells and blood basophils. Mast cells and basophils coated by IgE become sensitized. A later exposure to the same allergen cross-links the membrane-bound IgE present on previously sensitized mast cells and basophils. This results in **degranulation** of these cells. Degranulation is marked by the release

of pharmaceutically active mediators like histamine, serotonin, leukotrienes, prostaglandins etc. These mediators act on local tissues present in the vicinity of the reaction site and result in vasodilation, urticaria, muscle contraction and tissue damage.



**Figure 5: Fc RI and Fc RII**

### 13.3.2.1 Allergens involved

Various common allergens giving rise to Type I hypersensitivity are listed in table 3. Most of these allergens possess multiple epitopes and hence contain multiple antigenic components. It is a debatable question that certain allergens (pollens, food stuffs, drugs) possess potent allergic potential while many others are not. The basic logic behind allergenicity seems to be a complex series of interactions depending upon the chemical nature of the allergen, its dose, the route of administration, sometimes an adjuvant, and the genetic constitution of the recipient. The route of administration is important because it determines the means by which the antigens are presented to the immune system. It has been observed that most allergens are soluble and have a molecular weight between 10000–40000 Da, however no common minimum chemical or physiological property associated with the allergic property of substances has been found till date. Many allergens however share some features. First one is the intrinsic enzymatic activities shown dominantly by some allergens. The enzymatic activity is known to affect the cells and molecules of the immune system. Extracts from many fungi, bacteria, cockroaches, dust mites have moderately high

protease activity. Proteases are known to disrupt epithelial cell junctions. This permit the allergens to access the internal cells and tissues involved in immune responses. Some allergens (like dust mite) are known to show protease activity, which in one way or another, cleaves and activates complement system at mucosal surfaces. Proteases are also known to activate specific receptors on the surfaces of inflammation associated immune cells. Secondly, many allergens entering the host via mucosal routes influences the immune response of the individual in such a way that they mount a  $T_H2$  response which in turn leads to the production of IgE by B cells. Thirdly, some potent allergens are known to carry pathogen associated molecular patterns (PAMPs). PAMPs are capable of eliciting a cascade of immunological responses.

Generally, type I hypersensitivity reactions are elicited either by inhalation or ingestion, however other routes like dermal contact with sensitized person are also possible. The route of injections is one of the most efficient modes for an allergen to cross epithelial obstacles. Many environmental allergens cause reactions in the respiratory tract and may result in asthma, respiratory failures etc. Now-a-days, one of the most common reasons of "hay fever" or allergic asthma is the antigen *Der p 1* found in fecal particles of the house dust mite (*Dermatophagoides pteronyssinus*).

Food allergens usually results in allergic reactions of gastrointestinal tract with typical symptoms like diarrhea, nausea and vomiting. They may also cause problems in other organs like organs of respiratory tract and skin. Many drugs like penicillin administered either orally or by injection, can also result in building up of type I hypersensitivity reactions. Such reactions may be systemic or localized.

<b>Drugs</b>	<b>Foods</b>	<b>Fungal spores</b>	<b>Proteins</b>
Sulfonamides, Penicillin, Local Anesthetics, Salicylates	Eggs: Ovalbumin, Beans, Seafood, Peas, Milk: casein, Nuts, and $\beta$ -Lactoglobulin, Fruits like apple, kiwi Vegetables like celery	<i>Aspergillus</i> , <i>Alternaria</i> , <i>Cladosporum</i>	Foreign serum Vaccines
<b>Plant pollens</b>	<b>Insect products</b>	<b>Animal antigens</b>	<b>Miscellaneous</b>

Rye grass, Ragweed, Timothy grass, Birch trees, Mugwort, Ribwort/plantain	Wasp venom, Ant venom, Cockroach calyx, Dust mites	Epithelia of cats and dogs, Parakeet dung, Feathers, Animal hair and dander	House dust mite antigen (antigen <i>Der p 1</i> )
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Table 3: Common allergens

### 13.3.2.2 Mechanism of type I hypersensitivity

A large number of individuals mount IgE response (formerly known as **reagin antibody**) or show class switching from IgG to IgE, against many parasitic allergens. The IgE binds to the mast cells and basophils and cause their degranulation (figure 6). The level of serum IgE remains elevated until the allergen is cleared from the body. However, in some individuals, called the **atopic individuals** (individuals having hereditary predisposition towards the development of immediate hypersensitivity against common environmental antigens), the level of serum IgE increases many folds. The atopy is partial genetic problem which often runs in families. Atopic individuals are characterized by abnormally high level of IgE and eosinophil's. The normal concentration of IgE in blood is 0.1–0.4 µg/ml while for an atopic individual this concentration raises to more than 1 µg/ml. Two loci responsible for atopic condition rest on chromosome 5q and chromosome 11q.

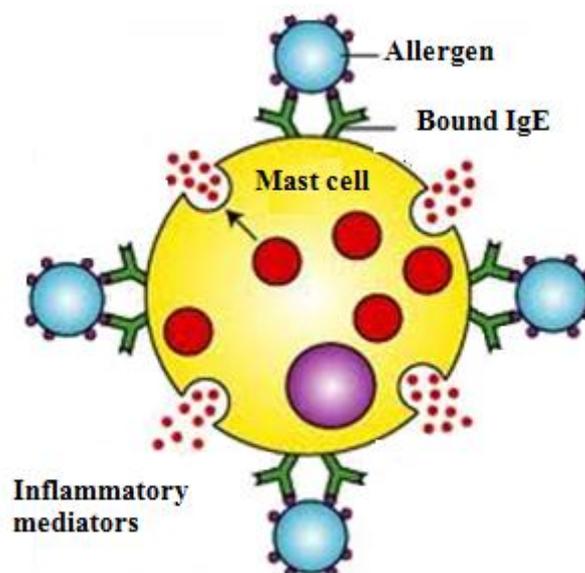


Figure 6: Type I hypersensitivity: (a) binding of

**allergens to sensitized IgE molecules; (b) release of vasoactive substances.**

An IgE molecule is composed of four constant region domains (in place of three constant domains in IgG). This additional domain ( $C_{H4}$ ) enables IgE to bind to the Fc RI surface glycoprotein receptors of mast cells and basophils (figure 6). In fact the presence of additional domain 'CH4' alters the conformation of Fc portion of the IgE molecule. The altered conformation enables the IgE molecule to bind with receptors of mast cell and basophils. In general, the half- life of IgE antibody in serum is 2-3 days, but when it binds to the mast cell receptor, its life span enhances many folds. IgE production is stimulated by activated allergen specific  $T_H2$  cells. In addition to IgE production,  $T_H2$  cells also secrete many cytokines like IL-4, IL-5, and IL-13. These cytokines are responsible for most of the clinical manifestations of type I hypersensitivity. IL-4 stimulates allergen specific B cells to undergo class switching (heavy-chain) to IgE. IL-5 recruits and activates eosinophils and IL-13 stimulates mucus secretion from epithelial cells.

The population of mast cells and basophils is very high in skin and mucous membrane surfaces of the respiratory and gastrointestinal tracts. Their number differs significantly in different body parts. Both mast and basophil cells contain a large number of membrane bound granules distributed throughout the cytoplasm. These granules contain pharmacologically active mediators like serotonin, leukotrienes and prostaglandins. Mast cells, after activation, release these mediators which in turn results in clinical manifestation of the Type I hypersensitivity. Mast cells are also known to secrete variety of cytokines which affect a number of processes involved hypersensitive reactions vasoactive substances.

### **13.3.2.3 Sequence of events**

Type 1 immune responses can be understood in two parts. Part one comprises of “*sensitization phase*” while part two comprises of the “*reaction phase*” (figure 7).

#### ***Sensitization Phase***

- (a) First exposure of multivalent allergen results in the production of IgE by activation of  $T_H2$  cells. In an atopic person the level of IgE production is many folds high as compared to a normal individual.
- (b) The excess of IgE binds with high affinity Fc receptors present on the

surface of the mast cells. Binding with Fc receptor enhances the life span of the bound IgE. This binding results in **sensitization** of mast cells (figure 7).

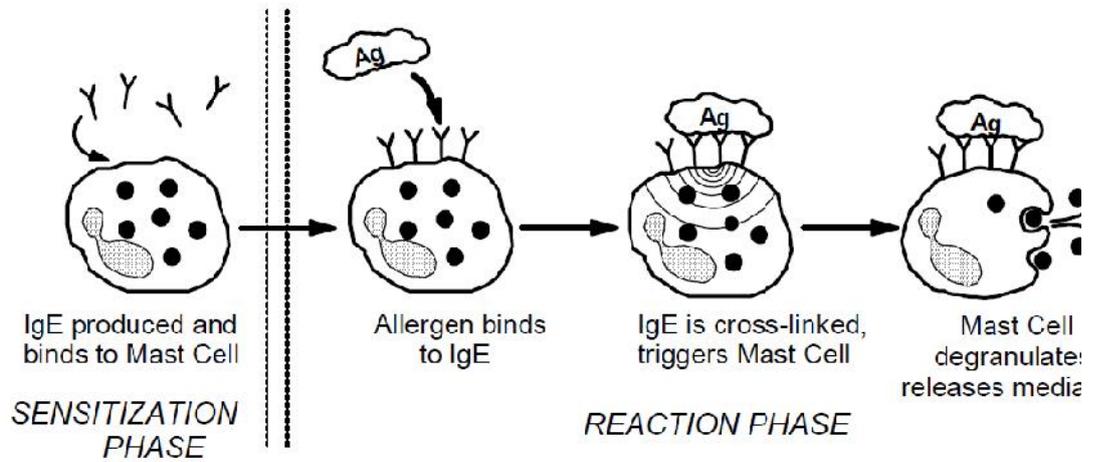


Figure 7: Different phases of type I hypersensitivity

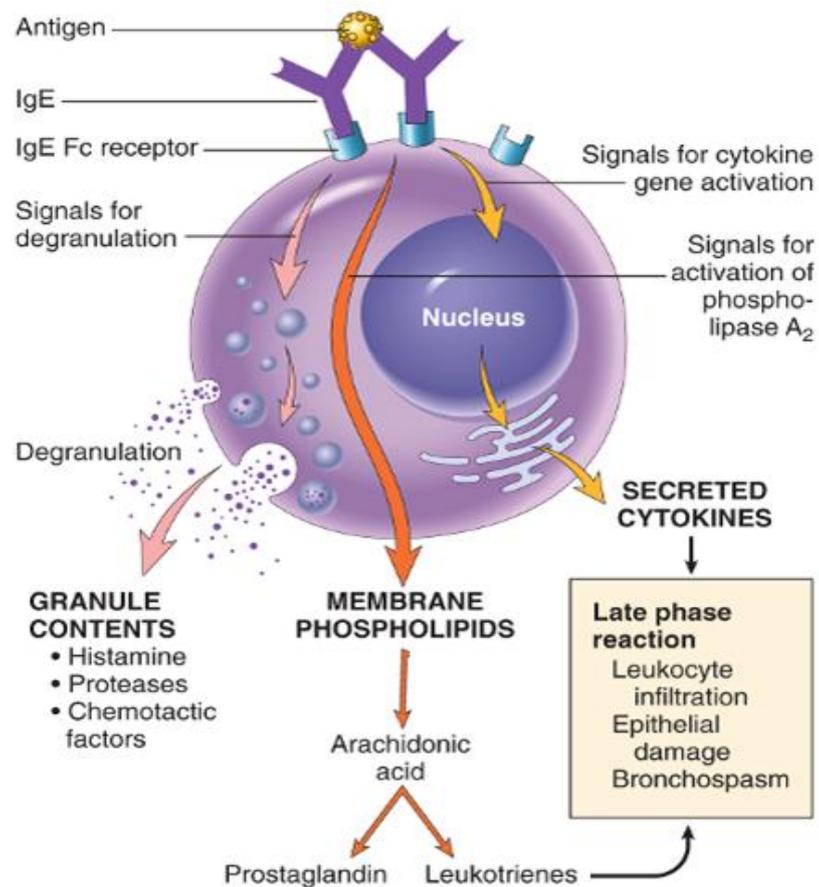
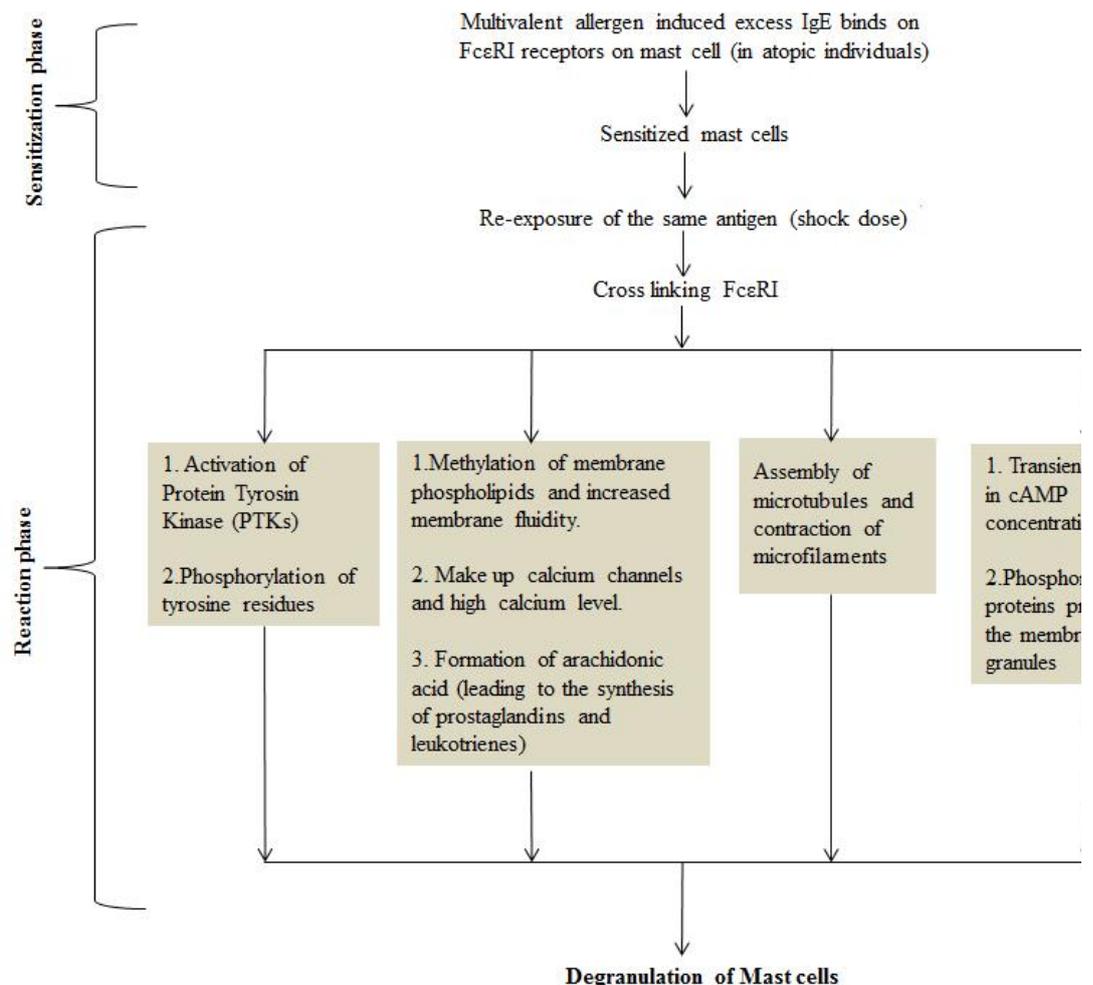


Figure 8: Mast cell degranulation in type I hypersensitivity

*Reaction Phase (figure 7 and 8)*

- 1) Re-exposure to the same multivalent allergen results in cross linking of IgE antibody molecules bound to the mast cells. This is known as **shock dose**.
- 2) The cross linking leads to aggregation of the Fc RI receptors and ultimately stimulation of a cascade of events which results in degranulation of mast cells. The detailed process of degranulation is as below (figure 9):
  - a) The cytoplasmic domains of the receptor Fc RI, present on mast cells remain associated with Protein Tyrosine Kinases (PTKs). Crosslinking of Fc RI activates the PTKs resulting in rapid phosphorylation of tyrosine residues present within the immuno-receptor tyrosine based activation motifs (ITAMs) found on  $\beta$  subunit of Fc RI. PTKs then phosphorylates **phospholipase C** which converts **phosphatidylinositol-4,5 bisphosphate** ( $PIP_2$ ) into **diacylglycerol** (DAG) and **inositol triphosphate** (IP3). Phosphorylation induces production of secondary messages which results in degranulation.



### Figure 9: Events Taking place in Type I hypersensitivity

- b) Fc RI crosslinking cause methylation of various membrane phospholipids. In-fact cross linking activates some enzymes which convert **phosphatidylserine (PS)** into **phosphatidylethanolamine (PE)**. PE so produced is methylated by the **phospholipid methyl transferase enzymes I and II (PMT I and II)** to form **phosphatidylcholine (PC)**. Accumulation of PC on exterior surface of plasma membrane results in increase of membrane fluidity. It also leads to the formation of calcium channels and make up of calcium concentration. This is due to the uptake of calcium from outside and release of calcium from cellular pools. IP<sub>3</sub> is a potent mobilizer of intracellular Ca<sup>2+</sup> stores. High concentration of calcium leads to the activation of **phospholipase A2** that promotes the breakdown of PC into **lysophosphatidylcholine (lyso PC)** and **arachidonic acid**. Arachidonic acid is further converted to **Prostaglandins** and **Leukotrienes**.
- c) Increased level of calcium promotes: i) assembly of **microtubules** and ii) contraction of **microfilaments**. Both these steps are necessary for the movement of granules towards plasma membrane. DAG produced in step “a” activates **protein kinase C (PKC)**. PKC along with Ca<sup>2+</sup> is necessary for microtubular assembly and the fusion of the granules with the plasma membrane.
- d) Phospholipid methylation and high calcium level leads to the transient increase in the activity of membrane bound enzyme adenylyate cyclase. Active **adenylyate cyclase** converts ATP to cAMP, leading to buildup of cAMP concentration. This activates **cAMP dependent protein kinases**. Protein kinases thus produced phosphorylate proteins present on the membrane of granules and hence alter the membrane permeability towards water and calcium. Change of membrane permeability results in swelling of the granules and facilitates fusion of granules with the plasma membrane.
- e) The increase in the concentration of cAMP is transient i.e. drops down quickly. This drop in concentration is a necessary step for degranulation process to proceed (for degranulation to proceed, momentarily high concentration of cAMP is needed; if high cAMP concentration persists for long, the degranulation process is blocked).

Increased membrane fluidity, formation of calcium channels, contraction of microtubules and microfilaments, changed granular permeability and transient buildup of cAMP collectively leads to the fusion of granules with plasma membrane and release of pharmacologically active mediators.

#### **13.3.2.4 Mediators of type I hypersensitivity**

Mast cell degranulation begins within seconds after cross linking of Fc RI receptor, releasing an array of preformed and newly generated inflammatory mediators (table 4). Inflammatory mediators are soluble, diffusible molecules that act locally (and at distant sites also) at the site of tissue damage and infection. These chemical factors brings about the vascular and cellular alterations resulting in vasodilation, increased vascular permeability, contraction and spasm of smooth muscle cells of the bronchioles, small arteries and gastrointestinal tract linings. They can act locally or systemically. When these mediators are generated in response to a parasitic infection, they help in maintaining body's defense processes, however if induced by inappropriate allergens, they results in extraordinary increase in tissue damaging vascular permeability and inflammation

Depending upon whether they are synthesized after sensitization of mast cells or are present in preformed state, the inflammatory mediators are classified as primary or secondary respectively. Primary mediators like histamine, proteases, heparins and various chemotactic factors are present in the granules in preformed state i.e. before cell activation Secondary mediators like prostaglandins, leukotriene's do not exist in preformed state rather are synthesized after activation of target cells during degranulation process, and many of them are released by the breakdown of membrane phospholipids during the degranulation process.

##### ***a) Primary mediators***

The primary mediators present in the mast cell granules include histamine, proteases, eosinophil chemotactic factor, neutrophil chemotactic factor and heparin (table 4). These are stored preformed in granules of mast cells, basophils and platelets. Since they are present in preformed state, their effect can be observed within a short span of time.

**Histamine** is among the most important primary mediators which make up 10% weight of the granule in which they remain stored. They are formed by the decarboxylation of amino acid histidine in a reaction catalyzed by the enzyme L-histidine decarboxylase. Its presence results in increased capillary

permeability, arteriolar dilation, eosinophil chemotaxis, and contraction of nonvascular smooth muscle cells. They can also stimulate nociceptors (receptors of sensory neuron) responsible for the pain response. In addition to immunological and inflammatory role, histamine also works to regulate the physiological functions in gut. Moreover, it also acts as a neurotransmitter. Histamine increases the capillary permeability towards leukocytes and some proteins. Increased vascular permeability causes fluid to escape from capillaries into the tissues. As a result the classic symptoms of an allergic reaction appear- a runny nose and watery eyes.

Histamine acts on the body cells through receptors. Different body cells express different receptors for histamine. These receptors can be of four types - H1, H2, H3 and H4. Histamine induced acute vascular effects, smooth muscle constriction in the bronchi and stimulation of eosinophil chemotaxis are mediated by H1 receptors. H2 receptors are known to cause vasodilation. They are important for their role in histamine-induced gastric secretions. These receptors are known to mediate a number of anti-inflammatory effects, like the inhibition of eosinophil chemotaxis. Histamine when binds to H2 receptors present on mast cells and basophils cause suppression of degranulation process; thus, a negative feedback is exerted. The H3 receptor is mainly involved in regulating the release of histamine by different producing cells. H4 receptors are normally found on cells of hematopoietic origin and mediate mast cell chemotaxis.

Another important vasoactive mediator very similar to histamine is **Serotonin** (5-hydroxytryptamine). It is a primary mediator normally found in platelets and mast cells present in the CNS and GI tract. Serotonin is known to dilate blood capillaries, increase vascular permeability and cause contraction of nonvascular smooth muscle. Serotonin is predominant vasoactive amine in rodents and some domestic ruminants.

Both histamine and serotonin results in a sudden drop in blood pressure occurs, followed by circulatory collapse and shock.

#### ***b) Secondary Mediators***

Some enzymes especially cellular phospholipases like phospholipase A<sub>2</sub> and C get activated during the inflammation process. These enzymes degrade phospholipids (which are major constituent of cell membrane) to **arachidonic acid**. Arachidonic acid has a short half-life and can be metabolized through two major pathways- the **lipoxygenase** and **cyclo-oxygenase** pathways.

Prostaglandins, prostacyclin and thromboxanes are produced by cyclooxygenase pathway while the lipoxygenase pathway is responsible for the production of leukotrienes and the branched lipoxins. Many cytokines also work as secondary mediators (table 4). Since the secondary mediators are synthesized by a biochemical cascade (after IgE cross linking), manifestation of their biological effects takes a long time. However, their effects are more pronounced and longer lasting, than those of histamine (primary mediators).

The *prostaglandins* are a group of lipid-soluble molecules produced by monocytes, macrophages and other cell types. They are derived from essential fatty acids and consist of a unique group of 20 carbon polyunsaturated, hydroxylated fatty acids called **eicosanoids** which are composed of a cyclopentanone core with two side chains. Prostaglandins, on the basis of the number of double bonds and the fatty acid from which they are derived, are classified into three classes. First class of prostaglandins has one double bond and is derived from dihomo- $\gamma$ -linolenic acid. Class two of prostaglandins has two double bonds and is derived from **arachidonic acid**. Class three prostaglandins have three double bonds and are derived from **eicosapentaenoic acid**.

Prostaglandins are not secreted from any specific gland rather they can be made in nearly all the organs of the body as per the need of time. They act as a signaling molecule and control a number of processes in various body organs in which they are produced. They are generally made at sites of infection or tissue damage, where they cause pain, fever and inflammation as part of the curative process. Prostaglandins regulate the contraction and relaxation of the muscles in the gut and the airways. They can enhance vascular permeability, are pyrogenic and increase sensitivity towards pain. They can also stimulate the buildup of leukocyte cAMP, which has an important suppressive effect on the release of mediators by mast cells, lymphocytes and phagocytes.

Leukotrienes are synthesized by oxidation of arachidonic acid by the enzyme arachidonate 5-lipoxygenase. Some families of leukotrienes are also synthesized from dietary omega 3 fatty acids by the action of cyclooxygenases. These are inflammatory molecules which are released by mast cells during an asthma attack and are primarily responsible for the bronchoconstriction. They act as a strong chemo-attractant for polymorphonuclear leukocyte movement.

There are two groups of leukotrienes. The first group largely acts during neutrophil dependent inflammations such as psoriasis, inflammatory bowel

disease and cystic fibrosis. The second group (cysteinyl-leukotrienes) is chiefly concerned with eosinophil and mast cell induced reactions like bronchoconstriction in asthma. The cysteinyl leukotrienes can also stimulate pro-inflammatory activities like endothelial cell adherence and chemokine production by mast cells. They bind to highly selective G protein coupled receptors on bronchial smooth muscle of windpipe and other airway tissues, and results in contraction of these muscles. They also results in increased secretion of mucus, mucus accumulation and infiltration of inflammatory cells in the airway wall. Their overproduction leads to asthmatic reactions. Compared to histamine, which also results in constriction of airways and edema formation, leukotrienes result in more long lasting reactions with 3-4 times more potency.

**Cytokines**, like interleukins 1–10, tumor necrosis factor (TNF- ) and interferon (INF- ) play a complex role in allergic reactions. They control the activity and functions of cells participating in allergic reactions. Cytokines can be synthesized by a number of cells however macrophages and T-lymphocytes are the chief cells producing them.

Interleukins and TNF can mobilize and activate leukocytes, enhance B and T cell proliferation and boost NK cell mediated cytotoxicity. Some of them can mediate the acute phase responses (like synthesis of complement components, coagulation factors, protease inhibitors and metal-binding proteins) and pyrexia. They can also increase the intracellular calcium ion concentration in leukocytes and hence lead to the production of prostaglandins. In chronic inflammatory conditions they activate fibroblasts and osteoblasts and release enzymes like **collagenase** and **stromelysin** that can cause cartilage and bone resorption.

In addition to the primary and secondary mediators, the plasma contains four interrelated protein systems *viz* **complement**, **kinins**, **fibrinolytic system** and the **coagulation factors**. All of them can generate different mediators of inflammation. Activated components of complement acts as chemotactic factors for neutrophils, stimulate the release of histamine from mast cells and increase vascular permeability. They are known to mark the target for phagocytosis by adhering to the surface of pathogens including bacteria. The kinin system produces substances which can increase the vascular permeability. Kinin system itself gets activated by coagulation factor XII. Bradykinin is the most important kinin and is largely responsible for the itching and pain experienced

during inflammation. The coagulation system of the body can convert fibrinogen (protein found in plasma) into fibrin. Fibrin is a major component of the fluid exudate. The fibrinolytic system can result in plasmin production. The fibrin and plasmin so produced interacts with each other and converts fibrin into a number of products that adversely affect vascular permeability. Hence coagulation and fibrinolytic system works in close association with each other and strengthen the inflammation process.

<b>Mediator</b>	<b>Physiological effect</b>
<b>Preformed mediators in granules (Primary mediators)</b>	
Histamine	Bronchoconstriction, mucus secretion, vasodilation, vascular permeability
Tryptase	Proteolysis
Kininogenase	Kinins and vasodilation, vascular permeability, edema
ECF-A (tetrapeptides)	Attract eosinophil and neutrophils
<b>Newly formed mediators (Secondary mediators)</b>	
Leukotriene B <sub>4</sub>	Basophil attractant
Leukotriene C <sub>4</sub> , D <sub>4</sub>	Same as histamine but 1000x more potent edema and pain
Prostaglandins D <sub>2</sub>	Platelet aggregation and heparin release: microthrombi
Cytokines	Enhance lymphocyte proliferation, mediate acute phase response

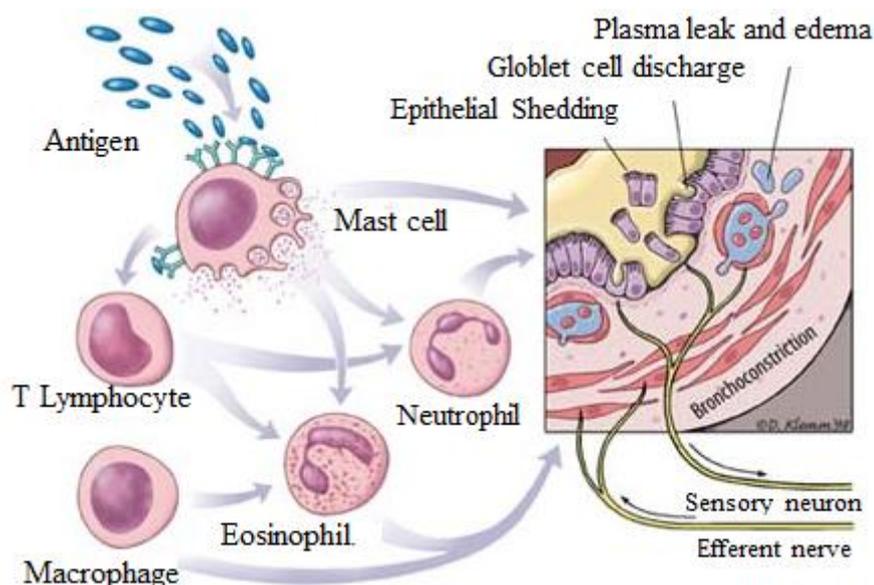
**Table 4: Physiological effects of various pharmacologically important mediators in hypersensitivity.**

#### **13.3.2.5 Examples of type I hypersensitivity**

The clinical manifestation of type I hypersensitivity ranges from localized atopic reactions to life threatening systemic anaphylaxis.

##### **(a) Asthma**

Asthma is a partially reversible disease of airway which results due to obstruction to air flow owing to the compression of neighboring smooth muscles, overdeveloped mucus glands, bronchial inflammation and bronchoconstriction. The bronchial inflammation may also result in narrowing of the airflow pathway due to buildup of edema and mucus and hence intensifies the breathing problem. It is a common localized manifestation. Asthma is of two types; the **allergic asthma** and **intrinsic asthma**. The allergic asthma results by airborne or blood borne allergens like dust, fumes, pollens, animal and insect products, and microbial products while an asthmatic reaction resulting from climate change or excessive cold or vigorous exercise is termed as **intrinsic asthma** (intrinsic asthma is independent of allergen stimulation). Intrinsic asthma results when the air tracks that bring air in and out of lungs constrict. People who are very sensitive to both low temperatures and dry air are especially prone to intrinsic asthma.



**Figure 10: Pathogenesis of Asthma**

The asthmatic attack is triggered by the degranulation of sensitized mast cells. The degranulation process develops in lower respiratory tract and results in constriction of bronchial smooth muscle cells, edema formation, inflammation, buildup of mucus and airway obstruction. The asthmatic patient's display abnormal level of neuropeptide receptors which are involved in relaxation and contraction of muscle cells, for example - such patients have high level of receptors for substance *P*, a neuropeptide which contracts smooth muscles, while decreased expression of receptors for many intestinal peptides which relaxes smooth muscles (figure 10).

Asthmatic reactions can be divided into **early (primary) response** and **delayed (secondary) response**. The **early response** manifests within minutes after exposure to the allergen and involves the manifestation of primary mediators like histamines and secondary mediators like leukotrienes and prostaglandins. Generally, cytokines play a negligible role in early asthmatic response. The **secondary response** occurs after a lapse of 8-10 hours and is mediated by various cytokines like Interleukin 4, 5, 10, 16, Tumor Necrosis Factor (TNF- $\alpha$ ), eosinophil chemotactic factors released by already present activated mast cells during and after immediate response. Delayed response occurs in 50% of the patients. The mast cell degranulation is the chief reason for manifestation of early response and the symptoms involved are bronchoconstriction, vasodilation and buildup of mucus. However, the late response involves inflammatory cells like eosinophils and neutrophils. The cytokines listed above, results in the recruitment of eosinophils and neutrophils at the site of inflammation. Both of these cells release toxins, oxygen radicals and many cytokines. The overall manifestation of these mediators is in the form of blocking of bronchial lumen with mucus, cellular debris, epithelium sloughing, thickening and contraction of basement membrane of diaphragm, edema etc. Contraction of basement membrane of diaphragm severely impairs the process of respiration. Thus, it could be concluded safely that the asthmatic late phase reactions are often more devastating and long lasting than the early phase.

***(b) Allergic Rhinitis***

It is one of the most common types of atopic disorder which is commonly known as **hay fever**. In this reaction the airborne allergens like pollens, dust particles induce sensitized mast cells to release pharmacologically active mediators like histamines, leukotrienes and prostaglandins. These mediators are released in the conjunctivae and nasal mucosa, where they cause localized vasodilation and increased capillary permeability. The overall effect is seen in the form of following symptoms:

- ✓ Sneezing and coughing.
- ✓ Excessive secretions of mucus glands in the nose.
- ✓ Obstruction in nasal airflow due to congestion of large veins.
- ✓ Irritation in throat, nose and eyes by allergic inflammation of sensory nerves.

- ✓ Watery exudation of nose, conjunctivae and upper respiratory tract.
- ✓ Watery exudation of nose, conjunctivae and upper respiratory tract.

**(c) Food allergy**

The allergic reactions caused by some food stuffs results in localized anaphylactic reactions. Such reactions normally occurs in and around the upper and lower gastrointestinal tract. Like other type I hypersensitivity reactions, these allergens binds to the sensitized mast cells resulting in IgE cross linking and ultimately leading to their degranulation. The mediators released as a consequence of mast cell degranulation cause contraction of smooth muscles and vasodilation in gastrointestinal tract leading to vomiting and/or diarrhea. Other symptoms include asthmatic attack and urticaria after ingestion of certain foods. Sometimes the increased permeability of mucus membrane cause allergens to enters the bloodstream.

**13.3.2.6 Late phase reactions**

A typical Type I reaction of hypersensitivity is characterized by a late phase reaction which follows the early phase reaction. Such reactions begin to develop after a lapse of 6-8 h of initial reaction and are different from the late phase reactions observed during asthma. Actually this late phase reaction is a result of infiltration of lymphocytes, macrophages, neutrophils, basophils and eosinophils at the site of reaction. This infiltration is mediated by the chemotactic chemicals released during mast cell degranulation. The tumor necrosis factors and interleukins enhance the expression of cell adhesion molecules on endothelial surfaces of venular tissues. Hence, the population of macrophages, neutrophils, basophils and eosinophils builds up on the surface of such cells.

The Late-phase reactions are associated with a second phase of smooth muscle contraction, sustained edema and tissue remodeling such as smooth muscle hypertrophy (an increase in size due to cell growth) and hyperplasia (an increase in the number of cells). The late phase reactions results in chronic allergic inflammation and have long term consequences. Thus they contribute to very serious long-term illness, such as chronic asthma.

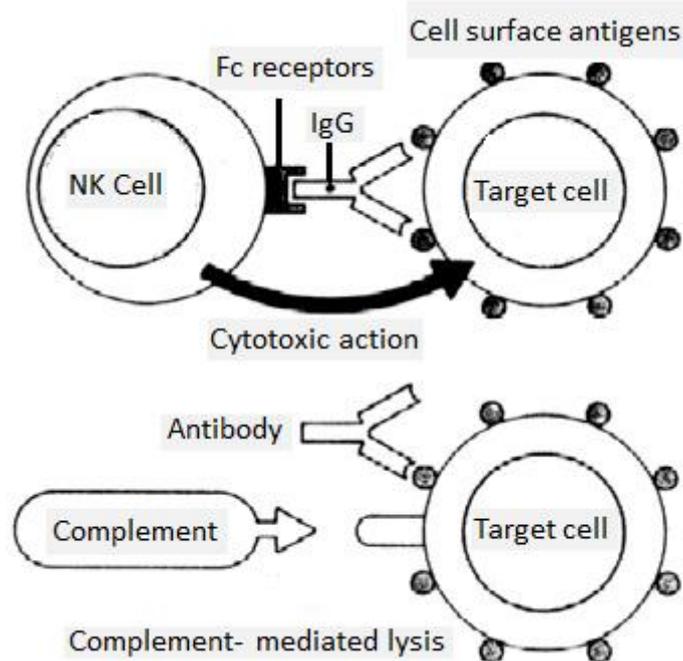
Eosinophils play a major role in the late-phase reactions. They are the most abundant cells which, by the action of eosinophil chemotactic factors released by the degranulating mast cells, aggregates at the site of infection. The Fc receptors for IgE and IgG present on the surface of eosinophils binds with the

IgE-allergen complexes. Binding of Ab-allergen complex on Fc R receptors on eosinophil results in their activation and degranulation, ultimately leading to the release of inflammatory chemicals. After degranulation, the eosinophils releases platelet-activation factors, leukotrienes, eosinophil cationic protein and some neurotoxins.

Apart from eosinophils, neutrophils also play a critical role in late phase reactions. The neutrophil chemotactic factors, released by the degranulating mast cells during the early phase of type I reaction, attract the neutrophils at the site of inflammation. Some other chemokines like IL-8 activate neutrophils that in turn results in the discharge of lytic enzymes, leukotrienes and other vasoactive agents.

### **13.3.3 Type II hypersensitivity reactions**

Type II hypersensitivity, also known as **antibody mediated cytotoxic hypersensitivity** can affect a large number of tissues and organs. Normally, endogenous antigens are associated with type II hypersensitivity, although exogenous chemicals (haptens) attached to cell membranes can also elicit these reactions. Such reactions are **primarily mediated by antibodies of IgM or IgG class and complement system**. The most common reactions involve antibody-mediated destruction of blood cells. An antigen present on the surface of a cell on combination with antibody IgG or IgM will boost the demise of that cell by promoting contact with phagocytic cells, natural killer cells and T cytotoxic cells. In some cases cell death may also occur through activation of complement system that creates pores in the cell membrane resulting in membrane damage.



**Figure 11: Pathogenesis of type II hypersensitivity**

In type II reactions, antibodies are directed against antigens present on the body cells (like circulating red blood cells) or extracellular materials. The IgG and IgM antibodies bind to these antigens to form Ag- Ab complexes. These complexes activate the classical pathway of complement, generating membrane attack complexes. This ultimately leads to elimination of antigenic cells or cells presenting foreign antigens, either by lysis or extracellular tissue damage (figure 11). In addition to the complement, several mediators of acute inflammation are also generated at the site of infection. The surface bound IgG or IgM and complement components (which can act as opsonins) are recognized by the Fc receptors of phagocytic cells (like macrophages) and/or lytic cells (like natural killer cells) resulting in **Opsonization** and **Antibody Dependent Cell Mediated Cytotoxicity (ADCC)** respectively (figure 11). Moreover in many cases macrophages and dendritic cells, that acts as antigen presenting cells, recognize the antigens present on the surface of the cells and hence mount a B cell response (in the form of antibody production) against them. In other words type II hypersensitivity induced pathogenesis is a result of role of antibodies in targeting cells for phagocytosis, activation of the complement system and interference with normal cellular functions.

#### **13.36.3.1 Examples of type II hypersensitivity**

Type II hypersensitivity may result in a number of diseases like Erythroblastosis Fetalis, Blood Transfusion Reactions and Rheumatic Fever.

***(a) Hemolytic Disease of the Newborn***

Hemolytic disease of the newborn is also known as **erythroblastosis fetalis**. This is a type of alloimmune disease (an immune response against foreign antigens (alloantigens) received from the individuals of the same species) which begins when the maternal IgG antibodies developed against Rh antigenic factor present on fetal RBC passes to the fetus through placenta. These antibodies then attacks on the Rh proteinic antigens present on fetal RBCs. This leads to the destruction of RBCs ultimately leading to severe anemia and reticulocytosis. The overall response of the disease may range from mild to lethal.

This disease develops when a pregnant mother is Rh negative and fetus is Rh positive. During the pregnancy period, trophoblast (a placental layer) separates the fetal RBC from mother's circulation. Presence of trophoblast prevents Rh<sup>-</sup> mother carrying an Rh<sup>+</sup> fetus to come in contact with Rh antigen during her first pregnancy. Hence, no Rh specific antibody is synthesized in mother's body during the first pregnancy period, however during the delivery time, the blood from umbilical cord mixes with the blood of mother's circulation (figure 12). As a result, the Rh<sup>+</sup> blood cells cause the mothers immune system to synthesize anti Rh IgM antibodies which clears the circulating Rh<sup>+</sup> RBCs from the blood. These antibodies get cleared from the circulation in few days' time, however the memory cells remains in the circulation for years (figure 12). In the subsequent pregnancy, the memory cells gets activated and by the process of class switching results in the formation of IgG antibodies which can cross the placenta. These anti Rh IgG antibodies can cause the complement mediated destruction of fetal RBC cells. As a result, the fetus faces mild to severe problem of anemia. Moreover the hemoglobin released from RBCs converts into bilirubin which gets accumulated in different organs and result in organ failure.

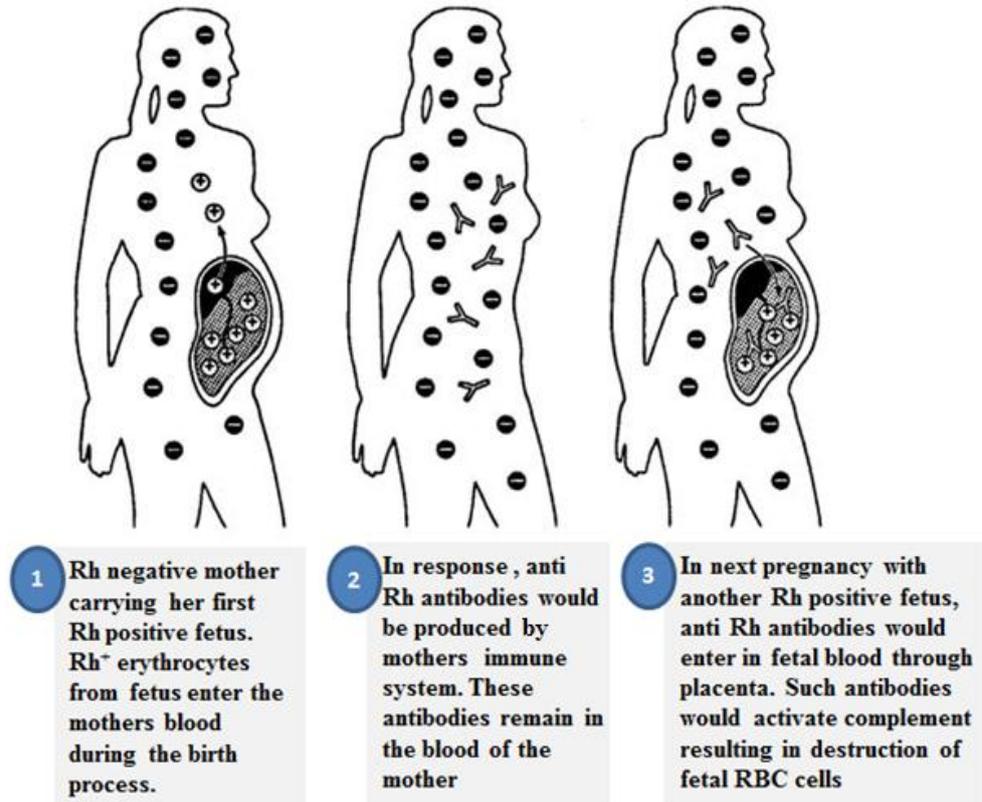


Figure 12: The cytotoxic reaction in Hemolytic Disease of the Newborn

- a) The treatment of erythroblastosis fetalis of new born caused by the Rh incompatibility depends upon the severity of the reaction. The following treatments are employed for the treatment:
- b) Administration of **Rhogam** antibodies (which are directed against Rh antigen), in mothers blood, within 24-48 hours of the birth process. This can immediately clear all the Rh<sup>+</sup> RBCs present in mother's blood (before the development of B cells) and hence no memory cell develops. The use of rhogam antibodies thus eliminates any chance of complication in the subsequent pregnancy.
- c) For an adverse condition, **intrauterine blood-exchange transfusion** is done to replace fetal Rh<sup>+</sup> red blood cells with Rh<sup>-</sup> cells. This may be done repeatedly in every one to three weeks' time.
- d) In less severe cases, toxic effects of bilirubin (produced due to breakdown of RBC cells and accumulation of hemoglobin) could be reduced by the use of low dose of UV light, which breaks bilirubin and prevents its accumulation in body organs.
- e) **Plasmapheresis** is another treatment which is done during the pregnancy period. In this method blood of the mother is separated into

two fractions *viz* plasma and cells. The plasma containing anti-Rh antibodies is replaced with fresh one.

In addition to Rh incompatibility, the hemolytic disease is also caused by the *ABO* blood group incompatibility between mother and fetus. *ABO* incompatibility results when *O* type mother carries *A* or *B* fetuses. In such a case mother develops either anti *A* or *B* antibody. Usually fetal anemia resulting from *ABO* incompatibility is mild and results in a slight elevation of bilirubin, resulting in jaundice. Depending on the severity of the anemia and jaundice, a blood-exchange transfusion or UV treatment may be required in these infants.

### ***(b) Blood Transfusion Reactions***

The attack of recipient's immune system on the red blood cells received from the donor is called **hemolytic reaction**. This reaction takes place only when the blood of recipient and the donor are not compatible. However if this sort of reaction is targeted against the donors WBC's then this is called the **febrile reaction**.

Human RBC membrane has a number of different polymorphic constituents (proteins and glycoproteins), the *ABO* being the dominant one. The antigenic groups *A* and *B* are coded by allelic form of blood group antigen i.e. *A* and *B*. These alleles encode specific glycosyltransferases which acts on different substrates to form antigen *A* and *B*. Individuals with *A* type blood group possesses *A* type antigen on the surface of blood cells while individuals of *B* blood group possesses *B* antigen. An *AB* individual possesses both the blood antigens i.e. *A* and *B* while an individual of *O* type blood group do not have either *A* or *B* antigen. An individual possessing one allelic form of a blood-group antigen can recognize other allelic forms on transfused blood cells as foreign and can mount an immune response.

In most of the cases, antibodies against the blood group antigen are induced and boosted by natural exposure to similar antigenic determinants (which are structurally similar to the blood group carbohydrates) on a variety of microorganisms present in the gut (as resident micro-flora). In fact human gut has few bacteria with cell wall antigens very similar to *A* and *B* blood group antigen. Thus an individual having *A* blood group will treat *A* like antigenic epitopes present on intestinal microorganisms as self and a state of tolerance will be developed. However, antibodies would be raised against *B* like epitopes present on other intestinal microorganisms. Similarly, an individual with *B* blood group will have antibodies against *A* like epitopes present on intestinal

microflora while a state of tolerance would exist against *B* type epitopes present on microbial surface. In this situation antibodies present in the recipient's blood can attack the donor blood if the two are not compatible, resulting in cross reaction. The antibodies so formed are IgM and are called as **isohemagglutinins**.

If a type *A* individual is transfused with type *B* blood, a transfusion reaction will occur in which the anti-B isohemagglutinins (already present in recipient's blood) bind to the *B* type blood cells and cause their destruction by means of complement-mediated lysis. A similar reaction would occur if the case is reversed i.e. a *B* type individual is transfused with blood containing *A* type RBC cells.

The clinical manifestations of the blood transfusion reactions may vary from mild to serious. Mild reactions include chill, fever, urticaria and pruritus while severe condition includes acute kidney failure, anemia, lung problems (pulmonary edema), severe shortness of breath, red urine, high fever, loss of consciousness and shock—life-threatening condition resulting from lack of adequate blood flow.

In addition to A, B antigens, blood cells have many other surface receptors/antigens. Most commonly found membrane antigens are Kidd, Kell and Duffy. These antigens, however, exhibit minor allelic differences within the population. The repeated transfusion reactions result in stimulation of antibody production, in this case IgG are synthesized. This reaction manifests in 3-6 days after the transfusion resulting in **delayed hemolytic transfusion reactions**. These reactions develop in patients who are treated by repeated transfusion of *ABO* compatible blood that is incompatible for other blood group antigens. The type of IgG produced in such reactions is less effective in activating complement. They display a milder reaction which results in incomplete lysis of RBC cells. They also result in stimulation of reactions like agglutination and opsonization which may result in macrophage mediated phagocytosis at extravascular sites. The late reaction is characterized by mild to high fever, increased bilirubin, mild anemia and jaundice, low level of hemoglobin etc.

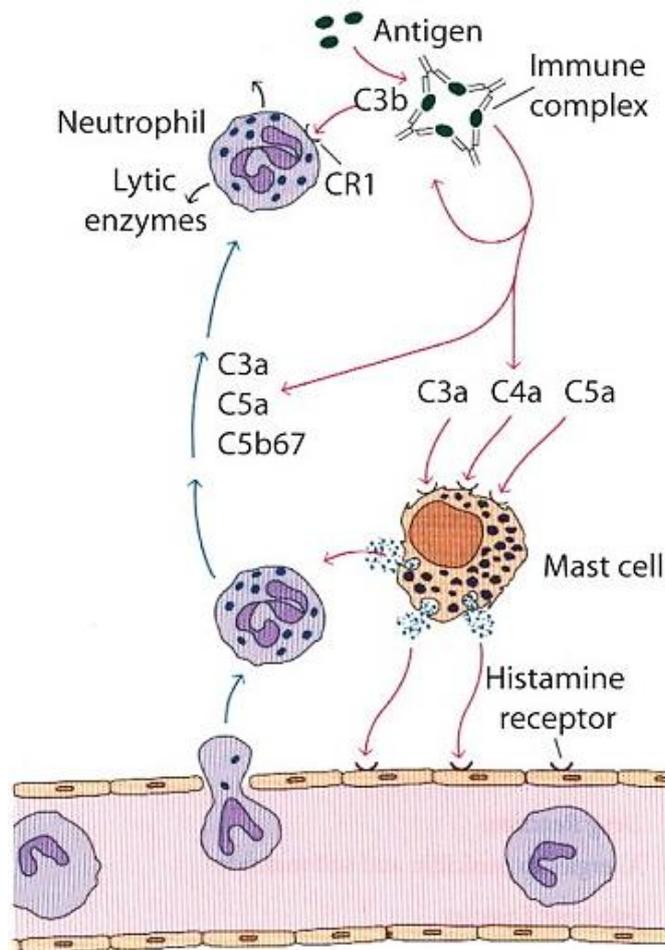
#### **13.3.4 Type III hypersensitivity reactions**

The Type III hypersensitivity is mediated by immune complex (Ag-Ab complexes). In general antibodies get bound to antigens and form immune complexes. These complexes help to remove antigens by phagocytosis. If such complexes are formed in excessively high amounts, they may lead to a tissue

destructive hypersensitive reaction. The manifestation of these reactions occurs by the activation of complement system. The extent of complexity associated with this hypersensitivity depends upon the magnitude of immune complexes and their body distribution. If the complexes remain confined to a single organ, localized hypersensitivity develops, however a systemic reaction can develop if these complexes are distributed in large number of organs or if they enter in the blood (reaction can develop wherever these complexes are deposited). The antibody affinity and size of immune complexes are important in development of disease and defining the tissue involved.

Normally, the antibody involved in such reactions is IgG, however IgM may also get involved. The damage further intensifies by the action of platelets and neutrophils.

The type III reaction differs from type II hypersensitivity (which also involves IgG and IgM). Unlike type II hypersensitivity, surface bound antigens are not involved in type III reactions rather Ag-Ab complexes deposited in various tissues elicit the reaction.



**Figure 13: Pathogenesis of type III hypersensitivity**

The type III hypersensitivity is manifested by the attack of large amount antigens into the tissues. These antigens then attach to the antibodies (IgG or IgM) and forms immune complexes. Some of the larger complexes so formed are internalized by the macrophages and hence get cleared. Smaller to intermediate sized complexes are also formed and they are difficult to clear. Some large to intermediate sized complexes deposits on the basement membrane of blood vessel walls or kidney glomeruli while the smaller complexes pass across the basement membrane and get deposited in the sub-epithelium surfaces. They can also migrate to various other sites. Wherever these immune complexes settle, they results in tissue destructive type-III hypersensitivity reactions. The immune complexes so formed have IgG or IgM attached with the antigen. Both of these antibodies have unique capacity to activate the complement system through classical pathway. The complement split products like C3a, C5a, and C5b67 acts as chemotactic factors for the neutrophils while other products like C3a, C4a, and C5a acts as anaphylatoxins which can result in localized degranulation of mast cells and enhanced vascular permeability (figure 13). The neutrophils accumulate in large numbers at the site of immune-complex deposition and the granular release from these cells cause tissue damage (figure 13). The C3b complement component also acts as an opsonin which coats the immune complexes. The C3b coated immune complexes attach and deposits over the surfaces like basement membrane of blood vessels or kidney or any other organ. In this situation, neutrophils become unable to phagocytose surface attached C3b coated immune complexes. However during their unsuccessful attempts to ingest the immune complexes, neutrophils releases a large quantities of lytic enzymes. Some of the complement components induce platelet aggregation. The aggregated platelets releases clotting factors resulting in the formation of micro-thrombi.

#### **13.3.4.1 Examples of type III hypersensitivity**

Type II hypersensitivity can be manifested in the form of a number of reactions like Arthus reaction, Serum sickness reactions etc.

##### ***(a) Arthus reaction***

Nicolas Maurice Arthus (1903) described about the **Arthus reaction**. During his experiments he repeatedly injected horse serum in rabbits and reported the formation of subcutaneous edema at the site of injection. He observed that repeated injections eventually led to the formation of gangrene. In this reaction, the antigen/antibody complexes are formed after the intradermal injection of an

antigen. Such a reaction begins rapidly in the previously sensitized persons. It is a local vasculitis reaction (cluster of inflammatory disorders leading to blood vessel damage) which destroys the cutaneous blood vessels. Complement activation and presence of polymorphonuclear leukocytes results in an inflammatory response.

Arthus reaction is characterized by swelling, induration, hemorrhage, edema, severe pain and sometimes necrosis.

***(b) Serum sickness***

Many times antitoxins like antitetanus or antidiphtheria administered in human, for different reasons, are mistaken as antigens by the immune system of recipient person and the complication which result is termed as **serum sickness**. In such a case the recipient produces antibodies against these antigens and forms antigen-antibody complexes that circulate along with the blood. After the lapse of a week's time, the individual starts to show a number of symptoms typical of serum sickness like skin eruptions (urticaria), joint pain, fever, weakness, edema, arthritis and sometimes lymphadenopathy. The severity of disease depends upon the number of immune complexes, size of complexes and the site of deposition.

The Small immune complexes, so formed, do not initiate inflammation and larger complexes get cleared by the reticuloendothelial system. It is the intermediate sized complexes which deposits at the blood vessel surfaces and nearby tissues leading to vascular necrosis and tissue damage by the activation of complement, recruitment and degranulation of granulocytic cells. The endothelial cells present in adjoining tissues shows elevated expression of adhesion molecules. The monocytes and the matured macrophages release pro-inflammatory cytokines which along with complement split products stimulates neutrophil migration and adherence at the site of immune complex deposition. The mast cells also get aggregated at this site and release a number of activated vasoactive amines which alter the vascular permeability. Within a short interval of time, a large number of inflammatory cells move at the site of immune complex deposition and cause a serious vascular necrosis.

All such immunological events continuously clear the free antigens from the blood, however the process of antibody production and formation of large immune complexes continues. A stage comes when detectable antigens disappear from the circulation and the antibody titer continues to rise in blood. A secondary serum sickness is characterized by a shorter latent period,

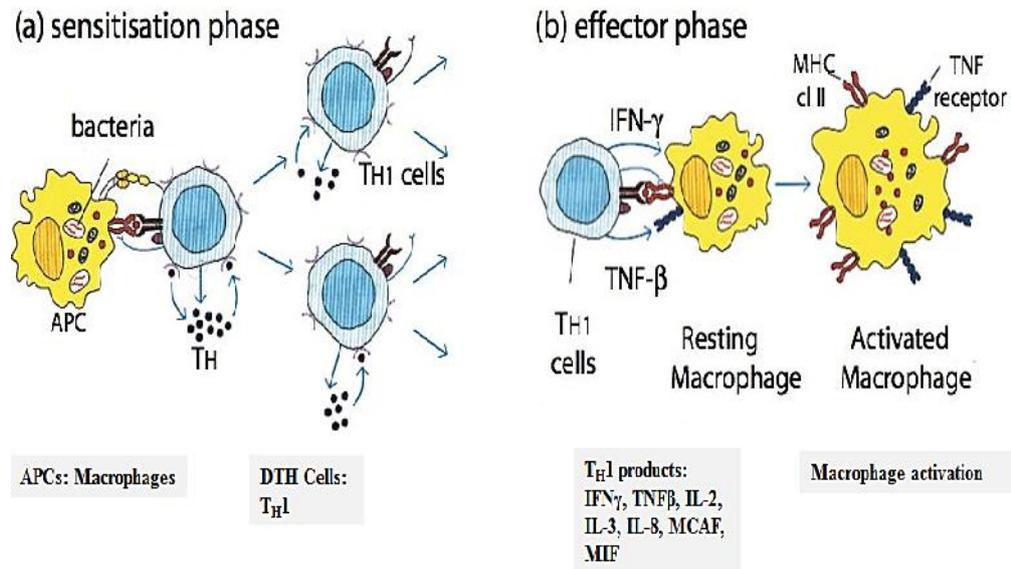
exaggerated symptoms and a brief clinical course.

Most common cause of serum sickness is hypersensitivity reaction towards the administration of drugs having proteins derived from other animal species like-Antitoxins, Antivenins, Hormones derived from various animal species, Streptokinase and Vaccines. Many polyclonal and monoclonal antibodies derived from animal sources like rabbit, horse and mouse serum are also known to cause serum sickness. In addition, many other antibiotics and antimicrobial compounds that can cause serum sickness includes -Ciprofloxacin, Streptomycin, Indomethacin, Tetracyclins, Cephalosporins, Metronidazole, Carbamazepine and many others.

In addition to serum sickness, the circulating immune complexes may cause many other manifestations like autoimmune diseases (Rheumatoid arthritis, Systemic lupus erythematosus, Goodpasture's syndrome), infectious diseases (Hepatitis, Meningitis, Mononucleosis, Malaria), allergies to drugs and food stuff.

#### **13.3.5 Type IV hypersensitivity reactions**

Type IV hypersensitivity, also called as **delayed type hypersensitivity**, is a cell-mediated immune reaction which primarily involves the participation and interaction of circulating  $T_H$  cells (in particular  $T_{H1}$  cells) with antigens and depends upon their density. This reaction mounts after a lapse of some 24-72 hours and hence called as delayed type hypersensitivity. In this reaction, some specific subtypes of  $T_{H1}$  cells (previously called as  $T_{DTH}$ ), after encountering certain type of antigens gets activated. The activated T cells, in response, produce a localized (at time systemic also) inflammatory response characterized by the presence of many non-specific inflammatory cells like macrophages.



**Figure 14: Pathogenesis of Type IV hypersensitivity**

The type IV reaction is marked with an initial sensitization phase of 1–2 weeks duration. Sensitization phase begins after primary contact with an antigen followed by an effector phase. The antigen presented on the surface of an antigen presenting cell (APC) together with the MHC II molecule interacts with the specific  $T_H$  cells. Such interactions results in activation and clonal expansion of  $T_H$  cells (figure 14). In few cases  $CD8^+$  cells are also activated. Generally, macrophages and langerhans cells (dendritic cells found in epidermis) works as APC in type IV hypersensitivity reactions. Sometimes, vascular endothelial cells also express MHC II and works as APC. During the sensitization phase APCs picks up the antigen (which enters through the epidermis) and carry them to the regional lymph nodes. Picked antigen joins and complexes with MHC II of antigen presenting cells. Within the lymph node, antigen-MHC II complex interacts with  $T_H$  cells. This results in activation of specific subsets of  $T_H1$  cells (figure 14). Activated  $T_H1$  cell, during the sensitization phase, releases cytokines which leads to the recruitment of immunological cells and the degradation of pathogens.

Effector phase is marked by the second exposure to the antigen. In this phase, the  $T_H1$  cell secretes a variety of cytokines that cause the recruitment and activation of many immunological cells (specific and non-specific). The effector phase is marked after a lapse of 48-72 hours past the second exposure. This delay is justified because of the time required by the cytokines to recruit the inflammatory cells at the site of inflammation. The cells normally employed for this purpose includes macrophages and polymorphonuclear leucocytes. The

number of such cells amplifies tremendously and within a short period of time, overshadows the number of  $T_H1$  cells. The monocytes deployed during this process adhere to the vascular endothelial cells and drifts from the blood into the surrounding tissues. With the lapse of time, cytokines released by  $T_H1$  cells converts the monocytes into fully developed and matured macrophages. The activated macrophages exhibits: (a) high level of phagocytosis, (b) release of large amount of cytotoxic mediators and (c) increased level of class II MHC molecules. In this way, the level of antigen presentation by macrophages boosts up by many times. All the above events lead to nonspecific destruction of pathogenic cells. If the pathogen is not cleared in short time, the DTH response mounts for a longer periods and becomes excessively destructive. This leads to the development of a visible granulomatous reaction due to the close adherence (sometimes fusion) of activated macrophage cells. The resultant bulky cell mass displaces the normal tissues, forms tumor like structures, releases very high concentration of lytic enzymes and vasoactive substances which can destroy the surrounding blood vessels and cause extensive tissue necrosis. Monocyte chemotactic factor, monocyte activating factor (MAF), migration-inhibition factor (MIF), interleukin-2, TNF / , interferon-gamma are among the major lymphokines involved in type IV reactions.

### 13.3.5.1 Examples of type IV hypersensitivity

Two most common examples of DTH which illustrates consequences of type IV reactions are **contact dermatitis and tuberculin reactions (table 5)**.

Type	Reaction time	Clinical appearance	Histology	Antigen and site
Contact	48-72 hr	Eczema	lymphocytes, followed by macrophages; edema of epidermis	Epidermal (organic chemicals, poison ivy, heavy metals, etc.)
Tuberculin	48-72 hr	local induration	lymphocytes, monocytes, macrophages	Intradermal (tuberculin, lepromin)

Table 5: Types of delayed hypersensitivity reactions

**(a) Contact dermatitis**

T cells mediate many contact dermatitis reactions, including the response to many reactants like trinitrophenol, formaldehyde, nickel, cosmetics and hair dyes, poison oak and others. These substances complex with skin proteins. Such complexes are internalized by Langerhans cells. They are then processed and presented along with class II MHC molecules to T<sub>DTH</sub> cells, causing activation of sensitized T cells. This reaction can also be used for determining the DTH reactions and observing the development of characteristic skin lesions.

**(b) Tuberculin reaction**

Tuberculin reaction is used as a test to determine whether the patient has previously been infected with *Mycobacterium tuberculosis*, the causal agent of tuberculosis. This is an effective test based upon the concept of delayed hypersensitivity reaction. A previously infected individual becomes sensitized. This test is marked by the inoculation of small amounts of protein extracted from the *Mycobacterium* into the skin. If positive, i.e. reactive T cells are present—redness and swelling would appear at the site of injection which increases in 24 hours and then disappears slowly. A positive reaction is characterized by the presence of lymphocytes and monocytes, edema, tissue fluid, dead cells etc.

Now after discussing the mechanism of manifestation of all the four types of hypersensitivities, it could be concluded that all of them differs significantly from each other in many respects, as is summarized in table 6. In addition to the above four types, one more type of hypersensitivity reaction has also been described. This reaction is called as **type V hypersensitivity**.

Characteristics	Type-I (anaphylactic)	Type-II (cytotoxic)	Type-III (immune complex mediated)	Type-IV (delayed type)
Antibody	IgE	IgG, IgM	IgG, IgM	None
Antigen	exogenous	cell surface	soluble	tissues & organs
Response	15-30 minutes	minutes-hours	3-8 hours	48-72

time				hours
Appearance	wheal & flare	lysis and necrosis	erythema and edema, necrosis	erythema and induration
Histology	basophils and eosinophil	antibody and complement	complement and neutrophils	monocytes and lymphocytes
Transferred with	Antibody	antibody	antibody	T-cells
Examples	allergic asthma, hay fever	erythroblastosis fetalis, Goodpasture's nephritis	SLE, farmer's lung disease	tuberculin test, poison ivy, granuloma

**Table 6: Comparison of Different Types of hypersensitivities**

### 13.3.6 Type V hypersensitivity reactions

Type V hypersensitivity reactions (also known as stimulatory hypersensitivity) have been described later and are in addition to the reactions described by Gell and Coombs. These immune responses stimulate or obstruct the functionality of endocrine receptors. Most of these responses are found in autoimmune disease. This is another type of antibody mediated (IgM or IgG) hypersensitivity which resembles Type II hypersensitivity in many ways but differs significantly in the mechanism. In place of binding to components of cell surface, the antibodies (produced in response to specific stimuli), binds to the receptors (including hormone receptors) present on the cells surface. This prevents the general working of the receptors (i.e binding with various ligands, interacting with environmental stimulus) and results in impairment of cell signaling processes.

In other words, these reactions do not kill or destroy the cell rather induce the dysfunctioning of the affected organ/tissue. The classical example of this reaction is Grave's disease which is caused by stimulation of the thyroid-stimulating hormone receptors on the surface of follicular cells. This results in over-activity of the thyroid gland leading to symptoms of hyperthyroidism.

Type-V hypersensitivity is not always stimulative rather it inhibitory also. For example, myasthenia gravis, an autoimmune disease in which antibodies are directed against acetylcholine receptors of neuro-muscular plates. Here, antibodies inhibit the neuromuscular transmissions. This results in weakening of muscles and paralysis. In few cases, the anti-acetylcholine receptor antibodies activate the complement system, leading to damage of cells present in neuromuscular areas.

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## **13.4 Immune response against infection**

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Human body is constantly engaged in warfare with the microbes which surrounds us. Many of the microbes have enough means of evading our defense mechanism and exhibiting their presence in the body. Emergence of new infections and the re-emergence of some old infections is a continuous process.

During first invasion by a pathogen, clinical symptoms may vary from nil to non-specific reactions to specific diseases and beyond that. In any case, however, immune response works for boosting up the body defense. An infection or disease condition arises only when this defense system is breached by the invaders. In fact development of infectious disease involves a set of complex interactions between the invading microbe and the host immune system.

Infection is established in many stages which start from microbial entry, its invasion and colonization in the host tissues. This is followed by breaching of host immunity leading to functional impairment and/or injury. Some microbes liberate toxins that cause tissue damage, functional disorders and at times death of the host cells. Many functional and structural features of microorganisms add to their virulence which itself is under the control of diverse mechanism.

### **13.4.1 General mechanism of immune response against infections**

In general there are many broad and varied responses which work for the providing immunity against microbes. These responses are mounted against the invader pathogens which may result in an infection:

### ***(1) Effector mechanisms of innate and adaptive immunity***

For any infection, the first line of defense is provided by the innate immune system. Innate immunity works in a non-specific manner and is executed at different levels. It includes the general defense by physiological and mechanical barriers like skin, action of sneezing and coughing and action of cilia and flagella. Many chemicals produced by body cells during their normal activities (like hydrochloric acid in stomach, saliva, interferon, mucopolysaccharides, zinc present in semen and many others) have antagonistic effect on the growth of microorganisms. The presence of resident microflora on different body parts exhibits negative impact on the growth of microorganisms. In addition, many immunological cells work for innate immunity. These cells possess genetically encoded receptors which are called TLRs (Toll like receptors). TLRs detect general danger caused by the pathogens. Presence of TLRs enables these cells to broadly recognize fungi, bacteria, viruses and noninfectious agents. This identification is not very specific and the organisms cannot distinguish between the specific genus and species. Some of these cells like macrophages, dendritic cells and polymorphonuclear leukocytes are also involved in specific immunological functions and can cause activation of adaptive immunity. In many cases, innate immunity is mediated by alternative pathway of complement. The complement activation also helps in activation of specific antibodies and secretion of specific cytokines from macrophages. Absence of innate immunity is crucial for host defense and susceptibility towards infection.

The adaptive immune system, however, provides a more persistent and solid response. Many microbes are capable to evade and resist innate immunity and the host immunity is predominantly dependent upon acquired immunity. Adaptive immune responses recruit a vast variety of effector cells- lymphocytic and non- lymphocytic, that works to eliminate invading microbes along with their products. In this process they produce memory cells which functions to protect individuals from future repeated infections.

### ***(2) Different infectious agents are treated in specialized ways for optimal protection***

The invading microbes differ in patterns of host invasion and colonization. Each of them exhibits varied mechanism of pathogenesis. Their removal from the host requires different effector systems. The extent and nature of immune response against infectious agents determines the course and outcome of the infection. The specialization of adaptive immunity permits the host to respond

to different types of microbes like bacteria, viruses, protozoans and fungi in specialized and specific manner. This could be understood by looking into the fact that the immune system generates various subsets of  $T_H1$ ,  $T_H2$ , and  $T_H17$   $CD4^+$  T cells along with the production of different isotypes of antibodies. This is why adaptive immunity is also called as specific immunity. Being specific, adaptive immunity counters the ill effects of pathogens.

**(3) *Equilibrium of microbial resistance towards body's defense mechanism and host immune system determines the extent of pathogenicity***

Any invading infectious agent applies a number of strategies to combat the host defense mechanism. It is the balance of immune system and pathogenic resistance towards host defense which determines the degree of pathogenicity.

Microbial strategies generally used for the evading the host defense are as below:

- A. ***Adhesion to the host cell surface by bacterial surface components.*** This promotes bacterial entry into the host cells. Presence of these components is important to inactivate complement and its components. Sialic acid which is present in many bacteria, for example, inhibits the activation of complement by alternative pathway. Many proteins, present on bacterial cell surface are responsible for resisting phagocytosis. Many bacteria that are rich in polysaccharide capsules resist phagocytosis and thus are better adapted in the host body. For the obvious reasons, such microbes are highly virulent as compared to their counterparts which lack capsular polysaccharides.
- B. ***Genetic variation of surface antigens.*** This mechanism is specifically important for pathogens to escape antibody attack. Many bacteria have remarkable capacity to undergo extensive genetic alterations of the genes responsible for the determination of antigenic property. This results in a comprehensive diversity of the surface antigenic molecules. As a result, antibodies produced against one antigenic determinant become insignificant as the pathogen very frequently changes the epitopic composition. This could be understood by following examples:
- a) In *Haemophilus influenza*, very rapid change in the production of glycosyl synthetases leads to significant alterations of antigenic molecules like surface lipopolysaccharides and other polysaccharides.
  - b) Pili of bacteria like *E.coli* and *Gonococi*, which is involved in bacterial adhesion to the host, contains a protein called **pilin** which happens to be a major proteinic antigen. The pilin genes undergo a very rapid alteration,

producing about  $10^6$  antigenically different pilin molecules. This enables bacteria possessing pilin protein to escape host defense.

***(4) Sometimes immune system can-not eliminate the pathogens rather it checks their growth***

In such a case microbes establishes a latent phase in which microbes survives but do not spread infection. This is true of some intracellular bacteria and many DNA viruses (especially belonging to herpesvirus and poxvirus families). Sometimes the bacterial infection becomes latent or persistent (like in tuberculosis). In such a case bacteria survives within the endosomal vesicles of the host. These infections are opportunistic and as the immune system of the host weakens or becomes faulty (due to varied reasons), the latent or persistent microbes becomes reactivated and starts to propagate the infection.

**13.4.2 Immunity against intracellular parasites**

Several diseases such as Leishmaniasis, Trypanosomiasis and Malaria are caused by protozoan parasites. Here, in general the word parasite refers towards infectious animal parasites like protozoa, helminthes, arthropods like ticks and mites. Many of them are toxic or relatively infective or may be both. Many protozoan infections often follow chronic passages. These parasites are responsible for a-great morbidity and mortality (especially in developing countries). In fact parasites accounts for highest morbidity and mortality, even more than any other infectious agent. Malarial parasite itself is among the largest affecting infectious parasitic.

Most of the parasites show a complex life style in which they live a part of their life in human (or any other vertebrate) and other part in an intermediate host like flies, mosquitoes, ticks etc. Mostly parasitic pathogenesis in human is caused by bites by intermediate hosts, like in malaria and trypanosomiasis. In some cases it is transmitted by sharing habitat with infected intermediate host (for example, Schistosomiasis).

The transmission and survival of pathogenic protozoans largely depends on their capability to evade host's nonspecific and specific immune responses. Innate immunity against parasitic infections is weak and most of the protozoans have capacity to resist elimination by host adaptive immune responses. Thus the infections caused by parasitic pathogens are chronic. Even, at times many anti-parasitic drugs are not effective in killing the organisms.

Persistence of parasitic infections may also cause some chronic immunological reactions, lead to tissue injury and immune regulation abnormalities. Thus some

of the manifestations seen after parasitic infections are result of host reaction only.

#### **13.4.2.1 Innate immune response**

Various mechanisms of innate immunity work against parasites protozoan and helminthic parasites. However, many parasites are able to adopt themselves so as to combat the nonspecific resistance offered by the host and begins to replicate. The adaptation for survival varies from parasite to parasite.

The primary innate immune response to protozoa is phagocytosis by macrophage, but many of them are unique in the sense they can replicate within the macrophages and evade phagocytosis. Some protozoans have membrane receptors which makes them to be recognized by T lymphocytic cell receptors (TLRs). TLRs are responsible for activating phagocytosis. Many of the parasites (like *Plasmodium* species that cause malaria, *Toxoplasma gondii* that result in toxoplasmosis and *Cryptosporidium* species that is responsible for causing diarrhea) express glycosyl phosphatidylinositol lipids on their membrane which can activate TLR 2 and TLR 4 present on T cells. A few helminthic parasites are too large to be phagocytosed however they activate phagocytic cells. These phagocytic cells secrete microbicidal substances to kill organisms which are too large to be phagocytosed. However, many helminthic parasites possess a very thick tegument that makes them resistant to cytotoxic substances released by neutrophils and macrophages.

Some of the parasites which spend intermediate stage of their life cycle in invertebrate host can activate alternative pathway of complement and get lysed by membrane attack complex. However, those which spend a part of their life in vertebrates resist lysis by complement. The reasons for this resistance can be: (a) the loss of surface proteins responsible for binding of complement proteins and/ or (b) acquisition of host regulatory proteins like decay acceleration factor (DAF).

#### **13.4.2.2 Adaptive immune responses**

Specific immunity depends upon various structural and biochemical properties along with the mechanism of pathogenesis executed by the pathogen. It is an established fact that parasites like protozoa and helminths vary greatly in their life cycle, structural and biochemical properties and pathogenic mechanism. Some of the parasites, like pathogenic protozoa live within the cells as intracellular parasites while some others like helminthes survive in extracellular spaces. Hence the response of the specific components of immune system also

varies significantly. In general, intracellular parasites are eliminated using cell mediated immunity while others are eliminated by special types of antibody responses (table 7). Specific immune responses to parasites can also result in significant tissue injury. Some parasites and their products induce granulomatous responses with simultaneous fibrosis. Granulomas are generally induced by TH1 responses against persistent antigens (figure 15).

**(a) Defense mechanism against protozoa (in general)**

The general mechanism of defense against protozoa is mediated by activated T<sub>H</sub>2 cells. The activated TH2 cell produces IgE antibodies which cause to activation of eosinophils and mast cells. Parasites like helminths presented by specific APCs stimulate T cells to get differentiated into TH2 subset which on activation releases IL-4 and IL-5. IL-4 stimulates production of IgE which has affinity to bind with Fc receptor of eosinophils and mast cells. As a result eosinophils and mast cells get activated. IL-4 also contributes to the expulsion of many nematodes, however the mechanism is not always IgE dependent. IL-4 is also known to assist in defense mechanism by enhancing the muscular movement's especially peristaltic movements etc. IL-5 released here is known for the development and activation of eosinophils and mast cells. Binding of IgE (along with antigen) to mast cells and eosinophils results in cross linking of Fc receptors. Receptor cross linking stimulates these cells to degranulate and release vasoactive substances, which in turn, can kill large diversity of pathogens. This could be considered as special type of antibody dependent cell mediated cytotoxicity (ADCC) in which IgE antibodies binds to surface of helminthes followed by attachment to eosinophils.

<b>Parasite</b>	<b>Diseases</b>	<b>Principal Mechanisms of Protective Immunity</b>
<b>Protozoa</b>		
<i>Plasmodium</i> species	Malaria	Antibodies and CD8 <sup>+</sup> CTLs
<i>Leishmania donovani</i>	Leishmaniasis (mucocutaneous dissemination )	CD4 <sup>+</sup> TH1 cells activate macrophages to kill phagocytosed parasites
<i>Trypanosoma brucei</i>	African trypanosomiasis	Antibodies

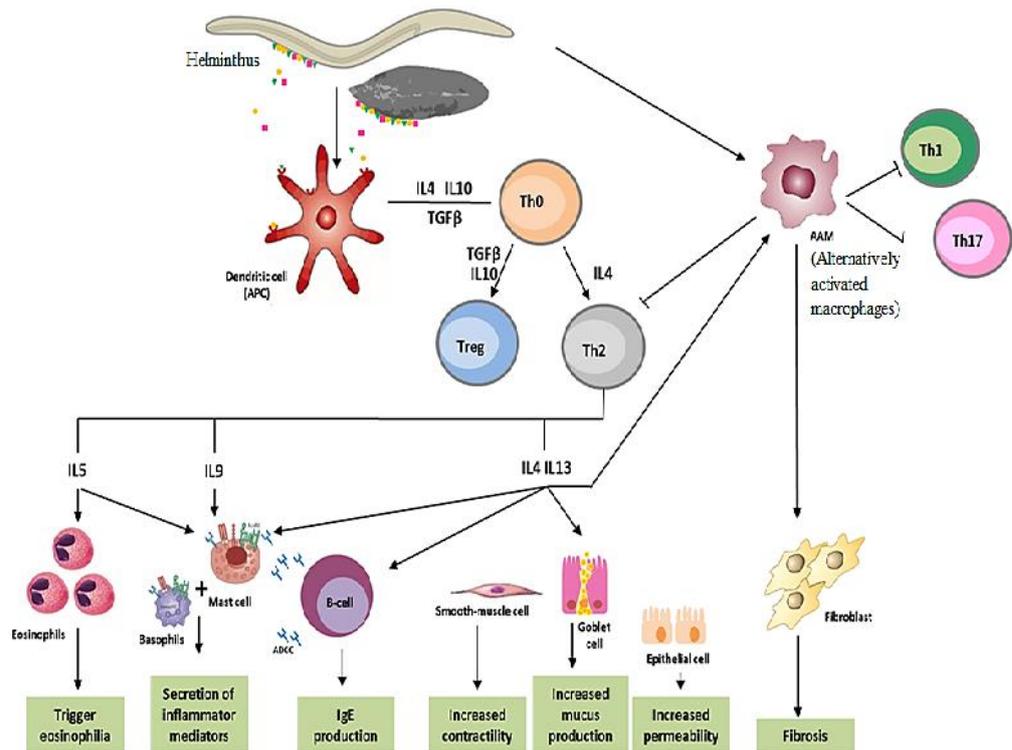
<i>Entamoeba histolytica</i>	Amoebiasis	Antibodies, phagocytosis
<b>Metazoa</b>		
<i>Schistosoma</i> species	Schistosomiasis	Killing by eosinophils, and macrophages
Filaria, e.g., <i>Wuchereria bancrofti</i>	Filariasis	Cell-mediated immunity; role of antibodies.

**Table 7: Immune Responses to Disease-Causing Parasites**

The joint efforts of mast cells and eosinophils ultimately results in removal of parasites from different body parts including the intestine (barrier immunity). The secretion of many basic proteins, which are more toxic for helminthes as compared to proteolytic enzymes and reactive oxygen species produced by many leukocytes like neutrophils and macrophages, make eosinophils more effective for killing helminthes (figure 15).

***(b) Defense mechanism against protozoa that live within macrophages***

Such protozoa that live within the macrophages execute cell mediated immunity wherein macrophages are activated by cytokines released by CD4<sup>+</sup> T cells. It is the dominance of cascade of cytokines released by subsets of activated T<sub>H</sub> cells (T<sub>H</sub>1 or T<sub>H</sub>2) which is responsible for the fate of parasites (resistance or susceptibility) inside the body. In principle, the activation of specific T<sub>H</sub>1 subset of T cells results in resistance against protozoan parasites like *Leishmania major* that survives within the macrophages. These strains cause *Leishmania* specific T<sub>H</sub>1 cells to produce IFN $\gamma$ . IFN $\gamma$  in turn results in the activation of macrophages that are capable of destroying intracellular parasites. Activation of T<sub>H</sub>2 cells, on the other hand, results in better survival of the parasite. The strains that promote *leishmaniasis* causes T<sub>H</sub>2 cells to releases many cytokines (like IL-4). The released cytokines are antagonistic to the activation and functioning of macrophages.



**Figure 15: Immune response against helminths**

The control and balance of immune responses against intracellular parasites (defensive and destructive) is managed by a complex set of several genes. The identification and detailed mechanism of these genes is still not known. CTL responses are generally mounted against protozoa that replicate inside the host cells and lyse them. In addition, these cells also stimulate specific antibodies. One such parasite is malarial parasite *Plasmodium* which lives inside red blood cells and hepatocytic cells. CTL response is the chief mechanism of defense against the spread of infection by this protozoan. In many cases, IFN- $\gamma$  plays a crucial protective role against many protozoan infections including malaria, toxoplasmosis and cryptosporidiosis.

#### 13.4.2.3 Immune evasion by parasites

Many parasites have unique ability to evade the immune system by one way or another. Many mechanisms executed by parasites for combatting the immunogenicity have been worked upon (table 8):

Mechanism of Immune Evasion	Examples
Antigenic variation	Trypanosomes, <i>Plasmodium</i>

Acquired resistance to complement, CTLs	Schistosomes
Inhibition of host immune responses	Filaria (secondary to lymphatic obstruction), trypanosomes
Antigen shedding	Entamoeba

Table 8: Mechanisms of Immune Evasion by Parasites

- a) ***Protozoa show anatomic sequestrations.*** Some protozoa survive and replicate inside the cell, while some others develop immunologically resistant cysts. Some others like helminthes reside in lumen of the intestine where they are protected from cell mediated immunity.
- b) ***Parasites mask their surface with a coat of host proteins.*** Many times surface of parasites (and their larvae) gets coated with ABO blood group glycolipids, MHC molecules and some other host molecules. This coating masks the parasitic antigens present on their surface. In this situation the organism is seen as self by the host immune system and thus no immune response is mounted. For example, larvae of schistosome which enters in the body through skin, invade the lungs before coming to the circulation and during this transport many host proteins get attached to its surface.
- c) ***Inside the vertebrate hosts, parasites adopt themselves to resist immune effector mechanism.*** During their stay in the vertebrate, many parasites develop strategies to combat the adverse effects of host immune reactions. Sometimes they develop tough teguments which are resistant to the damage by complement, CTL cells and antibodies. This tegument brings biochemical change in the surface coat. Secondly some of the parasites produce membrane glycoproteins which can inhibit complement activation. These factors mimics decay acceleration factors. Sometimes these glycoproteins can induce breakdown of membrane attack complexes limiting the success of complement mediated lysis.
- d) Some of the parasites are known to escape killing by macrophages. They employ various strategies. *Toxoplasma gondii* inhibits phagolysosome fusion while *T. cruzi* is known to lyse the membrane of the phagosomes. Some of the parasites have been reported to produce ectoenzymes that can cleave bound antibodies. In such cases, parasites become resistant to

antibody dependent effector mechanisms.

- e) ***Parasites can shed their antigenic coat.*** Ones inside the host, parasites like *Entamoeba histolytica*, *Trypanosomonas* spp. can shed their active membrane either spontaneously or after binding to specific antibodies. This results in loss of surface antigens and confers resistance to host defense mechanisms.
- f) ***Parasites vary their surface antigens during their life cycle in vertebrate host.*** Generally two forms of antigenic variation are well defined. The first change in antigen expression is specific to growth stage. It is such that the parasite produces different form of antigens in mature and infective stages. For example, the merozoite stage of malarial parasite, which is responsible for chronic infection, is antigenically distinct from infective sporozoite stage. Initially, immune system responds to antigens in sporozoite stage, but by the time it activates and become functional, the parasite differentiates into merozoite stage which has a different set of antigens. Thus the parasite evades from the host defense mechanism.

In the second way, parasite like *Trypanosoma brucei* and *Trypanosoma rhodesiense* show unremitting variation of major surface antigens. This is due to preset variation in expression of the genes coding for the major surface antigen. Thus, by the time the host mounts immune response against a parasite, an antigenically different form of organism comes out.

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### 13.5 Summary

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Immune system is defense mechanism which works to protect us from the ill effects of invading pathogens. A specific surveillance mechanism allows the immune cells to differentiate between the self and non-self-antigens. As a result, immune system can identify the antigens of foreign origin only. This mechanism is called tolerance. Tolerance generally works at two levels- central and peripheral. The central tolerance remains associated with primary lymphoid organs while peripheral tolerance is associated with peripheral lymphoid organs.

At times an exaggerated immune response against certain antigens is seen i.e. immune system becomes violent and results in damage of innocent body cells. This response is called hypersensitivity. Depending upon the mechanism of action, the reactions of hypersensitivity are of five major types viz type I, II, III, IV and V. Type I reactions are mediated by IgE antibody, type II reactions are

manifested by either IgG or IgM antibodies while type III are mediated by Ag-Ab complexes. Type IV is a cell mediated reaction. Type V is a stimulatory reaction which stimulate (at times obstructs also) functionality of endocrine receptors. Just like type II hypersensitivity, the type V reactions could be manifested by IgM or IgG.

Immune system is unique in the types of interactions it manifests against the pathogen colonization. In fact a number of strategies of antagonistic nature are exhibited by both – the immune system and the pathogen. Immune system works to reduce the ill effects of the invading pathogen while pathogen works (for the opposite goal) to remain in the host tissue.

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## 13.6 Glossary

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- **AIRE:** A protein present in primary lymphoid organs that regulates expression of tissue specific antigens in the thymus and play a very important role in T cell central tolerance. It is expressed by a subset of medullary epithelial cells.
- **Allergen:** an allergen is an antigen that induces an inappropriate exaggerated immune response in contrast to a classic response produced by the recipient host in response to most immunogens.
- **Anaphylaxis:** Anaphylaxis stands for “anti or opposite to protection” and is defined as extravagant reaction of an organism to a foreign substance to which it has previously become sensitized resulting from the release of histamine, serotonin and other vasoactive substances. It is an immediate type I hypersensitivity reaction, which is triggered by IgE-mediated mast cell degranulation.
- **Arthus reaction:** A localized experimental immune complex-mediated vasculitis reaction induced by injection of an antigen subcutaneously into a previously immunized animal or into an animal that has been given intravenous antibodies specific for the antigen. Circulating antibodies bind to the injected antigen and form immune complexes at the walls of small arteries at the site of injection and give rise to a local cutaneous vasculitis reaction with necrosis.
- **Asthma:** Asthma is a partially reversible type I hypersensitivity disease of airway which results due to obstruction to air flow owing to the compression of neighboring smooth muscle, overdeveloped mucus glands,

inflammation and bronchoconstriction.

- **Autoimmunity:** An abnormal immune response against self-antigens. It results by the breakdown of self- tolerance mechanism.
- **Central tolerance:** A form of self- tolerance which leads to elimination of self-reactive lymphocytes from primary generative organs such as the bone marrow and the thymus.
- **Delayed type of hypersensitivity:** A type IV hypersensitive response mediated by sensitized TH cells, which release various cytokines and chemokines. It is a type of immune reaction in which T cell–dependent macrophage activation and inflammation cause tissue injury.
- **Double-positive (DP) thymocyte:** A subset of developing T cells in the thymus (thymocytes) that express both CD4 and CD8 receptors and are at an intermediate developmental stage.
- **Erythroblastosis fetalis:** Type II hypersensitivity reaction in which maternal antibodies against fetal Rh antigens cause hemolysis of the erythrocytes of a newborn.
- **Hypersensitivity:** It is an exaggerated immune response disorder that causes damage to the individual. It can also be defined as a phenomenon where in immune system goes undesirably wild and causes injury to the individual. It may be damaging, uncomfortable and occasionally fatal.
- **Immunological tolerance:** Unresponsiveness of the adaptive immune system to self-antigens.
- **Peripheral tolerance:** Process by which self-reactive lymphocytes in the circulation (or peripheral secondary organs) are eliminated, rendered anergic, or otherwise inhibited from inducing an immune response.
- **Plasmapheresis:** A technique which is generally done during pregnancy when the Rh<sup>-</sup> mother makes anti-Rh antibodies that react with the blood cells of the Rh<sup>+</sup> fetus. This procedure involves separation of blood into two components - plasma and cells. The plasma is removed and cells are returned to the individual.
- **Rhogam antibodies:** Antibody against Rh antigen that is used to prevent erythroblastosis fetalis.

- **Serum sickness:** It is a type III hypersensitivity reaction that develops when antigen is administered intravenously, resulting in the formation of large amounts of antigen-antibody complexes and their deposition in tissues.
- **Tolerogens:** Antigens that induce tolerance rather than immune reactivity.
- **Tuberculin reaction:** A test that is used to determine whether the patient has previously been infected with *Mycobacterium tuberculosis*, the causal agent of tuberculosis.

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### 13 .7 Self-Learning Exercise

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#### Section -A (Very Short Answer Type)

1. Define tolerogen.
2. Which organ remains associated with B cell tolerance?
3. Why is receptor editing done?
4. What is AIRE?
5. Define systemic hypersensitivity reaction.
6. Which antibody is called as regain antibody?
7. Name any two secondary mediators of type I hypersensitivity.
8. Who discovered Arthus reaction?
9. Name any two type II hypersensitivity reactions.
10. According to Gel and Coomb, which hypersensitivity reaction is delayed type hypersensitivity?

#### Section -B (Short Answer Type)

1. Write short note on T cell tolerance.
2. Describe different classification criteria of hypersensitivity.
3. Write short note on primary mediators of type I hypersensitivity.
4. Explain: a) Rhogam antibodies and b) Serum sickness.
5. Write a short note on general features of immune response against microbes.

#### Section - C (Long Answer Type)

1. Write an explanatory note on tolerance.

2. Discuss detailed mechanism of type I hypersensitivity.
3. How is type III hypersensitivity different from type IV hypersensitivity?
4. Write short notes on:
  - a) Type V hypersensitivity
  - b) Role of innate immunity against microbial invasion
5. Write short notes on:
  - a) Adaptive immune response against parasites.
  - b) Immune evasion by parasites.

### **Answer Key of Section-A**

1. Antigen that induces tolerance is called as tolerogen.
2. Bone marrow.
3. Receptor editing is done to replace the self-reactive receptors on B cell with a new copy of normal receptors.
4. It is a unique protein expressed in epithelial cells that regulates expression of tissue specific antigen in thymus.
5. Hypersensitivity reactions which manifests over a large portion of the body or all over the body.
6. IgE.
7. Prostaglandin and Leukotrienes.
8. Nicolas Maurice Arthus (1903).
9. Erythroblastosis Fetalis, Blood Transfusion Reactions, Rheumatic Fever etc.
10. Type IV.

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## Unit-14

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# Techniques based on antigen-antibody interaction

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### Structure of Unit

- 14.0 Objective
- 14.1 Introduction
- 14.2 Precipitation Reactions
  - 14.2.1 Quantitative precipitin curve
  - 14.2.2 Precipitation methods in gel
  - 14.2.3 Immuno-electrophoresis
- 14.3 Agglutination Reactions
- 14.4 Radioimmunoassay
- 14.5 Enzyme-linked immunosorbent assay (ELISA)
- 14.6 Chemiluminescence
- 14.7 ELISpot Assay
- 14.8 Western Blotting
- 14.9 Immunofluorescence
- 14.10 Flow Cytometry
- 14.11 Summary
- 14.12 Glossary
- 14.13 Self Learning Exercises
- 14.14 References

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### 14.0 Objectives

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After going through this unit you will be able to understand:

- What is antigen-antibody interaction
- Which techniques are based on antigen-antibody interaction
- What is precipitation, Immuno-electrophoresis
- What is RIA, ELISA?

- What is Immunofluorescence, Western blotting, Flow cytometry?

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## 14.1 Introduction

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Antigens are macromolecules of natural or synthetic origin, chemically they consist of various polymers like proteins, polypeptides, polysaccharides or nucleoproteins. Antigens display two essential properties:

- 1.) They are able to evoke a specific immune response, either cellular or humoral type.
- 2.) They specifically interact with products of this immune response, i.e. antibodies or immunocompetent cells.

Antibodies are produced by plasma cells that result from differentiation of B lymphocytes following stimulation with antigen. Antibodies are heterogeneous group of animal glycoproteins and are also called immunoglobulins (Ig).

Immunochemical techniques are based on a reaction of antigen with antibody. The antigen antibody interaction is a bimolecular association similar to an enzyme-substrate interaction, with an important difference that it does not lead to an irreversible chemical alteration in either the antibody or the antigen. The association between an antibody and an antigen involves various non-covalent interactions between the antigenic determinant, or epitope, of the antigen and the variable-region (VH/VL) domain of the antibody molecule, particularly the hypervariable regions, or complementarity -determining regions (CDRs). The specificity of antigen-antibody interactions has led to the development of a variety of immunologic assays, which can be used to detect the presence of either antibody or antigen. Immunoassays have played vital roles in diagnosing diseases, monitoring the level of the humoral immune response, and identifying molecules of biological or medical interest. These assays differ in their speed and sensitivity. Some are strictly qualitative, others are quantitative.

The antibodies used in these reactions are of two types and are produced by various ways.

1. **Monoclonal antibodies:** These are products of a single clone of plasma cells derived from B-lymphocytes, prepared in the laboratory by hybridoma technology, based on cellular fusion of tumour (myeloma) cells with splenic lymphocytes of immunised mice. Monoclonal antibodies are directed against single epitope; and are all identical copies of immunoglobulin molecule with the same primary structure and

specificity of antigen binding site. They typically display excellent specificity, but poor ability to precipitate antigen.

2. **Polyclonal antibodies:** These are conventional antibodies, and are prepared by immunisation of animals (rabbits, goats, sheep) with the antigen. Blood serum of the immunised animal that contains antibodies against the antigen used, is called an antiserum. If one antigen (e.g. one protein) is used for immunisation, monospecific antibodies (antiserum) result.

The noncovalent interactions that form the basis of antigen antibody (Ag-Ab) binding include hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions. Because these interactions are individually weak (compared with a covalent bond), a large number of such interactions are required to form a strong Ag-Ab interaction. Furthermore, each of these noncovalent interactions operates over a very short distance, generally about

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10<sup>-7</sup> mm (1 angstrom, Å); consequently, a strong Ag-Ab interaction depends on a very close fit between the antigen and antibody. Such fits require a high degree of complementarity between antigen and antibody, a requirement that underlies the exquisite specificity that characterizes antigen-antibody interactions.

Although Ag-Ab reactions are highly specific, in some cases antibody elicited by one antigen can cross-react with an unrelated antigen. Such cross-reactivity occurs if two different antigens share an identical or very similar epitope. In the latter case, the antibody's affinity for the cross-reacting epitope is usually less than that for the original epitope.

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## 14.2 Precipitation Reactions

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Antibody and soluble antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate. Antibodies that aggregate soluble antigens are called precipitins. Although formation of the soluble Ag-Ab complex occurs within minutes, formation of the visible precipitate occurs more slowly.

Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen:

The antibody must be bivalent, a precipitate will not form with monovalent Fab fragments.

The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope, or have different epitopes that react with different antibodies present in polyclonal antisera.

#### 14.2.1 Quantitative precipitin curve:

When precipitation reaction occurs in fluid it results formation of precipitin curve. The measure of the strength of the binding of antigen to antibody is called affinity, and it is usually expressed in terms of the concentration of an antibody-antigen complex measured at equilibrium. It is measured by quantitative precipitin curve (basis for many immunochemical techniques) proposed by Heidelberger and Kendall in 1935.

Quantitative precipitin curve describes the relationship between the antigen concentration and the amount of precipitate for a constant quantity of an antibody. Three zones can be distinguished from the precipitin curve: (Fig.1)

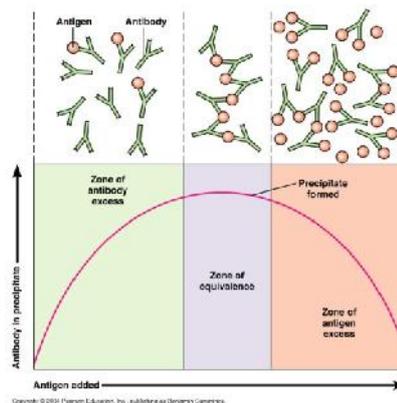


Fig. 1 Quantitative Precipitin Curve

Three zones can be distinguished on the precipitin curve:

**The antibody excess zone:** The amount of precipitate increases proportionally as the concentration of antigen increases. If the antibody is present in an excess, all the antigen binding sites are covered with the antibody and only small soluble antigen-antibody complexes are formed. No free antigen is detected in the supernatant, but free (unbound) antibodies can be found. Such conditions are useful for *immunoturbidimetry*, *immunonephelometry* and *non-competitive immunoassays*.

**The equivalence zone:** Molecules of antigen and antibody are cross-linked forming large, insoluble complexes. The complexes further aggregate and

precipitate. Neither free antigen nor free antibody can be detected in the supernatant. Equivalence is reached in *immunodiffusion techniques*.

**The antigen excess zone:** The amount of precipitate decreases due to the high antigen concentration. Large aggregated immunocomplexes decay. As all the antibody sites are saturated by antigen, small soluble complexes prevail. No free antibody but an increasing amount of free antigen may be found in the liquid phase. Excess of free antigen is required for *competitive immunoassays*.

The precipitin curve forms the basis of most of the immunochemical techniques that can be performed in clinical laboratories. When precipitation reaction is performed in gel it yields visible precipitin lines.

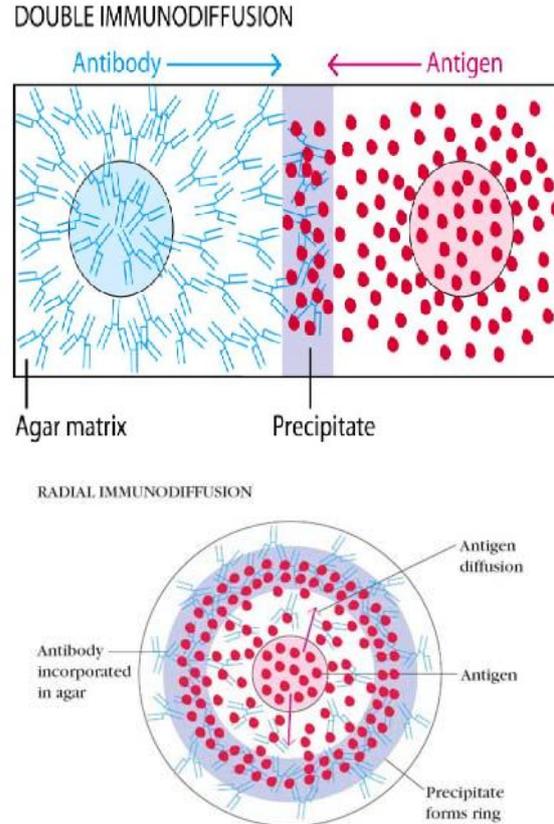
#### **14.2.2 Precipitation methods in gel:**

Two types of immunodiffusion reactions can be used to determine relative concentrations of antibodies or antigens in gel, to compare antigens, or to determine the relative purity of an antigen preparation. They are:

1. Radial immunodiffusion (Mancini method)
2. Double immunodiffusion (Ouchterlony method)

Both are carried out in a semisolid medium such as agar.

In radial immunodiffusion, only one component (i.e. antigen or antibody) diffuses from the place of sample application, while the other reaction partner is dispersed evenly in the gel. If both components of the immunochemical reaction diffuse in the gel against each other from places of their application, the technique is called double immunodiffusion. In the area of antigen antibody reaction a precipitation zone appears as a line, crescent or circle.



### 14.2.3 Immuno-electrophoresis:

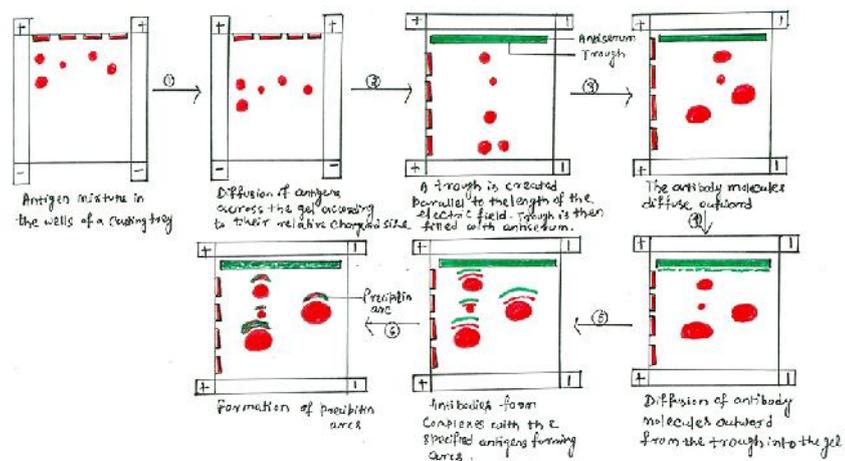
In immuno-electrophoresis, the antigen mixture is first electrophoresed to separate its components by charge. Troughs are then cut into the agar gel parallel to the direction of the electric field, and antiserum is added to the troughs. Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropriate proportions. Immuno-electrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum. A sample of serum is electrophoresed, and the individual serum components are identified with antisera specific for a given protein or immunoglobulin class. This technique is useful in determining whether a patient produces abnormally low amounts of one or more isotypes, characteristic of certain immunodeficiency diseases. It can also show whether a patient overproduces some serum protein, such as albumin, immunoglobulin, or transferrin. The immuno-electrophoretic pattern of serum from patients with multiple myeloma, for example, shows a heavy distorted arc caused by the large amount of myeloma protein, which is monoclonal Ig and therefore uniformly charge. Because immuno-electrophoresis is a strictly qualitative technique that only detects relatively high antibody, its utility is limited to the

detection of quantitative abnormalities only when the departure from normal is striking, as in immunodeficiency states and immunoproliferative disorders.

A related quantitative technique, rocket electrophoresis, does permit measurement of antigen levels. In rocket electrophoresis, a negatively charged antigen is electrophoresed in a gel containing antibody. The precipitate formed between antigen and antibody has the shape of a

rocket, the height of which is proportional to the concentration of antigen in the well. One limitation of rocket electrophoresis is the need for the antigen to be negatively charged for electrophoretic movement within the agar matrix.

Figure:3 Technique of Immunelectrophoresis



### 14.3 Agglutination Reactions

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Agglutination reactions are similar in principle to precipitation reactions; they depend on the crosslinking of polyvalent antigens. Just as an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions; this inhibition is called the prozone effect. Because prozone effects can be encountered in many types of immunoassays, understanding the basis of this phenomenon is of general importance. Bacterial agglutination is used to diagnose infection, Hemagglutination is used in blood typing. Antibody can also cross-link cells or beads. Cross-linking of red cells is called hemagglutination. Non-cross-linked cells settle in a bead to the bottom of the well. Cross-linked cells settle in a diffuse pattern. Which is used to measure antibody presence and level (titre). Used to measure antibodies to red cell antigens or to other antigens bound to the surface of red cells.

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## 14.4. Radioimmunoassay

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One of the most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA). The technique was first developed in 1960 by two endocrinologists, S. A. Berson and Rosalyn Yalow. The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody.

The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts. The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites. The decrease in the amount of radiolabeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample.

The antigen is generally labeled with a gamma-emitting isotope such as  $^{125}\text{I}$ , but beta-emitting isotopes such as tritium ( $^3\text{H}$ ) are also routinely used as labels. The radiolabeled antigen is part of the assay mixture; the test sample may be a complex mixture, such as serum or other body fluids, that contains the unlabeled antigen. The first step in setting up an RIA is to determine the amount of antibody needed to bind 50%–70% of a fixed quantity of radioactive antigen ( $\text{Ag}^*$ ) in the assay mixture. This ratio of antibody to  $\text{Ag}^*$  is chosen to ensure that the number of epitopes presented by the labeled antigen always exceeds the total number of antibody binding sites. Consequently, unlabeled antigen added to the sample mixture will compete with radiolabeled antigen for the limited supply of antibody. Even a small amount of unlabeled antigen added to the assay mixture of labeled antigen and antibody will cause a decrease in the amount of radioactive antigen bound, and this decrease will be proportional to the amount of unlabeled antigen added.

To determine the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen (antigen not bound to Ab), and the radioactivity in the precipitate is measured. A standard curve can be generated using unlabeled antigen samples of known concentration (in place of the test sample), and from this plot the amount of antigen in the test mixture may be precisely determined.

Various solid-phase RIAs have been developed that make it easier to separate the Ag-Ab complex from the unbound antigen. In some cases, the antibody is covalently crosslinked to Sepharose beads. The amount of radiolabeled antigen bound to the beads can be measured after the beads have been centrifuged and washed. Alternatively, the antibody can be immobilized on polystyrene or polyvinylchloride wells and the amount of free labeled antigen in the supernatant can be determined in a radiation counter. In another approach, the antibody is immobilized on the walls of microtiter wells and the amount of bound antigen determined. RIA screening of donor blood has sharply reduced the incidence of hepatitis B infections in recipients of blood transfusions.

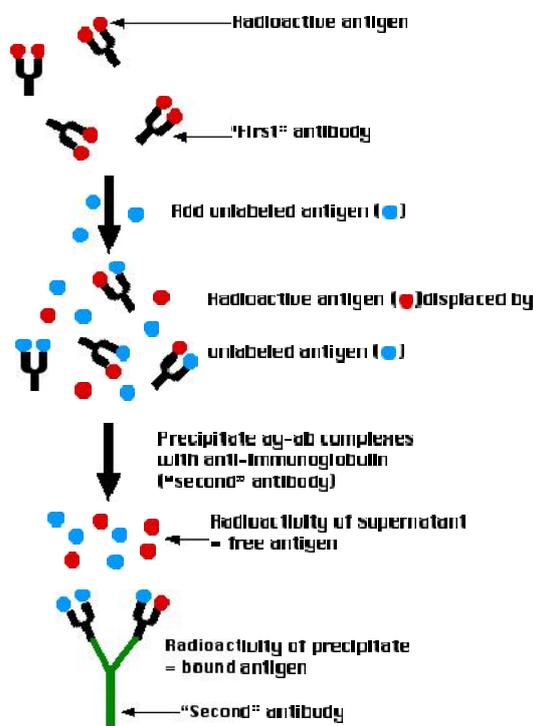


Figure:4 The technique of Radioimmuno Assay

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## 14.5 Enzyme-linked immunosorbent assay (ELISA)

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Enzyme-linked immunosorbent assay, commonly known as ELISA (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called a chromogenic substrate. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and -galactosidase. These assays

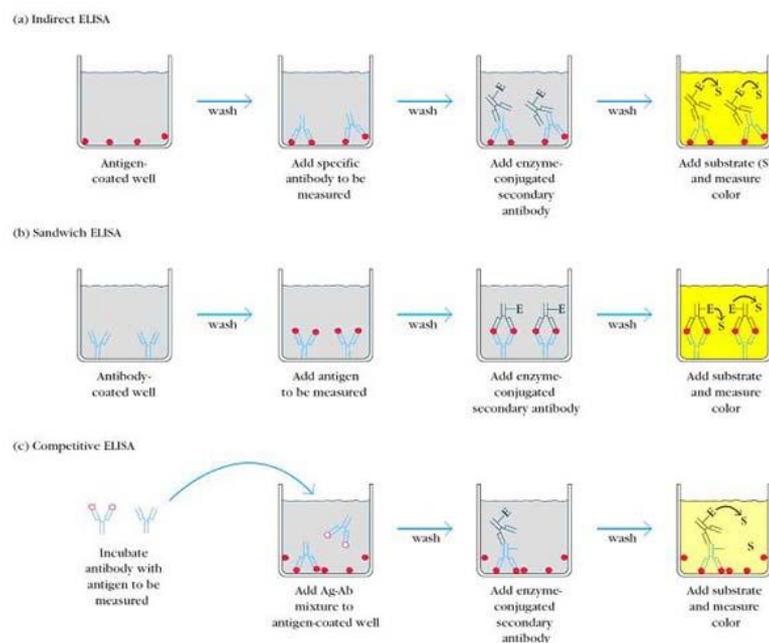
approach the sensitivity of RIAs and have the advantage of being safer and less costly.

Types of ELISA:

A number of variations of ELISA have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody. Each type of ELISA can be used qualitatively to detect the presence of antibody or antigen. Alternatively, a standard curve based on known concentrations of antibody or antigen is prepared, from which the unknown concentration of a sample can be determined.

1. Indirect ELISA: Antibody can be detected or quantitatively determined with an indirect ELISA. Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds.

Figure:5 Various types of ELISA



## 2. Sandwich ELISA:

Antigen can be detected or measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

### 3. Competitive ELISA:

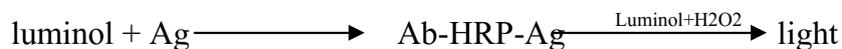
Another variation for measuring amounts of antigen is competitive ELISA. In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen coated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.

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## 14.6 Chemiluminescence

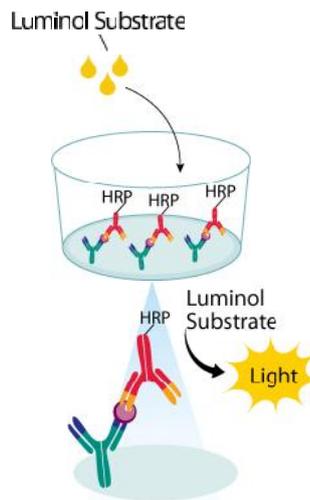
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Measurement of light produced by chemiluminescence during certain chemical reactions provides a convenient and highly sensitive alternative to absorbance measurements in ELISA assays. In versions of the ELISA using chemiluminescence, a luxogenic (light-generating) substrate takes the place of the chromogenic substrate in conventional ELISA reactions. For example, oxidation of the compound luminol by  $H_2O_2$  and the enzyme horseradish peroxidase (HRP) produces light:



The advantage of chemiluminescence assays over chromogenic ones is enhanced sensitivity. In general, the detection limit can be increased at least ten-fold by switching from a chromogenic to a luxogenic substrate, and with the addition of enhancing agents, more than 200-fold.

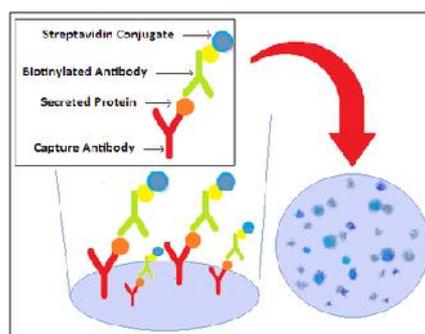
Figure:6 Chemiluminescence



## 14.7 ELISPOT Assay

A modification of the ELISA assay called the ELISPOT assay allows the quantitative determination of the number of cells in a population that are producing antibodies specific for a given antigen or an antigen for which one has a specific antibody. In this approach, the plates are coated with the antigen (capture antigen) recognized by the antibody of interest or with the antibody (capture antibody) specific for the antigen whose production is being assayed. A suspension of the cell population under investigation is then added to the coated plates and incubated. The cells settle onto the surface of the plate, and secreted molecules reactive with the capture molecules are bound by the capture molecules in the vicinity of the secreting cells, producing a ring of antigen-antibody complexes around each cell that is producing the molecule of interest. The plate is then washed and an enzyme-linked antibody specific for the secreted antigen or specific for the species (e.g., goat anti-rabbit) of the secreted antibody is added and allowed to bind. Subsequent development of the assay by addition of a suitable chromogenic or chemiluminescence-producing substrate reveals the position of each antibody- or antigen-producing cell as a point of color or light.

Figure:7 ELISpot Assay



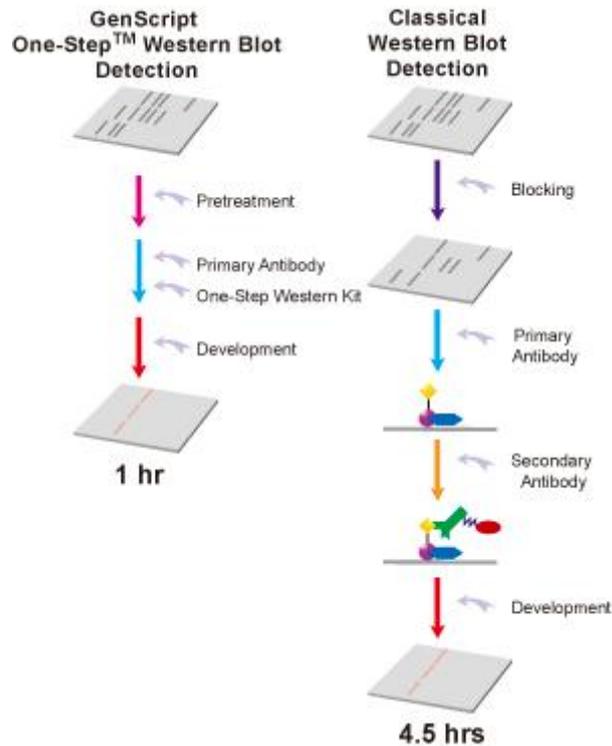
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## 14.7 Western Blotting

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Identification of a specific protein in a complex mixture of proteins can be accomplished by a technique known as Western blotting, named for its similarity to Southern blotting, which detects DNA fragments, and Northern blotting, which detects mRNAs. In Western blotting, a protein mixture is electrophoretically separated on an SDS-polyacrylamide gel (SDS-PAGE), a slab gel infused with sodium dodecyl sulfate (SDS), a dissociating agent. The protein bands are transferred to a nylon membrane by electrophoresis and the individual protein bands are identified by flooding the nitrocellulose membrane with radiolabeled or enzyme-linked polyclonal or monoclonal antibody specific for the protein of interest. The Ag-Ab complexes that form on the band containing the protein recognized by the antibody can be visualized in a variety of ways. If the protein of interest was bound by a radioactive antibody, its position on the blot can be determined by exposing the membrane to a sheet of x-ray film, a procedure called autoradiography. However, the most generally used detection procedures employ enzyme-linked antibodies against the protein. After binding of the enzyme-antibody conjugate, addition of a chromogenic substrate that produces a highly colored and insoluble product causes the appearance of a colored band at the site of the target antigen. The site of the protein of interest can be determined with much higher sensitivity if a chemiluminescent compound along with suitable enhancing agents is used to produce light at the antigen site. Western blotting can also identify a specific antibody in a mixture. In this case, known antigens of well-defined molecular weight are separated by SDS-PAGE and blotted onto nitrocellulose. The separated bands of known antigens are then probed with the sample suspected of containing antibody specific for one or more of these antigens. Reaction of an antibody with a band is detected by using either radiolabeled or enzyme-linked secondary antibody that is specific for the species of the antibodies in the test sample. The most widely used application of this procedure is in confirmatory testing for HIV, where Western blotting is used to determine whether the patient has antibodies that react with one or more viral proteins.

**Figure 8: Western Blotting**



## 14.9 Immunofluorescence

In 1944, Albert Coons showed that antibodies could be labeled with molecules that have the property of fluorescence. Fluorescent molecules absorb light of one wavelength (excitation) and emit light of another wavelength (emission). If antibody molecules are tagged with a fluorescent dye, or fluorochrome, immune complexes containing these fluorescently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wavelength. Antibody molecules bound to antigens in cells or tissue sections can similarly be visualized. The emitted light can be viewed with a fluorescence microscope, which is equipped with a UV light source. In this technique, known as immunofluorescence, fluorescent compounds such as fluorescein and rhodamine are in common use, but other highly fluorescent substances are also routinely used, such as phycoerythrin, an intensely colored and highly fluorescent pigment obtained from algae. These molecules can be conjugated to the Fc region of an antibody molecule without affecting the specificity of the antibody. Each of the fluorochromes absorbs light at one wavelength and emits light at a longer wavelength, some common fluorochromes are:

- (a) Fluorescein: It is an organic dye that is the most widely used label for immunofluorescence procedures, absorbs blue light (490 nm) and emits an intense yellow-green fluorescence (517 nm).

- (b) Rhodamine: It is also an organic dye, absorbs in the yellow-green range (515 nm) and emits a deep red fluorescence (546 nm). Because it emits fluorescence at a longer wavelength than fluorescein, it can be used in two-color immunofluorescence assays. An antibody specific to one determinant is labeled with fluorescein, and an antibody recognizing a different antigen is labeled with rhodamine. The location of the fluorescein-tagged antibody will be visible by its yellow-green color, easy to distinguish from the red color emitted where the rhodamine-tagged antibody has bound. By conjugating fluorescein to one antibody and rhodamine to another antibody, one can, for example, visualize simultaneously two different cell-membrane antigens on the same cell.
- (c) Phycoerythrin: It is an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence, stimulating its wide use as a label for immunofluorescence.

Fluorescent-antibody staining of cell membrane molecules or tissue sections can be direct or indirect. In direct staining, the specific antibody (the primary antibody) is directly conjugated with fluorescein; in indirect staining, the primary antibody is unlabeled and is detected with an additional fluorochrome-labeled reagent. A number of reagents have been developed for indirect staining. The most common is a fluorochrome-labeled secondary antibody raised in one species against antibodies of another species, such as fluorescein-labeled goat anti-mouse immunoglobulin. Indirect immunofluorescence staining has two advantages over direct staining: 1. The primary antibody does not need to be conjugated with a fluorochrome. Because the supply of primary antibody is often a limiting factor, indirect methods avoid the loss of antibody that usually occurs during the conjugation reaction.

2. Indirect methods increase the sensitivity of staining because multiple molecules of the fluorochrome reagent bind to each primary antibody molecule, increasing the amount of light emitted at the location of each primary antibody molecule.

A major application of the fluorescent-antibody technique is the localization of antigens in tissue sections or in subcellular compartments. Because it can be used to map the actual location of target antigens, fluorescence microscopy is a powerful tool for relating the molecular architecture of tissues and organs to their overall gross anatomy.

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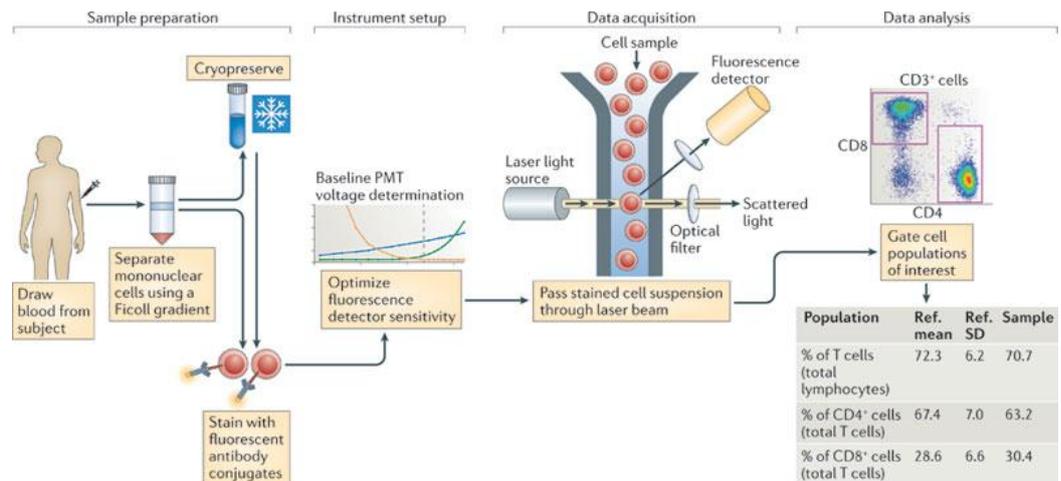
## 14.10 Flow Cytometry

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The fluorescent antibody techniques described are extremely valuable qualitative tools, but they do not give quantitative data. This shortcoming was remedied by development of the flow cytometer, which was designed to automate the analysis and separation of cells stained with fluorescent antibody. The flow cytometer uses a laser beam and light detector to count single intact cells in suspension. Every time a cell passes the laser beam, light is deflected from the detector, and this interruption of the laser signal is recorded. Those cells having a fluorescently tagged antibody bound to their cell surface antigens are excited by the laser and emit light that is recorded by a second detector system located at a right angle to the laser beam. The simplest form of the instrument counts each cell as it passes the laser beam and records the level of fluorescence the cell emits; an attached computer generates plots of the number of cells as the ordinate and their fluorescence intensity as the abscissa. More sophisticated versions of the instrument are capable of sorting populations of cells into different containers according to their fluorescence profile. Use of the instrument to determine which and how many members of a cell population bind fluorescently labeled antibodies is called analysis; use of the instrument to place cells having different patterns of reactivity into different containers is called cell sorting. The flow cytometer has multiple applications to clinical and research problems. A common clinical use is to determine the kind and number of white blood cells in blood samples. By treating appropriately processed blood samples with a fluorescently labeled antibody and performing flow cytometric analysis, one can obtain the information about how many cells express the target antigen as an absolute number and also as a percentage of cells passing the beam, the distribution of cells in a sample population according to antigen densities as determined by fluorescence intensity. It is thus possible to obtain a measure of the distribution of antigen density within the population of cells that possess the antigen. This is a powerful feature of the instrument, since the same type of cell may express different levels of antigen depending upon its developmental or physiological state, the size of cells. This information is derived from analysis of the light-scattering properties of members of the cell population under examination.

Flow cytometry also makes it possible to analyze cell populations that have been labeled with two or even three different fluorescent antibodies. Flow cytometry now occupies a key position in immunology and cell biology, and it has become an indispensable clinical tool as well.

### **Figure 9 Flow cytometry**



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## 14.11 Summary

Antigen-antibody interactions depend on four types of noncovalent interactions, i.e. hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions. The interaction of a soluble antigen and precipitating antibody in a liquid or gel medium forms an Ag-Ab precipitate. Electrophoresis can be combined with precipitation in gels in a technique called immunoelectrophoresis. The interaction between a particulate antigen and agglutinating antibody (agglutinin) produces visible clumping, or agglutination that forms the basis of simple, rapid, and sensitive immunoassays. Radioimmunoassay (RIA) is a highly sensitive and quantitative procedure that utilizes radioactively labeled antigen or antibody. The enzyme-linked immunosorbent assay (ELISA) depends on an enzyme-substrate reaction that generates a colored reaction product. ELISA assays that employ chemiluminescence instead of a chromogenic reaction are the most sensitive immunoassays available. In Western blotting, a protein mixture is separated by electrophoresis; then the protein bands are electrophoretically transferred onto nitrocellulose and identified with labeled antibody or labeled antigen. Flow cytometry provides an unusually powerful technology for the quantitative analysis and sorting of cell populations labeled with one or more fluorescent antibodies.

## 14.12 Glossary

- **ELISA:** Enzyme-linked immunosorbent assay
- **RIA:** Radioimmunoassay

- **Agglutination:** The interaction between a particulate antigen and agglutinating antibody (agglutinin) produces visible clumping.
- **Immunoelectrophoresis:** Electrophoresis can be combined with precipitation in gels in a technique called immunoelectrophoresis.

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### 14.13 Self-Learning Exercise

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#### Section -A (Very Short Answer Type)

1. ELISA is .....
2. RIA is.....
3. In ..... a protein mixture is separated by electrophoresis; then the protein bands are electrophoretically transferred onto nitrocellulose and identified with labeled antibody or labeled antigen.
4. .... provides an unusually powerful technology for the quantitative analysis and sorting of cell populations labeled with one or more fluorescent antibodies.
5. Immunochemical techniques are based on a reaction of.....
6. Electrophoresis can be combined with precipitation in gels in a technique called.....

#### Section -B (Short Answer Type)

1. Define fluorescence and name any 3 fluorescence dyes used in fluorescence assay.
2. What do you understand with precipitation reaction?
3. What do you understand with agglutination reaction?
4. Define ODD and RID.
5. Discuss in brief about Immunoelectrophoresis.

#### Section -C (Long Answer Type)

1. Explain in detail about precipitation curve.
2. Discuss in detail about ELISA.
3. Write short notes on- (a) Flow cytometry (b) RIA
4. Write in detail about role of fluorescence in immunofluorescence.
5. Explain in detail about chemiluminiscence.

#### Answer Key of Section-A

1. Enzyme-linked immunosorbent assay

2. Radioimmunoassay
  3. Western Blotting
  4. Flowcytometry
  5. antigen with antibody
  6. immunoelectrophoresis
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## **14.14 References**

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- Roitt I.M, Brostoff, J., Male D.K. (2001). Immunology (Illustrated Publisher, Mosby)
- T. J. Kindt, R.A. G. B. A. Osborne, J. Kuby (2006). Immunology (W.H. Freeman and Company, New York)

# Unit 15

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## Immunological disease, AIDS, Arthrites, SARS, Mad Cow diseases, Swine Flue

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### Structure of the unit

- 15.1 Objectives
- 15.2 Introduction
- 15.3 AIDS
  - 15.3.1 Signs and symptoms
  - 15.3.2 Transmission of HIV
  - 15.3.3 Causal organism: HIV
  - 15.3.4 HIV mode of action
  - 15.3.5 Diagnosis of HIV infection
  - 15.3.6 Treatment of AIDS
- 15.4 Rheumatoid Arthritis (RA)
  - 15.4.1 Signs and symptoms of RA
  - 15.4.2 Causes of RA
  - 15.4.3 Pathogenicity
  - 15.4.4 Treatment of RA
- 15.5 Severe acute respiratory syndrome (SARS)
  - 15.5.1 Symptoms of SARS
  - 15.5.2 Causal organism
  - 15.5.3 Pathogenicity
  - 15.5.4. Treatment of SARS
- 15.6 variant Creutzfeldt-Jakob Disease (vCJD)
  - 15.6.1 Symptoms of vCJD
  - 15.6.2 Causal agent: prion
  - 15.6.3 Pathogenicity
  - 15.6.4 Treatment of vCJD

## 15.7 Swine Flu

### 15.7.1 Symptoms of swine flu

### 15.7.2 Causal agent: H1N1

### 15.7.3 Pathogenicity

### 15.7.4 Prevention and Treatment of swine flu

## 15.8 Summary

## 15.9 Glossary

## 15.10 References

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# 15.1 Objectives

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After going through this unit you will be able to understand:

- Immunological diseases
- Symptoms, cause and pathogenicity of AIDS
- Symptoms, causes and pathogenicity of rheumatoid arthritis
- Symptoms, causes and pathogenicity of SARS
- Symptoms, causes and pathogenicity of rheumatoid arthritis
- Symptoms, causes and pathogenicity of swine flu

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# 15.2 Introduction

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A disease can be defined as an abnormal, pathogenic condition affecting the sufferer mentally, physically and physiologically. Diseases may be caused by some external source (bacteria, virus) or due to malfunctioning of the body's systems (autoimmune diseases). Humans encounter large number of disease causing factors/ agents in their environment. The immune system is a versatile defense system that functions to protect animals from invading pathogenic microorganisms and cancer. It generates an enormous variety of cells and molecules capable of specifically recognizing and eliminating a variety of foreign invaders. Functionally, an immune response can be divided into two related activities—recognition and response. Immunerecognition is remarkable for its specificity. The immune system is able to recognize subtle chemical differences that distinguish one foreign pathogen from another. Furthermore, the system is able to discriminate between foreign molecules and the body's own cells and proteins. Once a foreign organism has been recognized, the immune system recruits a variety of cells and molecules to mount an

appropriate response, called an effector response, to eliminate or neutralize the organism. In this way the system is able to convert the initial recognition event into a variety of effector responses, each uniquely suited for eliminating a particular type of pathogen. Later exposure to the same foreign organism induces a memory response, characterized by a more rapid and heightened immune reaction that serves to eliminate the pathogen and prevent disease.

An immune disorder/disease is a dysfunction of the immune system. An immunodeficiency disorder makes the body considerably more susceptible to catching viruses and bacterial infections. These disorders can be characterized in several different ways: Primary immune deficiencies are disorders in which part of the body's immune system is missing or does not function normally. A secondary immunodeficiency occurs when the immune system is compromised due to an environmental factor. Examples of these outside forces include HIV, chemotherapy, severe burns or malnutrition. Secondary immune disorders include AIDS, cancers of the immune system, such as leukemia. The following sections describes some human diseases which

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### **15.3 AIDS- Acquired Immuno Deficiency Syndrome**

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AIDS was first reported in the United States in 1981 and has since then become a major worldwide epidemic. AIDS is caused by the human immunodeficiency virus, or HIV. During initial stage of infection, a person experience a brief period of influenza-like illness. This is typically followed by a prolonged period without symptoms. As the infection progresses, it interferes more and more with the immune system, making the person much more susceptible to common infections like tuberculosis, as well as opportunistic infections and tumors that do not usually affect people who have working immune systems. The late symptoms of the infection are referred to as AIDS. This stage is often complicated by an infection of the lung known as pneumocystis pneumonia, severe weight loss, a type of cancer known as Kaposi's sarcoma, or other AIDS-defining conditions.

Since its discovery, AIDS has caused an estimated 36 million deaths worldwide (as of 2012). As of 2012, approximately 35.3 million people are living with HIV globally. HIV/AIDS is considered a pandemic—a disease outbreak which is present over a large area and is actively spreading.

#### **15.3.1 Signs and symptoms**

HIV infection comes in three stages. The first stage is called acute infection and it typically happens within a month or two of HIV entering the body, 40% to 90% of people experience flu like symptoms known as acute retroviral syndrome (ARS). This is the stage when the body's immune system fights with the antigen. The most common symptoms include fever, large tender lymph nodes, throat inflammation, rashes, headache, and/or sores of the mouth and genitals. Some people may also develop opportunistic infections at this stage. Gastrointestinal symptoms such as nausea, vomiting or diarrhea may occur, along with this neurological symptoms may also develop. Due to their nonspecific character, these symptoms are not often recognized as signs of HIV infection.

After the first stage, the immune system loses the battle with HIV and symptoms go away. HIV infection goes into its second stage, which can be a long period without symptoms, called the asymptomatic period or clinical latency. This is when people may not know they are infected and can pass HIV on to others. While typically there are few or no symptoms at first, near the end of this stage many people experience fever, weight loss, gastrointestinal problems and muscle pains. Between 50% to 70% people also develop persistent generalized lymphadenopathy, characterized by unexplained, non-painful enlargement of more than one group of lymph nodes for over three to six months.

### **15.3.2 Transmission of HIV**

Although the precise mechanism by which HIV-1 infects an individual is not known, epidemiological data indicate that common means of transmission include homosexual and heterosexual intercourse, receipt of infected blood or blood products, and passage from mothers to infants.

**Sexual contact:** Sexual contact with an infected person is the most frequent mode of HIV transmission. In the worldwide epidemic, it is estimated that 75% of the cases of HIV transmission are attributable to heterosexual contact.

**Blood or blood products:** Exposure to infected blood accounts for the high incidence of AIDS. Blood-borne transmission can be through needle-sharing during intravenous drug use, needle stick injury, transfusion of contaminated blood or blood product, or medical injections with unsterilised equipment. HIV is transmitted in about 93% of blood transfusions using infected blood. In developed countries the risk of acquiring HIV from a blood transfusion is

extremely low (less than one in half a million) where improved donor selection and HIV screening is performed.

**Mother to child:** Infants born to mothers who are infected with HIV-1 are at high risk of infection, unless infected mothers are treated with anti-viral agents before delivery. Possible vehicles of passage from mother to infant include blood transferred in the birth process and milk in the nursing period.

Transmission from an infected to an uninfected individual is most likely by transmission of HIV-infected cells—in particular, macrophages, dendritic cells, and lymphocytes.

### **15.3.3 Causal organism: HIV**

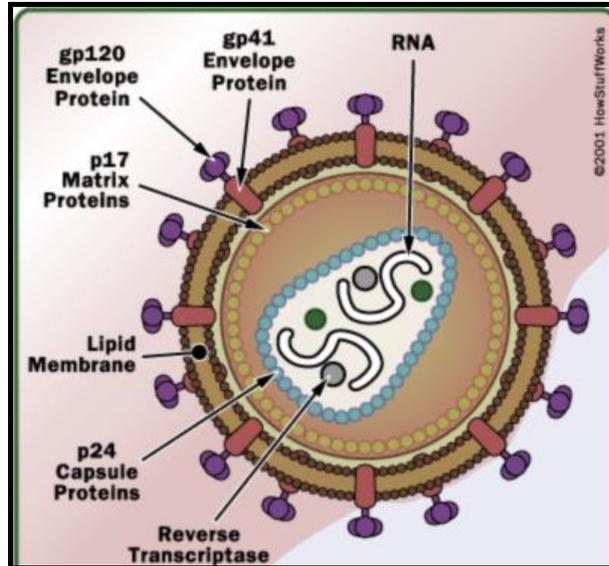
The causal agent of Acquired Immunodeficiency Syndrome (AIDS) was discovered independently by Luc Montagnier of France and Robert Gallo of the US in 1983-84. HIV is a retrovirus and belongs to the family lentivirus. Retroviruses carry their genetic information in the form of RNA. When the virus enters a cell, the RNA is reverse transcribed to DNA by a virally encoded enzyme, reverse transcriptase (RT). As the name implies, RT reverses the normal transcription process and makes a DNA copy of the viral RNA genome. This copy, which is called a provirus, is integrated into the cell genome and is replicated along with the cell DNA. When the provirus is expressed to form new virions, the cell lyses. Alternatively, the provirus may remain latent in the cell until some regulatory signal starts the expression process.

HIV infects mainly the CD4<sup>+</sup> lymphocytes (T cells), but also to a lesser degree monocytes, macrophages, and dendritic cells. Once infected, the cell turns into an HIV-replicating cell and loses its function in the human immune system.

The viral envelope, the outer coat of the virus, consists of two layers of lipids; different proteins are embedded in the viral envelope, forming "spikes" consisting of the outer glycoprotein (gp) 120 and the transmembrane gp41. The lipid membrane is borrowed from the host cell during the budding process (formation of new particles). gp120 is needed to attach to the host cell, and gp41 is critical for the cell fusion process.

The viral core (or capsid) is made from the protein p24. Inside the core are three enzymes required for HIV replication called reverse transcriptase, integrase and protease. Also present within the core is HIV's genetic material, which consists of two identical strands of RNA. The HIV matrix proteins (consisting of the p17 protein), lie between the envelope and core.

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was originally discovered, it is more virulent, more infective and is the cause of the majority of HIV infections globally.



**Fig. Structure of HIV**

#### **15.3.4 HIV mode of action**

The first step in HIV infection is viral attachment and entry into the target cell. HIV-1 infects T cells that carry the CD4 antigen on their surface; in addition, certain HIV strains will infect monocytes and other cells that have CD4 on their surface. The preference for CD4 cells is due to a high-affinity interaction between a coat (envelope or env) protein of HIV-1 and cell-surface CD4. Although the virus binds to CD4 on the cell surface, this interaction alone is not sufficient for entry and productive infection. Expression of other cell-surface molecules, co-receptors present on T cells and monocytes is required for HIV-1 infection.

After the virus has entered the cell, the RNA genome of the virus is reverse transcribed and a cDNA copy (provirus) integrates into the host genome. The integrated provirus is transcribed and the various viral RNA messages spliced and translated into proteins, which along with a complete new copy of the RNA genome are used to form new viral particles. The gag proteins of the virus are cleaved by the viral protease into the forms that make up the nuclear capsid in a mature infectious viral particle. CXCR4 and CCR5 serve as co-receptors for HIV-1 on T cells and macrophages, respectively.

Diagnosis of AIDS includes evidence for infection with HIV-1 (presence of antibodies or virus in blood), greatly diminished numbers of CD4T cells (200 cells/mm<sup>3</sup>), impaired or absent delayed-hypersensitivity reactions, and the occurrence of opportunistic infections. Patients with AIDS generally succumb to tuberculosis, pneumonia, severe wasting diarrhea, or various malignancies. The time between acquisition of the virus and death from the immunodeficiency averages nine to twelve years.

### **15.3.5 Diagnosis of HIV infection**

Tests used for the diagnosis of HIV infection in a particular person require a high degree of both sensitivity and specificity. In the United States, this is achieved using an algorithm combining two tests for HIV antibodies. If antibodies are detected by an initial test based on the ELISA method, then a second test using the Western blot procedure determines the size of the antigens in the test kit binding to the antibodies. The combination of these two methods is highly accurate.

### **15.3.6 Treatment of AIDS**

Till date there is no cure for AIDS, but medications are effective in fighting HIV and its complications. In 1987, a drug called AZT became the first approved treatment for HIV disease. Since then, approximately 30 drugs have been approved to treat people living with HIV/AIDS, and more are under development. There are currently five different "classes" of HIV drugs. Each class of drug attacks the virus at different points in its life cycle.

1. Nucleoside Reverse Transcriptase Inhibitors (NRTI): These drugs interrupt the virus from duplicating, which may slow the spread of HIV in the body. They include:

- Abacavir (Ziagen, ABC)
- Didanosine (Videx, dideoxyinosine, ddI)
- Emtricitabine (Emtriva, FTC)
- Lamivudine (Epivir, 3TC)
- Stavudine (Zerit, d4T)

2. Protease Inhibitors (PI): These FDA-approved drugs interrupt virus replication at a later step in the virus life cycle. Protease inhibitors include:

- Amprenavir (Agenerase, APV)
- Atazanavir (Reyataz, ATV)
- Fosamprenavir (Lexiva, FOS)

- Indinavir (Crixivan, IDV)
- Lopinavir (Kaletra, LPV/r)
- 3. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI): These block the infection of new cells by HIV. NNRTIs include:
  - Delvaridine (Rescriptor, DLV)
  - Efavirenz (Sustiva, EFV)
  - Nevirapine (Viramune, NVP)
- 4. Entry/Fusion Inhibitors: These medications work to block the virus from ever entering cells in the first place. The group of drugs includes Enfuvirtide, also known as Fuzeon or T-20.
- 5. Highly Active Antiretroviral Therapy (HAART): In 1996, highly active antiretroviral therapy (HAART) was introduced for people with HIV and AIDS. HAART, often referred to as the anti-HIV "cocktail" is a combination of three or more drugs, such as protease inhibitors and other anti-retroviral medications. The treatment is highly effective in slowing the rate at which the HIV virus replicates itself, which may slow the spread of HIV in the body. The goal of HAART is to reduce the amount of virus in the body, or the viral load, to a level that can no longer be detected with blood tests.

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## **15.4 Rheumatoid Arthritis (RA)**

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At times the immune system can go awry and, instead of reacting against foreign antigens, could focus its attack on self-antigens. There are mechanisms of self-tolerance which normally protect an individual from potentially self-reactive lymphocytes, failures do occur. Such failure result in an inappropriate response of the immune system against self-components termed autoimmunity. In the 1960s, it was believed that all self-reactive lymphocytes were eliminated during their development in the bone marrow and thymus and that a failure to eliminate these lymphocytes led to autoimmune consequences. Since the late 1970s, a broad body of experimental evidence has countered that belief, revealing that not all self-reactive lymphocytes are deleted during T-cell and B-cell maturation. Instead, normal healthy individuals have been shown to possess mature, recirculating, self-reactive lymphocytes. The presence of these self-reactive lymphocytes in the periphery does not inevitably result in autoimmune reactions, their activity must be regulated in normal individuals. A breakdown in this regulation can lead to activation of self-reactive clones of T or B cells, generating humoral or cell-mediated responses against self antigens. These

reactions can cause serious damage to cells and organs, sometimes with fatal consequences.

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that primarily affects joints. It may result in deformed and painful joints, which can lead to loss of function. The disease may also have signs and symptoms in organs other than joints. Rheumatoid arthritis is a common autoimmune disorder, most often affecting women from 40 to 60 years old. The major symptom is chronic inflammation of the joints, although the hematologic, cardiovascular, and respiratory systems are also frequently affected. Many individuals with rheumatoid arthritis produce a group of auto-antibodies called rheumatoid factors that are reactive with determinants in the Fc region of IgG. The classic rheumatoid factor is an IgM antibody with that reactivity. Such auto-antibodies bind to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a type III hypersensitive reaction, which leads to chronic inflammation of the joints.

#### **15.4.1 Symptoms of RA**

Rheumatoid arthritis tends to begin slowly with minor symptoms that come and go, usually on both sides of the body, and progress over a period of weeks or months. Symptoms of this chronic disease vary from person to person and can change from day to day. Bouts of disease activity are called flare-ups, while inactive periods are called remission. The common symptoms observed include: fatigue, morning stiffness, joint pain, joint stiffness, joint swelling, numbness, fever, decrease in the range of motion.

#### **15.4.2 Causes of RA**

Although the exact cause of RA remains unknown, recent findings suggest a genetic basis for disease development. It is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DR4. Smoking is the most significant non-genetic risk with RA being up to three times more common in smokers than non-smokers. Epidemiological studies have confirmed a potential association between RA and two herpes virus infections: Epstein-Barr virus (EBV) and Human Herpes Virus 6.

#### **15.4.3 Pathogenicity**

T cells, B cells and the orchestrated interaction of pro-inflammatory cytokines play key roles in the pathophysiology of RA. The cytokines most directly implicated in this process are TNF- $\alpha$  and IL-6; IL-1 and IL-17 may also play important roles. Various immune modulators (cytokines and effector cells) and

signalling pathways are involved in the pathophysiology of RA. The complex interaction of immunomodulators is responsible for the joint damage that begins at the synovial membrane and covers most IA structures. Synovitis is caused by the influx or local activation, or both, of mononuclear cells (including T cells, B cells, plasma cells, dendritic cells, macrophages and mast cells) and by angiogenesis. The synovial lining then becomes hyperplastic, and the synovial membrane expands and forms villi. The osteoclast-rich portion of the synovial membrane, destroys bone, whereas enzymes secreted by neutrophils, synoviocytes and chondrocytes degrade cartilage.

#### **15.4.4 Treatment of RA**

There is no cure for RA, but treatments can improve symptoms and slow the progress of the disease. Disease-modifying treatment has the best results when it is started early and aggressively. The goals of treatment are to minimize symptoms such as pain and swelling, to prevent bone deformity, and to maintain day-to-day functioning. Following types of medications are generally used:

- Nonsteroidal anti-inflammatory drugs (NSAIDs): These can relieve pain and reduce inflammation. Over-the-counter NSAIDs include ibuprofen (Advil, Motrin IB) and naproxen sodium (Aleve).
- Steroids. Corticosteroid medications, such as prednisone, reduce inflammation and pain and slow joint damage. Side effects may include thinning of bones, weight gain and diabetes.
- Disease-modifying antirheumatic drugs (DMARDs). These drugs can slow the progression of rheumatoid arthritis and save the joints and other tissues from permanent damage. Common DMARDs include methotrexate (Trexall), leflunomide (Arava), hydroxychloroquine (Plaquenil) and sulfasalazine (Azulfidine).

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### **15.5 Severe acute respiratory syndrome- SARS**

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Severe acute respiratory syndrome (SARS) has become a priority for health-care agencies around the world because of its transmissibility, associated mortality and the potential for pandemic spread. As of 31 December 2003, 8096 cases had been identified worldwide and 774 people had died, a mortality rate of about 9.6% (World Health Organization statistics). SARS is a viral respiratory disease of zoonotic origin caused by the SARS coronavirus (SARS-CoV).

### 15.5.1 Symptoms of SARS

Following exposure to the virus, the symptoms of SARS tend to develop within 2-7 days. During the incubation period before SARS manifests itself, the disease is not contagious. Most cases of SARS begin with a high fever, with a temperature exceeding 100.4<sup>0</sup>F (38<sup>0</sup>C). Other early symptoms include those common to flu, such as aches, chills, diarrhea, dry coughing and shortness of breath. These develop over the course of a week. Most patients go on to develop pneumonia, an infection of the lungs, which is the leading cause of death in children below the age of 5 worldwide.

### 15.5.2. Causal organism

A novel coronavirus, SARS coronavirus (SARS CoV), was identified as the causative agent of SARS. Virions are roughly 90 to 120 nm in diameter and contain a lipid bilayer surrounding a helical nucleocapsid structure that protects the genome. The virus genome is a positive-strand RNA of 29 kilobases, which encodes a RNA-dependent RNA polymerase. Several structural proteins are encoded within the intact virion, and these include the 180/90-kDa spike(S) protein, a 50- to 60-kDa nucleocapsid (N) protein, an 8-kDa envelope (E) protein, and the 23-kDa membrane (M) protein.

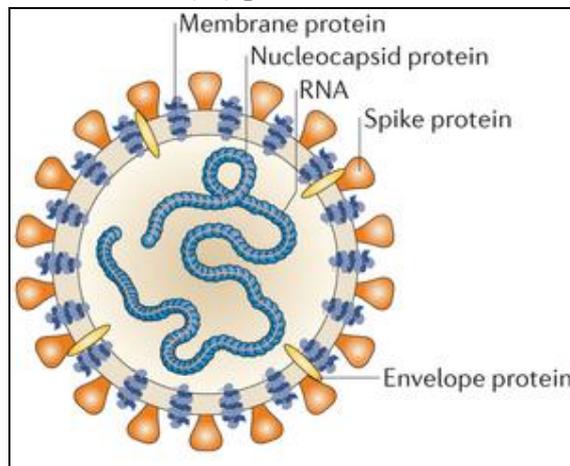


Fig. Structure of corona virus.

### 15.5.3 Pathogenicity of corona virus

A number of complete and partial autopsies of SARS patients have been reported since the first outbreak in 2003. The predominant pathological finding in these cases was diffuse alveolar damage (DAD). This severe pulmonary injury of SARS patients is caused both by direct viral effects and immunopathogenetic factors. The target organ of severe acute respiratory syndrome (SARS) is widely believed to be the lungs. However, other organ dysfunction, including gastrointestinal symptoms, abnormal liver function,

splenic atrophy, and lymphadenopathy have also been observed. This may reflect widespread immunopathology or the presence of extrapulmonary SARS-coronavirus (CoV) dissemination and replication. The innate immune response functions to prevent the viral replication and disease potential. From interferon (IFN) induction and secretion to the recruitment of macrophages and Dendritic Cells to sites of infection, the system functions to restrict tissue tropism and spread, dampen virus replication efficiency, and eliminate virally infected cell.

#### **15.5.4 Treatment and prevention of SARS**

Currently, no definitive medication protocol specific to SARS has been developed, although various treatment regimens have been tried without proven success. The Center for Disease Control and Prevention (CDC) recommends that patients suspected of or confirmed as having SARS receive the same treatment that would be administered if they had any serious, community-acquired pneumonia. SARS being a viral disease cannot be treated by antibiotics. Treatment of SARS is largely supportive with antipyretics, supplemental oxygen and mechanical ventilation as needed.

Treatment may include:

- Antibiotics to treat bacteria that cause pneumonia.
- Antiviral medications (although how well they work for SARS is unknown).
- High doses of steroids to reduce swelling in the lungs.
- Oxygen, breathing support (mechanical ventilation), or chest therapy.

Public health policies have been effective at controlling outbreaks. Many nations have stopped the epidemic in their own countries. All countries must continue to be careful to keep this disease under control. Viruses in the coronavirus family are known for their ability to change (mutate) in order to spread among humans.

There is no vaccine to date. Isolation and quarantine remain the most effective means to prevent the spread of SARS. Following preventive measures may be adopted:

- Reducing contact with people who have SARS.
- Avoid travel to places where there is an uncontrolled SARS outbreak.

- When possible, avoid direct contact with persons who have SARS until at least 10 days after their fever and other symptoms are gone.
- Hand hygiene is the most important part of SARS prevention.
- Covering mouth and nose while sneezing or coughing.
- Cleaning of commonly touched surfaces with a disinfectant.

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## **15.6 Variant Creutzfeldt-Jakob disease (vCJD)**

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Bovine spongiform encephalopathy (BSE), also known as mad cow disease, and variant Creutzfeldt-Jakob disease (CJD) are related disorders. They belong to the family of diseases known as the transmissible spongiform encephalopathies (TSEs). TSEs are caused by a transmissible proteinaceous particle, prion.

Variant Creutzfeldt-Jakob disease (vCJD) is a rare and fatal human neurodegenerative condition which is classified as a Transmissible Spongiform Encephalopathy (TSE) because of its ability to be transmitted and the characteristic spongy degeneration of the brain that it causes. vCJD was first described in the United Kingdom in March 1996 and has been linked with exposure to a TSE of cattle called Bovine Spongiform Encephalopathy (BSE), also known as Classical BSE<sup>1</sup>, which was first reported in the United Kingdom in 1986.

### **15.6.1 Symptoms of vCJD**

The first symptom of vCJD is rapidly progressive dementia, leading to memory loss, personality changes and hallucinations. Other frequently occurring features include anxiety, depression, obsessive-compulsive symptoms, and psychosis. This is accompanied by physical problems such as speech impairment, jerky movements (myoclonus), balance and coordination dysfunction (ataxia), changes in gait, rigid posture, and seizures. As the illness progresses, and, by the time of death, patients become completely immobile and mute. The duration of the disease varies greatly, but sporadic (non-inherited) vCJD can be fatal within months or even weeks.

The symptoms of CJD are caused by the progressive death of the brain's nerve cells, which is associated with the build-up of abnormal prion proteins forming amyloids. When brain tissue from a vCJD patient is examined under a microscope, many tiny holes can be seen where whole areas of nerve cells have

died. The word "spongiform" in "transmissible spongiform encephalopathies" refers to the sponge-like appearance of the brain tissue.

### **15.6.2 Cause of vCJD**

The infectious agent of vCJD is believed to be a specific type of misfolded protein called a prion. Prions are disease-causing form of a normal protein called cellular prion protein (PrPC) that is located primarily on the surface of central nervous system cells but also in other tissues of the body in mammals. The specific function of the normal prion protein (PrPC) is not clearly understood, but in experimental models it appears to play a role in protecting cells and helping them respond to oxygen deficiency. Stanley B. Prusiner coined the term prion for proteinaceous infective particle and changed to prion to sound rhythmic. Prions are not considered living organisms but are misfolded protein molecules which may propagate by transmitting a misfolded protein state. Prions are extremely small, smaller than viruses, and even through an electron microscope only aggregations (clusters), not individual prions, can be seen.

### **15.6.3 Pathogenicity**

All known mammalian prion diseases are caused by the prion protein, PrP. The endogenous, properly folded form is denoted PrPC (C for Common or Cellular), whereas the disease-linked, misfolded form is denoted PrP<sup>Sc</sup> (for Scrapie, after one of the diseases first linked to prions and neurodegeneration). The precise structure of the prion is not known, though they can be formed by combining PrPC, polyadenylic acid, and lipids in a Protein Misfolding Cyclic Amplification (PMCA) reaction. Upon entry into a healthy organism prion induces existing, properly folded proteins to convert into the disease-associated, misfolded prion form; the prion acts as a template to guide the misfolding of more proteins into prion form. These newly formed prions can then go on to convert more proteins themselves; this triggers a chain reaction that produces large amounts of the prion form. All known prions induce the formation of an amyloid fold, in which the protein polymerises into an aggregate consisting of tightly packed beta sheets. Amyloid aggregates are fibrils, growing at their ends, and replicating when breakage causes two growing ends to become four growing ends. These aggregates are extremely stable and accumulates in infected tissue, causing tissue damage and cell death. Prions are resistant to denaturation by chemical and physical agents, making disposal and containment of these particles difficult. The principal target of prion pathology

is the brain, yet most TSEs also display prion replication at extra-cerebral locations, including secondary lymphoid organs and sites of chronic inflammation.

#### **15.6.4 Treatment of vCJD**

Till date there is no cure for Creutzfeldt-Jakob Disease; only treatment for relieving symptoms is available. Antiviral drugs such as amantadine which have proved helpful in treating other neurodegenerative diseases like Parkinson's are under clinical trials. Some patients taking these antivirals have shown brief periods of improvement, with no harmful side effects. However, no treatment has been discovered that stops CJD completely. It is invariably fatal, often within a year of the onset of symptoms.

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### **15.7 Swine flu**

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Swine flu, also known as the H1N1 virus, made headlines in 2009 when it was declared a pandemic. Pandemics are contagious diseases affecting people throughout the world or on multiple continents at the same time. Swine influenza, also called pig influenza, swine flu, hog flu and pig flu, is an infection caused by any one of several types of swine influenza viruses. Swine flu is a contagious disease, and spreads in the same way as the seasonal flu, generally through coughing and sneezing of the infected people.

#### **15.7.1 Symptoms of swine flu**

The symptoms of swine flu are similar to those of seasonal flu. They include cough, fever, sore throat, stuffy or runny nose, body aches, headache, chills, and fatigue. The most common cause of death in persons suffering from swine flu is respiratory failure. Other causes of death are pneumonia (leading to sepsis), high fever (leading to neurological problems), dehydration (from excessive vomiting and diarrhea), electrolyte imbalance and kidney failure. Fatalities are more likely in young children and the elderly.

#### **15.7.2 Causal organism**

The most common cause of human influenza (flu) in 2009 was H1N1 virus, the subtype of influenza A virus. It is an Orthomyxovirus that contains the glycoproteins haemagglutinin (HA) and neuraminidase. For this reason, they are described as H1N1, H1N2 depending on the type of H or N antigens they express. Haemagglutinin causes red blood cells to clump together and binds the virus to the infected cell. Neuraminidase are a type of glycoside hydrolase enzyme which help to move the virus particles through the infected cell and

assist in budding from the host cells. In June 2009, the World Health Organization (WHO) declared the H1N1 strain of swine-origin as a pandemic. This strain is often called swine flu by the public media.

### **15.7.3 Pathogenicity**

Different influenza viruses possess differing antigenic properties, specifically for binding to the sialyl moiety (tetrasaccharide carbohydrate attached on the surface of cells) of host cells. With the aid of envelope glycoprotein HA, influenza viruses can easily bind to cell-surface glycosylated oligosaccharides that have sialic acids at their terminals, although this binding is very specific. This specificity acts as a host barrier against viral transmission. Some strains have the capacity to bind to both glycan linkages, making them more virulent and resulting in various intestinal illnesses. Mutations in the HA region of a virus may alter its binding affinity to the host through antigenic shift, leading to differential pathogenesis among different strains. HA helps the virus to attach to the sialic acid receptor of the host cell, which is followed by endocytosis. Viral RNA polymerase transcribes the viral genome and releases the mRNA for further processing. Viral genomic ssRNA synthesis takes place after mRNA release by exploiting host cellular machinery. This is then followed by assembly, maturation and ultimately by the production of progeny virions via budding from the infected host cell membrane.

### **15.7.4 Prevention and Treatment**

Control measures include simple practices such as avoiding contact with infected people by staying away from crowded places and public gatherings, or avoiding infected aerosols by using hygienic masks or a simple nose cover. Since the transmission of influenza infection occurs through bioaerosols, personal hygiene is an important factor. Individuals suspected of being infected should be kept under medical supervision, and should be specifically isolated from young children and immunocompromised individuals. Travelers from pandemic or endemic regions should be examined before and after their journey, and quarantine measures are needed at airports.

Several antiviral drugs are available for the treatment of influenza. One of the important actions of these antivirals is inhibiting the action of neuraminidase, thereby interfering with the release of progeny virions from the surface of the infected host cell. These antivirals include drugs like zanamivir, peramivir and favipiravir, which are administered intravenously, and oseltamivir, which is administered orally. Combination drugs have also been introduced. For

example, favipiravir is administered in addition to oseltamivir and zanamivir, a drug which more efficiently inhibits the RNA polymerase present in influenza viruses. Favipiravir has a significant role in inhibiting the replication of H1N1, drug-resistant viruses. Additionally, current studies have shown the role of interferon-inducible transmembrane protein family membranes 3 (IFITM3), as a potential candidate for curbing influenza infection.

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## 15.8 Summary

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A disease can be defined as an abnormal, pathogenic condition affecting the sufferer mentally, physically and physiologically. Diseases may be caused by some external source (bacteria, virus) or due to malfunctioning of the body's systems (autoimmune diseases). The immune system is a versatile defense system that functions to protect animals from invading pathogenic microorganisms and cancer. It generates an enormous variety of cells and molecules capable of specifically recognizing and eliminating a variety of foreign invaders.

AIDS, a major worldwide epidemic is caused by the human immunodeficiency virus (HIV). HIV is a retrovirus which carries its genetic information in the form of RNA. The virus infects mainly the CD4<sup>+</sup> lymphocytes (T cells), but also to a lesser degree monocytes, macrophages, and dendritic cells. The common means of HIV transmission include homosexual and heterosexual intercourse, receipt of infected blood or blood products, and passage from mothers to infants.

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that primarily affects joints. It may result in deformed and painful joints, which can lead to loss of function. It is a common autoimmune disorder, most often affecting women from 40 to 60 years old. The exact cause of RA remains unknown, recent findings suggest a genetic basis for disease development. It is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DR4. There is no cure for RA, but treatments can improve symptoms and slow the progress of the disease.

SARS is a viral respiratory disease of zoonotic origin caused by the SARS coronavirus (SARS-CoV). Symptoms of SARS are common to flu, such as aches, chills, diarrhea, dry coughing and shortness of breath. Most patients develop pneumonia, an infection of the lungs.

Variant Creutzfeldt-Jakob disease (vCJD) is a rare and fatal human neurodegenerative condition. Symptoms of vCJD include rapidly progressive

dementia, leading to memory loss, personality changes and hallucinations. The infectious agent of vCJD is believed to be a specific type of misfolded protein called a prion. Prions are resistant to denaturation by chemical and physical agents, making disposal and containment of these particles difficult. The principal target of prion pathology is the brain. Till date there is no cure for Creutzfeldt-Jakob Disease; only treatment for relieving symptoms is available.

Swine influenza, also called pig influenza, swine flu, and pig flu, is an infection caused by any one of several types of swine influenza viruses. Swine flu is a contagious disease, and spreads in the same way as the seasonal flu, generally through coughing and sneezing of the infected people. Swine flu is caused by H1N1 virus, the subtype of influenza A virus. It is an Orthomyxovirus that contains the glycoproteins haemagglutinin (HA) and neuraminidase.

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## 15.9 Glossary

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- **Kaposi's sarcoma:** A tumor caused by infection with human herpesvirus 8 (HHV8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV).
- **Pandemic:** A pandemic is an epidemic of infectious disease that has spread through human populations across a large region; for instance multiple continents, or even worldwide
- **AZT:** Azidothymidine (AZT) is a nucleoside analog reverse-transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of HIV/AIDS infection. AZT inhibits the enzyme (reverse transcriptase) that HIV uses to synthesize DNA, thus preventing viral DNA from forming.
- **Complement system:** The complement system is a part of the immune system that helps or complements the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the innate immune system.
- **Inflammation:** Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective response involving host cells, blood vessels, and proteins and other mediators that is intended to eliminate the initial cause of cell injury, as well as the necrotic cells and tissues and to initiate the process of repair.

- **Interferons:** Interferons (IFNs) are glycoproteins made and released by host cells in response to the presence of pathogens, such as viruses, bacteria, parasites, or tumor cells.

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## 15.10 Self-Learning Exercise

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### Section -A (Very Short Answer Type)

1. What is the full form of HIV?
2. What are NRTIs ?
3. What do you mean by NSID?
4. What does SARS stands for?
5. Give one example of an auto immune disease
6. What is TSE?

### Section -B (Short Answer Type)

1. What are prions?
2. What are auto immune diseases?
3. What are the different classes of drugs used to control HIV?
4. What are the symptoms of rheumatoid arthritis?
5. What are the various modes of AIDS transmission?

### Section -C (Long Answer Type)

1. What is rheumatoid arthritis? Give an account of its pathogenicity and treatment.
2. Explain the structure and pathogenicity of HIV.
3. What is the causal agent of SARS? Draw a labeled diagram of the same. List the preventive measures to be taken to control SARS
4. Give a detailed account of the symptoms and causative agent of variable Creutzfeldt-Jakob Disease.
5. Describe the symptoms, causative agent and pathogenicity of swine flu? What are the preventive and treatment strategies adopted to control it.

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## 15.11 References

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