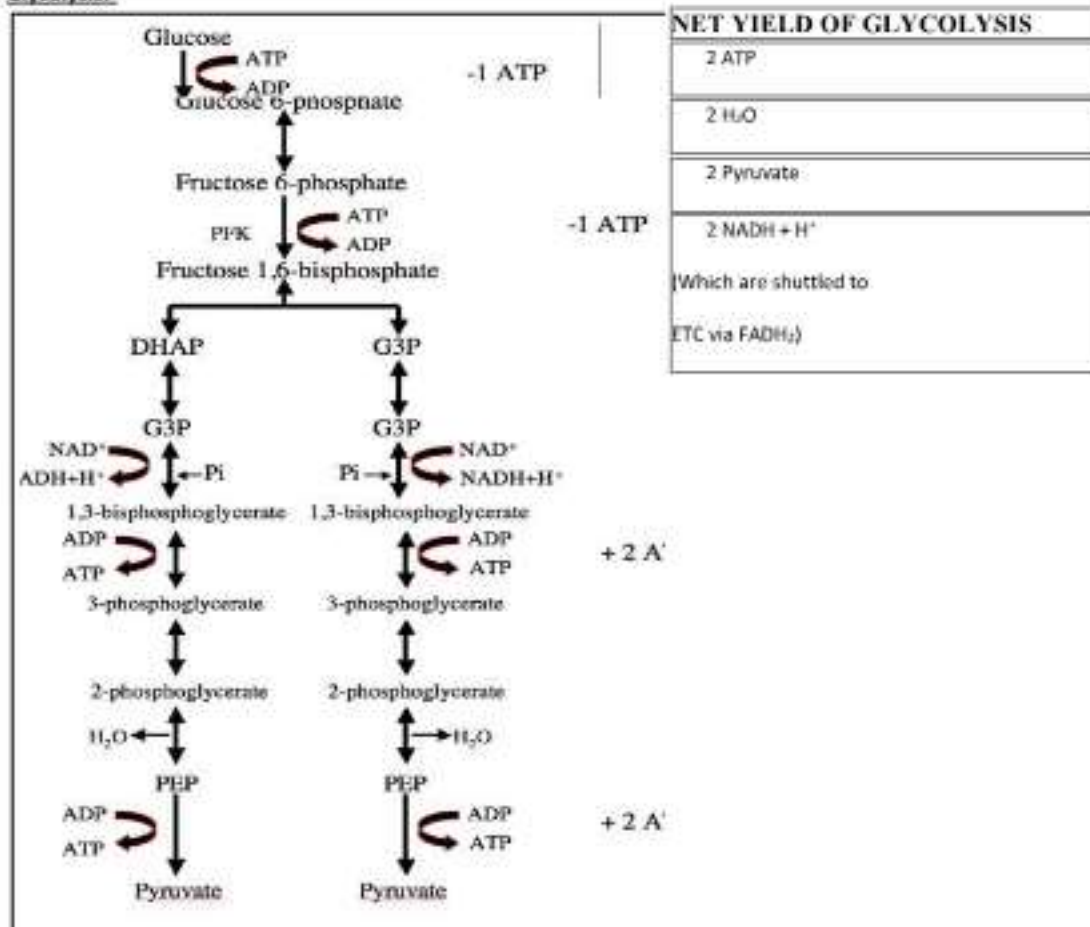


# CHAPTER: 7 CARBOHYDRATE METABOLISM

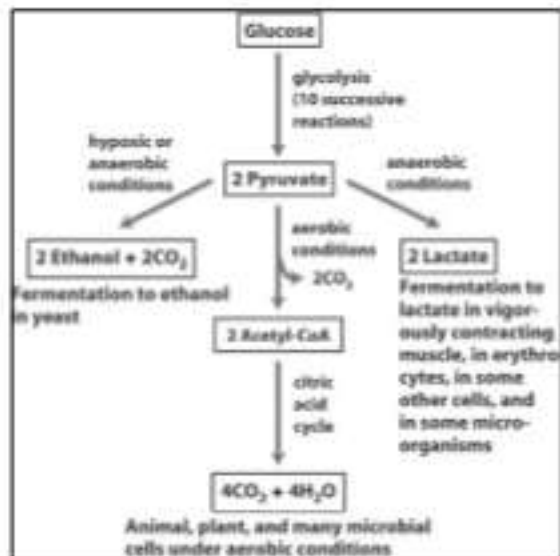
## Glycolysis:-



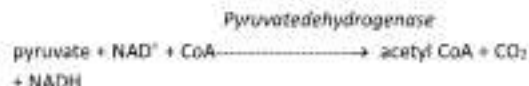
## Substrate-level phosphorylation:

There are two distinct methods by which cells synthesize ATP. **Oxidative phosphorylation**, involving the electron transport chain, the generation of ATP is linked to the oxidation of NADH and FADH<sub>2</sub> to NAD<sup>+</sup> and FAD respectively and occurs via the generation of a proton gradient across the inner mitochondrial membrane.

In contrast, the two ATP synthetic reactions in glycolysis (catalyzed by phosphoglycerate kinase and pyruvate kinase) involve the direct transfer of a phosphate from a sugar-phosphate intermediate to ADP; these reactions are examples of **substrate-level phosphorylation**. A third example of substrate-level phosphorylation is the synthesis of GTP by succinate dehydrogenase in the citric acid cycle. The GTP can be used to phosphorylate ADP to form ATP.

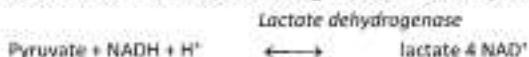
Fates of Pyruvate:

**1. Entry into the citric acid cycle.** Glycolysis releases relatively little of the energy present in a glucose molecule; much more is released by the subsequent operation of the citric acid cycle and oxidative phosphorylation. Following this route under aerobic conditions, pyruvate is converted to acetyl CoA by the enzyme **pyruvate dehydrogenase** and the acetyl CoA then enters the citric acid cycle. The pyruvate dehydrogenase reaction is an **oxidative decarboxylation**:



**2. Conversion to fatty acid or ketone bodies.** When the cellular energy level is high (ATP in excess), the rate of the citric acid cycle decreases and acetyl CoA begins to accumulate. Under these conditions, acetyl CoA can be used for fatty acid synthesis or the synthesis of ketone bodies.

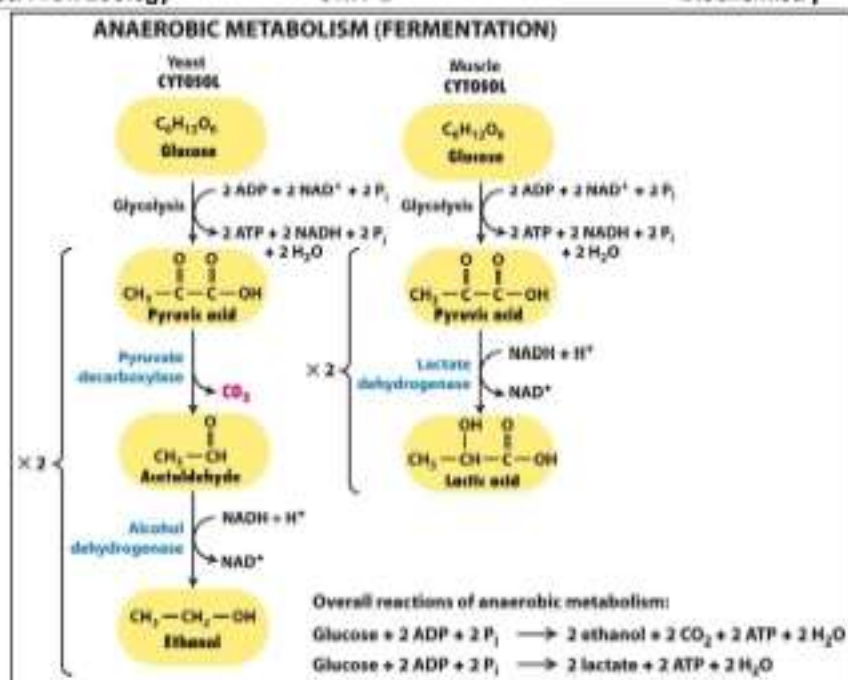
**3. Conversion to lactate:** The  $\text{NAD}^+$  used during glycolysis (in the formation of 1,3-bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase) must be regenerated if glycolysis is to continue. Under aerobic conditions,  $\text{NAD}^+$  is regenerated by the reoxidation of NADH via the electron transport chain. When oxygen is limiting, as in muscle during vigorous contraction, the reoxidation of NADH to  $\text{NAD}^+$  by the electron transport chain becomes insufficient to maintain glycolysis. Under these conditions,  $\text{NAD}^+$  is regenerated instead by conversion of the pyruvate to lactate by **lactate dehydrogenase**:



When sufficient oxygen becomes available once more,  $\text{NAD}^+$  levels rise through operation of the electron transport chain. The lactate dehydrogenase reaction then reverses to regenerate pyruvate that is converted by pyruvate dehydrogenase to acetyl CoA that can enter the citric acid cycle (see above). Thus the operation of lactate dehydrogenase in mammals is a mechanism for the reoxidation of NADH to  $\text{NAD}^+$ .

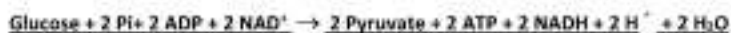
Hence allowing glycolysis to continue, and ATP to be made, under anaerobic conditions. The process is even more sophisticated in the case of vigorously contracting skeletal muscle. Here the lactate produced is transported in the bloodstream to the liver where it is converted back to glucose and can return once again via the bloodstream to the skeletal muscle to be metabolized to yield energy. This is called as Cori cycle. Finally, in some microorganisms, lactate is the normal product from pyruvate.

**4. Conversion to ethanol:** - In yeast and some other microorganisms under anaerobic conditions, the  $\text{NAD}^+$

**Energy yield:**

Early in glycolysis, two ATPs are required for the conversion of glucose to glucose 6-phosphate by hexokinase and for the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate by PFK.

However, fructose 1,6-bisphosphate then gives rise to two three-carbon units, each of which generates two ATPs in subsequent steps (catalyzed by phosphoglycerate kinase and pyruvate kinase) giving a net yield of two ATPs per original glucose molecule. The overall reaction is:



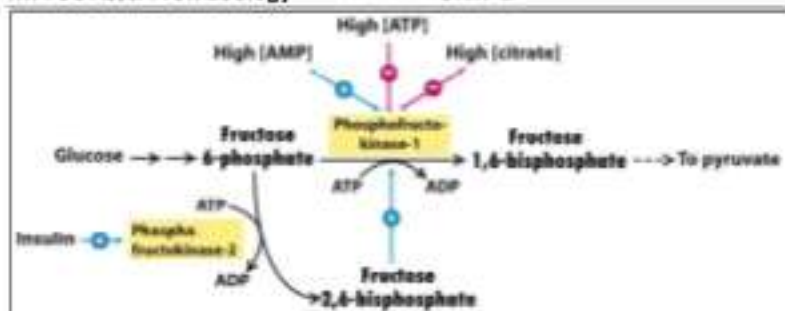
Note that, under aerobic conditions, the two NADH molecules that are synthesized are reoxidized via the electron transport chain generating ATP. Given the cytoplasmic location of these NADH molecules, each is reoxidized via the glycerol 3-phosphate shuttle and produces approximately two ATPs during oxidative phosphorylation or via the malate-aspartate shuttle and produces approximately three ATPs during oxidative phosphorylation.

**Regulation of glycolysis:****Phosphofructokinase**

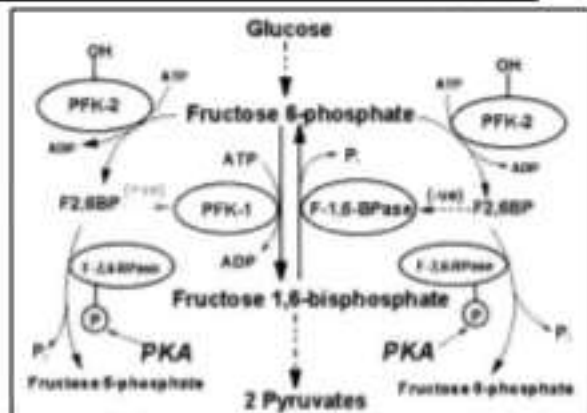
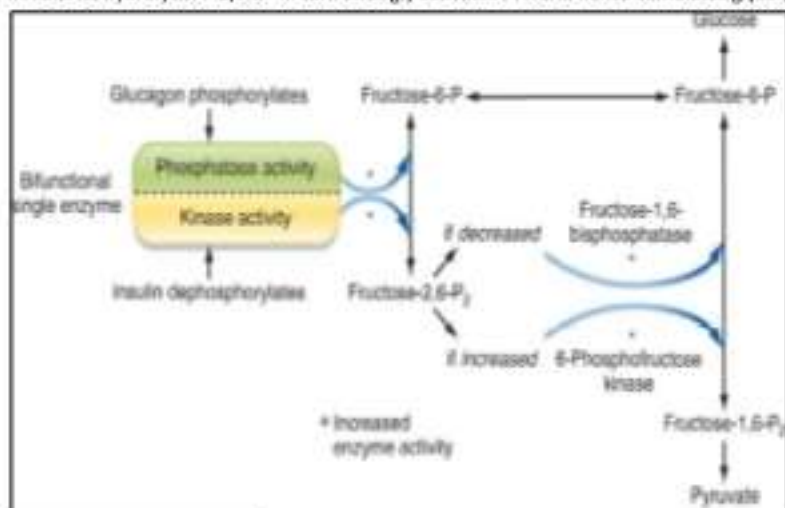
The most important control step of glycolysis is the irreversible reaction catalyzed by phosphofructokinase (PFK). The enzyme is regulated in several ways:

**1. ATP/AMP.** PFK is allosterically inhibited by ATP but this inhibition is reversed by AMP. This allows glycolysis to be responsive to the energy needs of the cell, speeding up when ATP is in short supply (and AMP is plentiful) so that more ATP can be made, and slowing down when sufficient ATP is already available.

**2. Citrate.** PFK is also inhibited by citrate, the first product of the citric acid cycle proper. A high level of citrate signals that there is a plentiful supply of citric acid cycle intermediates already and hence no additional breakdown of glucose via glycolysis is needed.



3. **Fructose 2,6-bisphosphate** Fructose 2,6-bisphosphate (F-2,6-BP) is synthesized from fructose 6-phosphate by an enzyme called phosphofructokinase-2 (PFK2), a different enzyme from PFK. F-2,6-BP is hydrolyzed back to fructose 6-phosphate by fructose biphosphatase 2 (FBPase2). Amazingly, both PFK2 and FBPase2 are activities catalyzed by the same polypeptide; hence this is a **bi-functional enzyme**. Fructose 6-phosphate stimulates the synthesis of F-2,6-BP and inhibits its hydrolysis. F-2,6-BP in turn strongly activates PFK and hence stimulates glycolysis.



The overall effect is that when fructose 6-phosphate levels are high, PFK (and hence glycolysis) is stimulated. PFK2 and FBPase2 are also controlled by covalent modification. When blood glucose levels fall, the hormone glucagon is released into the bloodstream and triggers a cAMP cascade that leads to phosphorylation of the PFK2/FBPase2 polypeptide at a single serine residue. This activates FBPase2 and inhibits PFK2, lowering the level of F-2,6-BP and hence decreasing the rate of glycolysis. The reverse is true as glucose levels rise; the phosphate group is removed from the PFK2/FBPase2 polypeptide by a phosphatase, thus inhibiting FBPase2 and activating PFK2, raising the level of F-2,6-BP and hence increasing the rate of glycolysis.

F-2,6-BP is also important in preventing glycolysis (glucose degradation) and gluconeogenesis (glucose synthesis) operating simultaneously. This is called reciprocal regulation.

**4. H<sup>+</sup> ions:** PFK is inhibited by H<sup>+</sup> ions and hence the rate of glycolysis decreases when the pH falls significantly. This prevents the excessive formation of lactate (i.e. lactic acid) under anaerobic conditions and hence prevents the medical condition known as acidosis (a deleterious drop in blood pH).

### Hexokinase

Hexokinase, which catalyzes the first irreversible step of glycolysis, is inhibited by glucose 6-phosphate. Thus when PFK is inhibited, fructose 6-phosphate builds up and so does glucose 6-phosphate since these two metabolites are in equilibrium via phosphoglucose isomerase.

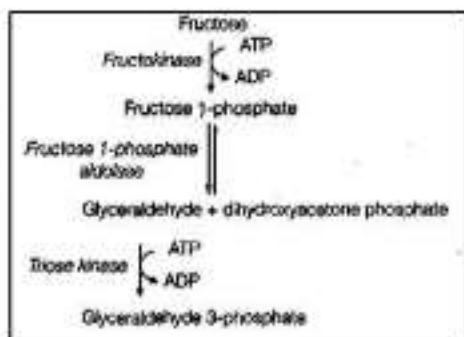
The hexokinase inhibition then reinforces the inhibition at the PFK step. At first sight this seems unusual since it is usually the first irreversible step of a pathway (the committed step) that is the main control step. On this basis, it may appear that hexokinase should be the main control enzyme, not PFK. However, glucose 6-phosphate, the product of the hexokinase reaction, can also feed into glycogen synthesis or the pentose phosphate pathway. Thus the first irreversible step that is unique to glycolysis is that catalyzed by PFK and hence this is the main control step.

### Pyruvate kinase

Pyruvate kinase catalyzes the third irreversible step in glycolysis. It is activated by fructose 1,6-bisphosphate. ATP and the amino acid alanine allosterically inhibit the enzyme so that glycolysis slows when supplies of ATP and biosynthetic precursors (indicated by the levels of Ala) are already sufficiently high. In addition, in a control similar to that for PFK (see above), when the blood glucose concentration is low, glucagon is released and stimulates phosphorylation of the enzyme via a cAMP cascade. This covalent modification inhibits the enzyme so that glycolysis slows down in times of low blood glucose levels.

### Metabolism of fructose:

Fructose is an abundant sugar in the human diet; sucrose (table sugar) is a disaccharide which when hydrolyzed yields fructose and glucose and fructose is also a major sugar in fruits and honey. There are two pathways for the metabolism of fructose; one occurs in muscle and adipose tissue, the other in liver:



### The fructose 1-phosphate pathway

1. **In muscle and adipose tissue,** fructose can be phosphorylated by hexokinase (which is capable of phosphorylating both glucose and fructose) to form fructose 6-phosphate which then enters glycolysis.

2. **In liver,** the cells contain mainly glucokinase instead of hexokinase and this enzyme phosphorylates only glucose. Thus in liver, fructose is metabolized instead by the fructose 1-phosphate pathway

\* Fructose is converted to fructose 1-phosphate by fructokinase.

\* Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate by fructose 1-phosphate aldolase.

The dihydroxyacetone feeds into glycolysis at the triose phosphate isomerase step

- The glyceraldehyde is phosphorylated by triose kinase to glyceraldehyde 3-phosphate and so also enters glycolysis.

#### Metabolism of galactose:

The hydrolysis of the disaccharide lactose (in milk) yields galactose and glucose. Thus galactose is also a major dietary sugar for humans. Galactose and glucose are epimers that differ in their configuration at C-4. Thus the entry of galactose into glycolysis requires an epimerization reaction. This occurs via a four-step pathway called the **galactose-glucose interconversion pathway**.

1. Galactose is phosphorylated by **galactokinase** to give galactose 1-phosphate.

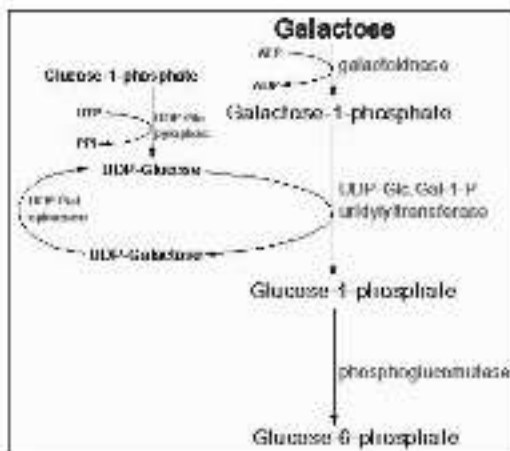
2. Galactose 1-phosphate uridylyl transferase catalyzes the transfer of a uridylyl group from UDP-glucose to galactose 1-phosphate to form UDP-galactose and glucose 1-phosphate.

#### The galactose-glucose interconversion pathway

3. The UDP-galactose is converted back to UDP-glucose by **UDP-galactose 4-epimerase**. Thus, overall, UDP-glucose is not consumed in the reaction pathway.

4. Finally the glucose 1-phosphate is converted to glucose 6-phosphate by **phosphoglucomutase**. The glucose 6-phosphate then enters glycolysis.

**Galactosemia** is a genetic disease caused by an inability to convert galactose to glucose. Toxic substances accumulate such as **galactitol**, formed by the reduction of galactose, and lead to dire consequences for the individual. Children who have the disease fail to thrive, may vomit or have diarrhea after drinking milk, and often have an enlarged liver and jaundice. The formation of cataracts in the eyes, mental retardation and an early death from liver damage are also possible.



Most cases of galactosemia are due to a deficiency of the **galactose-1-phosphate uridylyl transferase** enzyme and hence these individuals cannot metabolize galactose. The disease is treated by prescribing a galactose-free diet which causes all the symptoms to regress except mental retardation which may be irreversible. Since such patients have normal levels of **UDP-galactose 4-epimerase**, they can still synthesize UDP-galactose from UDP-glucose and so can still synthesize, for example, oligosaccharides in glycoproteins that involve Gal residues.

### GLUCONEOGENESIS

Gluconeogenesis **synthesizes glucose from noncarbohydrate precursors, including lactate and pyruvate, citric acid cycle intermediates, the carbon skeletons of most amino acids and glycerol**. This is extremely important since the brain and erythrocytes rely almost exclusively on glucose as their energy source under normal conditions. The liver's store of glycogen is sufficient to supply the brain with glucose for only about half a day during fasting.

Thus gluconeogenesis is especially important in periods of starvation or vigorous exercise. During starvation, the formation of glucose via gluconeogenesis particularly uses amino acids from protein breakdown and glycerol from fat breakdown. During exercise, the blood glucose levels required for brain and skeletal muscle function are maintained by gluconeogenesis in the liver using lactate produced by the muscle. The main site of gluconeogenesis is the liver, although it also occurs to a far lesser extent in the kidneys. Very little gluconeogenesis occurs in brain or muscle.

# OPERONS

## Control of Prokaryote Gene Expression

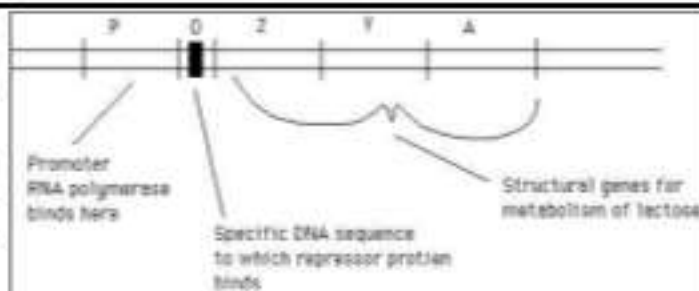
Genetic control in prokaryotes is exercised both at the level of transcription and at subsequent (post-transcriptional) stages of gene expression. Control of gene expression in eukaryotic cells is, in general, more complex than in prokaryotic cells, and involves a 'multilayered' approach in which diverse control mechanisms exert their effects at multiple levels.

The initiation of transcription is regulated primarily in a negative fashion by the synthesis of trans-acting repressor proteins, which bind to operator sequences upstream of protein coding sequences. **Collections of metabolically-related genes are grouped together and coordinately controlled as 'operons' in this way.** Transcription of these operons typically produces a polycistronic mRNA (multiple ORF) which encodes several different proteins.

## Induction of The Lac Operon:

Many protein-coding genes in bacteria are clustered together in operons, which serve as transcriptional units that are coordinately regulated.

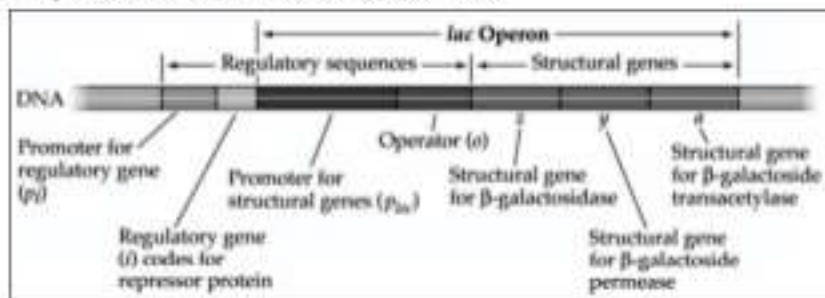
- The structural genes including the adjacent regulatory elements form a functional genetic unit known as the **Lac Operon**



**They are transcribed to yield a single polycistronic mRNA that is then translated to produce all three enzymes.**

The existence of a polycistronic mRNA ensures that the amounts of all three gene products are regulated coordinately. Transcription occurs from a single promoter ( $P_{lac}$ ) that lies upstream of these structural genes and binds RNA polymerase. However, also present are an operator site ( $O_{lac}$ ) between the promoter and the structural genes, and a **lac** gene that codes for the **lac repressor** protein.

The genes which regulate the lactose metabolic enzymes include:



### 3 Structural genes :-

- galactosidase (Z) which hydrolyzes lactose to glucose and galactose
- galactoside permease OR lactose permease (Y) it transports lactose into the cell across the cell membrane
- transacetylase (A) the role of this enzyme is not clear.

### 3 regulatory elements :-

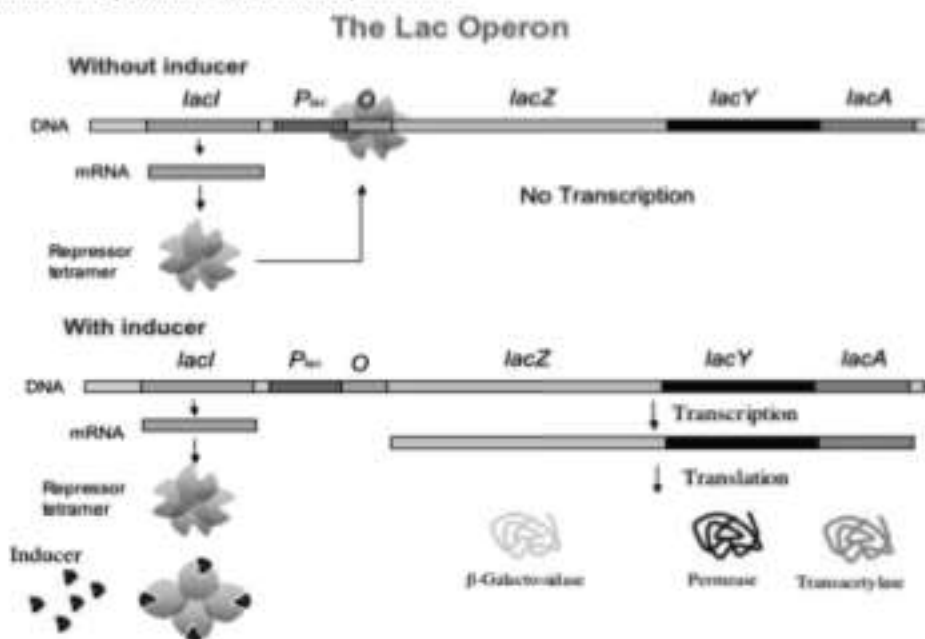
1. **Promoter:** (P) is acting element where RNA polymerase bind to initiate transcription
  2. **Operator(O):** this is the cis acting element present adjacent to promoter where repressor bind to hinder the movement of RNA polymerase. And prevent transcription of structural genes
  3. **Repressor (I)** By binding to the operator, the repressor prevents the initiation of transcription by the RNA polymerase.  
They continually monitor and respond to environmental conditions. In the case of the genes regulating lactose metabolism, the presence of lactose in the environment (media) is continually being monitored.
- The repressor protein proves to be the key element in regulating the operon with respect to environmental conditions.
  - It has two functional sites:
    - lactose binding site
    - operator binding site
  - If repressor is bound to the operator, the structural genes of the operon are not transcribed. When repressor protein is bound to the operator, it physically blocks RNA polymerase from transcribing the adjacent genes.
  - If repressor is not bound to the operator, the structural genes of the operon are transcribed:
  - when the repressor protein is bound to lactose (actually allolactose), it no longer binds to the operator. The binding of lactose to the repressor alters the conformation of the repressor so that it no longer has a high affinity for the operator and the repressor can no longer bind to the operator region and falls off. RNA polymerase can then bind to the promoter and transcribe the lac genes **Thus when lactose is present in the media the Lac Operon is ON and when Lactose is absent the Lac Operon is off**
  - Normally *E. coli* cells make very little of any of these three proteins but when lactose is available it causes a large and coordinated increase in the amount of each enzyme. Thus each enzyme is an **inducible enzyme** and the process is called **induction**. few molecules of  $\beta$ -galactosidase in the cell before induction convert the lactose to **allolactose**, which then turns on transcription of these three genes in the lac operon. Thus allolactose is an inducer. Allolactose is a disaccharide similar to lactose. It consists of the monosaccharides  $\beta$ -D-galactose and  $\beta$ -D-glucose linked through a  $\beta$  1-6 glycosidic linkage.
  - Another inducer of the lac operon is **isopropylthiogalactoside (IPTG)**. Unlike allolactose, this inducer is not metabolized by *E. coli* and so is useful for experimental studies of induction (**gratituos inducer**).

Many protein-coding genes in bacteria are clustered together in operons, which serve as transcriptional units that are coordinately regulated.

#### The Lac Repressor:

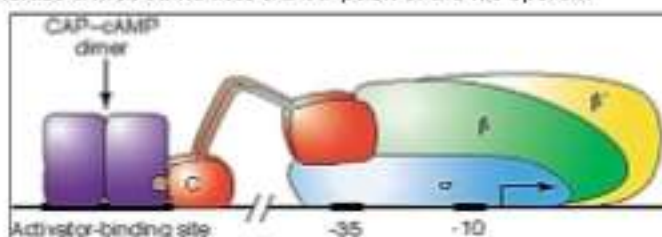
- The *lacI* gene has its own promoter ( $P_{lacI}$ ) that binds RNA polymerase and leads to transcription of lac repressor mRNA and hence production of lac repressor protein monomers.
- Four identical repressor monomers come together to form the active tetramer, which can bind tightly to the lac operator site,  $O_{lac}$ . The  $O_{lac}$  sequence is **palindromic**, that is it has the same DNA sequence when one strand is read 5' to 3' and the complementary strand is read 5' to 3'.
- This symmetry of the operator site is matched by the symmetry of the repressor tetramer.
- In the absence of an inducer such as allolactose or IPTG, the *lac I* gene is transcribed and the resulting repressor protein binds to the operator site of the lac operon,  $O_{lac}$  and prevents transcription of the *lacZ*, *lacY* and *lacA* genes.

- During induction, the inducer binds to the repressor. This causes a change in conformation of the repressor that greatly reduces its affinity for the *lac* operator site. The *lac* repressor now dissociates from the operator site and allows the RNA polymerase (already in place on the adjacent promoter site) to begin transcribing the *lacZ*, *lacY* and *lacA* genes. This yields many copies of the polycistronic mRNA and, after translation, large amounts of all three enzymes.
- If inducer is removed, the *lac* repressor rapidly binds to the *lac* operator site and transcription is inhibited almost immediately.
- The *lacZYA* RNA transcript is very unstable and so degrades quickly such that further synthesis of the  $\beta$  galactosidase, permease and transacetylase ceases.



#### CRP/CAP:

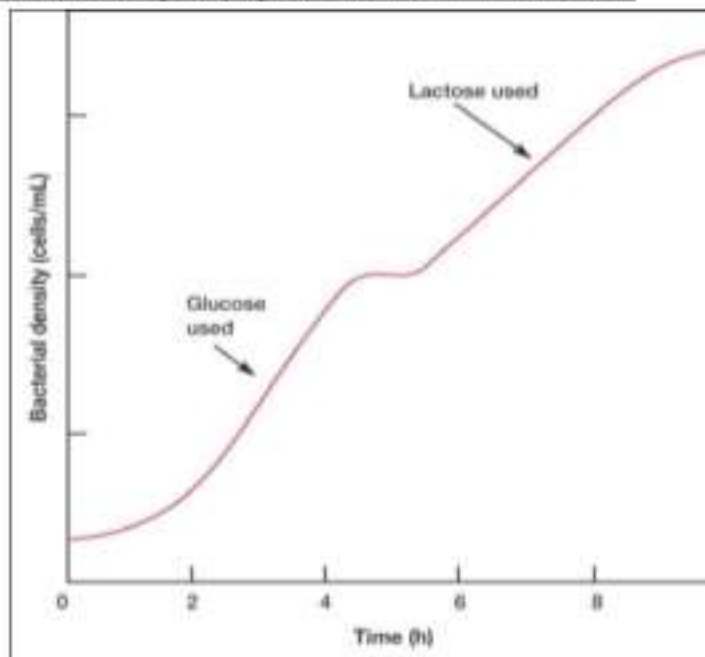
High-level transcription of the *lac* operon requires the presence of a specific activator protein called **catabolite activator protein (CAP)**, also called **cAMP receptor protein (CRP)**. This protein, which is a dimer, cannot bind to DNA unless it is complexed with 3'5' cyclic AMP (cAMP). The CRP-cAMP complex binds to the *lac* promoter just upstream from the binding site for RNA polymerase. It increases the binding of RNA polymerase and so stimulates transcription of the *lac* operon.



Whether or not the CRP protein is able to bind to the *lac* promoter depends on the carbon source available to the bacterium. When glucose is present, *E. coli* does not need to use lactose as a carbon source and so the *lac* operon

does not need to be active. Thus the system has evolved to be responsive to glucose. Glucose inhibits adenylate cyclase, the enzyme that synthesizes cAMP from ATP. Thus, in the presence of glucose the intracellular level of cAMP falls, so CRP cannot bind to the *lac* promoter, and the *lac* operon is only weakly active (even in the presence of lactose). When glucose is absent, adenylate cyclase is not inhibited, the level of intracellular cAMP rises and binds to CRP.

Therefore, when glucose is absent but lactose is present, the CRP-cAMP complex stimulates transcription of the *lac* operon and allows the lactose to be used as an alternative carbon source. In the absence of lactose, the *lac* repressor of course ensures that the *lac* operon remains inactive. These combined controls ensure that the *lacZ*, *lacY* and *lacA* genes are transcribed strongly only if glucose is absent and lactose is present.



#### Positive and negative regulation:

The *lac* operon is a good example of **negative control (negative regulation)** of gene expression in that bound repressor prevents transcription of the structural genes. **Positive control (positive regulation)** of gene expression is when the regulatory protein binds to DNA and increases the rate of transcription. In this case the regulatory protein is called an activator. The CAP/CRP involved in regulating the *lac* operon is a good example of an activator. Thus the *lac* operon is subject to both negative and positive control.

► **LAC Operon** is an **INDUCIBLE operon** (i.e., exhibits negative control). It is always off and is turned on by an inducer molecule (allolactose)

► **CATABOLIC REPRESSION**...as long as glucose is present LAC operon is OFF, even if allolactose is present glucose prevents the action of the LAC operon through another regulator-like protein, the Catabolite Activator Protein or CAP and DNA binding site - CAP gene

► CAP is an allosteric protein, regulated by cAMP

**when glucose is low** - all the ATP is hydrolyzed favoring high cAMP amounts cAMP-CAP conformation can bind to CAP DNA region - favors rapid transcription

**when glucose is high** - lots of ATP & little cAMP

- IgE is homocytotropic; that is, it has an affinity for cells ("cytotropic") of the host species that produced it ("homo"). This affinity is particularly strong for tissue mast cells and blood basophils. Fixation to these cells occurs via a **cell membrane bound FcεR** (i.e., receptor for the Fc portion of the ε chain of IgE) reacting with the Fc fragment (CH3 and CH4 domains).
- On combining with allergens, IgE antibodies trigger the release of histamine and other mediators of atopic disease from the cells.
- IgE may also be important in immunity to certain helminthic parasites.
- IgE is unable to activate complement via the classical pathway.
- IgE has a vascular half-life of 2 to 3 days and is heat-labile at 56°C.

**Production:** IgE is produced by B cells and plasma cells in the spleen, in lymphoid tissue of the tonsils and adenoids, and in the respiratory and gastrointestinal mucosa. IgE does not cross the placenta. IgE production begins in the fetus early in gestation.

#### 5. Immunoglobulin D (IgD):-

It was first discovered in a patient developed multiple myeloma. They have serum concentrate of 30 µg/ml (approximately 3 to 5 mg/dl) and constitute about .2% of human serum. They are present extensively on PM of B-cells. Their exact function is unknown but probably they play a role in Antigen dependent B-cells differentiation i.e. it eliminates B-cells which can produce self reactive antibodies. IgD exists as a monomer

## CHAPTER: 17 MONOCLONAL ANTIBODIES

An antibody produced by a single clone of cells (specifically, a single clone of hybridoma cells) and therefore a single pure homogeneous type of antibody. Monoclonal antibodies can be made in large amounts in the laboratory and are a cornerstone of immunology. The term "monoclonal" pertains to a single clone of cells, a single cell and the progeny of that cell.

**Köhler and Milstein found a way to combine**

- the unlimited growth potential of myeloma cells with
- the predetermined antibody specificity of normal immune spleen cells.

They did this by literally fusing myeloma cells with antibody-secreting cells from an immunized mouse. The technique is called **somatic cell hybridization**. The result is a **hybridoma**

### The procedure

Mix the following spleen cells from a mouse that has been immunized with the desired antigen with myeloma cells.

Use an agent to facilitate fusion of adjacent plasma membranes like PEG. Even so, the success rate is so low that there must be a way to **select for the rare successful fusions**.

So, use myeloma cells that have:

- lost the ability to synthesize **hypoxanthine-guanine-phosphoribosyltransferase (HGPRT)**.

This enzyme enables cells to synthesize purines using an extracellular source of **hypoxanthine** as a precursor.

Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an alternate pathway that they can use to synthesize purines.

However, **when cells are exposed to aminopterin (a folic acid analog), they are unable to use this other pathway and are now fully dependent on HGPRT for survival.**

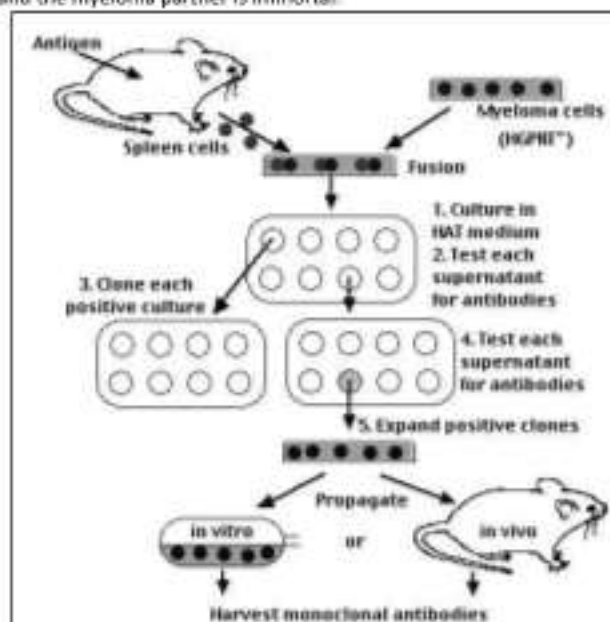
- lost the ability to synthesize any antibody molecules of their own (so as not to produce a hybridoma producing two kinds of antibody molecules).

1. The first property is exploited by transferring the cell fusion mixture to a culture medium - called **HAT medium** because it contains:

- hypoxanthine
- aminopterin
- the pyrimidine thymidine

### The logic:

- Unfused myeloma cells cannot grow because they lack HGPRT.
- Unfused normal spleen cells cannot grow indefinitely because of their limited life span. However,
- Hybridoma cells (produced by successful fusions) are able to grow indefinitely because the spleen cell partner supplies HGPRT and the myeloma partner is immortal.



2. Test the supernatants from each culture to find those producing the desired antibody.

3. Because the original cultures may have been started with more than one hybridoma cell, you must now isolate single cells from each antibody-positive culture and subculture them.
4. Again, test each supernatant for the desired antibodies. Each positive subculture having been started from a single cell - represents a clone and its antibodies are monoclonal. That is, each culture secretes a single kind of antibody molecule directed against a single determinant on a preselected antigen.
5. Scale up the size of the cultures of the successful clones.

Hybridoma cultures can be maintained indefinitely:

- *In vitro*; that is, in culture vessels. The yield runs from 10-60 µg/ml.
- *In vivo*; i.e., growing in mice. Here the antibody concentration in the serum and other body fluids can reach 1-10 mg/ml. [When the hybridoma cells are injected in mice (in the peritoneal cavity, the gut), they produce tumors containing an antibody-rich fluid called ascites fluid.]

**Catalytic Antibodies:**

Antibodies have been selected for their affinity for the antigen rather than for the transition state of any reaction the antigen might undergo. If the immunogen were a transition state or a transition-state analogue, however, antibodies should catalyze the appropriate reaction. If this were the case, it should be possible to make antibodies with catalytic activity to order.

Monoclonal antibodies directed against various transition-state analogues have been found to have some of the expected catalytic activities and specificities. Such antibodies have many of the characteristics of enzymes in that they accelerate reactions up to  $10^5$ -fold over the noncatalyzed rate, show comparable substrate specificities, exhibit a Michaelis  $K_m$  values for substrates, and are subject to competitive inhibition. The catalytic activities of the antibodies generated thus far are still lower than of natural enzymes, however, probably as a result of deficiencies in the way the analogues mimic the true transition states.

Other approaches can be taken to generate catalytic antibodies. Bisubstrate inhibitors can generate antibodies that bind the two individual substrates and enhance reaction between them simply due to their proximity in the antibody combining sites. Reactive groups can be generated in combining sites by using the appropriate immunogen, by mutation of the antibody gene, or by chemical modification of the antibodies.

## CHAPTER: 18 ANTIGEN-ANTIBODY INTERACTIONS

**I. IN VITRO ANTIGEN-ANTIBODY REACTIONS** (i.e., serologic reactions) provide methods for the diagnosis of disease and for the identification and quantitation of antigens and antibodies.

**A.** The titer, or level, of antibody in serum can be measured by using known antigens, and such titers can be of diagnostic and prognostic importance (e.g., a rise in antibody titer between acute and convalescent serum can be diagnostic for a specific disease). The titer of an antiserum usually is obtained by determining the greatest dilution of serum that reacts with the antigen.

**B. The forces involved in antigen-antibody interactions are profoundly affected by various environmental factors.** The antigen-antibody complex is not bound firmly together; it may even dissociate spontaneously. However, the equilibrium is far to the right, with a very large association constant ( $K_d$ ) of  $10^6$  to  $10^9$ .

1. Physiologic pH and salt concentration promote optimal union. Forces of attraction tend to be weaker in very acidic (i.e., below pH 4.0) and alkaline (i.e., above pH 10.0) conditions. High salt concentrations also can inhibit the interaction between an antigen and its homologous antibody.

2. Temperature also plays an important role: The higher the temperature (up to a maximum of 50°-55°C), the more rapid is the rate of reaction. This is due to the increase in kinetic motion of the reactants.

**C. Various forces act to hold the antigen-antibody complex together.** The maximum attractive forces stabilizing antigen-antibody complexes are Van der Waals forces and ionic bonds.

**Van der Waals forces** occur because of spatial fit. These forces hold antigen to antibody when the two molecules have complementary shapes. When the molecules have less similar shapes, these forces are less effective.

2. **Ionic bonds** (also called coulombic forces) are patterns of complementary electrical charge on the molecule. The electrostatic interactions tend to hold the molecules together.

**D. Affinity.** The strength of the attraction between a single epitope and its matching paratope (the antigen-binding site on the antibody molecule) is referred to as the affinity of the reaction between the two reactants. Antigen-antibody complexes of low affinity dissociate readily.

**E. Avidity** refers to the strength of the interaction between multivalent antigens and the population of antibodies that they have induced. **Avidity is influenced by the affinity of individual antibodies for their epitopes, the valence of the antigen, and the valence of the antibodies.**

**F. Studies using synthetic polypeptides have shown that only those amino acids that are spatially accessible because of tertiary protein structure are immunoreactive.**

1. Proteins can exist as globular or fibrous proteins or mixtures of both; the nature of the structure is important.

2. The ability of antibody to bind to antigenic sites can be affected by altering the tertiary structure.

a. The antigenic sites then would no longer be spatially arranged in such a way that antibody-antigen coupling could occur.

**b. Insulin molecules provide an illustration.**

► Insulin is composed of A and B chains. Antibody to either one of these chains can be produced by splitting the chains, purifying them, and injecting them into a foreign host (e.g., a pig). The host produces antibody to the particular chain that was injected.

► If the host's (i.e., the pig's) antibodies are injected back into the animal species that supplied the original insulin (i.e., man), the antibodies will not react with intact insulin molecules.

► The tertiary structure of native insulin is such that the epitopes on the A and B chains are not accessible.

**G. The physical state of the antigen** is responsible, in general, for the identification of antigen-antibody reactions and the naming of antibodies. The same antibody molecule could, in fact, be described by each of the following terms.

1. **Agglutinins** are antibodies that aggregate cellular antigens.

2. **Lysins** are antibodies that cause dissolution of cell membranes.

3. **Precipitins** are antibodies that form precipitates with soluble antigens.

4. **Antitoxins** are antibodies that neutralize toxins.

### PRECIPITATION REACTIONS

► The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops into a visible precipitate. Antibodies that thus aggregate soluble antigens are called precipitins.

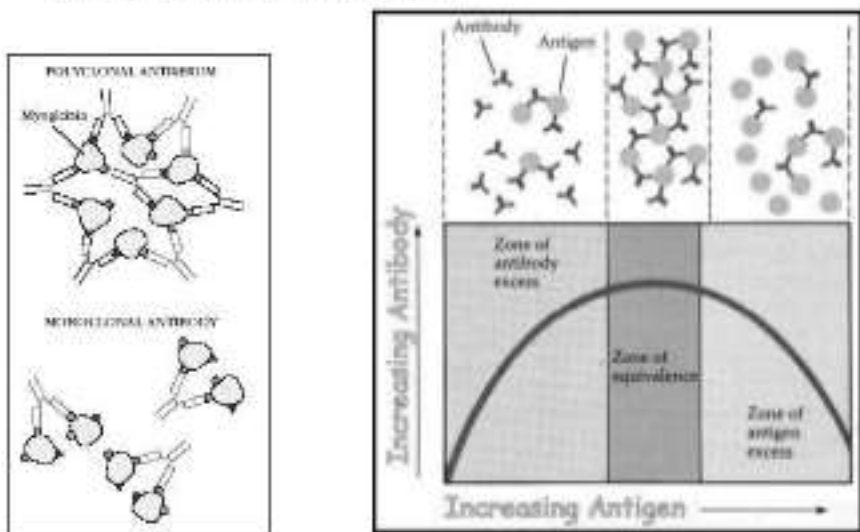
► The precipitate develops as neighboring antibody molecules within the lattice form ionic bonds with each other, causing the lattice to lose its charge and thus become insoluble.

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 either be 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.

#### Precipitation reactions.

- (a) **Polyclonal antibodies can form lattices, or large aggregates, that precipitate out of solution.** However, if each antigen molecule contains only a single epitope recognized by a given monoclonal antibody, the antibody can link only two molecules of antigen and no precipitate is formed.
- (b) **A precipitation curve for a system of one antigen and its antibodies.** This plot of the amount of antibody precipitated versus increasing antigen concentrations (at constant total antibody) reveals three zones: a zone of antibody excess, in which precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant; an equivalence zone of maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant; and a zone of antigen excess in which precipitation is inhibited and antigen not bound to antibody can be detected in the supernatant.



#### PRECIPITATION REACTIONS IN FLUIDS

► A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. After the precipitate forms, each tube is centrifuged to pellet the precipitate, the supernatant is poured off, and the amount of precipitate is measured.

► Plotting the amount of precipitate against increasing antigen concentrations yields a precipitin curve. Excess of either antibody or antigen interferes with maximal precipitation, which occurs in the so-called equivalence zone, when the ratio of antibody to antigen is optimal. As a large multimolecular lattice is formed at equivalence, the complex increases in size and precipitates out of solution.

► In the region of antibody excess, unreacted antibody is found in the supernatant along with small soluble complexes consisting of multiple molecules of antibody bound to a single molecule of antigen.

► In the region of antigen excess, unreacted antigen can be detected and small complex are again observed, this time consisting of one or two molecules of antigen bound to a single molecule of antibody.

#### PRECIPITATION REACTIONS IN GELS

##### RADIAL IMMUNODIFFUSION (MANCINI METHOD)

► The relative concentrations of an antigen can be determined by a simple quantitative assay in which an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum. As the antigen diffuses into the agar, the region of equivalence is established and a ring of precipitation forms around the well. The area of the precipitin ring is proportional to the concentration of antigen.

# CHAPTER: 11

## PATTERNING AND MORPHOGENESIS IN LIMB DEVELOPMENT

### Limb organizing centers

The limb has three organizing centers - the apical ectodermal ridge (AER), the zone of polarizing activity (ZPA) and the dorsal ectoderm - that help to pattern the proximodistal, anteroposterior and dorsoventral limb axes, respectively. As well as patterning the axes, these organizers also interact with each other to maintain limb outgrowth.

### Specifying the proximodistal axes

The proximo-distal limb axis emerges progressively as mesenchyme cells drop out of the progress zone and differentiate. The type of structure formed by these differentiating cells may depend on the amount of time they have spent in the progress zone, and hence the number of division cycles they have undergone in response to signaling from the AER.

### Specifying the anteroposterior axis

The anteroposterior limb axis is specified by the zone of polarizing activity in the posterior mesenchyme, as grafting this region to the anterior side of the limb bud can induce duplication and mirror image reversal of the axis. The secreted protein Sonic hedgehog can substitute for the activity of the ZPA and appears to act in a dose-dependent manner to specify the fates of anteroposterior structures, such as the different digits of the hand.

### Positional values along the proximodistal and anteroposterior axes

Along both the anteroposterior and proximodistal limb axes, the 5' *HoxA* and *HoxD* genes are expressed in overlapping concentric patterns. These *Hox* genes play an important role in the regional specification of different skeletal elements along the two axes, as gene knockouts cause the deletion or respecification of particular limb structures.

### Specifying the dorsoventral axis

The dorsoventral limb axis is specified by the secreted protein *Wnt7a*, which is synthesized in the dorsal ectoderm. This activates a transcription factor called *Lmx1* in the dorsal mesenchyme. The inactivation of either gene generates biventral limbs, while overexpression throughout the limb generates bidorsal limbs.

### Interaction and dependence in axis patterning

There is a significant interaction and interdependence between the three signaling pathways. The AER is maintained by Sonic hedgehog secreted by the ZPA. The maintenance of the ZPA is dependent on both FGFs secreted by the AER and *Wnt7a* secreted by the dorsal ectoderm. Furthermore, FGFs secreted by the AER are required in addition to Sonic hedgehog to establish the nested pattern of *HoxD* gene expression along the anteroposterior axis.

### Other patterning influences

There is evidence that the limb is pre-patterned before the induction of the three signaling centers, as the absence of the AER and ZPA in certain mutants does not prevent the regionalized expression of asymmetrically expressed genes, such as

those of the *HoxD* cluster. Furthermore, there is evidence that the limb has significant self-organizing ability, as shown by the development of recognizable digits in disaggregated and recombined limb buds.

#### **Morphogenesis in the limb**

The final structure of the limb depends largely on the regulation of cell death, controlled by the opposing activities of BMPs (which promote apoptosis) and BMP antagonists (which inhibit it). Complementary patterns of BMPs and their antagonists are seen in the limb, marking the interdigital and internal necrotic zones that separate the digits and the two bones of the zeugopodium, and the positions where joints will form.

#### **The vertebrate Hox genes**

Vertebrate genomes contain four copies of the *Drosophila* homeotic complex, designated Hox-A, Hox-B, Hox-C and Hox-D. Although there are significant differences between the vertebrate and fly complexes, the similarities are remarkable. The same types of homeobox gene are present, allowing classification into 13 cognate groups (paralogous subgroups) based on homeobox structure. Further more, the genes are arranged in more or less the same order along the chromosome and are expressed, in a similar manner, with the most 3' genes expressed in the most anterior domains and the most 5' genes in the most posterior domains. Since the divergence of *Drosophila* and vertebrates, the fly HOM-C has been split into two subcomplexes, whilst the vertebrate cluster has undergone a 5' end expansion and has been duplicated in its entirety to generate four complexes. Each of the four complexes has suffered individual losses, which may be different between species. For instance, the hatched boxes in the above figure represent Hox-C genes present in humans but missing in mice.

► The similarity between the *Drosophila* and vertebrate homeobox-containing genes, in terms of both structure and expression patterns is strong evidence for a conserved function. This has been confirmed by the use of cloned human HOX genes to rescue *Drosophila* homeotic mutants, and targeted disruptions of mouse Hox genes do indeed generate partial homeotic transformations. Deletion of the *Hoxc-8* gene, for instance, results in transformation of lumbar vertebrae into vertebrae with a more anterior characteristic (in this case, a thoracic vertebra, complete with a rib). The Paralogous Hox genes appear to have overlapping but nonidentical functions and may cooperate with each other in certain cases. Individual gene knock-outs of genes in paralogous subgroup 3, for instance, cause different types of disturbances to the structures of the neck, but when combined in the same mouse, severe defects are observed including missing vertebrae.

# CHAPTER: 12

## REGENERATION

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Regeneration is of three types:

### 1. Physiological Regeneration

There is a constant loss of many kinds of cells due to wear and tear caused by day-to-day activities. The replacement of these cells is known as physiological regeneration.

Example:

⇒ Replacement of R.B.C's

The worn out R.B.C's are deposited in the spleen and new R.B.C's regularly produced from the bone marrow cells, since the life span of R.B.C's is only 120 days.

⇒ Replacement of Epidermal Cells of the Skin

The cells from the outer layers of epidermis are regularly peeled off by wear and tear. These are constantly being replaced by new cells added by the malpighian layer of the skin.

### 2. Reparative Regeneration

This is the replacement of lost parts or repair of damaged body organs. In this type of regeneration, wound is repaired or closed by the expansion of the adjoining epidermis over the wound.

Example:

⇒ Regeneration of limbs in salamanders

⇒ Regeneration of lost tail in lizard

⇒ Healing of wound

⇒ Replacement of damaged cells.

### 3. Autotomy

In some animals like starfish, some part of the body is broken off on being threatened by a predator. This phenomenon of self-mutilation of the body is called autotomy.

Example:

⇒ Crabs break off their leg on approaching of the enemy

⇒ Holothurians throw off their internal viscera

⇒ Starfish breaks off an arm

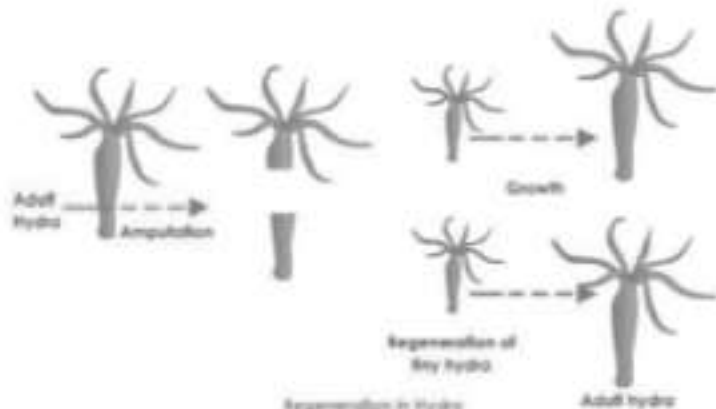
### Regenerative capacity in Animal Group

The capacity of regeneration varies in its extent in various animal groups. Regenerative capacity is very high among the protozoan, sponges and coelenterates.

#### Invertebrates

- In sponges, the entire body can be reconstructed from isolated body cells. The cells rearrange and reorganize to form bilayered sponge body wall.
- Regeneration was first discovered in hydra by Tremble (1740). Even 1/1000th part of the body regenerate into new organisms.
- In hydra and planaria, small fragments of the body can give rise to a whole animal. When a hydra or a planaria is cut into many pieces, each individual part regenerates into a whole individual.

- Some annelids like earthworms are able to regenerate some segments removed from the anterior and posterior ends of the body.
- Some molluscs can regenerate only the eyes and heads while squids can also regenerate their arms.



- Many arthropods (e.g., spiders, crustaceans, insect larvae, etc) can regenerate limbs only. Regeneration is faster in the young than in the adults. Regenerated part may not always be similar to the part lost. This type of regeneration is called heteromorphosis.
- Echinoderms (like starfish, brittle star, sea lily) exhibit autotomy. They can regenerate arms and parts of the body.
- **Vertebrates**
- **Fishes:** Lamprey can regenerate its lost tail. Some fishes have the ability to regenerate parts of its fins.
- **Amphibians:** The regeneration power is well marked in urodel amphibians like salamanders, newts and their axoloti larvae. They can regenerate limbs, tail, external gills, jaws, parts of eye like lens and retina. Tail and limb regeneration is found in the larval stages of frogs and toads.
- **Reptiles:** Lizards exhibit autotomy. When threatened, the lizard detaches its tail near the base to confuse its predator and later regenerates a new tail. The new tail differs from the old one in its shape, absence of vertebrae and the kind of scales covering it.
- **Birds:** Regeneration is restricted to parts of the beak.
- **Mammals:** Regeneration is restricted to tissues only. External parts are not regenerated. Skin and skeletal tissues possess great power of regeneration. The liver has the maximum capacity of regeneration. If one kidney is damaged or removed, the other enlarges to compensate the lost kidney. This is called as compensatory hypertrophy.
- Regeneration is an usual form of asexual reproduction in several lower groups of animals.

### Three Types of Regeneration based on Cellular Mechanism

1. The first mechanism involves the dedifferentiation of adult structures to form an undifferentiated mass of cells that then becomes respecified. This type of regeneration is called **epimorphosis** and is characteristic of regenerating limbs. 2. The second mechanism is called **morphallaxis**. Here, regeneration occurs through the re-patterning of existing tissues, and there is little new growth. Such regeneration is seen in hydras. 3. A third type of regeneration is an intermediate type, and can be thought of as **compensatory regeneration**. Here, the cells divide, but maintain their differentiated functions. They produce cells similar to themselves and do not form a mass of undifferentiated tissue. This type of regeneration is characteristic of the mammalian liver.

#### 1. Epimorphosis:

In contrast to morphallaxis, epimorphosis requires active cellular proliferation prior to the replacement of the lost body part.

Epimorphosis can be further subdivided into dedifferentiation-dependent and dedifferentiation-independent subclasses. Planarian, which are flatworms, regenerate using a dedifferentiation-independent mechanism in which preexisting stem cells, known as neoblasts, begin to proliferate and migrate to the injured site in response to injury.

These cells then form a mass of proliferating cells, known as the **regeneration blastema**, that will later differentiate into the specialized cells that comprise the regenerated structure.

Most tissue regeneration in mammals also belongs to the dedifferentiation-independent subclass. For example, mammals can regenerate their muscle, bone, epithelia of the skin and gut, blood, and some neurons by activating preexisting stem cells or progenitor cells.

Vertebrate limb regeneration involves cell dedifferentiation and growth.

#### Regeneration of a Limb of a Newt

When an adult salamander limb is amputated, the remaining cells are able to reconstruct a complete limb, with all its differentiated cells arranged in the proper order. In other words, the new cells construct only the missing structures and no more. For example, when a wrist is amputated, the salamander forms a new wrist and not a new elbow.

⇒ Upon limb amputation, a plasma clot forms, and within 6 to 12 hours, epidermal cells from the remaining stump migrate to cover the wound surface, forming the **wound epidermis**. This single-layered structure is required for the regeneration of the limb, and it proliferates to form the **apical ectodermal cap**. Thus, in contrast to wound healing in mammals, no scar forms, and the dermis does not move with the epidermis to cover the site of amputation. The nerves innervating the limb degenerate for a short distance proximal to the plane of amputation.

⇒ During the next 4 days, the cells beneath the developing cap undergo a dramatic dedifferentiation: bone cells, cartilage cells, fibroblasts, myocytes, and neural cells lose their differentiated characteristics and become detached from one another. The formerly well-structured limb region at the cut edge of the stump thus forms a proliferating mass of indistinguishable, dedifferentiated cells just beneath the apical ectodermal cap. This dedifferentiated cell mass is called the **regeneration blastema**. These cells will continue to proliferate, and will eventually redifferentiate to form the new structures of the limb.

⇒ The creation of the blastema depends upon the formation of single, mononucleated cells. It is probable that the macrophages that are released into the wound site secrete metallo-proteinases that digest the extracellular matrices holding epithelial cells together. The proliferation of the salamander limb regeneration blastema is dependent on the presence of nerves; a minimum number of nerve fibers must be present for regeneration to take place. It is thought that the neurons release mitosis-stimulating factors that increase the proliferation of the blastema cells.

⇒ **Glial growth factor (GGF)** is known to be produced by newt neural cells, is present in the blastema, and is lost upon denervation. When this peptide is added to a denervated blastema, the mitotically arrested cells are able to divide again.

⇒ **A fibroblast growth factor** may also be involved. FGFs infused into denervated blastemas are able to restore mitosis.

⇒ Another important neural agent necessary for cell cycling is **transferrin**, an iron-transport protein that is necessary for mitosis in all dividing cells (since ribonucleotide reductase, the rate-limiting enzyme of DNA synthesis, requires a ferric ion in its active site). When a hindlimb is severed, the sciatic nerve transports transferrin along the axon and releases large quantities of this protein into the blastema. Neural extracts and transferrin are both able to stimulate cell division in denervated limbs, and chelation of ferric ions from a neural extract abolishes its mitotic activity. Regeneration blastema resembles in many ways the progress zone of the developing limb.

⇒ The dorsal-ventral and anterior-posterior axes between the stump and the regenerating tissue are conserved, and cellular and molecular studies have confirmed that the patterning mechanisms of developing and regenerating limbs are

**Oblique Muscles:**

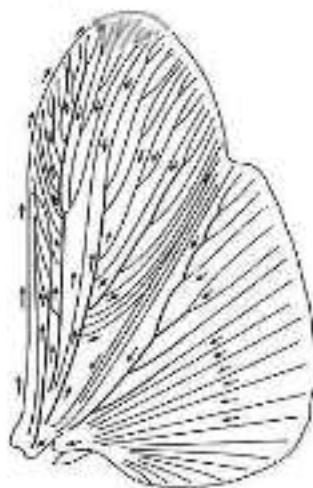
- These extrinsic muscles originate in the mid-ventral line of the body wall and are inserted dorsally and ventrally onto the parapodium.
- Typically, there are two pairs of these muscles in each segment, one pair anterior and one posterior.
- These are so arranged that they affect the anterior and posterior beat of parapodium.
- Moreover, contraction of these muscles cause retraction of parapodia.

**Intrinsic Muscles:**

- Intrinsic protractor and retractor muscles are responsible for the protrusion and withdrawal of the chaetae bundles and their supporting acicula.
- Besides these muscles, there are well developed acicular muscles that remain attached to the inner end of the acicula and inserted into the parapodial wall.
- The parapodium in *Neanthes* is clearly quite versatile in its mode of action.
- The development of extrinsic and intrinsic musculature fore-shadow a type of movement in which, as in arthropods, a balanced system of antagonistic muscles operate upon a hydrostatic skeleton.
- These properties of musculature and parapodia are so integrated that it enables *Neanthes* to enjoy diverse habitats.

**Locomotion in Cockroach with the Help of Wings:**

Cockroaches (*Periplaneta americana*), the pterygotan insect, are fast runners. Their ability to walk rather than run and fly rapidly is regarded as the defensive mechanism. They rarely resort to flight and can fly for a short duration with the help of hind wings.

**Description of Wings:**

- In cockroaches, the wings are modification of exoskeleton.
- In *P. americana*, two pairs of reddish brown coloured wings are present; one pair situated on the mesothorax and the other pair on the metathorax.
- The wings reach up to the tip of the abdomen in the females and a little beyond it in the males.
- The leathery mesothoracic forewings are not used in flight but serve to protect the hind wings at rest.
- They are hence called wing cover or tegmina or elytra. The hind wings are thin, membranous, transparent and delicate, and used in flight.
- Each wing is composed of two membranous layers of cuticle enclosing tubular tracheae. The chitin thickens around the tracheae and haemocoelomic space to form nervures or veins.
- These veins form effective supporting skeletal rods for the wings.
- In the mesothorax, the scutum on each side bears one anterior and one posterior tar- gal process for the articulation of the forewings.
- In metathorax, similar structures are also present on the lateral side of the scutum for the articulation of the hind wings.
- In cockroaches, direct muscles inserted on the basal sclerites of the wings are responsible mainly for the tilting or feathering of the wings.

- In males, the wing muscles are opaque and pink but in females, these muscles are hyaline and white.

#### Circulation in Wings:

- The haemolymph flows between the tracheae and the wall of the veins.
- It enters the wing by the costa as a rule and returns to the body by the posterior margin, following a fairly constant path along the largest channels in the wing.
- This circulation is necessary for the normal sclerotization of the wing and for maintaining the wings in a healthy condition.
- The parts of wing deprived of circulating haemolymph become dry, brittle and may often crack away.
- **Muscles:-** The muscles of wing fall into two classes;
  - (i) Direct muscles.
  - (ii) Indirect muscles.

**Direct Muscles:-** These muscles are inserted into the base of the wing basalar and subalar sclerites which are connected to the axillary sclerites by ligaments (Fig. 1) lateral view of the thorax showing the direct wing muscles.

These are of three types.

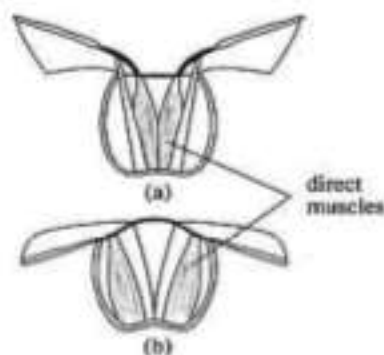
- (i) Flexor muscle arising on the pronotum and inserted into the third axillary sclerite (flexes the wing backward)
- (ii) Basalar Extensor/levator muscles arise from coxisternal sternum and coxae are inserted into the basalar. These cause extension of the wing from the flexed position.
- (iii) Subalar Depressor arising on the mesonotum is inserted into the subalar. These muscles extend and depress the wings.

**Indirect muscles :-** These are not directly associated with the wings, but move the wings as a result of the distortions which they produce in the shape of the thorax.

These are of three types :

1. Dorsolongitudinal muscles runs dorsally and longitudinally between the mesothorax and metathorax.
2. Dorsoventral/Tergosternal muscles run dorsoventrally between tergum and sternum from post phragma.
3. Oblique dorsal muscles are well developed only in Diptera and Cicadidae but absent altogether, often small in others.

#### Mechanism of Flight:



In cockroaches, the two pair of wings beat independently of each other, but the hind wings operate in the air turbulence created by the forewings.

During flight, each hind wing articulates with the edge of the tergum, but its inner end rests on a dorsal pleural process which act as a fulcrum

- Upward movement of the wings results indirectly from the contraction of vertical muscles within the thorax, depressing the tergum.
- Downward movement of the wings is produced directly, by contraction of muscles attached to the wing base.

- Thus, the flight of cockroach is direct. However, during flight, up and down movement alone is not sufficient for flight.
- The wings must at the same time be moved forward and backward. During a complete cycle of a single beat, wings are held at different angles to provide both lift and forward thrust.
- This insect can also achieve lift by raising and lowering wing veins, thereby changing the wing shape or contour.
- The raising or lowering of the wings results from the contraction of direct flight muscles attached to the base of wings.
- A very slight decrease in muscle length during contraction can bring about a large movement of the wings. The elastic nature of the thoracic skeleton and the joints of wing articulation also contribute to the beat motion.

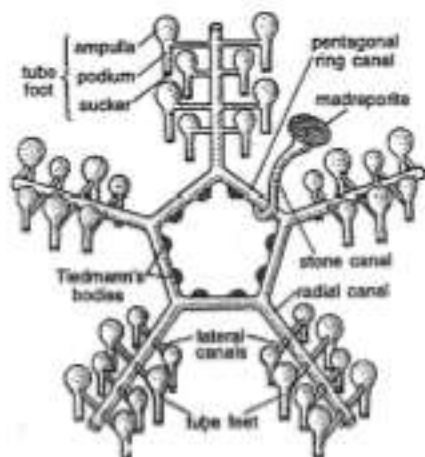
#### Control of Flight:

In cockroaches, there is no flight control centre in the nervous system. But the eyes and sensory receptors on the antennae, head and wings provide continual feedback information for flight control.

#### Locomotion in Pila:

- The foot of Pila helps in locomotion. The fat sole of the foot helps Pila to move very slowly by creeping on the substratum.
- During movement the foot is protruded through the opening of the shell and this extension is brought about by the sudden influx of blood into it.
- The glands present in the foot produce a slimy secretion that helps the animal to glide on dry surface.
- The foot is provided with vertical, longitudinal and transverse muscles.
- During locomotion the wave-like contractions on its surface are produced by the contraction of the vertical muscles.
- The contraction of the transverse muscles drives the blood forward which causes the extension of the foot in front. During this process the longitudinal muscles contract to pull the posterior end of the foot forward.

#### Echinoderms



Echinoderms or star fishes belong to a well characterized phylum in which hydrostatic principle has evolved perfectly for locomotion and feeding.

The principles involved in the use of tube feet for locomotion and prey capturing have been well studied in Asterias. A peculiar hydrostatic pressure mechanism is found in star fishes, which is known as water vascular or Ambulacral system, used for locomotion and prey capturing. This system is composed of following structures.

**(a) Madreporite :** It is a rounded, thick and sieve like calcareous plate, which is situated on the aboral surface on the central disc of star fishes. It possesses numerous fine radiating furrows having more or less 250 small bones and each pore leads into a small pore canal. All the pore canals unite to form larger collecting canal within the madreporite. All the collecting canals finally lead into a sac like ampulla below the madreporite. The ampulla is continued into a stone canal.

**(b) Stone canal :** This canal is also known as madreporic canal, which is a S-shaped tube like structure which opens into ring canal on the oral side. The wall of stone canal is supported by a series of calcareous rings, to provide it strength, hence it is known as stone canal. The cells lining the stone canal are provided by cilia or flagella which create water current to draw water in it. In the beginning or in young star fishes the stone canal is a simple tube but later on, two spirally coiled lamellae develop within its lumen.

**(c) Ring canal :** Ring canal is a wide ring like canal around the oesophagus. It is pentagonal in Asterias and each angle of pentagon lies in a radial position.

**(d) Tiedmann's bodies :** Also known as racemose glands, are small, rounded and glandular sac like structures, opening into ring canal. They are nine in number and the position of 10th is occupied by the stone canal, which opens into the ring canal. Each Tiedmann's body is consisted of outer peritomeum enclosing a stroma of connective tissue and some muscle fibres along with numerous tubules. Exact role of Tiedmann's bodies is still unknown, however according to some workers they are associated with filtration, while according to others these are mucous forming bodies or as lymphatic glands, manufacturing phagocytes, which are released into the water streams.

**(e) Foliar vesicles :** Foliar vesicles are thin walled contractile structures. They are located at each interradius and opening on outer surface of the ring canal. It is supposed that they store water and help in regulating water pressure. Foliar vesicles are absent in Asterias (Family : Asteridae).

**(f) Radial canal :** From the ring canal there arises a radial canal along each radius, which extends up to tip of corresponding arm. Each radial canal runs in the ambulacral groove and terminates as lumen the terminal tentacle.

**(g) Lateral canals :** In each arm the radial canal gives out two series of lateral or podial canals along its entire length. The lateral canals of two series are short and long alternately in such a way that a short canal has a long canal on its outer and inner side, but only a short canal on its opposite side. Each lateral canal opens into a tube foot.

**(h) Tube Feet :** As each lateral canal opens into a tube foot, there are two double rows of tube feet with respect to each series of alternately present short and long lateral canal. Each tube foot consists of three regions – viz a rounded sac-like ampulla, a middle tubular podium and a cup-like sucker at the terminal lower end of the podium.

Each podium is present above the ambulacral ossicle, and projecting into the coelom. The tubular podium extends through the ambulacral groove. The walls of the tube foot contains longitudinal muscles while the wall of the ampulla contains circular muscles.

The most peculiar function of this systems is locomotion by providing a hydraulic pressure mechanism. Tube feet also help in capturing prey. Thin wall of tube feet may also help in respiration by exchanging gases.

## Chapter 3 Types of nutrition in Invertebrates

Many types of nutrition are found in invertebrates:

**1. Holophytic nutrition** : this is a autotrophic (self feeding) type of nutrition in which animals synthesize the food by photosynthesis. Energy is obtained from the sun to synthesize the food. **This method is also called autotrophic phototrophy.**

**2. Holozoic nutrition** : most of invertebrates obtain their food by eating fully or parts of other organisms (plants or animals). Such mode of nutrition is called holozoic. **This is also named as heterotrophic (others feeding) mode of nutrition.**

**3. Parasitic nutrition** : this also a type of heterotrophic nutrition but very specialized in its operation. These organisms obtain their food from other living organisms by causing up or little harm to their host organisms.

There are two types of parasites: **ectoparasites** that live external y on the body of their host organisms to derive food, while **endoparasites** live inside the body of the host organism.

**4. Saprozoic nutrition** : this heterotrophic mode of nutrition involves absorption of food by osmosis, meaning through the body surface. **This is also called osmotrophy.**

Food consists largely of solution of organic matter processed by decomposing bacteria.

**5. Mixotrophic nutrition** : in this mode of nutrition, an animal adopts a combination of more than one mode of nutrition. For example, autotrophy is combined with phagotrophy or osmotrophy or parasitism.

### Digestion in Invertebrates

The process of physiology of digestion involves three basic steps

1. Ingestion (intake of food),
2. digestion (enzymatic breakdown of food stuff) and
3. egestion (discarding undigested part of food).

The details of the physiology differs in the invertebrates depending upon the mode of nutrition and type of food eaten.

If the animal is eating big pieces or whole of the organism (as food) then it will cut and masticate the food before digestion and have apparatus for food mastication and grinding.

If the animals is feeding upon small, minute particles of food then above is not needed.

Again if it is osmotroph or parasite absorbing pre digested food, then there no need of food digestion at all.

The general plan of digestion is :

- proteins (of food material) are digested in acidic medium (pH 4-6) under the action of proteolytic enzymes (proteases, for example zymase).
- Carbohydrates are digested in alkaline medium (pH above 7) under the action of amylolytic enzymes (amylases, ex. amylase). Lipid is digested by lipases.
- However, certain invertebrates only are reported to have the capacity to digest fats/lipids. It is also reported that mostly fat/lipid is egested undigested. Details of digestion differ in all the phyla and individual species.

## Chapter 6 Physiology of Excretion

**Excretion:** The process of removal of waste material from the body is termed as Excretion. Waste products are like urea, uric acid  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , Excess salt etc.

**Body has two types of fluids:**

1. **Intracellular fluid** - also called the cytoplasm.

2. **Extracellular fluid** = (ECF) This fluid found outside the cells like - tissue fluid Blood-plasma, Body cavity fluid, cerebrospinal fluid, Pleural fluid etc. Max. **Quantity of ECF is in the form of blood plasma.**

Claude - Bernard called the ECF as Milieu Interior or the internal environment of the body.

Baird Hastings called the cells of the body as islands and ECF as the internal - Sea.

► There is a continuous exchange of materials between ECF and intracellular fluid e.g. From the ECF Oxygen and many nutrients go into the ICF and many waste materials enter the ECF from the ICF. For the continuous exchange of materials between the ECF and ICF, maintenance of chemical composition of ECF is a must, this process is termed as **Homeostasis. The name Homeostasis was given by Walter-Cannon.**

⇒ Excretory organs help in maintaining the chemical-composition of ECF. They continuously remove the waste-materials formed during metabolism from the ECF, so excretory organ are also termed as **Organs of Homeostasis** -

### EXCRETORY ORGANS: FUNCTIONAL TYPES

#### Generalized excretory organs

Contractile vacuules of protozoans

Invertebrate nephridial organs

Malpighian tubules of insects

Vertebrate kidneys

#### Specialized excretory organs

Gills (crustaceans, fish)

Rectal glands (elasmobranchs)

Salt glands (reptiles, birds)

Liver (vertebrates)

Intestine (insects)

### Osmoconformers and Osmoregulators

An animal may be an osmoconformer or osmoregulator depending on how they balance water loss with water gain.

**Osmoconformers** = Animals that do not actively adjust their internal osmolarity. Most marine invertebrates are osmoconformers.

- Body fluids are isotonic to the environment.
- Body fluid composition usually differs from the external medium due to internal regulation of specific ions.
- Some vertebrates of the Class Agnatha (hagfishes) are also osmoconformers.

**Osmoregulators** = Animals that regulate internal osmolarity by discharging excess water or taking in additional water.

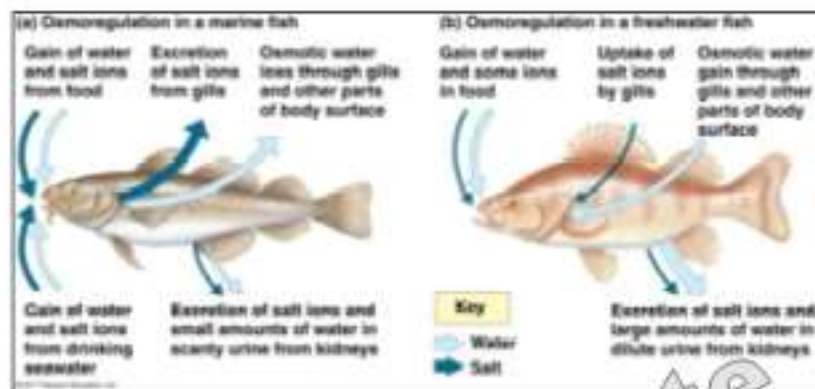
Many saltwater animals, all freshwater animals, and terrestrial animals

Net movement of water in or out, requires a concentration gradient - the maintenance of which requires energy.

Osmoregulation permits animals to live in a variety of habitats, but the tradeoff is that it requires an energy expenditure by the animal.

- ⇒ Most cartilaginous fishes, including sharks, maintain internal salt concentrations lower than sea water by pumping salt out through rectal glands and through the kidneys, yet their osmolarity is slightly hypertonic to seawater.
  - a. Sharks retain urea as a dissolved solute in the body fluids.
  - b. Sharks also produce and retain trimethylamine oxide (TMAO), which protects their proteins from denaturation by urea.
  - c. Retention of these organic solutes (urea, TMAO) in the body fluids actually makes them slightly hypertonic to seawater.

- Do not drink water, but balance osmotic uptake of water by excreting urine. Marine bony fishes are hypotonic to seawater.
- Compensate for osmotic water loss by drinking large amounts of seawater and pumping excess salt out with their gill epithelium.
- Excrete only a small amount of urine.



### TYPE OF ANIMALS ON THE BASIS OF EXCRETION

#### Ammonotelic

Animals excreting their nitrogenous waste in the form of ammonia are known as ammonotelic. Ammonia is highly soluble in water with which it forms ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) which injures cells directly by alkaline caustic action. Hence excretion of ammonia requires large amounts of water to be lost from the body. That is why such amonide is suitable for aquatic organisms which have a constant access to water.

⇒ No energy is required to produce ammonia. E.g. all aquatic invertebrates, bony fishes and aquatic amphibians.

⇒ In anurans (amphibians) the larval tadpoles excrete ammonia, while the adults produce urea.

#### Ureotelic

Animals that excrete their nitrogenous waste mainly in the form of urea are known as ureotelic.

⇒ Urea can be stored in body for considerable periods of time, and is least toxic. It is eliminated in the form of urine. Ureotelism is exhibited by semi-terrestrial animals, e.g. some earthworms, adult amphibians, elasmobranch (cartilaginous fishes) and mammals.

⇒ Frog like other amphibians is ammonotelic in tadpole state and ureotelic in mature state. Earthworm is similarly ammonotelic when sufficient water is available and ureotelic when water availability is reduced.

**Uricotelic:** Animals which excrete their nitrogenous waste mainly in the form of uric acid and urates are known as uricotelic. Terrestrial animals like insects, reptiles, and birds excrete uric acid. Uric acid ( $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$ ) (which require more energy) is produced by degradation of purines (e.g. guanine) in liver and kidneys to some extent.

⇒ In uricotelic animals, excess nitrogen is first used in synthesis of purines. A purine is changed to **xanthine** (from hypoxanthine or guanine) which is then **oxidised to uric acid**. Part of uric acid is **oxidised** further to form **allantoin** and **allantoic acid**.

⇒ Teleost fish excrete allantate or hydration product of allantoin. In most fishes and amphibians, allantate is hydrolysed to urea and glyoxylate.

**Aminotelism** is the excretion of amino acids which cannot be metabolised due to their being in excess. The animals performing aminotelism are called aminotelic, e.g. some molluscs (Pila, Unio, Limnaea) and some echinoderms (starfish, Holothuria).

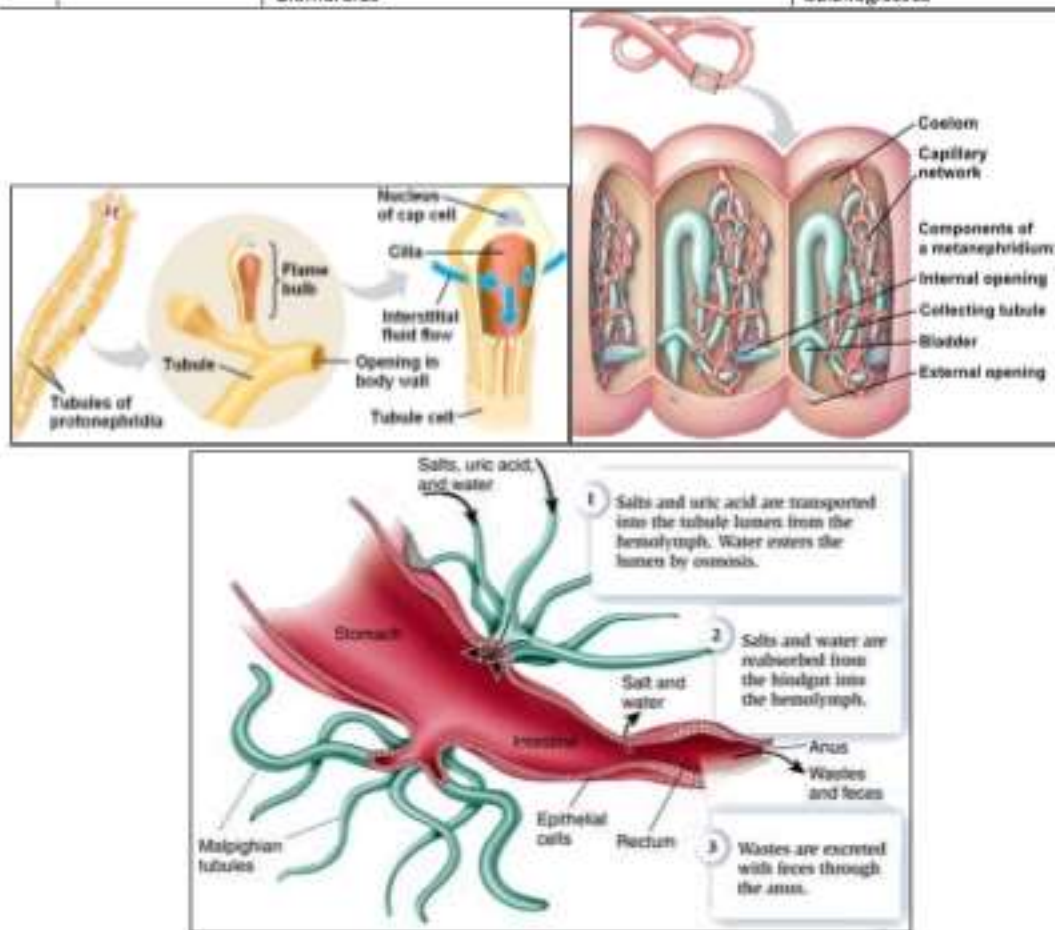
**Main Nitrogenous Waste-Materials:** Different type of animals excrete nitrogenous waste materials in various forms-like:

- Amino-acids:** Some animals excrete amino-acids. These are termed as Aminotelic e.g. Some molluscs (Unio, Limnia, Snails) Some echinoderms like Asterias.
- Ammonia:** Majority animals do the deamination of amino-acids. In this process the ammonia ( $\text{NH}_3$ ) is removed from the amino-acids. Majority aquatic animals excrete this ammonia from the body. Such animals are termed as **Ammonotelic**. Ammonia is highly soluble in water so it diffuses rapidly in water. To excrete ammonia, more amount of water is required. To excrete **1 gm of ammonia 300-500 ml. water is required**. Ammonia is highly toxic, so more amount of ammonia can't be kept inside the body.

3. **Urea:** Amphibians, Mammals and fishes of the Elasmobranchii group convert the ammonia obtained from deamination into urea. Urea is soluble in water but less soluble as compared to ammonia. Less water is required to excrete urea from the body. To excrete **1 gm of urea, 50 ml of water is required**. Urea is not toxic so some amount of urea can be circulated into the blood before excreting out. Urea is taken from the site of formation to the excretory organ through blood. In human blood 20-40 mg/100 ml urea is present. The amount of ammonia is very less or negligible i.e. 0.0001-0.0003 mg/ 100 ml of blood. This much amount of ammonia is not toxic for the body. Tadpole larva of amphibia is Ammonotelic and adult animal is Ureotelic.
4. **Uric-Acid:** Majority terrestrial animals which have a scarcity of water, convert the ammonia obtained from deamination into uric acid and excrete it in the form of uric-acid. These animals are termed as Uricotelic. Like-Reptiles, Birds and Insects. Uric acid is insoluble in water; so water is not required to excrete it. Uric-acid is excreted in the form of a paste. Uricotelism is an adaptation of the terrestrial habitat. Uric-acid is non-toxic. It is less nontoxic as compared to urea.
5. **Tri-methyl amine-oxide:** Some animals convert the ammonia into non-toxic tri-methyl amine oxide and excrete it. It has a typical fishy-smell, e.g. Marine-fishes, Marine molluscs and Marine crustaceans etc.
6. **Guanine:** Spiders convert ammonia into guanine and then excrete it. It is similar to uric acid; its structure is same as that of uric acid. It is insoluble in water. Guanine is excreted in the form of crystals. It is also an adaptation to check the water-loss.
7. **Allantoin:** Majority mammals convert the Purines and Pyrimidines to Allantoin and then excrete it. In man purines are excreted in the form of uric-acid and pyrimidines in the form of Alanine and Iso-butyric acid.
8. **Hippuric-acid:** In mammals, the Benzoic acid is excreted out in the form of Hippuric acid.  
 Benzoic acid + Glycine  $\longrightarrow$  Hippuric acid  
 But in birds, the benzoic acid is treated with Ornithine amino-acid.  
 Benzoic acid + Ornithine  $\longrightarrow$  Ornithuric acid
9. **Creatinine & Creatine:** Creatinine is the product of the breakdown of creatine. The amount of creatine excreted through the urine by an adult individual is about 1.2 to 2.7 g per 24 hours. **Creatinine** coefficient any be defined as the ratio between the amount excreted in 24 hours and the body weight in kilogram. It is commonly 20 to 26 mg per kg per 24 hours in normal man and 14 to 22 mg per kg in normal woman per 24 hours. **Creatine** is present in the urine of children and in much smaller amounts in a normal adult men. It is observed that normal males excrete about 5% of the total Creatinine output of creatine (60-150 mg/day). In females, this amount is higher than that of in males. Excretion of creatine is increased in pregnancy and is decreased in hypothyroidism. In normal urine, creatine is absent. But in newborn infants, pregnant and lactating females the urine contains creatine. Creatine is obtained in the liver from amino-acids in pathological conditions namely starvation, impaired carbohydrate metabolism, hyperthyroidism and certain myopathies creatinuria is also found.
- Creatinine:** It is formed in the muscles. In the muscles a high-energy compound called Phosphocreatinine. Creatinine is formed from its reduction. It is excreted along with urine.
10. **Oxalate:** Normal urine contains about 10-30 mg of oxalate per 24 hours. Excretion of oxalic acid is increased in diabetes, in certain liver diseases.
11. **Hippuric acid:** It is chemically benzoyl glycine. It is the detoxication products of benzoic acid with glycine. The quantity of hippuric acid excreted through the urine is about 0.7g (ranges about 0.1 to 1.0 g).
12. **Amino acids:** In adults about 150-200 mg of amino acids are excreted through the urine in 24 hours.

No.	Animals	Excretory organ	Examples
1.	Protozoans	Plasmalemma	Amoeba
2.	Porifera	General body surface	Sycon
3.	Coelenterates	General body surface	Hydra
In the above three, contractile vacuole is also there which is not really an excretory organ. It is specially for water balance & helps to get rid of extra water that diffuse into the cell.			
4.	Platyhelminthes	Flame cells (Solenocytes) Protonephridium	Taenia, planaria Larva of platyhelminth Miracidium, redia larva
5.	Aschelminthes	Renette cell (excretory cell)	Ascaris
6.	Annelida	Nephridia Chloragogen cells	Earthworm Earthworm megascolex
7.	Arthropoda	Malpighian tubules, uricose gland, Urate cells Coxal gland Green gland	Cockroach Spider, Scorpion (arachnida) Prawn (crustacea)
Special glands called rectal glands reabsorb water and ions and urine which are mixed with faeces. This is an adaptation of dry habitat.			

8.	Echinoderm	Tube feet (podia) & dermal branchia (thin walls of gills)	Starfish
9.	Protochordates	Solenocytes Neural gland Glomerulus	Amphioxus Hermatia Balanoglossus



#### LOCATION AND STRUCTURE OF KIDNEYS

A pair of kidneys is present in the dorsal part of the abdominal-cavity & lateral to vertebral column. In human being right kidney is slightly downwards than the left kidney (approx. 2.5 cm.).

⇒ In mammals, the kidneys are Bean-shaped or concavo-convex type. The small pit like structure is found on the medial surface called as hilum or hilus. From the Hilus part a ureter comes out.

⇒ Both the ureters open through separate openings into the urinary-bladder. The openings of these ureters into the bladder are oblique; so they prevent the backward flow of urine into the kidney. Bladder opens into the urethra.

⇒ Opening of urinary bladder is control by external & internal sphincter. Internal sphincter which is involuntary in nature while external sphincter voluntary nature. Normally it remains contracted; only at the time of micturition it remains relaxed. The diaphragm remains active during micturition and exerts pressure on the bladder. Urethra opens to the outside through the urinogenital aperture.

In human Male urethra is large than female urethra. It is approximately 20 cm, & divided into four parts

- Urinary urethra (preprostatic urethra).** It is 1.0-1.5 cm long part which lies between urinary bladder and point of union with ejaculatory ducts
- Prostatic urethra** - (2.5 cm.) run between prostatic lobe.
- Membranous urethra** - (2.5cm.) runs between perineal muscle.
- Penile urethra** - (15 cm.) It is largest part of male urethra & runs in corpus spongiosum part of penis & open outside in the form of external urethra orifice. While female urethra is short approx 4 cm. & open in anterior part of vaginal vestibule.

### DEVELOPMENT OF KIDNEY

During embryonic development nephrotome plate develops from mesoderm which is made up of fine tubules called **nephros**. Nephrotome develops into kidney while nephros develops into Nephrons or uriniferous tubules.

On the basis of development kidney are of 4 types:

(1) **Archinephros**: It is the basic and ancestral form. Such kidney is found today in larvae of certain cyclostomes (Myxine), but do not occur in any adult vertebrate. Glomeruli are only present in some of the posterior tubules.

(2) **Pronephric Kidney**: Pronephros the **most primitive excretory organs** that develop in vertebrate, corresponding to the first stage of kidney development. Develop from **anterior part of Nephrotome plate**. Its nephrons are in simple tubular shape. Nephrons are not differentiated.

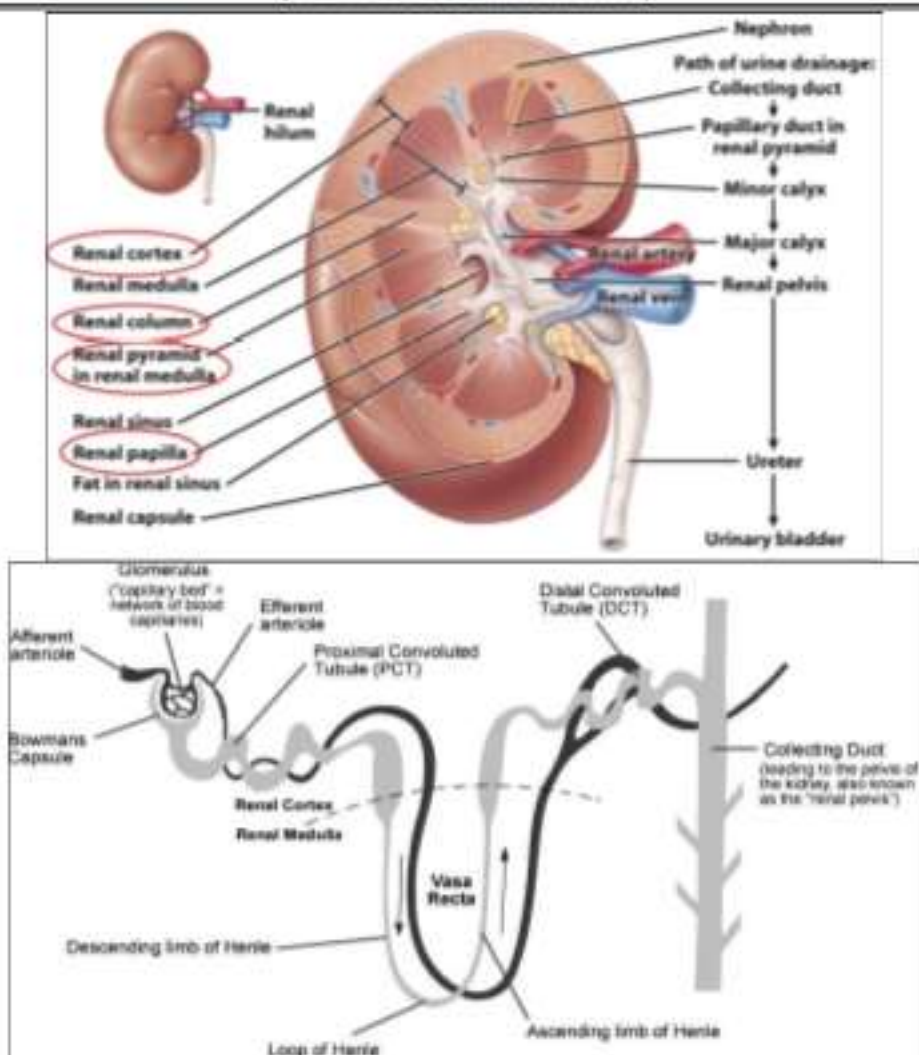
**Example**: It is present at the embryo of more advanced fish and at the larval stage of amphibians. In human beings, it is rudimentary and replaced by mesonephros after 3.5 weeks.

(3) **Mesonephric Kidney or opisthonephros**: develop from **middle part of Nephrotome plate** & remaining part of nephrotome is destroyed. Only Bowman's capsule is found in nephrons while remaining part is simple tubular.

**Example**: Most of the fishes & adult Amphibians but in reptiles, birds and mammals it is functional in embryo.

(4) **Metanephric Kidney**: Develops from **posterior part of nephrotome** while remaining part is destroyed. Nephrons are well differentiated in to Bowman's capsule PCT, DCT & loop of Henle's. **Example**: Reptile, Aves, Mammals.

### (INTERNAL STRUCTURE OF KIDNEYS)



Around each kidney, there is a coat of white fibrous connective tissue called the **Renal-capsule**. Inside of the kidney is filled with loose connective tissue. This connective tissue is divided into two - **Outer is Cortex and Inner Medulla**. Medulla is raised inside the cortex in the form of a dome shaped structure called the **Pyramid**.

➔ In each kidney of man, 8-12 pyramids are present. Projection of the cortex are embedded into medulla called as renal column of Bertini

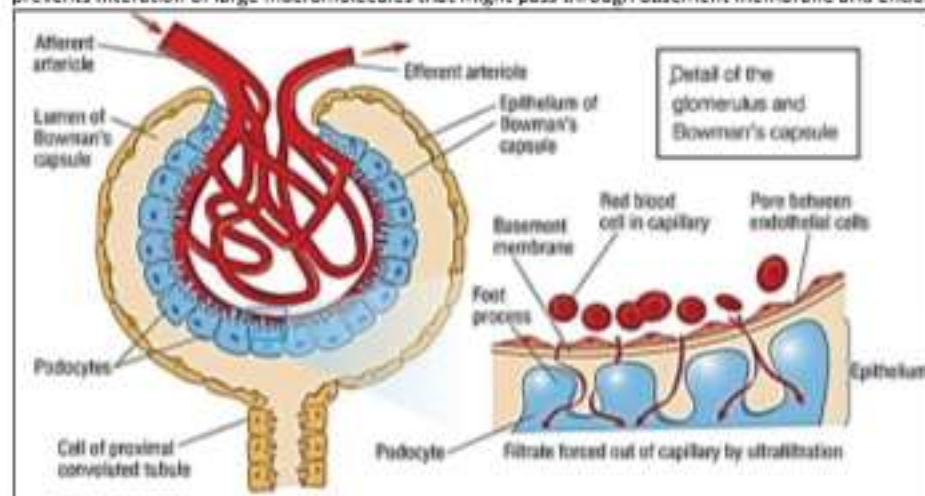
The Proximal part of the ureter is broad and funnel shaped; and is termed as the pelvis. Narrow apex of the pyramid called Renal-papilla. Their pelvis is divided into 3 or 4 branches. These branches are termed as Major calyx. Major-calyx is further divided into sub-branches called Minor-calyx. Minor-calyxes are expanded in the pyramids to collect urine.

**Minor & major calyces make the endoskeleton of the kidney**

In each kidney of man 10-12 lakh Nephrons are present. Nephron is a **functional & anatomical unit of kidney**. A nephron consists of **two parts**—an initial filtering component (the renal corpuscle) and a long tubule (**renal tubule**)—both made of simple cuboidal epithelium.

**Renal corpuscle:-**

- The renal corpuscle **filters out large solutes from the blood, delivering water and small solutes to the renal tubule for modification**. The renal corpuscle (or Malpighian corpuscle) is **composed of a glomerulus and Bowman's capsule**. Where blood is filtered to begin the process of urine formation. The nephron begins as a double-walled blind cup called **Bowman's capsule** (lined by squamous epithelium) which surrounds a network of capillaries known as **glomerulus**.
- **Glomerulus** is a capillary (fenestrated) tuft that **receives its blood supply from an afferent arteriole** of the renal circulation. Blood enters glomerular capillaries through **afferent arteriole** and leaves through **efferent arteriole**. The **diameter of afferent arteriole is much more than that of efferent arteriole**.
- Bowman's capsule is composed of **inner visceral (simple squamous epithelial cells) and outer parietal (simple squamous epithelial cells) layers**. The **visceral layer** lies just beneath the thickened glomerular basement membrane and is **made of podocytes** which send **foot processes** over the length of the glomerulus.
- **Foot processes** interdigitate with one another forming filtration slits that, in contrast to those in the glomerular endothelium, are spanned by diaphragms.
- The size of the filtration slits restricts the passage of large molecules (eg. albumin) and cells (e.g., red blood cells and platelets). As a result, the **filtrate** leaving the Bowman's capsule is **very similar to blood plasma** in composition as it passes into the proximal convoluted tubule. In addition, foot processes **have a negatively charged coat (glycocalyx)** that limits the filtration of negatively-charged molecules, such as albumin. The parietal layer of Bowman's capsule is lined by a single layer of squamous epithelium.
- **Unlike the visceral layer, the parietal layer does not function in filtration. Rather, the filtration barrier is formed by three components: the diaphragms of the filtration slits, the thick glomerular basement membrane, and the glycocalyx secreted by podocytes.**
- Podocytes are special, less flattened cells which line the concavity of Bowman's capsule. **Surface of Podocytes bear hooks that help in binding the capillaries of the glomerulus through a basement membrane**. Podocytes prevents filtration of large macromolecules that might pass through basement membrane and endothelium.



➔ After **Bowman's Capsule** the nephron is in the form of a straight tubule called the Neck of Nephron.

After the neck, the remaining part of the nephron is in the form of a highly coiled tubule, termed as the secretory-tubule.

All generic and specific epithets have authors, the name(s) of the person(s) who first officially described them in a publication. You will often see scientific names with an author's name following it. This is often confusing to non-taxonomists but is really important because it is very useful in tracing the history of applications of names through time. Scientific names with very similar spellings can usually be distinguished from one another when an author's names is included.

- *Rhinacloa pallipes* Reuter  
*Rhinacloa pallidipes* Maldonado

- **Dates of authorship**

Dates of official descriptions can also be included with scientific names to further clarify situations and locate relevant literature.

- *Macracoleus femoralis* Reuter, 1879  
*Cyrtocapsus femoralis* Reuter, 1892  
*Psallopsis femoralis* Reuter, 1901

- **Author's names in parentheses - typographical errors?**

No. If the species in question in a particular classification is in the genus in which it was described the author's name(s) do not appear in parentheses.

- *Notropis cardinalis* Mayden

However, if the species in a classification is in a genus other than the one in which it was described the author's name(s) appear in parentheses.

- *Luxilus cardinalis* (Mayden)

In the botanical literature the same applies but the author's name(s) in parentheses may be followed by a further name of the author who moved the species to its genus of current placement.

- *Cerataxomia boliviana* Brongn.  
*Zamia boliviana* (Brongn.) A. DC.

- **Different usages of the same name?**

In some instances in zoology authors may use a scientific name differently than the person (author) who originally described the species. In such a case the scientific name, as listed in catalogs and other writings, is separated from user's name by a colon.

*Phytocaris marmoratus* Blanchard  
*Phytocaris marmoratus*: Stonedahl.

## Basic Tenets of the Codes

While the codes differ in their organization and some rules the basic ideas behind all of the codes are outlined below. Each of these will be discussed in more detail below.

- **Priority**
- **Availability**
- **Typification**
- **Species-group names**
- **Genus-group names**
- **Homonymy**
- **Synonymy**

### Priority

- This is a simple concept: the first name applied to a taxon is the name that will be used.
- Often, taxonomists, systematists, ecologists, behavioral biologists, and others encounter multiple names that appear to relate to the same taxon, say a species. Which name is correct?
- While not part of his system, Linnaeus did endorse priority in principle but did not practice it.
- Such a dilemma typically is the result of early taxonomic studies where researchers were in different parts of the world or continent and were independently describing taxa without knowledge that another person was describing the same taxon.
- It can also result from researchers not fully understanding variation within a species or that the different "looking" things are different stages in a life cycle or different sexes.
- Example: **parrot fishes, aphids**
- Priority relates to date of publication or mailing date (public availability). Priority involves only date, not page or line precedence. If the day is not determinable then the accepted date is the 1st day of the smallest time unit (week, month, year) that can be determined. Older valid names have priority over newer valid names; the oldest valid name of a taxon takes precedence over all other names of a taxon. Generally this name is referred to as the senior name.
- In zoology priority extends to ranks of the Superfamily and below.
- Priority is not intended to upset stability because stability of a classification is one of the main objectives of biological classification. Thus, in instances where a name change would cause much confusion the codes provide provisions that permit the conservation of a younger and well established name. In zoology the ICZN has the power to suppress an older name and make the younger name the valid name for the taxon.
- Priority extends back to particular taxonomic works for each group of organisms. Names applied before these specified works are not considered valid names.
- The baseline priority for zoological nomenclature begins with Linnaeus' *Systema Naturae*, 10th edition, considered published 1 January 1758. Any works published in 1758 or after are considered published.
- For plants the baseline priority dates to the work of Clerck (1757).
- Baseline priority for botanical names dates to Linnaeus' *Species Plantarum* (1753)

### Simple example of priority:

#### Stoneroller

#### *Camptostoma anomalum* (Rafinesque)

*Rutilus anomalus* Rafinesque, 1820.

*Exoglossum spinicephalum* Valenciennes, 1844

*Exoglossum dubium* Kirtland, 1845

*Leuciscus prolixus* Storer, 1845

*Chondrostoma pullum* Agassiz, 1854

*Camptostoma nasutum* Girard, 1856

*Camptostoma formosulum* Girard, 1856

*Dionda plumbea* Girard, 1856

- These are all valid and available names for the same taxon (species).
- Using priority the correct name to be applied to the taxon is *Rutilus anomalus* Rafinesque but when placed in the genus *Camptostoma* the specific epithet must agree in gender with the genus. Hence, the name becomes *Camptostoma anomalum* (Rafinesque).

**More complex example of priority:**

- *Lygaeus saltitans* Fallén, 1807. *Chlamydatus* was described by Curtis (1883) and included a new species *marginatus* Curtis.
- Fieber (1858) described new genus *Agallistes* that included *saltitans* (Fallén), along with other species. The type of *Agallistes* was fixed as *saltitans* by Kirkaldy in 1906.
- Fallén synonymized *marginatus* Curtis with *saltitans* Fallén.
- Thus, *Chlamydatus saltitans* (Fallén) is the name that must be used on the basis of priority. Finally, there is a basic conflict between the objective of stability and the code of priority.

**Availability**

Whereas priority is a comparatively objective criterion, availability is more nebulous. With reference to the different codes most names would be considered "available" if they meet the following four criteria.

- Appear in a work published after 1753 for plants and 1758 for most animals.
- Meet the criteria for publication designated by the codes.
- Are written in the Latin alphabet (today in English except for plants).
- Are binominal (if referring to species).

The codes also require other things depending upon the code.

**Publication:****Okay publications -**

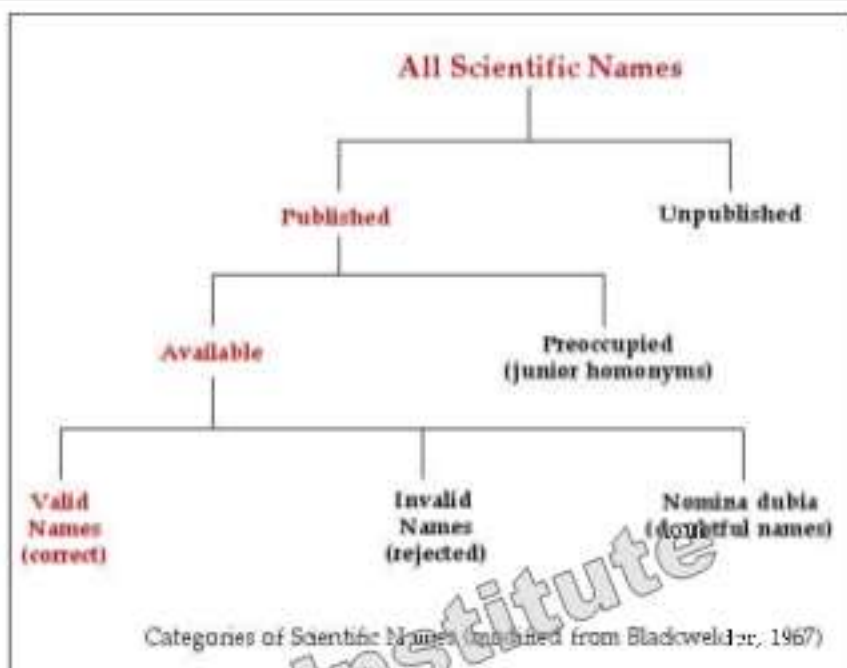
- Must be issued publicly for the purpose of providing a permanent scientific record.
- Must be obtainable when first issued, free of charge or by purchase.
- Must have been produced in an edition containing simultaneously obtainable copies by a method that assures numerous identical copies.
- Before 1986, must be via ink on paper, i.e. conventional printing or mimeographing (latter okay in zoology but not botany).

After 1986 can be via photocopying or any other "unconventional" method but must include a statement that nomenclatural content is for permanent, public, and scientific record therein.

**Forbidden publications -**

- Distribution on microfilm, computer printouts, or pre-1986 photocopies
- A mention of a name at a scientific meeting
- Labeling specimens deposited in a museum
- The distribution of proof sheets (zoology)
- Deposition of document (e.g., thesis) in a collection of documents, a library, or other archive
- Distribution only to colleagues or students of a note, even if printed, in explanation of an accompanying illustration.

Criteria for "publication" are being evaluated seriously now with the many more options for reproducing multiple copies available to the public for permanent record (dissertations through University Microfilms and WWW).



#### Typification

- The ideas of priority and availability were important considerations from the time of Linnaeus. However, the **type** concept was not.
- Early authors considered the most common species to be "typical" of the genus. Species were represented by a series of "typical" specimens. Linnaeus commonly replaced his typical specimens with "better" specimens. This is a big problem for many reasons.
- This was problematic: It became difficult for fixing generic limits or determining representatives of different species names.
- The **type** concept and requirement for designation of types partially solved this problem and assisted in the objective of stability; it was a major advance in the evolution of the codes. In zoology it was codified in the **Regulae** (the first code: International Nomenclature of Zoology adopted by 5th Congress, published under the 6th Congress in 1905). Mandatory designation of types in botany did not apply until Jan. 1958.
- This procedure ties names to specimens and is important.

#### The concept is not typological:

- The type specimen is not meant to be "typical"
- Types are not supposed to represent variation in a taxon.
- However, there are some biases practiced today.
- A "good" specimen
- An adult in breeding condition or with additional characters visible.

#### Types of Types:

There are two forms of types –

(1) names or (2) specimens.

#### ➤ Species-group types.

These represent a single specimen to which a name is attached. This provides an objective criterion for establishment of usage of that name. Species-group types are recognized in the codes as primary types and include the following possibilities.

**Reciprocal Altruism**

In evolutionary biology, **reciprocal altruism** is a behaviour whereby an organism acts in a manner that temporarily reduces its fitness while increasing another organism's fitness, with the expectation that the other organism will act in a similar manner at a later time. The concept was initially developed by Robert Trivers to explain the evolution of cooperation as instances of mutually altruistic acts. The concept is close to the strategy of "tit for tat" used in game theory.

## Theory

The concept of "reciprocal altruism", as introduced by Trivers, suggests that altruism, defined as an act of helping someone else although incurring some cost for this act, could have evolved since it might be beneficial to incur this cost if there is a chance of being in a reverse situation where the person whom I helped before may perform an altruistic act towards me.<sup>[1]</sup>

Putting this into the form of a strategy in a repeated prisoner's dilemma would mean to cooperate unconditionally in the first period and behave cooperatively (altruistically) as long as the other agent does as well. If chances of meeting another reciprocal altruist are high enough or the game is repeated for a long enough amount of time, this form of altruism can evolve within a population.

This is close to the notion of "tit for tat" introduced by Anatol Rapoport, although there still seems a slight distinction in that "tit for tat" cooperates in the first period and from thereon always replicates an opponent's previous action, whereas "reciprocal altruists" stop cooperation in the first instance of non-cooperation by an opponent and stay non-cooperative from thereon. This distinction leads to the fact that in contrast to reciprocal altruism, tit for tat may be able to restore cooperation under certain conditions despite cooperation having broken down.

Stephens shows a set of necessary and jointly sufficient conditions "... for an instance of reciprocal altruism:

1. the behaviour must reduce a donor's fitness relative to a selfish alternative;
2. the fitness of the recipient must be elevated relative to non-recipients
3. the performance of the behaviour must not depend on the receipt of an immediate benefit;
4. conditions 1, 2, and 3 must apply to both individuals engaging in reciprocal helping.

There are two additional conditions necessary "...for reciprocal altruism to evolve:"

- A mechanism for detecting 'cheaters' must exist.
- A large (indefinite) number of opportunities to exchange aid must exist.

The first two conditions are necessary for altruism as such, while the third is distinguishing reciprocal altruism from simple mutualism and the fourth makes the interaction reciprocal.

**Condition number five is required as otherwise non-altruists may always exploit altruistic behaviour without any consequences and therefore evolution of reciprocal altruism would not be possible.** However, it is pointed out that this "conditioning device" does not need to be conscious. Condition number six is required to avoid cooperation breakdown through backwards induction - a possibility suggested by game theoretical models.

## Examples

The following examples could be understood as altruism. However, showing reciprocal altruism in an unambiguous way requires more evidence as will be shown later.

### Cleaner fish

- An example of reciprocal altruism is cleaning symbiosis, such as between cleaner fish and their hosts, though cleaners include shrimps and birds, and clients include fish, turtles, octopuses and mammals.
- Aside from the apparent symbiosis of the cleaner and the host during actual cleaning, which cannot be interpreted as altruism, the host displays additional behaviour that meets the criteria for altruism:
- The host fish allows the cleaner fish free entrance and exit and does not eat the cleaner, even after the cleaning is done. The host signals the cleaner it is about to depart the cleaner's locality, even when the cleaner is not in its body. The host sometimes chases off possible dangers to the cleaner.

The following evidence supports the hypothesis:

- The cleaning by cleaners is essential for the host. In the absence of cleaners the hosts leave the locality or suffer from injuries inflicted by ecto-parasites.<sup>1</sup> There is difficulty and danger in finding a cleaner.
- Hosts leave their element to get cleaned. Others wait no longer than 30 seconds before searching for cleaners elsewhere.
- A key requirement for the establishment of reciprocal altruism is that the same two individuals must interact repeatedly, as otherwise the best strategy for the host would be to eat the cleaner as soon as cleaning was complete. This constraint imposes both a spatial and a temporal condition on the cleaner and on its host.

- Both individuals must remain in the same physical location, and both must have a long enough lifespan, to enable multiple interactions. Surprisingly, there is reliable evidence that individual cleaners and hosts do indeed interact repeatedly.
- This example meets some, but not all, of the criteria described in Trivers's model. In the cleaner-host system the benefit to the cleaner is always immediate.
- However, the evolution of reciprocal altruism is contingent on opportunities for future rewards through repeated interactions. In one study, nearby host fish observed "cheater" cleaners and subsequently avoided them.
- In these examples, true reciprocity is difficult to demonstrate since failure means the death of the cleaner.
- However, if Randall's claim that hosts sometimes chase off possible dangers to the cleaner is correct, an experiment might be constructed in which reciprocity could be demonstrated.

### Warning calls in birds

- Warning calls, although exposing a bird and putting it in danger, are frequently given by birds. An explanation in terms of altruistic behaviour is given by Trivers:
- It has been shown that predators learn specific localities and specialize individually on prey types and hunting techniques.
- It is therefore disadvantageous for a bird to have a predator eat a conspecific, because the experienced predator may then be more likely to eat him.
- Alarming another bird by giving a warning call tends to prevent predators from specializing on the caller's species and locality. In this way, birds in areas in which warning calls are given will be at a selective advantage relative to birds in areas free from warning calls.
- Nevertheless, this presentation lacks important elements of reciprocity. It is very hard to detect cheaters. Also, there is no evidence that a bird refrains from giving calls when another bird is not reciprocating.
- And there is no evidence that individuals interact repeatedly.
- Another explanation for warning calls is that these are not warning calls at all: A bird, once it has detected a bird of prey, calls to signal to the bird of prey that it was detected, and that there is no use trying to attack the calling bird.

Two facts support this hypothesis:

- The call frequencies match the hearing range of the predator bird.
- Calling birds are less attacked—predator birds attack calling birds less frequently than other birds.

### Nest Protecting

Red-winged blackbird males help defend neighbor's nests. There are many theories as to why males behave this way. One is that males only defend other nests which contain their extra-pair offspring. Extra-pair offspring is juveniles which may contain some of the male bird's DNA. Another is the tit-for-tat strategy of reciprocal altruism. A third theory is, males help only other closely related males.

### A study done by The Department of Fisheries and Wildlife provided evidence that males used a tit-for-tat strategy.

The Department of Fisheries and Wildlife tested many different nests by placing stuffed crows by nests, and then observing behavior of neighboring males. The behaviors they looked for included the number of calls, dives, and strikes.

- After analyzing the results, there was not significance evidence for kin selection; the presence of extra-pair offspring did not affect the probability of help in nest defense.
- However, males reduced the amount of defense given to neighbors when neighbor males reduced defense for their nests.
- This demonstrates a tit-for-tat strategy, where animals help those who previously helped them. This strategy is one type of reciprocal altruism.

### Vampire bats

- Vampire bats also display reciprocal altruism, as described by Wilkinson. The bats feed each other by regurgitating blood. Since bats only feed on blood and will die after just 70 hours of not eating, this food sharing is a great benefit to the receiver and a great cost to the giver.
- To qualify for reciprocal altruism, the benefit to the receiver would have to be larger than the cost to the donor. This seems to hold as these bats usually die if they do not find a blood meal two nights in a row. Also, the requirement that individuals who have behaved altruistically in the past are helped by others in the future is confirmed by the data.
- However, the consistency of the reciprocal behaviour, namely that a previously non-altruistic bat is refused help when it requires it, has not been demonstrated.

- Therefore, the bats do not seem to qualify yet as an example for reciprocal altruism. However, a closer look at the data shows that – except for a single interaction – all instances of feeding happened between individuals of the same group, who are on average cousins.
- Thus, it seems much more probable that this example is a case of kin selection than reciprocal altruism.

#### Primates

- Grooming in primates meets the conditions for reciprocal altruism according to some studies. One of the studies in vervet monkeys shows that among unrelated individuals, grooming induce higher chance of attending to each other's call for aids.
- However, vervet monkeys also display grooming behaviors within group members, displaying alliances.
- This would demonstrate vervet monkey's grooming behavior as a part of kin selection since the activity is done between siblings in this study.
- Moreover, following the criteria by Stephen, if the study is to be an example of reciprocal altruism, it must prove the mechanism for detecting cheaters.

#### SLEEP

- Sleep is a behavior.
- Sleep is not distinguished by movement.
- The best research on human sleep is conducted in a sleep laboratory.
- **During wakefulness the EEG of a normal person shows two basic patterns of activity: alpha activity and beta activity.**
- **Stage 1 sleep**, marked by a presence of some theta activity (3.5-7.5Hz). This stage is actually a transition between sleep and wakefulness; if we watch our volunteer's eyelids, we will see that from time to time they slowly open and close and that her eyes roll upward and downward. About 10 minutes later she enters stage 2 sleep.
- **Stage 2 sleep**, the EEG during this stage is generally irregular but contains periods of theta activity, sleep spindles, and K complexes. Sleep spindles are short bursts of waves of 12-14Hz that occur between two and five times a minute
- during stages 1-4 of sleep. Some investigators believe that sleep spindles represent the activity of a mechanism that decreases the brain's sensitivity to sensory input-disconnects the brain from the outside world, so to speak-and thus permits the person to enter deeper stages of sleep. The sleep of older people contains fewer sleep spindles and is generally accompanied by more awakenings during the night.
- K complexes are sudden, sharp wave forms, which, unlike sleep spindles, are usually found only during stage 2 sleep. Some investigators believe that they, too, represent mechanisms involved in keeping the person asleep.
- The subject is sleeping soundly now; but if awakened, she might report that she has not been asleep.
- **About 15 minutes later the subject enters stage 3**, signaled by the occurrence of high-amplitude delta activity (less than 3.5Hz). **The distinction between stage 3 and stage 4 is not clear-cut; stage 3 contains 20-50 percent of delta activity, and stage 4 contains more than 50 percent delta activity.**
- About 90 minutes after the beginning of sleep (and about 45 minutes after the onset of stage 4 sleep), the EEG suddenly becomes mostly desynchronized, with a sprinkling of theta waves, very similar to the record obtained during stage 1 sleep.
- **REM sleep** for the rapid eye movements that characterize it. REM sleep has also been called paradoxical sleep, because of the presence of beta activity, which is usually seen during wakefulness or stage 1 sleep. The term paradoxical merely reflects people's surprise at observing an unexpected phenomenon, but the years since its first discovery (reported by Aserinsky and Kleitman in 1955) have blunted the surprise value.
- **Stages 1-4 are usually referred to as non-REM sleep. Stages 3 and 4 are referred to as slow-wave sleep, because of the presence of delta activity.** As we will see, research has focused on the role of REM sleep and of slow-wave sleep; most investigators believe that the other stages of non-REM sleep, stages 1 and 2, are less important than the others. By some criteria, stage 4 is the deepest stage of sleep; only loud noises will cause a person to awake, and when awakened, the person acts groggy and confused. During REM sleep a person may not react to noises, but he or she is easily aroused by meaningful stimuli, such as the sound of his or her name. Also, when awakened from REM sleep, a person appears alert and attentive.
- The fact that REM sleep occurs at regular 90 minute intervals suggests that a brain mechanism alternately causes REM and slow wave-sleep. Normally, a period of slow-wave sleep must precede REM sleep. In addition, there seems to be a refractory period after each occurrence of REM sleep, during which time REM sleep cannot take place again. In fact, the cyclical nature of REM sleep appears to be controlled by a "clock" in the brain that also controls an activity cycle that continues through waking. The first suggestion that a 90-minute

activity cycle occurs throughout the day came from the observation that infants who are fed on demand show regular feeding patterns (Kleitman, 1961).

- During REM sleep we become paralyzed. Brain is very active, Cerebral blood flow and oxygen consumption are accelerated.
- If penile enlargement occurs during REM sleep, then his failure to obtain an erection during attempts at intercourse is not caused by physiological problems such as nerve damage or a circulatory disorder.
- **Electromyogram (EMG)** (*my oh gram*)- An electrical potential recorded from an electrode placed on or in a muscle.
- **Electro-oculogram (EOG)** (*ah kew loh gram*)- An electrical potential from the eyes, recorded by means of electrodes placed on the skin around them; detects eye movements.
- **Alpha activity**- Smooth electrical activity of 8-12Hz recorded from the brain; generally associated with a state of relaxation.
- **Beta activity**- Irregular electrical activity of 13-30Hz recorded from the brain; generally associated with a state of arousal.
- **Theta activity**- EEG activity of 3.5-7.5 Hz that occurs intermittently during early stages of slow-wave sleep and REM sleep.
- **Delta activity**- Regular, synchronous electrical activity of less than 3.5Hz recorded from the brain; occurs during the deepest stages of slow-wave sleep.
- **REM sleep**- A period of desynchronized EEG activity during sleep, at which time dreaming, rapid eye movements, and muscular paralyses occur; also called paradoxical sleep.
- **Non-REM sleep**- All stages of sleep except REM sleep.
- **Basic rest-activity cycle (BRAC)**- A 90-minute cycle (in humans) of waxing and waning alertness, controlled by a biological clock in the caudal brain stem; controls cycles of REM sleep and slow-wave sleep.

#### SUMMARY

Sleep is generally regarded as a state, but it is, nevertheless, a behavior. As we will see later in this chapter, we do not sleep because our brains "run down"; instead, active brain mechanisms cause us to engage in the behavior of sleep. The stages of non-REM sleep, stages 1-4, are defined by EEG activity. Slow-wave sleep (stages 3 and 4) comprises the two deepest stages. Alertness consists of desynchronized beta activity (13-30 Hz); relaxation and drowsiness consists of alpha activity (8-12 Hz); stage 1 sleep consists of alternating periods of alpha activity, irregular fast activity, and theta activity (3.5-7.5 Hz); the EEG of stage 2 sleep lacks alpha activity but contains sleep spindles (short periods of 12-14 Hz activity) and occasional K complexes; stage 3 sleep consists of 20-50 percent delta activity (less than 3.5 Hz); and stage 4 sleep consists of more than 50 percent delta activity. About 90 minutes after the beginning of sleep, people enter REM sleep. Cycles of REM and slow-wave sleep alternate in periods approximately 90 minutes. REM sleep consists of rapid eye movements, a desynchronized EEG, sensitivity to external stimulation, muscular paralysis, genital activity, and dreaming.

#### Why Do We Sleep?

##### Sleep as an Adaptive Response

Sleep is universal phenomenon among vertebrates. Only warm-blooded vertebrates (mammals and birds) exhibit unequivocal REM sleep, with a desynchronized EEG and rapid eye movements.

- Animals that have safe hiding places (such as rabbits) sleep a lot, unless they are very small and need to eat much of the time (such as shrews). Large predators such as lions can sleep safely wherever and whenever they choose, and indeed, they sleep many hours of the day. In contrast, large animals that are preyed upon and have no place to hide (such as cattle) sleep very little.
- The two cerebral hemispheres of some species of porpoises take turns sleeping.
- Undoubtedly, sleep does serve as a useful behavior. The fact that sleeping time varies with environmental engages in is somewhat flexible.

##### Sleep as a Restorative Process

###### Effects of Sleep Deprivation

*Studies with Humans.* Sleep deprivation did not interfere with people's ability to perform physical exercise.

- What happens to sleep-deprived subjects after they are permitted to sleep again? Most of them sleep longer the next night or two, but they never regain all of the sleep they lost.
- Both cerebral metabolic rate and cerebral blood flow decline during slow-wave sleep, falling to about 75 percent of the waking level during stage 4 sleep.