TSWRAFPDCW, Bhongir SUBJECT: Zoology

Immunology and Animal Biotechnology, PAPER - VII

FACULTY: K. Srilatha

Topic: Animal Cell Culture Media

Introduction

Cell culture is one of the major techniques in the life sciences. It is the general term used for the removal of cells, tissues or organs from an animal or plant and their subsequent placement into an artificial environment conducive to their survival and/or proliferation. Basic environmental requirements for cells to grow optimally are: controlled temperature, a substrate for cell attachment, and appropriate growth medium and incubator that maintains correct pH and osmolality. The most important and crucial step in cell culture is selecting appropriate growth medium for the *in vitro* cultivation. A growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms, cells, or small plants. Cell culture media generally comprise an appropriate source of energy and compounds which regulate the cell cycle. A typical culture medium is composed of a complement of amino acids, vitamins, inorganic salts, glucose, and serum as a

Types of Cell Culture Media

Animal cells can be cultured either using a completely natural medium or an artificial/synthetic medium along with some

Media Type		Examples	Uses
Natural media	Biological Fluids	plasma, serum, lymph, human placental cord serum, amniotic fluid	
	Tissue	Extract of liver, spleen, tumors, leucocytes and bone marrow, Extract of the bovine embryo and chick embryo	Extracts
	Clots	coagulants or plasma clots	
Artificial media	Balanced salt solutions	PBS, DPBS, HBSS, EBSS	Form the basis of complex media
	Basal media	MEM DMEM	Primary and diploid culture
	Complex	RPMI-1640, IMDM	Supports a wide

media	range of mammalian cells

Table 1. Types of natural and artificial media.

Natural media

Natural media consist solely of naturally occurring biological fluids. Natural media are very useful and convenient for a wide range of animal cell culture. The major disadvantage of natural media is its poor reproducibility due to lack of knowledge of the exact composition of these
natural media.

Advantages of serum in media	Disadvantages of serum in media
Serum contains various growth factors and hormones which stimulates cell growth and functions.	Lack of uniformity in the composition of serum
Helps in the attachment of cells	Testing needs to be done to maintain the quality of each batch before using
Acts as a spreading factor	May contain some of the growth inhibiting factors
Acts as a buffering agent which helps in maintaining the pH of the culture media	Increase the risk of contamination
Functions as a binding protein	Presence of serum in media may interfere with the purification and isolation of cell culture products
Minimizes mechanical damages or damages caused by viscosity	

Artificial media

Artificial or synthetic media are prepared by adding nutrients (both organic and inorganic),
vitamins, salts, O_2 and CO_2 gas phases, serum proteins, carbohydrates, cofactors. Different artificial media have been devised to serve one or more of the following purposes: 1) immediate survival (a balanced salt solution, with specific pH and osmotic pressure); 2) prolonged survival (a balanced salt solution supplemented with various formulation of organic compounds and/or serum); 3) indefinite growth; 4) specialized functions.

Artificial media are grouped into four categories:

1. **Serum containing media**

Fetal bovine serum is the most common supplement in animal cell culture media. It is used as a low-cost supplement to provide an optimal culture medium. Serum provides carriers or chelators

for labile or water-insoluble nutrients, hormones and growth factors, protease inhibitors, and binds and neutralizes toxic moieties.

2.Serum-free media

Presence of serum in the media has many drawbacks and can lead to serious misinterpretations in immunological studies. A number of serum-free media have been developed. These media are generally specifically formulated to support the culture of a single cell type, such as Knockout Serum Replacement and Knockout DMEM from Thermo Fisher Scientific, and mTESR1 medium from Stem Cell Technologies, for stem cells, and incorporate defined quantities of purified growth factors, lipoproteins, and other proteins, which are otherwise usually provided by the serum. These media are also referred to as 'defined culture media' since the components in these media are known.

3.Chemically defined media

These media contain contamination-free ultra pure inorganic and organic ingredients, and may also contain pure protein additives, like growth factors. Their constituents are produced in bacteria or yeast by genetic engineering with the addition of vitamins, cholesterol, specific amino

acids, and fatty acids. 4.**Protein-free media**

Protein-free media do not contain any protein and only contain non-protein constituents. Compared to serum-supplemented media, use of protein-free media promotes superior cell growth and protein expression and facilitates downstream purification of any expressed product. Formulations like MEM, RPMI-1640 are protein-free and protein supplement is provided when required.

TSWRAFPDCW – BHONGIR TEACHING MODULE

CLASS: BZC/MZC (BSc) DEPARTMENT: ZOOLOGY

Year ll (lV Sem) Paper: IV (Genetics)

Topic: CROSSING OVER

Lecturer Name: E. JYOTHI No. of Teaching Hours: 2

Objectives of the Topic:

- Student can able to understand the concept of crossing over
- Understand the overall idea of crossing over path way.
- Understand the Importance of crossing over
- Student can draw neat diagram of crossing over

PRE TEST: Understanding the student's basic knowledge by asking orally on the following

I)What is the importance of crossing over?

II)Types of crossing over?

III)Give some examples of crossing over? IV)What is the use of crossing over?

1. Crossing-over takes place in the

(a)Diakinesis stage

(b)Anaphase stage

(c)) Pachytene stage

(d)Leptotene stage

2.Crossing Over occurs when the homologous chromosomes contain

a) 1 Chromatid b)2 Chromatid

c)4 chromatid d)8 chromatid

3.The term crossing over is described by

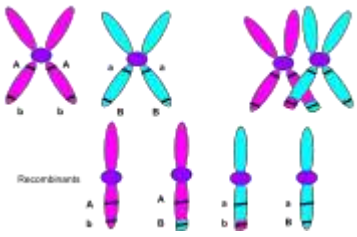
a) Mendal

b) Thomson Hunt Morgan

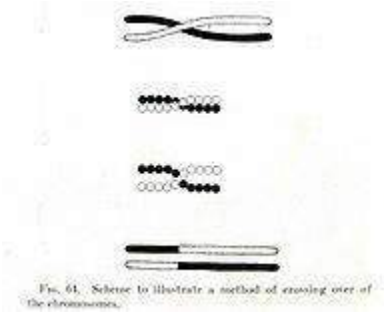
c) Watson and Crick

d) Lamarck

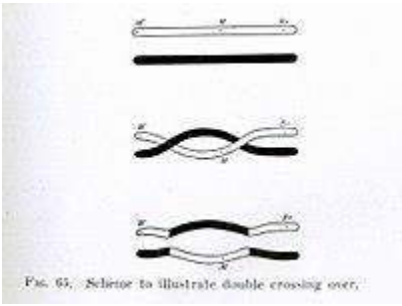
Module Content: Chromosomal crossover, or **crossing over**, is the exchange of genetic material during sexual reproduction between two homologous chromosomes' non-sister chromatids that results in recombinant chromosomes. It is one of the final phases of genetic recombination, which occurs in the *pachytene* stage of prophase I of meiosis during a process called synapsis. Synapsis begins before the synaptonemal complex develops and is not completed until near the end of prophase I. Crossover usually occurs when matching regions on matching chromosomes break and then reconnect to the other chromosome.



Crossing over occurs between prophase I and metaphase I and is the process where two homologous non-sister chromatids pair up with each other and exchange different segments of genetic material to form two recombinant chromosome sister chromatids. It can also happen during mitotic division,[1] which may result in loss of heterozygosity. Crossing over is essential for the normal segregation of chromosomes during meiosis.[*citation needed*] Crossing over also accounts for genetic variation, because due to the swapping of genetic material during crossing over, the chromatids held together by the centromere are no longer identical. So, when the chromosomes go on to meiosis II and separate, some of the daughter cells receive daughter chromosomes with recombined alleles. Due to this genetic recombination, the offspring have a different set of alleles and genes than their parents do. In the diagram, genes B and b are crossed over with each other, making the resulting recombinants after meiosis Ab, AB, ab, and aB.



Thomas Hunt Morgan's illustration of crossing over (1916)

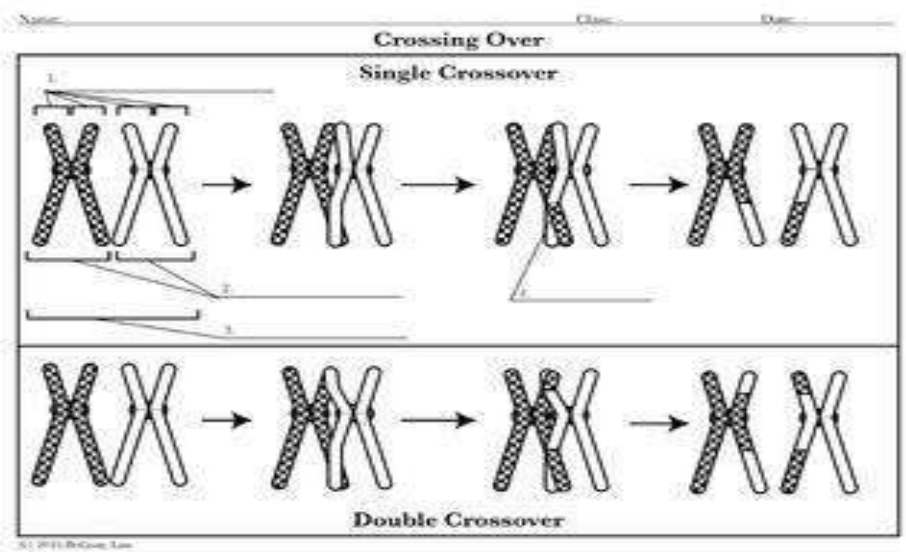


Types of Crossing over:

•Single cross **over**: Formation of single chiasma and involves only two chromatids out of four.

•Double cross **over**: Formation of two chiasmata and involves two or three or all four strands.

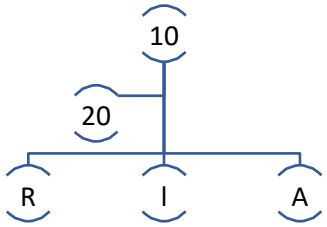
•Multiple cross **over**: Formation of more than two chiasmata and **crossing over** frequency is extremely low.

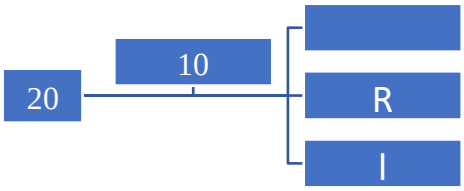


e)Given your answers to parts (c) and (d), what can you say about the relationship between the gene for wing length and the gene for wing surface?

You would expect he gene for wing length and the gene for wing surface to be linked and the distance is expected to be either approximately 10 cM or approximately 30 cM apart.

f)Based on the preceding, what are the two possible arrangements of the three genes? Indicate the map distance between genes for each arrangement.





g) In a final mapping experiment, you cross a true-breeding red-bodied, short and crinkle- winged male with a true breeding yellow-bodied, long and smooth-winged female. List the expected genotypes of the F1 progeny. What are the associated phenotypes?

P: RRllaa x rrLLAA

F1: RrLlAa red-bodied, long and smooth-winged

2.While working with a type of beetle that is normally smooth, large, and white, you discover three mutations that lead to the recessive phenotypes bumpy, small, and grey, respectively. You cross true-breeding smooth white beetles to true-breeding bumpy grey beetles and get all smooth white F1 beetles. Then you cross the F1 beetles to true-breeding bumpy grey beetles and, after analyzing 800 F2s, calculate a map distance of 5 cM between the smoothness and color loci.

a)What are the four phenotypic classes you got in the F2, and about how many of each did you get?

•For the body type (i.e., smooth or bumpy) use B or b to designate the alleles.

•For the color (i.e., white or grey) use G or g to designate the alleles.

•For the size (i.e., large or small) use L or l to designate the alleles.

*In each case, use the uppercase letter for the associated with the dominant phenotype and the lower case letter for the allele associated with the recessive phenotype.

Genotypic class	Phenotypic class	How many of the phenotype
Bbgg	Smooth white	380
bbGg	Bumpy grey	380
bbGb	Smooth grey	20
bbgg	Bumpy white	20

b) You cross two true-breeding parents to get all F1 beetles that are large and smooth. You cross the F1 to true breeding small, bumpy beetles and get: 228 large and smooth, 19 small and

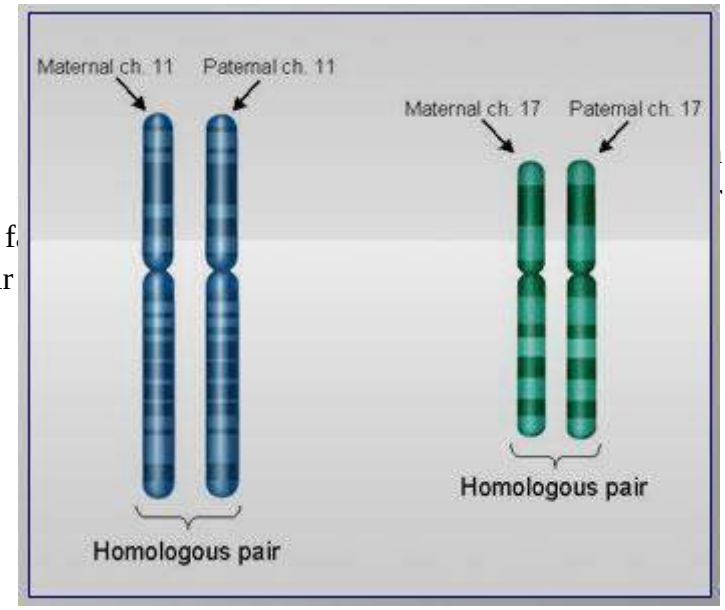
smooth, 16 large and bumpy, 237 small and bumpy. What were the genotypes and phenotypes of the two parental beetles (P generation)?

	Genotype	Phenotype
Parent 1	LLBB	Large smooth
Parent 2	llbb	Small bumpy

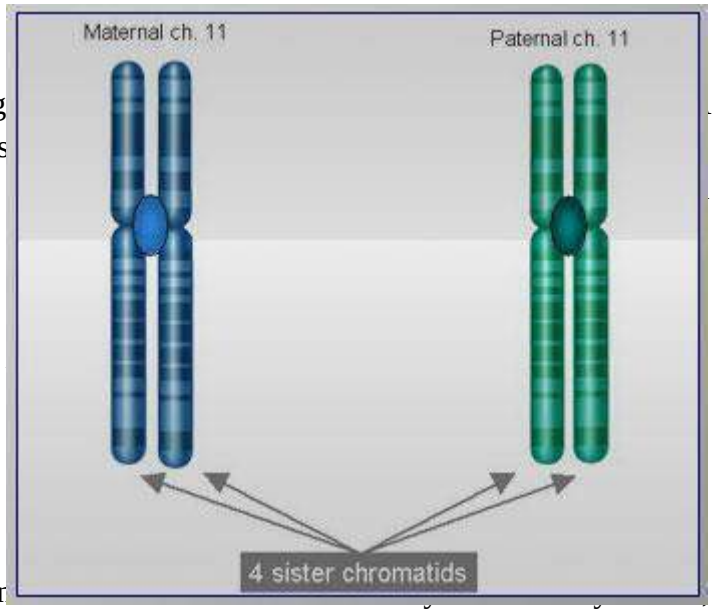
- ***Meiosis*** creates gametes (egg and sperm cells).
- During meiosis, genetic information is exchanged between the maternally and paternally inherited copies of a pair of chromosomes in order to create new combinations of genes. This process of ***genetic recombination*** helps to increase genetic variability within a species. It allows for the transmission of virtually limitless combinations of genes from parent to offspring.
- The resulting gametes have 23 new chromosomes, one member of each of the 23 pairs, representing unique combinations of the original maternal and paternal copies.

2. Meiosis Terminology—Homolog

- ***Homologous chromosomes***, chromosome pair. One member and the other member is from the f
- During meiosis, homologs pair



3. Meiosis Terminology—Chromatid



- A ***chromatid*** is formed during strands formed after DNA synthes
- Sister chromatids are from together at the centromere.
- The classic drawing of a chromosome shaped like the letter

Examples of crossing over:

For **example**, a DNA segment or although one chromosome may code for brown eyes and the other for blue eyes. ... **Crossing over** occurs most often between different alleles coding for the same gene

Student Activities:

- Group discussion on crossing over
- Seminars by Students
- Quiz will be conducted to the students
- Preparing the model of Crossing over

Assessment: Assessing the Student after completion of the Topic by conducting Oral Test or written test.

1..Explain the types of crossing over?

2 .Draw the crossing over diagram?

3 3.Write the importance of crossing over?

Reference Books:

★ Modern text book of zoology

Dr.N. Arum gam -SARAS PUBLICATION

★ Genetics text book

R.L. Kotpal

★ B.Sc. Telugu Academy

TSWRAFPDCW – BHONGIR

TEACHING MODULE

CLASS:BZC/MZC (BSC)

DEPARTMENT: ZOOLOGY

Year ll yr (lV sem)

Paper: IV (Genetics)

Topic: LINKAGE

Lecturer Name: E.JYOTHI

No. of Teaching Hours: 2

Objectives of the Topic:

- Student can able to understand the concept of linkage.
- Understand the overall idea of linkage path way.
- Understand the Importance of linkage.
- Student can draw neat diagram of linkage.

PRE TEST:

Understanding the students basic knowledge by asking orally on the following

I) What is importance of linkage?

II) Types of linkage?

III) Give some examples of linkage?

IV) What is the use of linkage?

Module Content:

The starting point of this paper is the classical notion of linkage (or liaison) of algebraicvarieties. It goes back to the late 19th and early 20th century, when M. Noether, Halphen,and Severi used it to study algebraic curves inP3. Linkage allows to pass from a givencurve to another curve, related in a geometric way to the original one. Iterating theprocedure one obtains a whole series of curves in the same "linkage class". The usefulnessof this technique is explained by two observations: (a) certain properties of the curve arepreserved under linkage, and (b) the resulting curves may be simpler, and thus easier tohandle, than the original one

Types of Genetic Linkage:

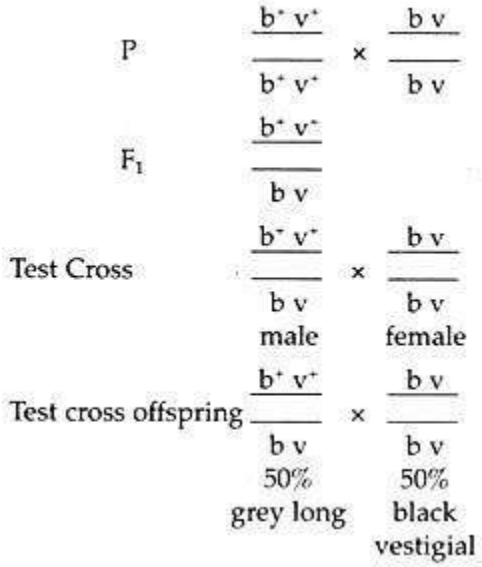
According to the degree, linkage may be classified into complete and incomplete types. Genes lying on the same chromosome might always be transmitted together and these genes might not undergo independent assortment. Linkage between these genes is considered as complete. Complete linkage between genes on the same chromosome is rare in most sexually reproducing species.

However, in Drosophila, there is complete absence of crossing-over in males; so in males there is 100% linkage in different genes of the same chromosome. Gene pairs in most link•age groups assort at least partially, indepen•dent of each other and these linked genes are not always transmitted together. Linkage between these genes is considered as incom•plete.

In Drosophila, gray body colour and long wing are two dominant linked characters; the genes for these characters may be indicated as b+ and v+ and their location on the chromo•some may be indicated as b+ v+. Again, black body and vestigial wing are two linked reces•sive characters.

These genes may be indicated as b and v and their location on chromosome as b v. Pure gray long fly is crossed with pure black vestigial fly. The F1 hybrids are phenotypically gray long and their genotype will be $\frac{b+\ v+}{b\ v}$. Now a male heterozygote fly is test crossed with a double recessive female. The test-cross offspring were gray long and black vestigial in the ratio of 1 : 1. In this case it is seen that two linked dominant characters gray and long remain together in the P1 flies, F1 hybrid flies and 50% of the test cross offspring's.

Again the two recessive linked characters black and vestigial remain together in the P1 black vestigial flies and 50% of the test cross offspring's. Thus, we get either grey long combination or black vestigial combination for more than one generation and do not get a mixture like grey vestigial or black long in any generation. Thus, this example shows complete linkage between two domi•nant traits grey and long and in between two recessive traits black and vestigial.



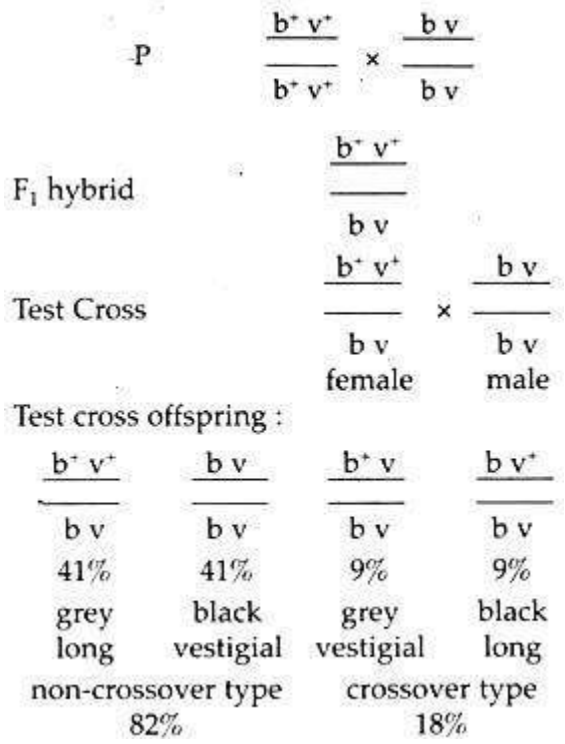
Complete Linkage in Drosophila:

It is important to note that for the test cross the male hybrid Drosophila is test crossed with a female, fly because only in males there is no cross-over and complete linkage can be shown. If the female hybrid is taken, complete linkage cannot be shown as crossing-over occurs in female.

A case of incomplete linkage can be illus•trated as follows: pure grey bodied long winged and black bodied vestigial winged were crossed. A female heterozygote grey long fly is crossed with double recessive black vestigial male.

In females, cross-over occurs as a result of which it produces four kind of gametes, two non-cross-over types of gametes are b+ v+ and b v, and two cross•over type of gamete are b+ v and b v+. In this case, 82% of the test cross offspring's are non-cross-over type, of which 41% are grey long and 41% are black vestigial; 18% of the total offspring's are cross-over type of which 9% grey vestigial and rest 9% black long.

The cross is shown below:



Incomplete Linkage in Drosophila:

In this case, the two dominant characters grey long remain together in P and F1; but after test cross, in addition to the original combination of characters, new combinations like, gray vestigial and black long are appeared i.e., dominant and recessive traits may appear together due to occurrence of crossing-over in the F1 hybrid females.

Thus, it is a case of incomplete linkage. It is important to note that in the test cross, the hybrid fly is female. A male hybrid cannot be selected for the test cross in this experiment, as in Drosophila, there occurs no crossing-over in males.

Example on Linkage: When two different varieties of sweet pea—one having red flowers and round pollen grain and other having blue flower and long pollen grain were crossed, the F1 plants were blue flowered with long pollen (blue long characters were respectively dominant over red and round characters). When these blue long (heterozygous) hybrids were crossed with double recessive red and round (homozygous) individuals (test cross), they failed to produce expected 1:1:1:1 ratio in F2 generation. These actually produced following four combinations in the ratio of 7 : 1 : 1 : 7 (7 blue long : 1 blue round : 1 red long : 7 red round) .

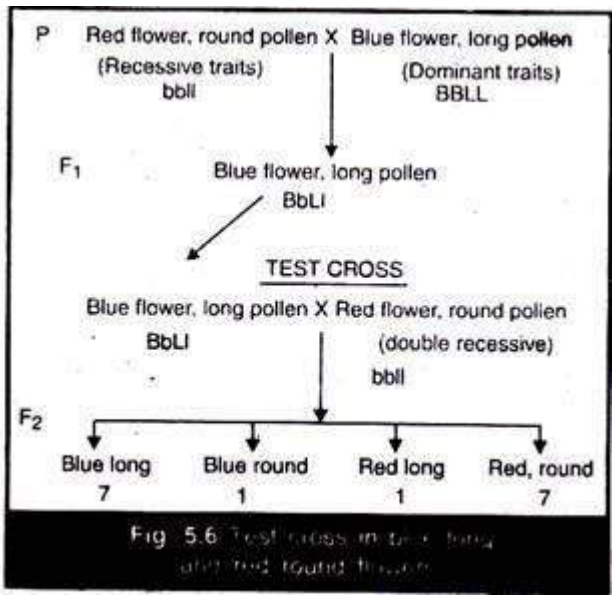


Fig 5.6

Linkage Content:

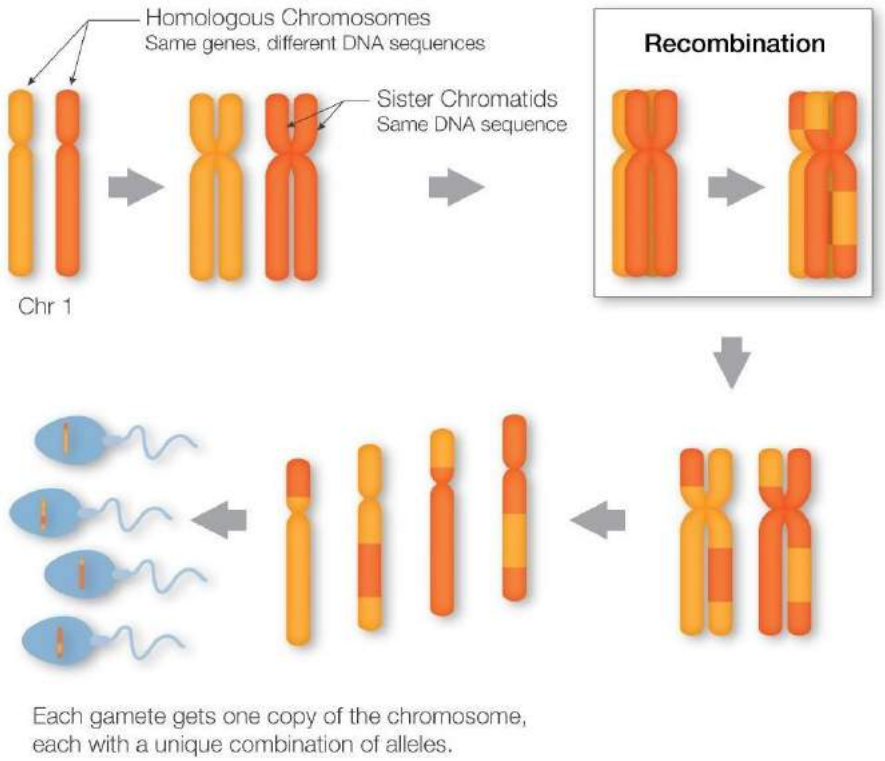
During the formation of gametes (eggs and sperm in people and pigeons), chromosomes go through a process called homologous recombination.

First, the cell makes an identical copy of each chromosome. Identical copies are called sister chromatids, and they remain attached to one another for now.

Next, all four copies—two identical copies of two homologous chromosome—line up next to one another, and they swap large sections of DNA. The DNA strands actually break and rejoin. After recombination, the chromosomes still have the same genes arranged in the same order, but the alleles have been rearranged.

Finally, the chromosomes are divvied up so that each gamete gets just one copy of each chromosome. While each gamete ends up with one copy of every gene, they have different combinations of alleles for those genes.

Recombination increases genetic diversity. The location of the chromosome break points is random (or nearly so), and each gamete receives a random copy of each recombined chromosome. All of this jumbling and mixing allows for a nearly infinite number of allele combinations.



Student Activities:

- Group discussion on Linkage
- Seminars by Students
- Quiz will be conducted to the students
- Preparing the model of Linkage

Assessment:

Assessing the Student after completion of the Topic by conducting Oral Test or written test.

i. Explain the types of Linkage?

ii. Draw the Linkage diagram?

iii. Steps involved in Linkage?

iv. Write the importance of the Linkage?

Reference Books:

★ Modern text book of zoology

Dr.N. Arumugam -SARAS PUBLICATION

★ Genetics text book

R.L.Kotpal

★ B.Sc Telugu Academi

TSWRDC BHONGIR

Affiliated to Mahatma Gandhi University Year: II YR (IV SEM); Class: BZC/MZC (BSC)
Department: ZOOLOGY Paper: (IV) GENETICS, Code: BS405

Name of the faculty: K. SRILATHA

Module: Mendal's Laws

No. of teaching hours: 05

Module Objectives: The main objective of this module is to introduce students to the principles of genetics.

✓To Predict the inheritance and expression patterns of alleles subject to the major categories of Mendelian & non-Mendelian behavior.

✓Law of Dominance and purity of gametes

✓Law of independent assortment and dihybrid cross

✓Monohybrid & Dihybrid test crosses

✓Back cross

✓Inheritance patterns – Phenotypic & Genotypic

Pre-test: Understanding the student's knowledge by asking GENETICS basic terms like

- What is Inheritance/Heredity?
- What are Variations?
- What is gene?
- What is Phenotype?
- What is genotype? (Homozygous & Heterozygous)
- Alleles & Allelomorphs?

Module Content:

Brief history about Mendel and his Hybridization Experiments:

Johann Gregor Mendel (1822–1884), often called the "father of genetics," was a teacher, lifelong learner, scientist, and man of faith. It would be fair to say that Mendel had a lot of grit: he persevered through difficult circumstances to make some of the most important discoveries in biology.

Gregor Mendel

Mendel had difficulty paying for his education due to his family's limited means, and he also suffered bouts of physical illness and depression; still, he persevered to graduate from high school and, later, university. After finishing university, he joined the Augustinian Abbey of St. Thomas in Brno, in what is now the Czech Republic. At the time, the monastery was the cultural and intellectual hub of the region, and Mendel was immediately exposed to new teachings and ideas.

His decision to join the order (against the wishes of his father, who expected him to carry on the family farm) appears to have been motivated in part by a desire to continue his education and pursue his scientific interests. Supported by the monastery, he taught physics, botany, and natural science courses at the secondary and university levels.

In 1856, Mendel began a decade-long research project to investigate patterns of inheritance. Although he began his research using mice, he later switched to honeybees and plants, ultimately settling on garden peas as his primary model system.

•Crossing of two genetically different individuals is called hybridization.

Example:

i.Crossing of homozygous tall and dwarf pisum plants.

ii.Crossing of homozygous black (BB) and white (bb) Guinea pigs.

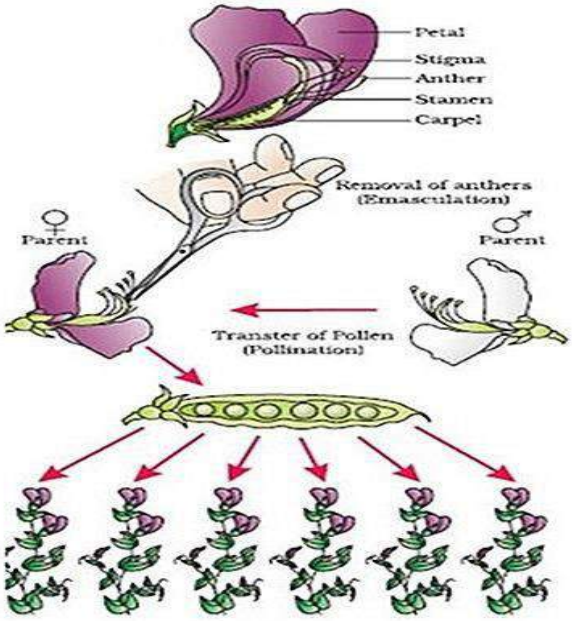


Figure 5.2 Steps in making a cross in pea

Why Mendel succeeded in proposing principles of inheritance?

1.His success mainly depends upon the selection of pea plants. They sharply differ in few characters.

2.The plants are annuals and produce many generations within a short span.

3.The plants are self-pollinated. The flowers facilitated cross pollination also. The cross pollination is carried out by removing anthers from the flowers of female parent by **emasculation**.

4.He studied the inheritance of one character at a time in the beginning and later two or more.

5.Mendel continued his experiments up to 'F3' generation (F = Filial or daughter generation)

6.He analyzed the results statistically which enabled him to derive the numerical ratios.

7."Selection of pure breeding strains as the starting material was important for his success".

8.Mendel conducted experiments on seven characters or traits, each of which exists in two forms.

9.Dominant and **Recessive**.

10.'F'generation

It is the filial generation produced due to hybridisation. In hybridisation experiment, first produced progeny is called 'F1' (1 - first ; f - filial) generation and second produced is called 'F2' (2 - second; F-filial) generation.

11.Hybrids

The first progeny ('F1' generation) produced due to hybridisation are called hybrids. These are always heterozygous.

The mechanism of **inheritance** was discovered even before the discovery of hereditdry material, D.N.A. The way of transmission of characters from one generation to another generation was

first demonstrated by **Gregor Johann Mendel** in 1866. He said that every cell of an organism contains two factors for each character. The factors seperate during gametogenesis. Now the Mendel factors are called genes. As the mechanism of inheritance was first discovered by Mendel, he is considered as the **father of genetics**. He worked on **pisum plant** for about '8' years from 1856 to 1864. Mendel published his findings in the magazine proceedings of the natural history society of Brunn in 1866. But his findings were unnoticed until his death. But in 1900 the Mendel's laws were rediscovered independently by **Devries, Correns and Tschermak**. Hence these three scientists are considered as rediscoverers of Mendelism or principles of inheritance.

Mendel selected pisum p

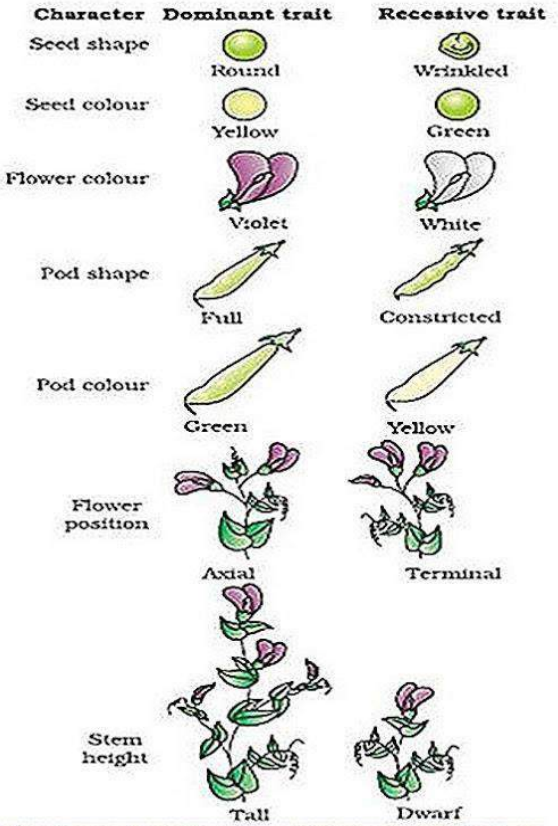


Figure 5.1 Seven pairs of contrasting traits in pea plant studied by Mendel

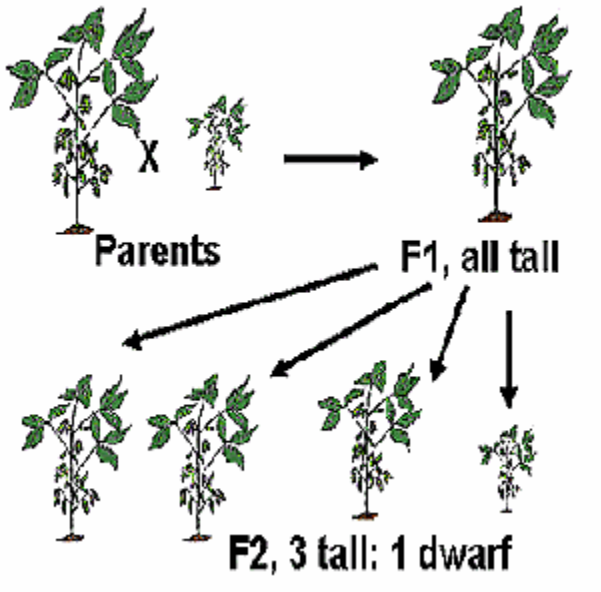
i. As it is suitable for easy cross pollination
ii. As it is a naturally self-pollinated plant.
iii. As it isan annual plant.
iv. As it has many contrasting forms.

The principles of inheritance or Mendel's laws are three.
1.Principle of dominance
2.Principle of segregation
3.Principle of independent assortment.

These were deduced by monohybrid and dihybrid crosses.

Mono Hybrid Cross

The principle of dominance and segregation were deduced from monohybrid cross.
DEFINITION
A cross made to study the inheritance of o forms or two different alleles is known as mor Crossing of pure tall (TT) and dwarf (tt) to character or two contrasting forms (Tall, Dwa Such crossing is called mono hybridisation an monohybrids.



PHENOTYPIC EXPLANATION OF MONO HYBRID CROSS

Mendel crossed pure tall plant with pure dwarf plant. To do this, he

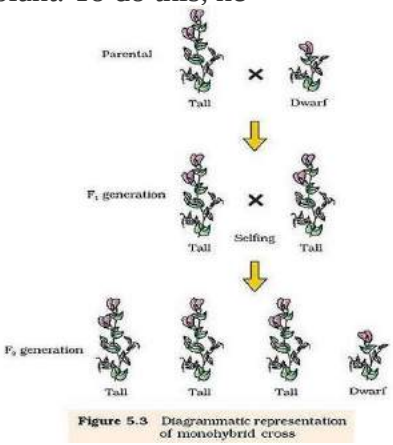


Figure 5.3 Diagrammatic representation of monohybrid cross

transferred pollen grains of tall plant (male parent) to the stigma of dwarf plant (female parent or emasculated parent). It is called normal cross.

In another cross, Mendel transferred the pollen grains of dwarf plant (male parent) to the stigma of tall plant (female parent) or emasculated parent). It is called reciprocal cross.

In both the above crosses, Mendel got all ‘F1’ monohybrid tall plants. Why ‘F1’ progeny is tall? This resulted him to propose the principle of dominance.

B.Law of Dominance - Definition:

Mendet proposed this principle by observing ‘F1’ progeny.

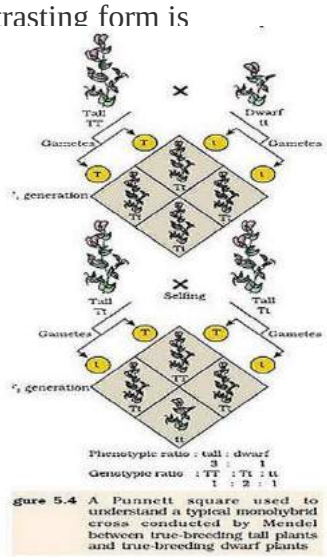
Law of dominance states that when a pair of contrasting forms (Tall, Dwarf) were crossed, the contrasting form that expressed (Tall) in ‘F1’ generation is called dominant contrasting form. The contrasting form that did not express in ‘F1’ generation is called recessive contrasting form (Dwarf).

In the above experiment tall is dominant over dwarf. What happened to the recessive allele, dwarf in ‘F1’?

To find out the answer for above Question, Mendel continued the experiment.

C.Self pollination of ‘F1’ individuals:

Mendel allowed self pollination in the ‘F1’ individuals. In ‘F2’ he got both tall and dwarf in the ratio of 3 : 1. So, the recessive contrasting form is



gure 5.4 A Punnett square used to understand a typical monohybrid cross conducted by Mendel between true-breeding tall plants and true-breeding dwarf plants

expressed in 114 ‘F2’ generation. The appearance of recessive progeny resulted the proposal of law of segregation.

D. Observation of ‘F3’ generation:

Mendel allowed self pollination in ‘F2’ individuals to observe ‘F3’ generation. ‘F2’ dwarf plants produced all dwarf plants. It indicates that the dwarf plant is a true breeding plant. 1/3 ‘F2’ tall plant produced only tall plants. It indicates that 1/3 among ‘F2’ tall plants is a true breeding plants. 2/3 ‘F2’ tall plants behaved like ‘F1’ by producing tall and dwarf in the ratio of 3:1.

E. Law of segregation:

It was explained by Mendel by observing recessive progeny of ‘F2’ generation. Law of segregation states that the two alleles of heterozygous or monohybrid or ‘F1’ are separated during gamete formation. Hence gametes are always pure. The law of segregation is also called law of purity of gametes. The gamete receives either dominant or recessive allele but

never both. In ‘F1’ hybrid tall, the alleles of tall and dwarf seperated and enter into two different gametes.

Dihybrid cross experiments of Mendel

Mendel had some Questions while doing breeding experiments. They are, do different for different characters also segregate or seperate? Whether the genes are character would alter f the genes of another character during their inheritance together?
To answer the above Questions, Mendel did dihybrid cross. He also deduced the law of independent assortment from dihybrid cross.

1.DIHYBRID CROSS -DEFINITION

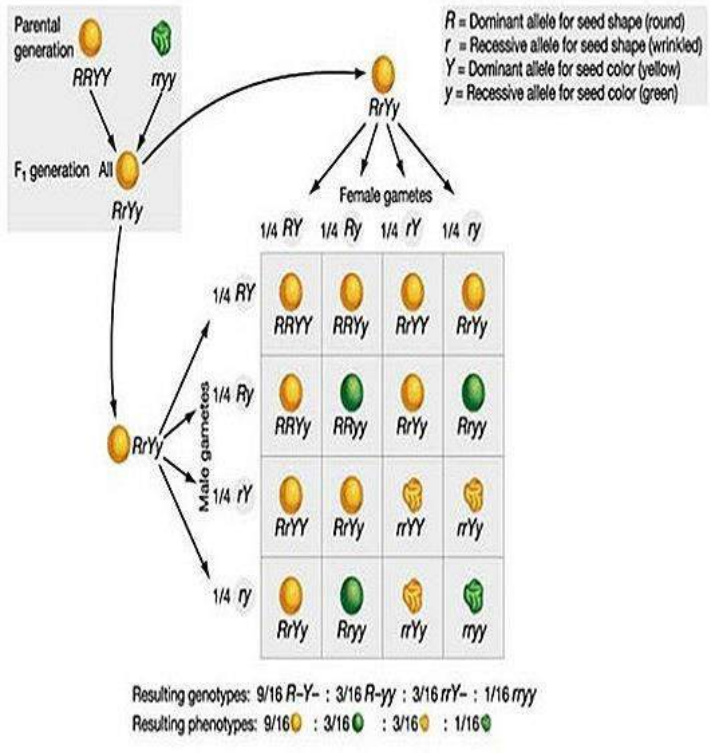
A cross made to study the inheritance of two characters or two pairs of contrasting forms or two pairs of different alleles is known as dihybrid cross.
Example:
Crossing of pure yellow, Round seeded plant with green, Wrinkled seeded pisum plants
Character -1 - seed colour Yellow --- green

Character - 2 - seed shape Round -----wrinkled

2.PHENOTYPIC HYPOTHESIS OF DIHYBRID CROSS

A. Crossing of ‘p’ generation:

Mendel crossed pure yellow, round seeded plant with pure green wrinkled



seeded plant. He got all yellow,Round seeded plants in ‘F1’ generation. These are dihybrids. The, ‘F1’ generation showed that yellow is dominant over green and round is dominant over wrinkled forms. This dominance was allready observed in monohybrid crosses.

B.Crossing of ‘F1’ individuals:

Mendel allowed ‘F1’ di hybrid yellow, round seeded plants for self pollination. He got different types of individuals in ‘F2’.
They are,

1. Yellow, Round (Double dominant)

2. Yellow,Wrinkled (Recombinant-I)

3. Green, Round (Recombinant-Il)

4. Green, Wrinkled (Double recessive)

The above ‘4’ types of inidividuals are in the ratio of 9/1 6: 3/16: 3/16: 1/16 respectively.
‘F2’ ‘dihybrid ratio led mendel to propose the law of independent assortment.

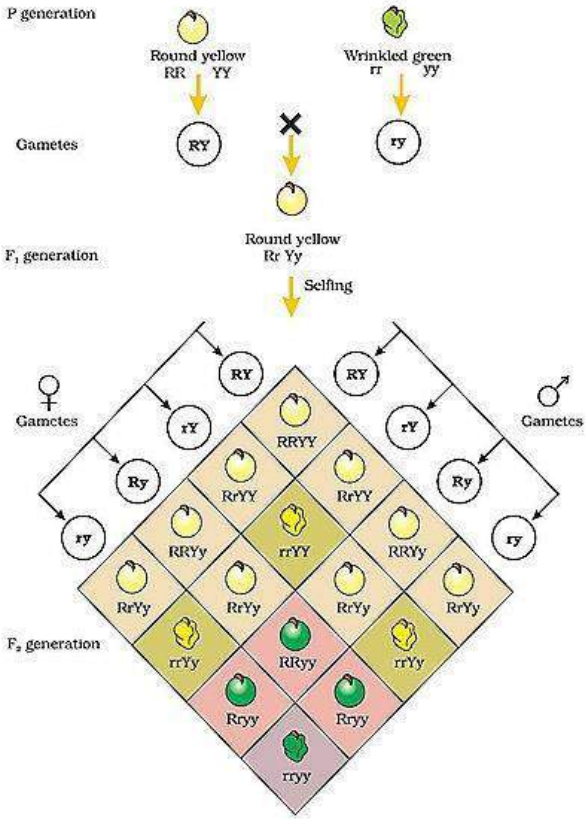


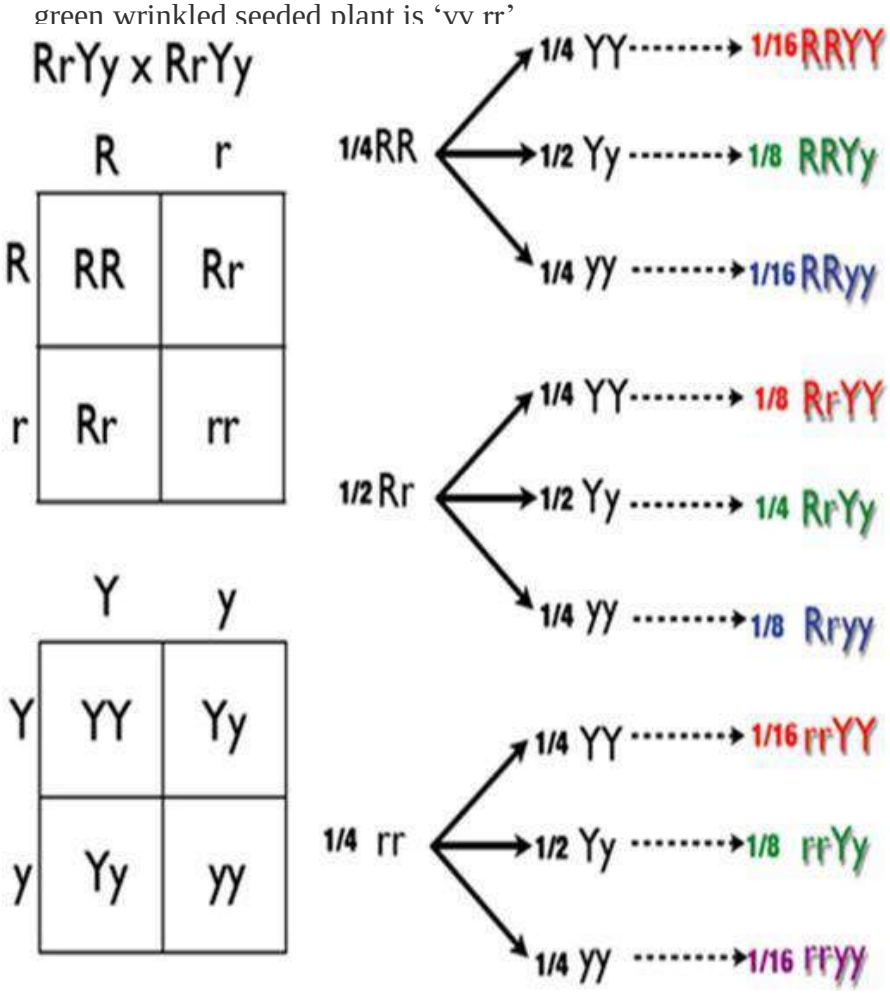
Figure 5.7 Results of a dihybrid cross where the two parents differed in two pairs of contrasting traits: seed colour and seed shape

3. GENE HYPOTHESIS OF DIHYBRID CROSS

The yellow colour controlling gene is represented as ‘Y’ . The green colour controlling gene is represented as ‘y’. The round shape controlling gene is represented as ‘R’. The wrinkled shape controlling gene is represented as ‘r’.

A. Genotypes of ‘P’generation:

The genotype of pure yellow round seeded plant is ‘YY RR’. The genotype of double recessive green wrinkled seeded plant is ‘yy rr’



B. Crossing of pure ‘P’-.generation:

Pure yellow round seeded plant (YY RR) produced only one type of gametes (YR) type. All gametes carried ‘Y’ and ‘ R’ genes to the F1 generation. Pure green wrinkled seeded plant (yy rr) also produced one type of gametes (yr). When ‘YR’ type of gametes were fertilized by ‘yr’ type of gametes, all F1 inidividuals produced were dihybrid yellow round seeded plants(YyRr). The F1 generation indicates that yellow colour (Y) and round shape (R) controlling genes are dominant over green colour (y) and wrinkled shape (r) controlling genes respectively like in monohybrid crosses.

C. Crossing of'F1'dyhibrid individuals:
Mendel allowed self pollination in dihybrid yellow round seeded plants (YyRr). The 'F1' female produced '4' types of ova. They. are YR, Yr, yR and yr. Like wise male also produced '4'types of pollen grains. They are YR, Yr, yR, and yr. When the above '4" types of ova were fertilized by the above '4' types of pollen grains, Mendel got '4' different phenotypes and '9' different genotypes in F2 generation.

The 'F2'generation is represented in punnet's squares or checker board.
4. 'F2' – PHENOTYPIC RATIO
In 'f2' generation, two parent phenotypes and two recombinats are produced. They are,

i.Double dominant (VeIIow,Round) -- 9/16.

ii.Recombinant - I (Yellow, Wrinkled) --- 3/16.

iii.Recombinant -Il (Green, Round) --- 3/16.

iv.Double rcessive (Green, Wrinkled) --- 1/16.

In 'F2' generation, if we observe the phenotypic ratio of one character that is either the ratio of yellow and green or Round and Wrinkled, it is 12/16: 4/16 or 3:1 . So, in dihybrid cross, the phenotypic ratio of one character is not effected by phenotypic ratio of another.

5. 'F2' - GENOTYPIC RATIO
In F2 generation the '1 6' individuals of 4 different phenotypes contain '9' different genotypes. They are 1 :2:1 :2:4:2:1 :2:1 or (1 : 2 : j)2 . The ' F2' different genotypes are The '9' double dominant yellow round seeded plants contain '4' different genotypes. They are 1/9 YYRR, 2/9 YyRR, 2/9 YYRr and '. 4/9YyRr. The '3' individuals of recombinant -I (yellow wrinkled) contain '2' different genotypes. They are 1/3 YYrr and 2/3 Yyrr. The '3' individuals of recombinant-IF (green, round) also contain 2 different genotypes. They are 1/3 yyRR and 2/3 yyRr. The double recessive green wrinkled always contains one genotype only. It is yyrr. If we observe the F2 genotypic ratio of dihybrid cross, the genotypic ratio of one character is not affected by the genotypic ratio of another character.

LAW OF INDEPENDENDENT ASSORTMENT'
As the recombinants (yellow wrinkled and green round) were produced in 'F2' generation of dihybrid cross, Mendel said that every allele behaves independently. Hence all possible combinations were produced in F2 generation. It is called independent assortment. As alleles behaved independently 'Y' is not only inherited along with 'R' but also with 'r' . Like wise 'y' is not only inherited along with 'r' but also with 'R' . This independent behavour of genes lead to the formation of '4' types of gametes from 'F1' and two recombinants.

Student activities:

- Motivate the students to collect more information on Mendal's plant hybridization experiments & related pictures.
- Conduct group discussion on how Mendel deduced the inheritance patterns.
- Give the problems on mendel's laws and usage of punnet square.

Assessment:

Assessing the students level of understanding the topic after completing by asking oral questions and conducting the slip tests.

Post test & answers:

Q1) Define the following terms:

Dominant – In genetics, the ability of one allelic form of a gene to determine the phenotype of a heterozygous individual, in which the homologous chromosomes carries both it and a different (recessive) allele.

Recessive – In genetics, an allele that does not determine phenotype in the presence of a dominant allele.

Phenotype – The observable properties of an individual resulting from both genetic and environmental factors.

Genotype – An exact description of the genetic constitution of an individual, either with respect to a single trait or with respect to a larger set of traits.

Alleles – The alternate forms of a genetic character found at a given locus on a chromosome.

Homozygous – In a diploid organism, having identical alleles of a given gene on both homologous chromosomes. An individual may be a homozygote with respect to one gene and a heterozygote with respect to another.

Heterozygous – Of a diploid organism having different alleles of a given gene on the pair of homologues carrying that gene.

Mendel's First Law – Law of Segregation - In genetics, the separation of alleles, or of homologous chromosomes, from one another during meiosis so that each of the haploid daughter nuclei produced by meiosis contains one or the other member of the pair found in the diploid mother cell, but never both.

Mendel's Second Law – Law of Independent Assortment - During meiosis, the random separation of genes carried on nonhomologous chromosomes.

Sex-Linked – The pattern of inheritance characteristic of genes located on the sex chromosomes of organisms having a chromosomal mechanism for sex determination.

Haploid – Having a chromosome complement consisting of just one copy of each chromosome; designated 1n or n.

Diploid – Having a chromosome complement consisting of two copies (homologues) of each chromosome. Designated 2n.

Q2) The gene for hair color in rabbits has two alleles Q and q. Q is dominant and codes for brown hair. q is recessive and codes for white hair. Write out all the possible genotypes and phenotypes.

There are three possible genotypes: QQ, Qq, qq There are two possible phenotypes: Brown and white

Q3) Using the above example, fill in the Punnett's Square of offspring genotypes if one parent is heterozygous and the other is white haired. If the pair of rabbits have a litter of 24 babies, write out the expected number of each genotype and phenotype in the table below.

		heterozygous parent	
		Q	q
white-haired parent	q	Qq	Qq
	q	Qq	Qq

Genotype	Phenotype	Expected Number
Qq	Brown	12
qq	White	12

Q4) The gene for plant height in sunflowers has two codominant alleles, T_1 for tall plants and T_2 for short plants. If a tall plant is crossed with a short plant, fill in the table below.

		tall parent	
		T_1	T_1
short parent	T_2	T_1T_2	T_1T_2
	T_2	T_1T_2	T_1T_2

Genotype: T_1T_2 Phenotype: Medium Height (100%)

Q5) Take any two of the seedlings from part 2A and cross them. Fill in the results below.

	T_1	T_2
T_1	T_1T_1	T_1T_2
T_2	T_1T_2	T_2T_2

Genotype	Phenotype	Expected Number
T_1T_1	Tall	25%
T_1T_2	Medium	50%
T_2T_2	Short	25%

MCQ's

1) The phenotypic ratio of Monohybrid cross is [ ]
(a) 3:1 (b) 2:1
(c) 1:1 (d) 9.3.3.1
Answe
r: A

2) The monohybrid test cross ratio is [ ]
(a) 3:1 (b) 2:1
(c) 1:1 Answer: C (d) 9.3.3.1

3) The genotypic ratio of monohybrid cross is [ ]
(a) 3:1 (b) 1:2:1
(c) 1:1 (d) 9.3.3.1
Answe
r: B

4) The crossing of F1 to homozygous recessive parent is called [ ]
(a) F1 cross (b) Back cross
(c) Test cross (d) None of the above
Answer: C

5) The test cross is used to determine the [ ]
(a) Genotype of the plant (b) Phenotype of the plant
(c) None of the above Answer: A (d) All of the above

6) The phenotypic dihybrid ratio is [ ]
(a) 9:3:3:1 (b) 9:3:2:1 (c) 9.3.1.2 (d) 9.1.3.2
Answer: A

7) Which of the following terms represent a pair of contrasting characters [ ]
(a) Allelomorphs (b) Heterozygous
(c) Homozygous (d) Co-dominant

Answer: A

8) Each gamete carry [ ]

(a) Only Dominant allele (b) Only Recessive allele

(c) Any one of the alleles (d) All of these

Answer: C

9) The dihybrid test cross ratio is [ ]

(b) 9:3:3:1 (b) 1:2:1:2

(c) 1:1:1:1 (d) None of the above

Answer: C

10) Which of the following statement is "true" regarding the law of independent assortment [ ]

(a)Independent assortment leads to variation

(b)Independent assortment leads to formation of new combinations of characters

(c)Factors assort independently (d) All of the above Answer: D

11) The best method is to determine the genotype of the dominant parent is by crossing with the

hybrid. This cross is called [ ]

(a) Cross fertilization (b) Test cross

(c) Back cross Answer: C (d) Selfing

12) All of these obeys Mendal's laws except [ ]

(a) Purity od gametes (b) Linkage

(c) Dominance (d) Independent assortment

Answer: B

13) The title of the Mendal's paper presented at Brunn's natural science socity in 1865 was [ ]

(a) Experiments on pea plants (b) Law of Heredity

(c) Law of Inheritance (d) Experiments in plant hybridization

Answer: D

14) The F1 generation is determined by crossing P/P with p/p. Then the progeny obtained from them were intercrossed. What will be the ratio of pure breeding flowers to not pure breeding flowers in F2? [ ]

a) 3:1 pure: non- pure

b) 1:3 pure: non- pure

c) 1:1 pure: non- pure

d) 1:2 pure: non- pure

Answer: C

15) In a cross between wild type pure breeding recessive and heterozygous dominant trait, if 4 progeny are found to be expressing the dominant phenotype, what will be the expected of recessive? [ ]

a) 1 b) 2 c) 3 d) 4

Answer: D

Reference books

1. Genetics by P.S.Verma & Agarwal
2. Principles of Genetics by Gardner & Simmons

TSWRDC BHONGIR

Affiliated to Mahatma Gandhi University

Year: III YR (V SEM); Paper: V (Animal Physiology & Biochemistry)

Class: BZC/MZC (BSC)

Department: ZOOLOGY Name of the faculty: K. SRILATHA

Module Topic: Electron Transport chain (Carbohydrate Metabolism)

No. of teaching hours: 02

Module Objectives: The main objective of this module is to introduce electron transport chain topic to the students.

✓To understand the role of mitochondria in health & disease.
✓To know about Electron transfer, oxidative phosphorylation and ATP synthesis.

Pre-test: Understanding the student's basic knowledge by asking orally on the following

- Glycolysis
- Citric acid cycle/Krebs Cycle
- Generation of ATP from these two pathways of cellular respiration
- Will these pathways generate ATP directly?

Answer: No

- How these two pathways are linked to ETC
- What are redox reactions?

Answer: Redox is a type of chemical reaction in which the oxidation states of atoms are changed. Redox reactions are characterized by the actual or formal transfer of electrons between chemical species, most often with one species undergoing oxidation while another species undergoes reduction.

Module Content:

Electron Transport Chain

You have just read about two pathways in cellular respiration—glycolysis and the citric acid cycle—that generate ATP. However, most of the ATP generated during the aerobic catabolism of glucose is not generated directly from these pathways. Rather, it is derived from a process that begins with moving electrons through a series of electron transporters that undergo redox reactions: **the electron transport chain**. This causes hydrogen ions to accumulate within the matrix space. Therefore, a concentration gradient forms in which hydrogen ions diffuse out of the matrix space by passing through ATP synthase. The current of hydrogen ions powers the catalytic action of ATP synthase, which phosphorylates ADP, producing ATP.

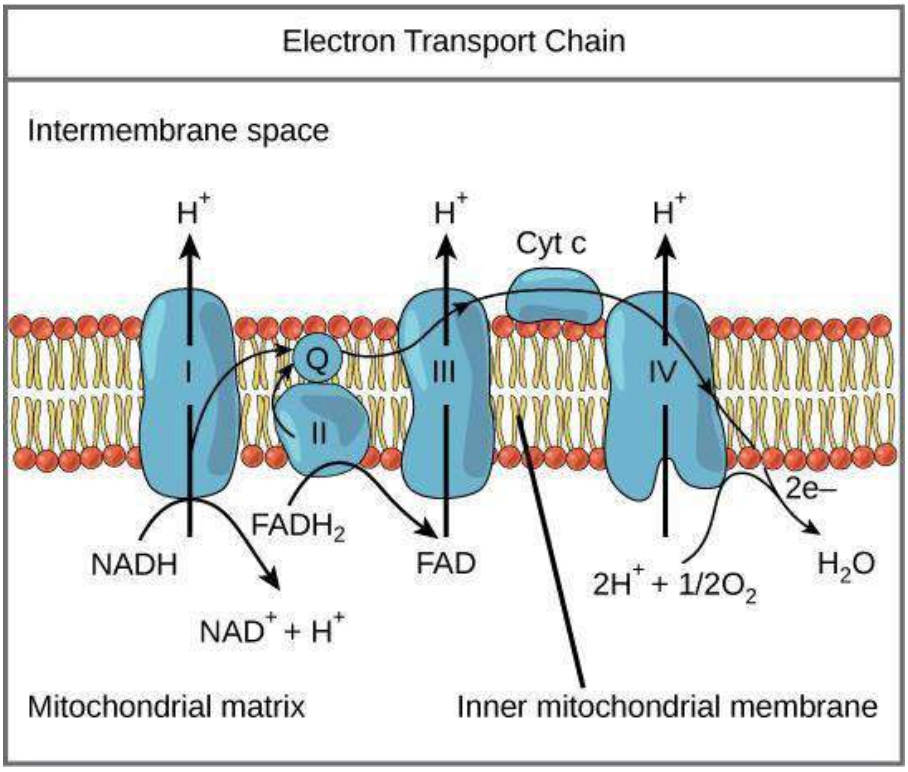


Figure 1. The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and $FADH_2$ to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space, and oxygen is reduced to form water.

The electron transport chain (Figure 1) is the last component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Oxygen continuously diffuses into plants; in animals, it enters the body through the respiratory system. Electron transport is a series of redox reactions that resemble a relay race or bucket brigade in that electrons are passed rapidly from one component to the next, to the endpoint of the chain where the electrons reduce molecular oxygen, producing water. There are four complexes composed of proteins, labeled I through IV in Figure 1, and the aggregation of these four complexes, together with associated mobile, accessory electron carriers, is called the electron transport chain. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes. Note, however, that the electron transport chain of prokaryotes may not require oxygen as some live in anaerobic conditions. The common feature of all electron transport chains is the presence of a proton pump to create a proton gradient across a membrane.

Complex I

To start, two electrons are carried to the first complex aboard NADH. This complex, labeled I, is composed of flavin mononucleotide (FMN) and an iron-sulfur (Fe-S)-containing protein. FMN, which is derived from vitamin B_2, also called riboflavin, is one of several prosthetic groups or co-factors in the electron transport chain. A **prosthetic group** is a non-protein molecule required for the activity of a protein. Prosthetic groups are organic or inorganic, non-peptide molecules bound to a protein that facilitate its function; prosthetic groups include co-enzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase and is a very large protein, containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space, and it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

Q and Complex II

Complex II directly receives $FADH_2$, which does not pass through complex I. The compound connecting the first and second complexes to the third is **ubiquinone** (Q). The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced, (QH_2), ubiquinone delivers its electrons to the next complex in the electron transport chain. Q receives the electrons derived from NADH from complex I and the electrons derived from $FADH_2$ from complex II, including succinate dehydrogenase. This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex. Since these electrons bypass and thus do not energize the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons. The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.

Complex III

The third complex is composed of cytochrome b, another Fe-S protein, Rieske center (2Fe-2S center), and cytochrome c proteins; this complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic group of heme. The heme molecule is similar to the heme in hemoglobin, but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced and oxidized as it passes the electrons, fluctuating between different oxidation states: Fe^{++} (reduced) and Fe^{+++} (oxidized). The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding them, giving slightly different characteristics to each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes (cytochrome c is the acceptor of electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time).

Complex IV

The fourth complex is composed of cytochrome proteins c, a, and a_3. This complex contains two heme groups (one in each of the two cytochromes, a, and a_3) and three copper ions (a pair of

Cu_A and one Cu_B in cytochrome a_3). The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water (H_2O). The removal of the hydrogen ions from the system contributes to the ion gradient used in the process of chemiosmosis.

Chemiosmosis

In chemiosmosis, the free energy from the series of redox reactions just described is used to pump hydrogen ions (protons) across the membrane. The uneven distribution of H^+ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient), owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane.

If the membrane were open to diffusion by the hydrogen ions, the ions would tend to diffuse back across into the matrix, driven by their electrochemical gradient. Recall that many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels. Similarly, hydrogen ions in the matrix space can only pass through the inner mitochondrial membrane through an integral membrane protein called ATP synthase (Figure 2). This complex protein acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient. The turning of parts of this molecular machine facilitates the addition of a phosphate to ADP, forming ATP, using the potential energy of the hydrogen ion gradient.

ATP Yield

The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport chain complexes can pump through the membrane varies between species. Another source of variance stems from the shuttle of electrons across the membranes of the mitochondria. (The NADH generated from glycolysis cannot easily enter mitochondria.) Thus, electrons are picked up on the inside of mitochondria by either NAD^+ or FAD^+. As you have learned earlier, these FAD^+ molecules can transport fewer ions;

consequently, fewer ATP molecules are generated when FAD^+ acts as a carrier. NAD^+ is used as the electron transporter in the liver and FAD^+ acts in the brain.

Another factor that affects the yield of ATP molecules generated from glucose is the fact that intermediate compounds in these pathways are used for other purposes. Glucose catabolism connects with the pathways that build or break down all other biochemical compounds in cells, and the result is somewhat messier than the ideal situations described thus far. For example, sugars other than glucose are fed into the glycolytic pathway for energy extraction. Moreover, the five-carbon sugars that form nucleic acids are made from intermediates in glycolysis. Certain nonessential amino acids can be made from intermediates of both glycolysis and the citric acid cycle. Lipids, such as cholesterol and triglycerides, are also made from intermediates in these pathways, and both amino acids and triglycerides are broken down for energy through these pathways. Overall, in living systems, these pathways of glucose catabolism extract about 34 percent of the energy contained in glucose.

Student activities:

- Motivate the students to collect more information on agents which inhibit the ETC and their mechanisms.
- Conduct group discussion on how Movement electrons takes place across the complexes
- Outline how energy is harvested and used to drive cellular reactions
- Describe and illustrate the metabolic pathways of; glycolysis, glycogenolysis, gluconeogenesis, the citric acid cycle, oxidative phosphorylation.
- Describe the interrelationships between the various metabolic pathways and outline their overall regulation

Assessment:

Assessing the students level of understanding the topic after completing by asking oral questions and conducting the slip tests.

Post-tests

Q1) Dinitrophenol (DNP) is an uncoupler that makes the inner mitochondrial membrane leaky to protons. It was used until 1938 as a weight-loss drug. What effect would you expect DNP to have on the change in pH across the inner mitochondrial membrane? Why do you think this might be an effective weight-loss drug?

Answer: After DNP poisoning, the electron transport chain can no longer form a proton gradient, and ATP synthase can no longer make ATP. DNP is an effective diet drug because it uncouples ATP synthesis; in other words, after taking it, a person obtains less energy out of the food he or she eats. Interestingly, one of the worst side effects of this drug is hyperthermia, or overheating of the body. Since ATP cannot be formed, the energy from electron transport is lost as heat.

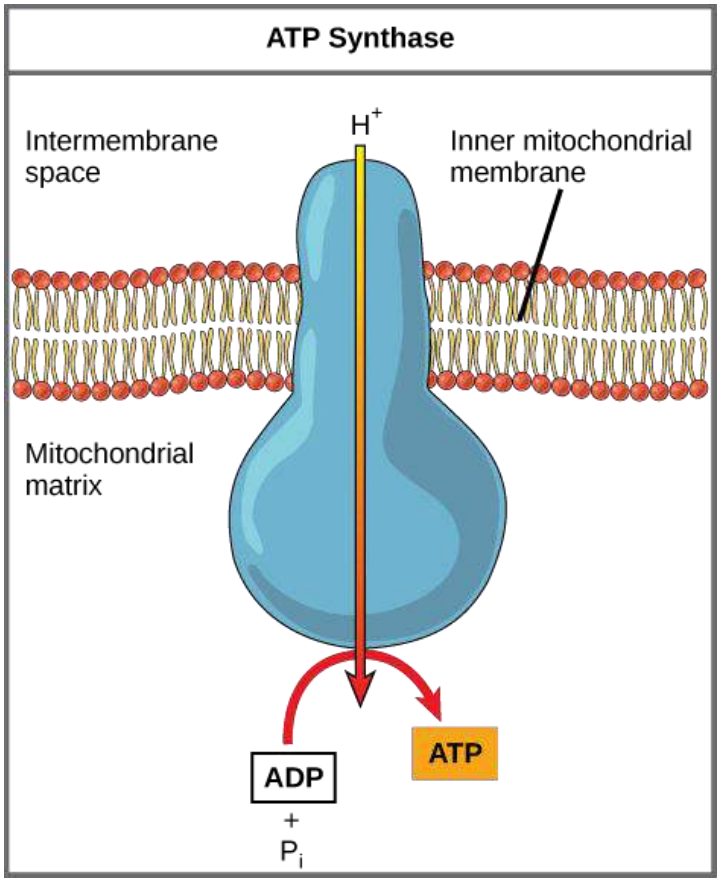


Figure 2. ATP synthase is a complex, molecular machine that uses a proton (H+) gradient to form ATP from ADP and inorganic phosphate (Pi). (Credit: modification of work by Klaus Hoffmeier)

Chemiosmosis (Figure 3) is used to generate 90 percent of the ATP made during aerobic glucose catabolism; it is also the method used in the light reactions of photosynthesis to harness the energy of sunlight in the process of photophosphorylation. Recall that the production of ATP using the process of chemiosmosis in mitochondria is called oxidative phosphorylation. The overall result of these reactions is the production of ATP from the energy of the electrons removed from hydrogen atoms. These atoms were originally part of a glucose molecule. At the end of the pathway, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen attract hydrogen ions (protons) from the surrounding medium, and water is formed.

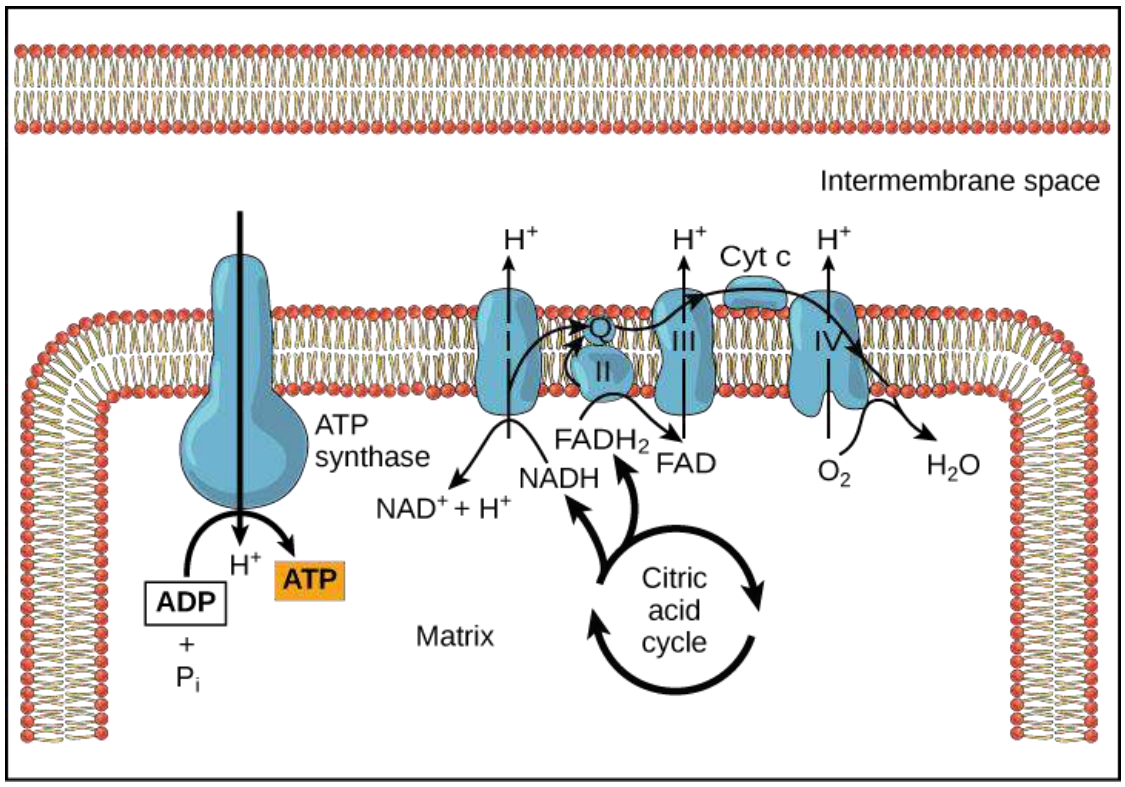


Figure 3. In oxidative phosphorylation, the pH gradient formed by the electron transport chain is used by ATP synthase to form ATP.

Q2) Cyanide inhibits cytochrome c oxidase, a component of the electron transport chain. If cyanide poisoning occurs, would you expect the pH of the intermembrane space to increase or decrease? What effect would cyanide have on ATP synthesis?

Answer: After cyanide poisoning, the electron transport chain can no longer pump electrons into the intermembrane space. The pH of the intermembrane space would increase, the pH gradient would decrease, and ATP synthesis would stop.

IN SUMMARY: ELECTRON TRANSPORT CHAIN

The electron transport chain is the portion of aerobic respiration that uses free oxygen as the final electron acceptor of the electrons removed from the intermediate compounds in glucose catabolism. The electron transport chain is composed of four large, multiprotein complexes embedded in the inner mitochondrial membrane and two small diffusible electron carriers shuttling electrons between them. The electrons are passed through a series of redox reactions, with a small amount of free energy used at three points to transport hydrogen ions across a membrane. This process contributes to the gradient used in chemiosmosis. The electrons passing through the electron transport chain gradually lose energy, High-energy electrons donated to the chain by either NADH or $FADH_2$ complete the chain, as low-energy electrons reduce oxygen molecules and form water. The level of free energy of the electrons drops from about 60 kcal/mol in NADH or 45 kcal/mol in $FADH_2$ to about 0 kcal/mol in water. The end products of the electron transport chain are water and ATP. A number of intermediate compounds of the citric acid cycle can be diverted into the anabolism of other biochemical molecules, such as nonessential amino acids, sugars, and lipids. These same molecules can serve as energy sources for the glucose pathways.

Reference books

1. Biochemistry by U. Satyanarayana

2. Telugu Academy “Animal Physiology & Biochemistry”

TSWRAFPDCW, Bhongir SUBJECT: Zoology

PAPER- GE (Tools and Techniques in Biology) FACULTY:

K. Srilatha

Topic: Colorimeter Principles and Applications

Colorimeter is a light-sensitive device that helps certain solutions absorb a particular wavelength of light in colorimetry. It is used to measure the absorbance and transmittance of light that passes through a liquid. Colorimeter can also be used to determine the concentration of a coloured compound in a solution. The principle of colorimeter is based on the fact that coloured compounds can absorb a certain wavelength of light when monochromatic light is passed through them. The working of a colorimeter is based on the concept of Beer-Lambert's law. It was invented by Louis J Duboscq in the year 1870.

Principle of Colorimeter : The principle of Colorimeter is based on the photometric technique that states when an incident light of intensity (I_0) passes through a solution, then

- Part of the incident light is reflected (I_r)
- Part of the incident light is transmitted (I_t)
- Part of the incident light is absorbed (I_a)

Therefore,

$I_0 = I_r + I_t + I_a$

Here, the value of reflected light (I_r) is eliminated as I_0 and I_t values are enough to calculate I_a. The values for the amount of light absorbed and transmitted are measured by keeping I_r constant. The principle of colorimeter is based on two fundamental laws of photometry that establish the relationship between the amount of light absorbed and the concentration of the substance.

Colorimeter

Beer-Lambert Law

Beer's Law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution.

$Log_{10}\ I_0/\ I_t\ = a_sc$ Where,

$a_s \rightarrow$ Absorbency Index

$c \rightarrow$ Concentration of solution

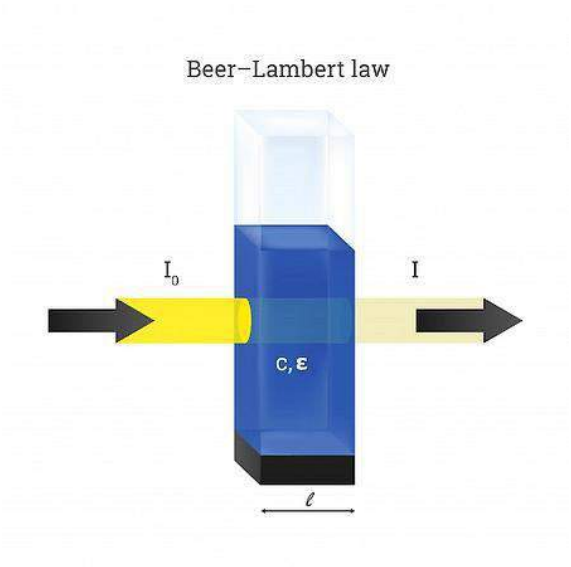
Lambert's Law states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.

$A = \log_{10} I_0/I_t\ = a_sb$ Where,

$A \rightarrow$ Test of Absorbance

$a_s \rightarrow$ Absorbance of standard solution

$b \rightarrow$ length or thickness of the solution



Beer-Lambert Law

The combined mathematical expression of Beer-Lambert law is: $Log_{10}\ I_0 / I_t = a_s bc$

If b is kept constant by taking a cuvette or a standard cell, then $Log_{10}\ I_0 / I_t = a_s c$

Where Absorption index is given by $a_s = A/cl$

Where,

A → Absorbance or optical density of the solution

c → Concentration of the absorbing material (gm/lit)

l → distance travelled by light in solution (cm)

In simple terms, the combined principle of Beer-Lambert's law states that the amount of light absorbed by a colour solution is directly proportional to the concentration of the solution and the length of the light path through the solution.

$A \propto cl$ $A = \in cl$

Where,

$\in \rightarrow$ Absorption Coefficient

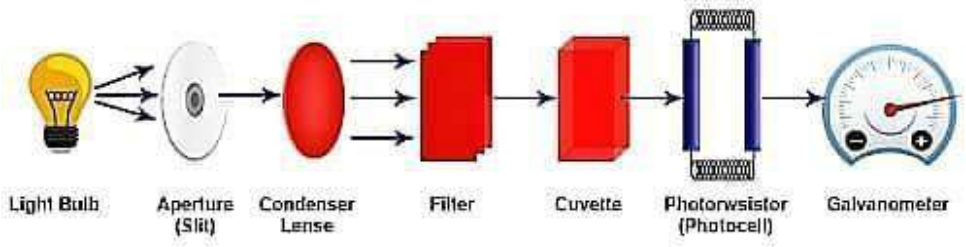
Applications of Colorimeter

1.Colorimeter is most commonly used to determine the concentration of a coloured compound by measuring the absorbance or optical density.
2.In the case of colourless compounds, a suitable reagent is introduced which when mixed, would result in a coloured compound. This is then measured in the colorimeter against the known values of the standard solution.
3.The course of a reaction can be determined in a colorimeter by measuring the rate of formation and disappearance of the light-absorbing compound.
4. Colorimeter can also act in the reverse process by which it can identify a compound by measuring the absorption index.

Components Of Colorimeter

The main parts that make up the colorimeter are as follows:

- Source of Light: Tungsten filament is commonly used as a light source in colorimeters.
- Monochromator: It is used to split the light into different wavelengths and select the particular wavelength under observation.
- Sample Holder: This is where the cuvettes or test tube containing the colour sample solution is placed. These are made of glass at visible wavelengths.

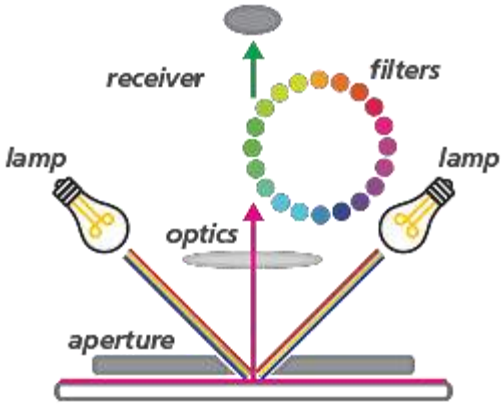


Components of Colorimeter

- Photo Detector System: This system produces an electric signal when light falls into it which is in turn reflected as a reading in the galvanometer.
- Measuring Device: The galvanometer is used as the measuring device where it converts the electrical signals into readings that correspond to the intensity of light.

Working Of Colorimeter

1. Colorimeter has to be calibrated first by using the standard solutions of the known concentration of the solute that is to be determined in the test solution. The standard solutions are poured into the cuvettes which are then placed in the sample holder.



Working of Colorimeter

1. A beam of light of a certain wavelength specific to the assay is directed towards the test solution. Before it reaches the test solution, it passes through a series of lenses and filters. The lens helps in accurate navigation of the beam of light. The filters split the incoming light into different wavelengths and allow the required wavelength to reach the cuvette containing the test solution.

1. The monochromatic light (light of one wavelength) reaches the test solutions and some of the light gets reflected, some would get absorbed and the remaining would pass through the test solution and falls onto the photodetector. The photodetector sends the pulses to the galvanometer. The galvanometer reads the electrical signals from the detector and displays them in digital form. The reading corresponds to the absorbance or the optical density of the test solution.

1. To determine the absorbance or optical density of the test solution, the following formula is used.

We know that, $A = \in cl$

For standard and test solutions, $\in$ and l are constant Therefore,

$A_T = C_T$ …(i)

$A_s = C_s$ …(ii)

Cross multiplying (i) and (ii) $A_T \times C_s = C_T \times A_s$

$C_T = (A_T / A_s) \times C_s$ Where,

$C_T \rightarrow$ Concentration of test solution

$C_s \rightarrow$ Concentration of standard solution

$A_T \rightarrow$ Absorbance or Optical density of test solution

$A_s \rightarrow$ Absorbance or Optical density of the standard solution

Uses Of Colorimeter

- Colorimeter is widely used in the medical industry to estimate biochemical samples such as blood, urine, cerebral spinal fluid, plasma, serum, etc.
- They are used to analyse the colour contrast and brightness in mobile, computer and television screens to provide users with the best viewing experience.
- It also finds its application in the paints and textile industries.
- Colorimeter is used in the food and food processing industry.
- It is used in the printing industry to measure the quality of print paper and printing ink.

Uses of Colorimeter

- They are also used to test the water quality and screen for the identification of chemical substances such as chlorine, fluorine, cyanide, iron, molybdenum, etc.
- They are used in jewellery to measure diamond quality.
- Colorimeter is used to measure the concentration of haemoglobin in blood samples.
- It helps to monitor the nutrient concentration in the soil for plant growth.
- Colorimeter is also used in the pharmaceutical industry to identify substandard products and drugs.

Advantages of Colorimeter

The benefits of using a colorimeter are:

- Colorimeter is a cheap and efficient method of quality analysis.
- Portable colorimeters are available which makes them convenient to use.
- Quantitative analysis of coloured compounds can be easily done by using Colorimeter.

Disadvantages of Colorimeter

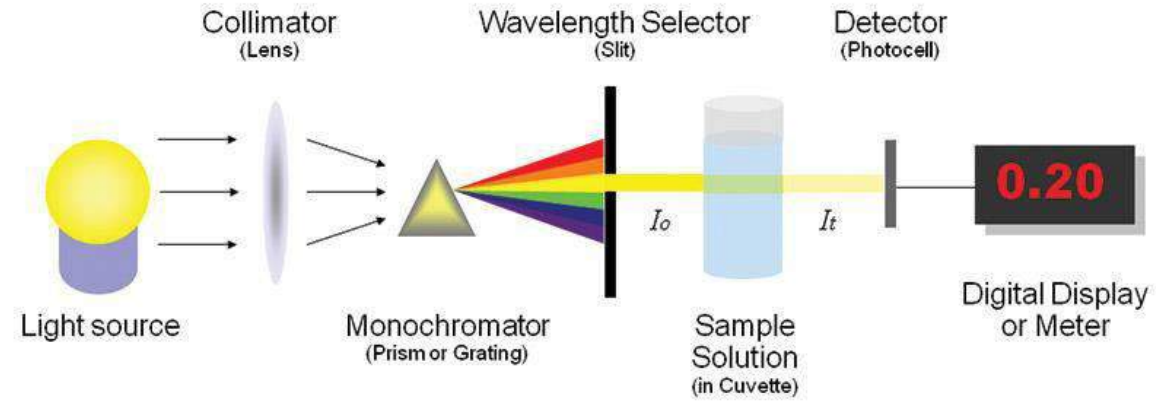
- It becomes a tedious process to identify the concentration of colourless compounds.
- Since Colorimeter measures the absorbance of wavelength only in the visible spectrum of light (400nm to 700nm), it does not work in the ultraviolet and infrared spectrum.
- It is not possible to set a specific wavelength; rather a range of spectrum has to be set to measure the absorbance.

Spectrophotometer- Principle and Applications

- A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.
- Spectrophotometer techniques are mostly used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer.
- Scientist Arnold J. Beckman and his colleagues at the National Technologies Laboratory (NTL) invented the Beckman DU spectrophotometer in 1940.

Spectrophotometer

Principle, Instrumentation, Applications



Principle of Spectrophotometer

The spectrophotometer technique is to measure light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a graph on the detector and then on the display device.

1.In the spectrophotometer, a prism (or) grating is used to split the incident beam into different wavelengths.

2.By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm.

3.The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

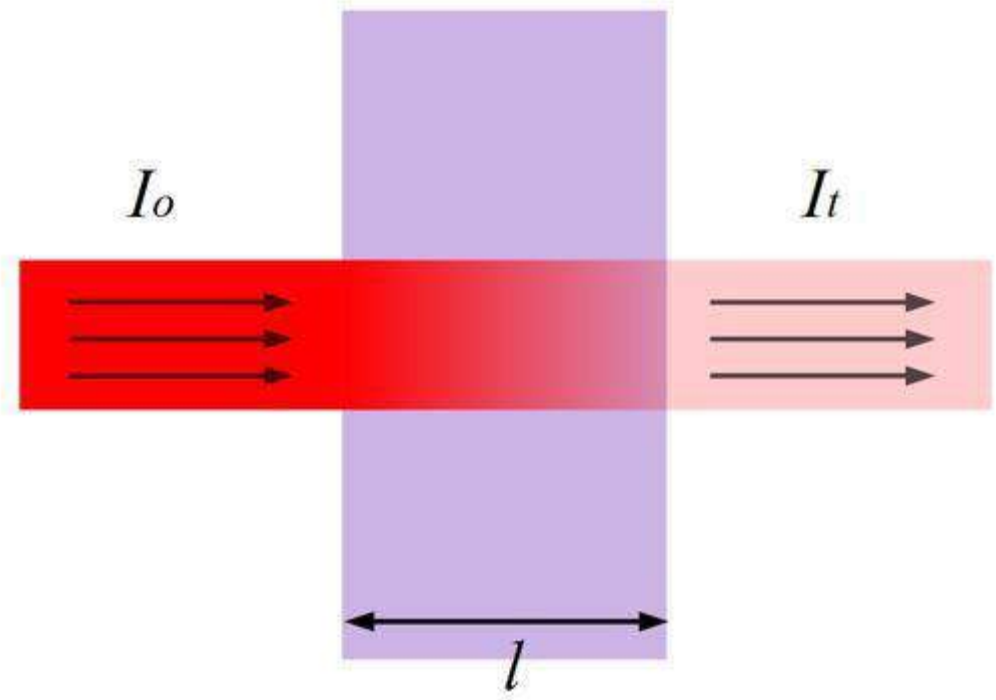
the amount of photons that goes through the cuvette and into the detector is dependent on the length of the cuvette and the concentration of the sample. Once you know the intensity of light after it passes through the cuvette, you can relate it to transmittance (T). Transmittance is the fraction of light that passes through the sample. This can be calculated using the equation

Transmittance(T)=It/Io Transmittance(T)=It/Io

Where I_t is the light intensity after the beam of light passes through the cuvette and I_o is the light intensity before the beam of light passes through the cuvette. Transmittance is related to absorption by the expression:

Absorbance(A)=−log(T)=−log(It/Io)

Where absorbance stands for the amount of photons that is absorbed. With the amount of absorbance known from the above equation, you can determine the unknown concentration of the sample by using Beer-Lambert Law.



Beer-Lambert Law

Beer-Lambert Law (also known as Beer's Law) states that there is a linear relationship between the absorbance and the concentration of a sample. For this reason, Beer's Law can *only* be applied when there is a linear relationship. Beer's Law is written as:

$A=\epsilon lc$ where

- AA is the measure of absorbance (no units),
- $\epsilon\epsilon$ is the molar extinction coefficient or molar absorptivity (or absorption coefficient),
- ll is the path length, and
- cc is the concentration.

The molar extinction coefficient is given as a constant and varies for each molecule. Since absorbance does not carry any units, the units for $\epsilon\epsilon$ must cancel out the units of length and concentration. As a result, $\epsilon\epsilon$ has the units: $L \cdot mol^{-1} \cdot cm^{-1}$. The path length is measured in centimeters. Because a standard spectrometer uses a cuvette that is 1 cm in width, ll is always

assumed to equal 1 cm. Since absorption, $\epsilon\epsilon$, and path length are known, we can calculate the concentration cc of the sample.

Instrumentation of Spectrophotometer

The essential components of spectrophotometer instrumentation include:

1. A table and cheap radiant energy source

•Materials that can be excited to high energy states by a high voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.

1. A monochromator, to break the polychromatic radiation into component wavelength (or) bands of wavelengths.

•A monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands.

Prisms:

•A prism disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent

•Two types of Prisms are usually employed in commercial instruments. Namely, 600 cornu quartz prism and 300 Littrow Prism.

Grating:

- Gratings are often used in the monochromators of spectrophotometers operating ultraviolet, visible and infrared regions.

1. Transport vessels (cuvettes), to hold the sample

•Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as “CUVETTES”.

•Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz.

1. A Photosensitive detector and an associated readout system

•Most detectors depend on the photoelectric effect. The current is then proportional to the light intensity and therefore a measure of it.

•Radiation detectors generate electronic signals which are proportional to the transmitter light.

•These signals need to be translated into a form that is easy to interpret.

•This is accomplished by using amplifiers, Ammeters, Potentiometers and Potentiometric recorders.

Applications

Some of the major applications of spectrophotometers include the following:

•Detection of concentration of substances
•Detection of impurities
•Structure elucidation of organic compounds
•Monitoring dissolved oxygen content in freshwater and marine ecosystems
•Characterization of proteins
•Detection of functional groups
•Respiratory gas analysis in hospitals

- Molecular weight determination of compounds
- The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations.

TSWRAFPDCW, Bhongir SUBJECT: Zoology

PAPER- GE (Tools and Techniques in Biology), VI Sem

FACULTY: Dr. K. Srilatha

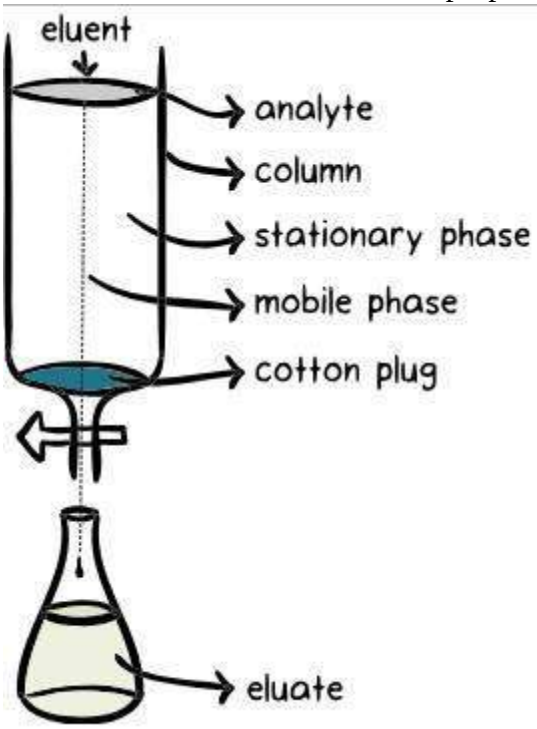
Topic: Chromatography

What is chromatography?

- Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.
- The Russian botanist Mikhail Tswett coined the term chromatography in 1906.
- The first analytical use of chromatography was described by James and Martin in 1952, for the use of gas chromatography for the analysis of **fatty acid** mixtures.
- A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties to separate materials.

owerful separation tool that is used in all branches of
and is often the
only means of separating components from complex mixtures.

ple of Chromatography (how does chromatography work)



- Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.
- The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights.

- Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into the mobile phase, and leave the system faster.

Three components thus form the basis of the chromatography technique.

1. **Stationary phase:** This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface solid support".
2. **Mobile phase:** This phase is always composed of "liquid" or a "gaseous component."
3. **Separated molecules**

The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on the separation of

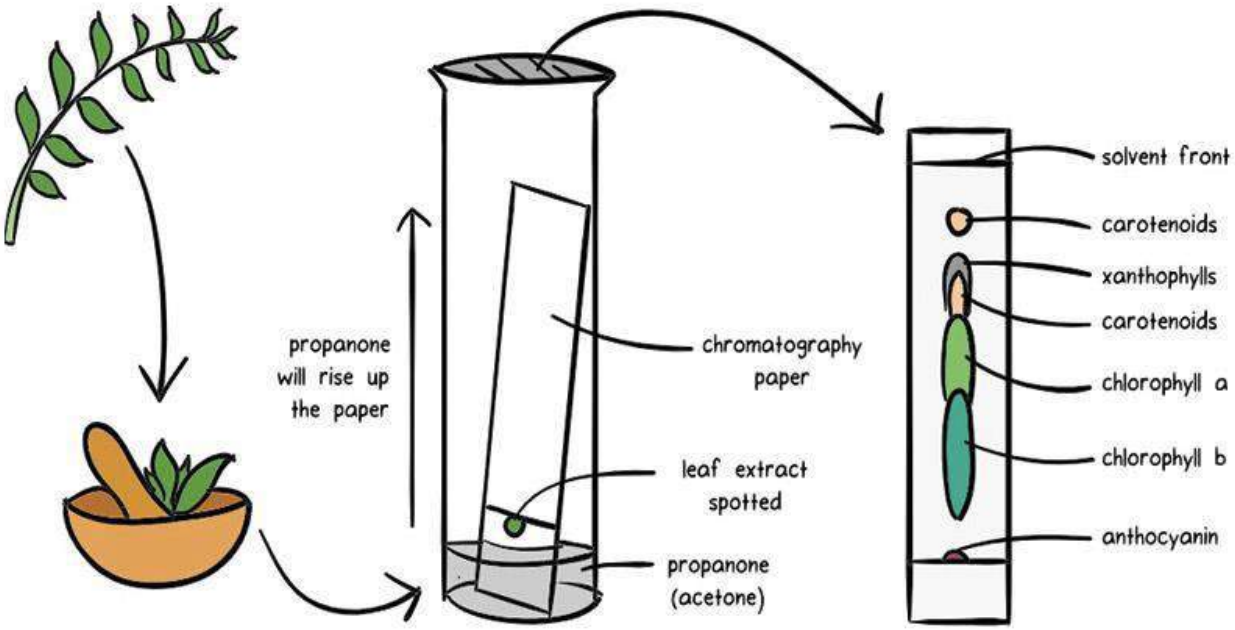


Image Source: Khan Academy

Types of Chromatography

•Substances can be separated on the basis of a variety of methods and the presence of characteristics such as size and shape, total charge, hydrophobic groups present on the surface, and binding capacity with the stationary phase.

•This leads to different types of chromatography techniques, each with their own instrumentation and working principle.

•For instance, four separation techniques based on molecular characteristics and interaction type use mechanisms of ion exchange, surface adsorption, partition, and size exclusion.

•Other chromatography techniques are based on the stationary bed, including column, thin layer, and paper chromatography.

Commonly employed chromatography techniques include:

1.Column chromatography
2.Ion-exchange chromatography
3.Gel-permeation (molecular sieve) chromatography
4.Affinity chromatography

5. Paper chromatography
6. Thin-layer chromatography
7. Gas chromatography (GS)
8. Hydrophobic interaction chromatography
9. High-pressure liquid chromatography (HPLC)

Affinity chromatography

Affinity chromatography is a separation technique where the components of a mixture are separated based on their affinity towards the stationary phase of the system.

Principle of Affinity chromatography

- This chromatography technique is based on the principle that components of a mixture are separated when the element having an affinity towards the stationary phase binds to the stationary phase. In contrast, other components are eluted with the mobile phase.
- The substrate/ ligand is bound to the stationary phase so that the reactive sites for the binding of components are exposed.
- Now, the mixture is passed through the mobile phase where the components with binding sites for the substrate bind to the substrate on the stationary phase while the rest of the components are eluted out with the mobile phase.
- The components attached to the stationary phase are then eluted by changing the pH,

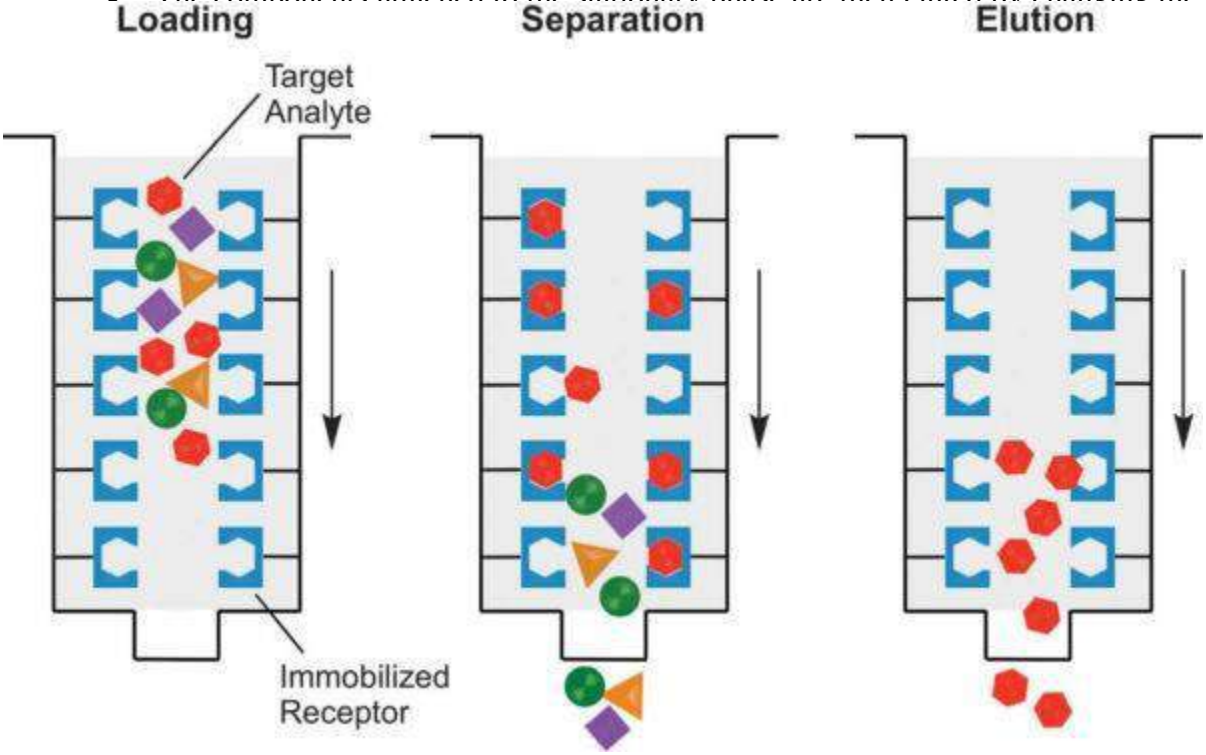


Figure: Affinity chromatography. Image Source: Creative Biostructure. Steps of Affinity chromatography

- The column is prepared by loading it with solid support like agarose or cellulose, onto which the substrate/ ligand with the spacer arm, is attached.

- The mobile phase containing the mixture is poured into the column at a constant rate.
- Once the process is complete, the ligand-molecule complex is eluted from the stationary phase by changing the conditions that favor the separation of ligand and components of the mixture.

Uses of Affinity chromatography

- Affinity chromatography is used as a staple separation technique from enzymes and other proteins.
- This principle is also applied in the in vitro antigen-antibody reactions.
- This technique is used for the separation of components as well as the removal of impurities from a mixture.
- Affinity chromatography can be used in the detection of mutation and nucleotide polymorphisms in nucleic acids.

Examples of Affinity chromatography

- The purification of coli β-galactosidase from a mixture of proteins using the p- aminophenyl-1-thio-β-D-galactopyranosyl agarose as the affinity matrix.
- The removal of excess albumin and α_2-macroglobulin from the serum albumin.

Column chromatography

Column chromatography is the separation technique where the components in a mixture are separated on the basis of their differential adsorption with the stationary phase, resulting in them moving at different speeds when passed through a column.

It is a solid-liquid chromatography technique in which the stationary phase is a solid & mobile phase is a liquid or gas.

Principle of Column chromatography

- This technique is based on the principle of differential adsorption where different molecules in a mixture have different affinities with the absorbent present on the stationary phase.
- The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column.
- However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions.
- Here, the stationary phase in the column chromatography also termed the absorbent, is a solid (mostly silica) and the mobile phase is a liquid that allows the molecules to move through the column smoothly.

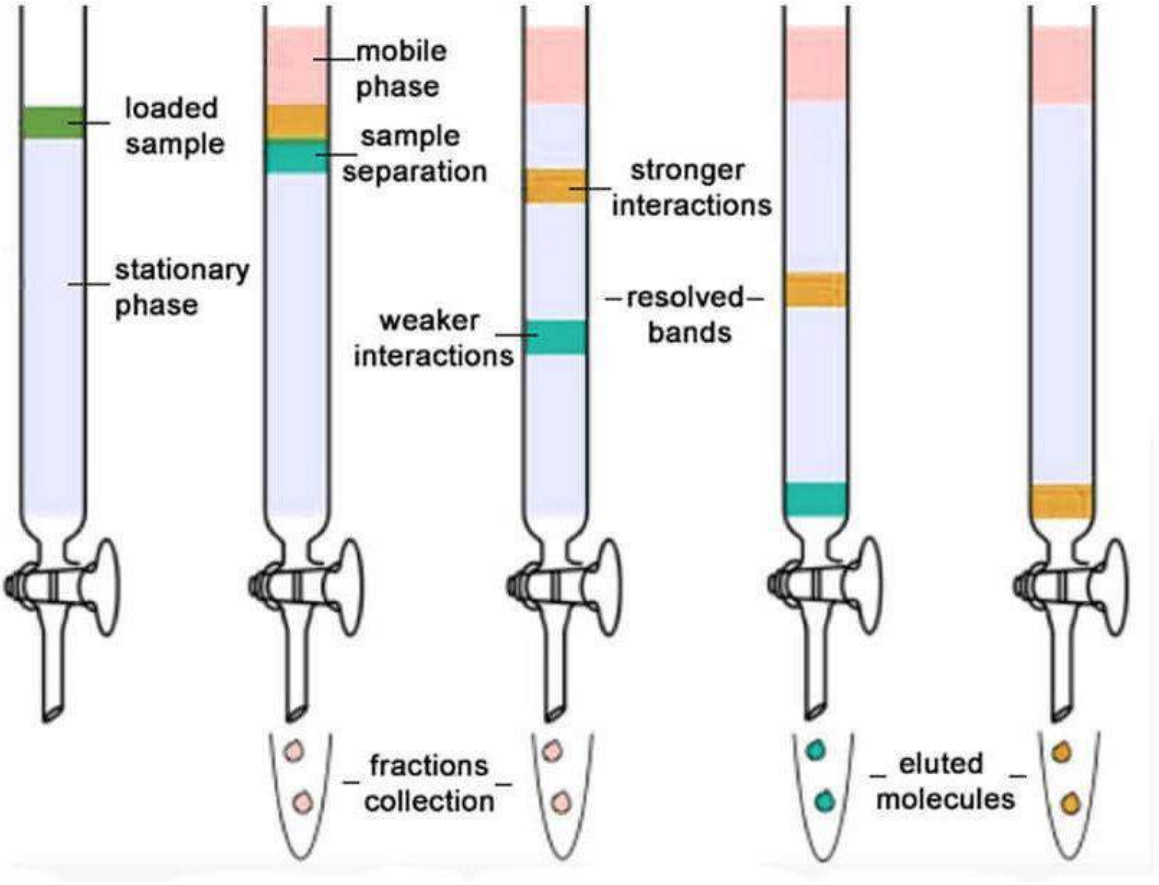


Figure: Column chromatography. Image Source: PrepGenie. Steps of Column chromatography

- The column is prepared by taking a glass tube that is dried and coated with a thin, uniform layer of stationary phase (cellulose, silica).
- Then the sample is prepared by adding the mixture to the mobile phase. The sample is introduced into the column from the top and is allowed to pass the sample under the influence of gravity.
- The molecules bound to the column are separated by elution technique where either solution of the same polarity is used (isocratic technique), or different samples with different polarities are used (gradient technique).
- The separated molecules can further be analyzed for various purposes. Uses of Column chromatography
- Column chromatography is routinely used for the separation of impurities and purification of various biological mixtures.
- This technique can also be used for the isolation of active molecules and metabolites from various samples.
- Column chromatography is increasingly used for the detection of drugs in crude extracts. Examples of Column chromatography
- Extraction of pesticides from solid food samples of animal origin containing lipids, waxes, and pigments.

- Synthesis of Pramlintide which is an analog of Amylin, a peptide hormone, for treating **type 1 and type 2 Diabetics**.
- Purification of bioactive glycolipids, showing antiviral activity towards HSV-1 (Herpes Virus).

Gas chromatography Gas chromatography is a separation technique in which the molecules are separated on the basis of their retention time depending on the affinity of the molecules to the stationary phase.

The sample is either liquid or gas that is vaporized in the injection point. Principle of Gas chromatography

- Gas chromatography is based on the principle that components having a higher affinity to the stationary phase have a higher retention time as they take a longer time to come out of the column.
- However, the components having a higher affinity to the stationary phase have less retention time as they move along with the mobile phase.
- The mobile phase is a gas, mostly helium, that carries the sample through the column.
- The sample once injected in converted into the vapor stage is then passed through a detector to determine the retention time.
- The components are collected separately as they come out of the stationary phase at different times.

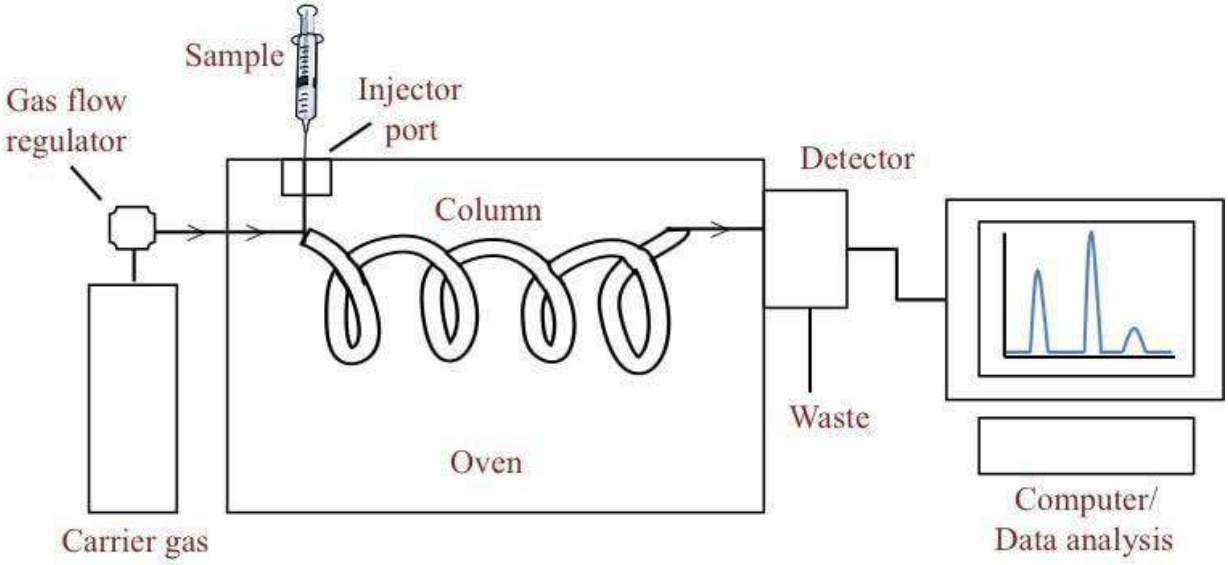


Figure: Gas chromatography. Image Source: Bitesize Bio. Steps of Gas chromatography

- The sample is injected into the column where it is vaporized into a gaseous state. The vapourised component than mixes with the mobile phase to be carried through the rest of the column.
- The column is set with the stationary phase where the molecules are separated on the basis of their affinity to the stationary phase.
- The components of the mixture reach the detector at different times due to differences in the time they are retained in the column.

Uses of Gas chromatography

- This technique is used to calculate the concentration of different chemicals in various samples.
- This is used in the analysis of air pollutants, oil spills, and other samples.
- Gas chromatography can also be used in forensic science to identify and quantify various biological samples found in the crime scene.

Examples of Gas chromatography

- The identification of performance-inducing drug in the athlete's urine.
- The separation and quantification of a solid drug in soil and water samples.

Gel filtration chromatography/ Gel permeation chromatography/ Size exclusion chromatography/ Molecular sieve chromatography

Gel-filtration chromatography is a form of partition chromatography used to separate molecules of different molecular sizes.

This technique has also frequently been referred to by various other names, including gel-permeation, gel-exclusion, size- exclusion, and molecular- sieve chromatography.

Principle

- Molecules are partitioned between a mobile phase and a stationary phase as a function of their relative sizes.
- The stationary phase is a matrix of porous polymer which have pores of specific sizes.
- When the sample is injected with the mobile phase, the mobile phase occupies the pores of the stationary phase.
- If the size of the molecules is appropriate enough to enter the pores, they remain in the pores partly or wholly.
- However, molecules with a larger size are retained from entering the pores, causing them to be moved with the mobile phase, out of the column.
- If the mobile phase used in an aqueous solution, the process is termed gel filtration chromatography.
- If the mobile phase used is an organic solvent, it is termed as gel permeation chromatography.

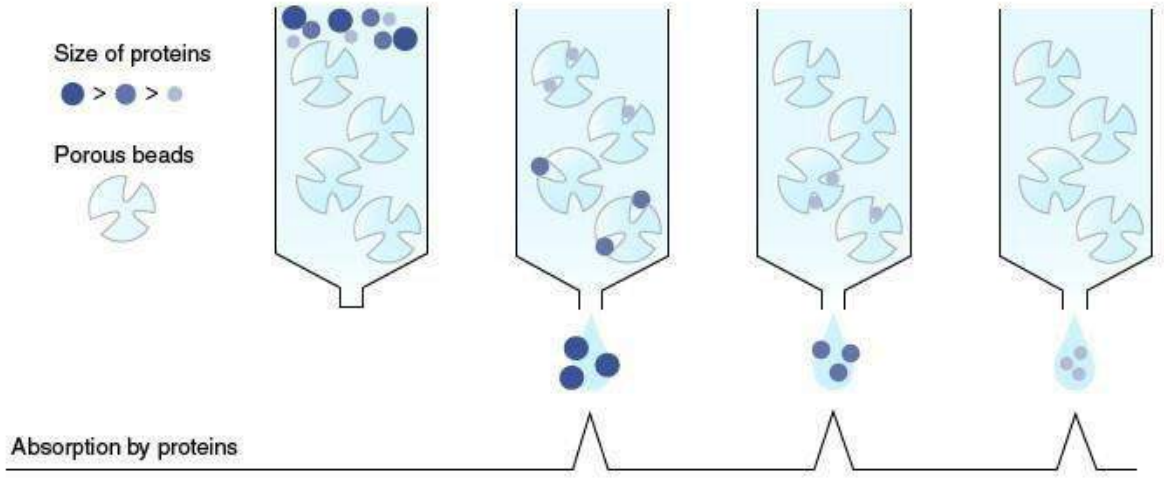


Figure: Gel-filtration chromatography. Image Source: MBL Life Science.

Steps

- The column is filled with semi-permeable, porous polymer gel beads with a well-defined range of pore sizes.
- The sample, mixed with the mobile phase, is then injected into the column from the top of the column.
- The molecules bound to the column are separated by elution solution where either solution of the same polarity is used (isocratic technique), or different samples with different polarities are used (gradient technique).
- Elution conditions (pH, essential ions, cofactors, protease inhibitors, etc.) can be selected, which will complement the requirements of the molecule of interest.

Uses

- One of the principal advantages of gel-filtration chromatography is that separation can be performed under conditions specifically designed to maintain the stability and activity of the molecule of interest without compromising resolution.
- The absence of a molecule-matrix binding step also prevents unnecessary damage to fragile molecules, ensuring that gel-filtration separations generally give high recoveries of activity.
- Because of its unique mode of separation, gel-filtration chromatography has been used successfully in the purification of proteins and peptides from various sources.
- Gel-filtration chromatography has been used to separate various nucleic acid species such as DNA, RNA, and tRNA as well as their constituent bases, adenine, guanine, thymine, cytosine, and uracil.

Examples

- The separation of recombinant human granulocyte colony-stimulating factor (rhG- CSF) from inclusion bodies in high yield by urea-gradient size-exclusion chromatography.
- The separation of hen egg lysozyme using both acrylamide- and dextran-based gel columns.

High-performance liquid chromatography (HPLC)

High-performance liquid chromatography is a modified form of column chromatography where the components of a mixture are separated on the basis of their affinity with the stationary phase.

Principle of HPLC

- This technique is based on the principle of differential adsorption where different molecules in a mixture have a varying degree of interactions with the absorbent present on the stationary phase.
- The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column.
- However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions.
- This process is slightly different from the column chromatography as in this case; the solvent is forced under high pressures of up to 400 atmospheres instead of allowing it to drip down under gravity.

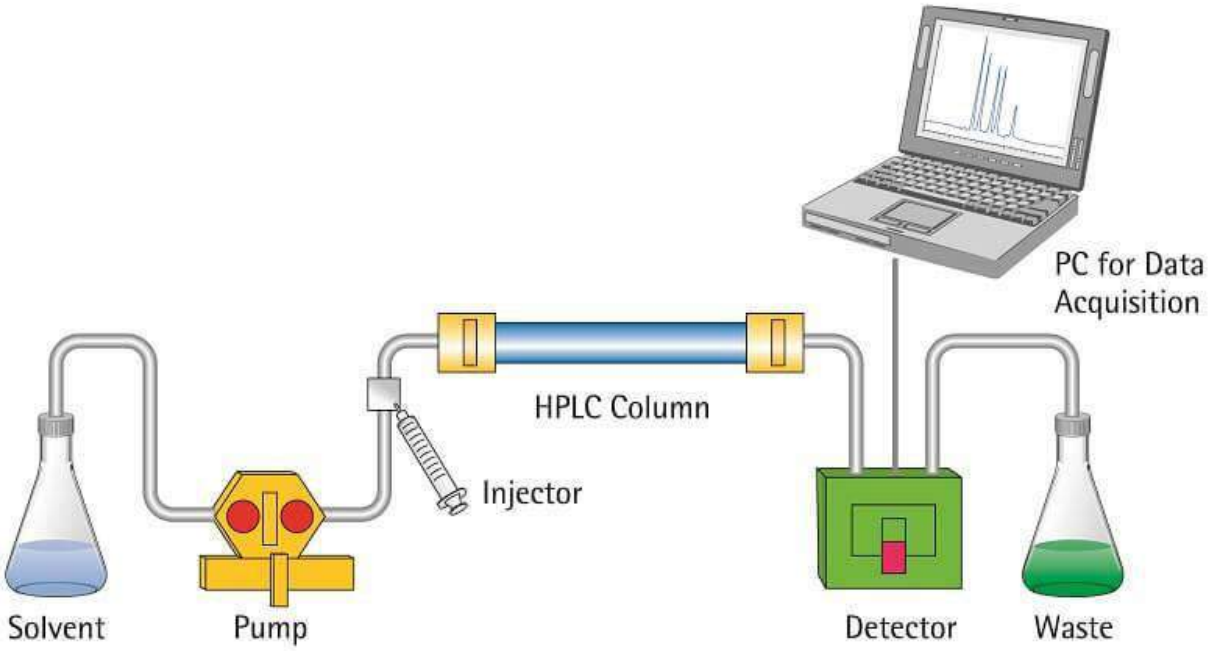


Figure: High-performance liquid chromatography (HPLC). Image Source: Toppr. Steps of HPLC

- The column is prepared by taking a glass tube that is dried and coated with a thin, uniform layer of stationary phase (cellulose, silica).
- Then the sample is prepared by adding the mixture to the mobile phase. The sample is introduced into the column from the top, and a high-pressure pump is used to pass the sample at a constant rate.
- The mobile phase then moves down to a detector that detects molecules at a certain absorbance wavelength.
- The separated molecules can further be analyzed for various purposes. Uses of HPLC
- High-performance liquid chromatography is used in the analysis of pollutants present in environmental samples.
- It is performed to maintain product purity and quality control of various industrial productions.
- This technique can also be used to separate different biological molecules like proteins and nucleic acids.
- The increased speed of this technique makes the process faster and more effective. Example of HPLC
- High-performance liquid chromatography has been performed to test the efficiency of different antibodies against diseases like Ebola.

Hydrophobic interaction chromatography Hydrophobic interaction chromatography is the separation technique that separates molecules on the basis of their degree of hydrophobicity.

Principle of Hydrophobic interaction chromatography

- The principle of hydrophobic interaction chromatography is based on the interaction between two molecules with hydrophobic groups.

- Here, the stationary phase is solid support applied with both hydrophobic and hydrophilic groups.
- The solvent molecules containing hydrophobic regions interact with the hydrophobic groups, thus separating them from the molecules with hydrophilic groups.
- The interaction is then reversed by applying an elution solution with decreasing salt gradient, which causes the molecules with hydrophobic groups to be separated from the stationary phase.

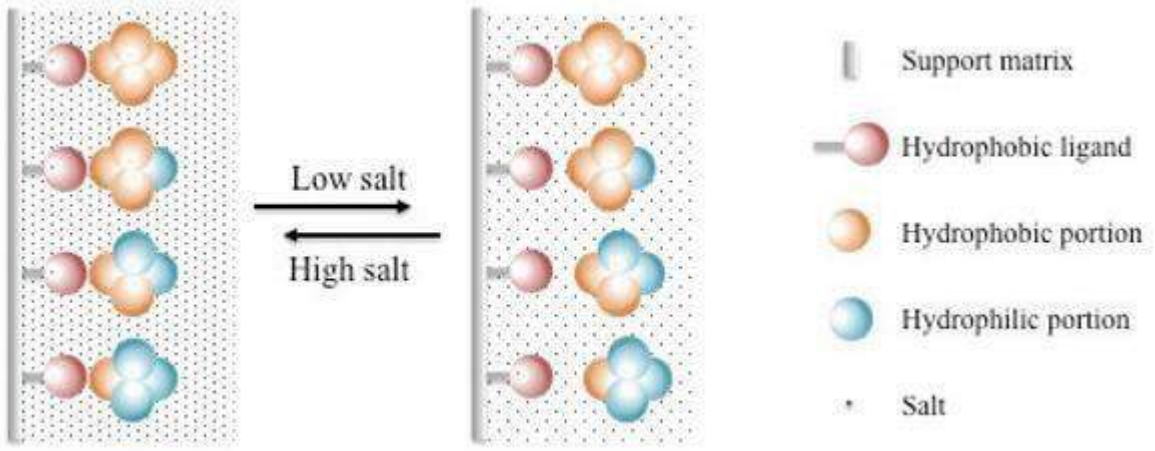


Figure: Hydrophobic interaction chromatography. Image Source: American Pharmaceutical Review.

Steps of Hydrophobic interaction chromatography

- The column is prepared with a glass tube applied with solid support like silica gel, upon which hydrophobic groups like phenyl, octyl butyl, are attached.
- The sample is prepared by adding the mixture to the mobile phase.
- The sample is then injected into the column from the top of the column.
- The molecules with hydrophobic groups form an interaction with the hydrophobic groups of the stationary phase. In contrast, the molecules without such groups move out of the column with the mobile phase.
- Then a particular elution solution with decreasing salt gradient is then passed into the column that removes the bound molecules from the stationary phase.

Uses of Hydrophobic interaction chromatography

- Hydrophobic interaction chromatography is extremely important for the separation of proteins with hydrophobic groups.
- This technique is more appropriate than other methods, as this technique results in minimum denaturation activities.
- Similarly, this method can also be applied to the separation of other organic compounds with hydrophobic groups.
- This allows the separation of hydrophilic and hydrophobic biological molecules from each other.

Example of Hydrophobic interaction chromatography

- The separation of plant proteins from the crude extracts.

Ion exchange chromatography Ion exchange chromatography is the separation technique for charged molecules by their interaction with the oppositely charged stationary phase in the form of ion-exchange resin.

Principle of Ion exchange chromatography

- This technique is based on the principle of attraction of charged resin and the oppositely charged analyte. Here the exchange of negatively/ positively charged ions takes place to remove the charged molecules.
- The stationary phase is first coated with particular charges where the components of the mixture with opposite charges will bind.
- A cation or anion exchange resin with a higher affinity to the charged components then binds the components, displacing the oppositely charged resin.
- The cation or anion exchange resin-component complex then is removed by using different buffers.

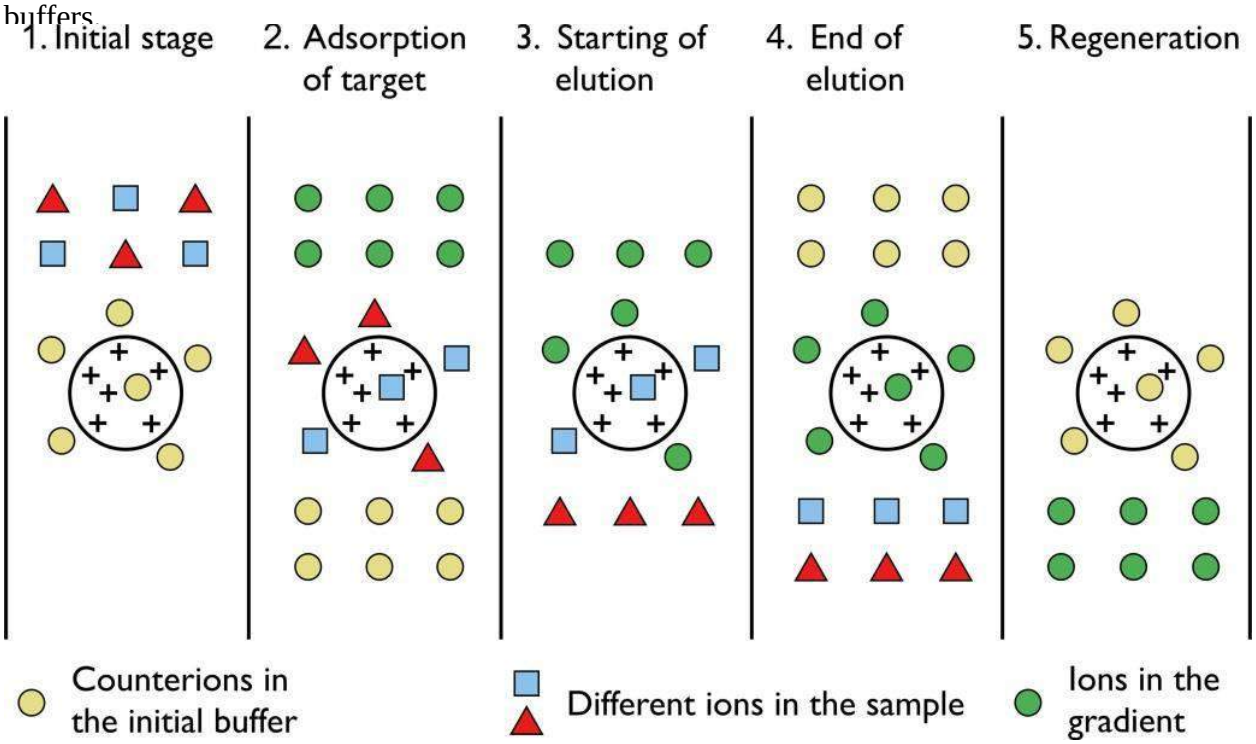


Figure: Ion exchange chromatography. Steps of Ion exchange chromatography

- A column packed with charged resin that can either be positively charged or negatively charged is taken as the stationary phase.
- The mixture with the charged particles is then passed down the column where the charged molecules bind to the oppositely charged resins.
- If a cation exchange resin is used, the positively charged molecules now bind to the cation exchange resin displacing the negatively charged resin.
- Similarly, if an anion exchange resin is used, the negatively charged molecules bind to the anion exchange resin displacing the positively charged resin.
- Now an appropriate buffer is applied to the column to separate the complex of charged exchange resins and the charged molecules.

Uses of Ion exchange chromatography

- Ion exchange chromatography is used in the purification of water where the positively charged ions are replaced by hydrogen ions, and the negatively charged ions are replaced by hydroxyl ions.

- This method also works as an effective method for the analysis of the products formed after hydrolysis of nucleic acids.
- The separation of metals and other inorganic compounds is also facilitated by the ion-exchange chromatography.

Examples of Ion exchange chromatography

- The separation of positively charged lanthanoid ions obtained from the earth's crust.
- The separation of proteins from the crude mixture obtained from the blood serum.

Liquid chromatography Liquid chromatography is a separation technique where the mobile phase used is liquid, and the separation can take place either in a column or a plain surface.

Principle of Liquid chromatography

- The process of liquid chromatography is based on the principle for the affinity of the molecules to the mobile phase.
- If the components to be separated have a higher affinity to the mobile phase, the molecules move along with the mobile phase and come out of the column faster.
- However, if the components have a lower degree of interaction with the mobile phase, the molecules move slowly and thus come out of the column later.
- Thus, if two molecules in a mixture have different polarities and the mobile phase is of a distinct polarity, the two molecules will move at different speeds through

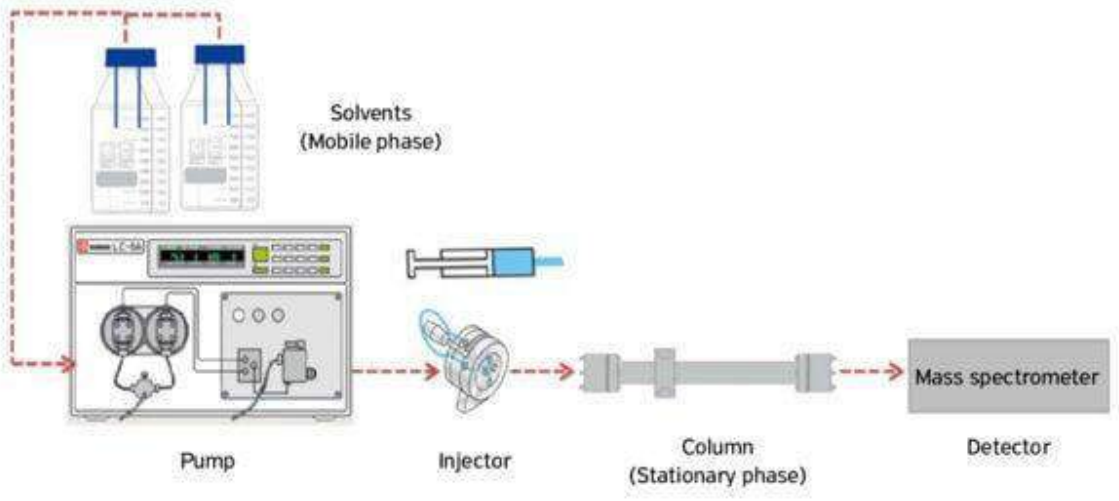


Figure: Liquid chromatography. Image Source: Vânia Margaret Flosi Paschoalin (Researchgate).

Steps of Liquid chromatography

- The column or paper is prepared where the stationary phase (cellulose or silica) is applied on the solid support.
- The sample is added to the liquid mobile phase, which is then injected into the chromatographic system.
- The mobile phase moves through the stationary phase before coming out of the column or the edge of the paper.
- An elution solution is applied to the system to separate the molecules from the stationary phase.

Uses of Liquid chromatography

- Liquid chromatography is an effective method for the separation of a colored solution as they form two separate bands after separation.
- This method can also be used over other techniques as it is quite simple and less expensive.
- It can be used for the separation of solid molecules that are insoluble in water. Examples of Liquid chromatography
- High-performance liquid chromatography is a modified form of liquid chromatography that is used in the research regarding biological molecules.

Paper chromatography Paper chromatography is a separation technique where the separation is performed on a specialized paper.

Principle of Paper chromatography

- Paper chromatography is of two types based on two different principles.
- The first is the paper adsorption chromatography that is based on the varying degree of interaction between the molecules and the stationary phase.
- The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column.
- However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions.
- The second type of paper chromatography is the paper partition chromatography. It is based on the principle that the moisture on the cellulose paper acts as a stationary phase for the molecules moving with the mobile phase.
- The separation of the molecules is thus based on how strongly they adsorb onto the stationary phase.
- An additional concept of 'retention factor' is applied during the separation of molecules in the paper chromatography.
- The retention value for a molecule is determined as a ratio of distance traveled by the molecule to the distance traveled by the mobile phase.
- The retention value of different molecules can be used to differentiate those molecules.

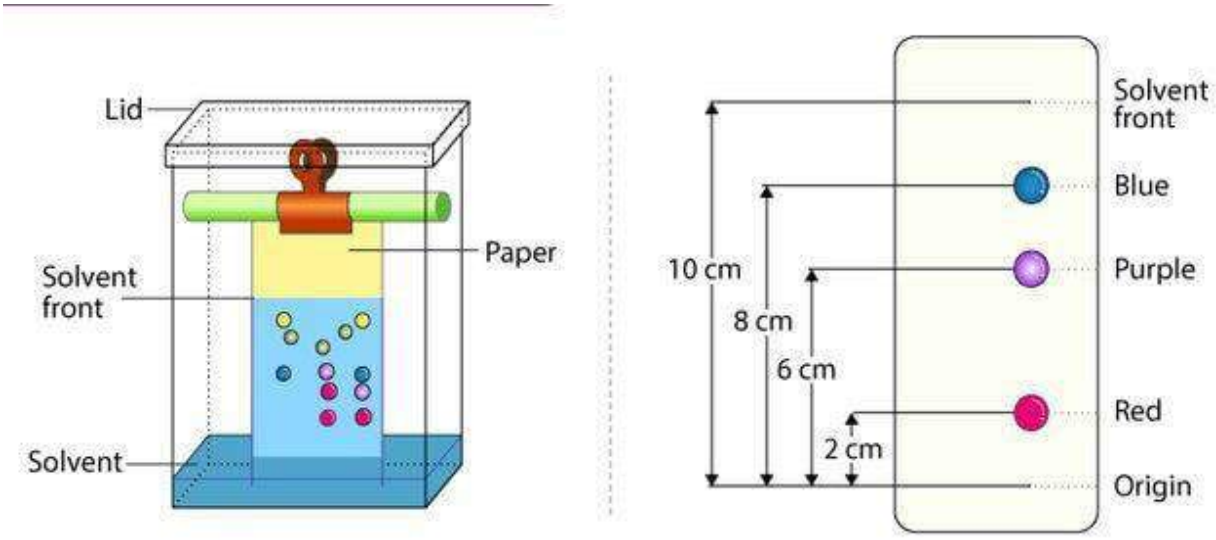


Figure: Paper chromatography. Steps of Paper chromatography

- The stationary phase is selected as a fine quality cellulosic paper.
- Different combinations of organic and inorganic solvents are taken as the mobile phase.
- About 2-200 µl of the sample solution is injected at the baseline of the paper, and it is allowed to air dry.
- The sample loaded paper is then carefully dipped into the mobile phase not more than the height of 1 cm.
- After the mobile phase reaches near the edge of the paper, the paper is taken out.
- The retention factor is calculated, and the separated components are detected by different techniques.

Uses of Paper chromatography

- Paper chromatography is performed to detect the purity of various pharmaceutical products.
- It can also be employed to detect contamination in various samples, like food and beverages.
- This method can also be used for the separation of impurities from various industrial products.
- The analysis of the reaction mixtures in chemical labs is also conducted via paper chromatography.

Examples of Paper chromatography

- Paper chromatography is used in the separation of mixtures of inks or other colored drinks.

Thin-layer chromatography (TLC) Thin-layer chromatography is a separation technique where the stationary phase is applied as a thin layer on a solid support plate with a liquid mobile phase.

Principle of Thin-layer chromatography (TLC)

- This chromatography technique is based on the principle that components of a mixture are separated when the component having an affinity towards the stationary

phase binds to the stationary phase. In contrast, other components are eluted with the mobile phase.

- The substrate/ ligand is bound to the stationary phase so that the reactive sites for the binding of components are exposed.
- Now, the mixture is passed through the mobile phase where the components with binding sites for the substrate bind to the substrate on the stationary phase while the rest of the components are eluted out with the mobile phase.
- After separation, the molecules are seen as spots at a different location throughout the stationary phase.
- The detection of molecules is performed by various techniques.

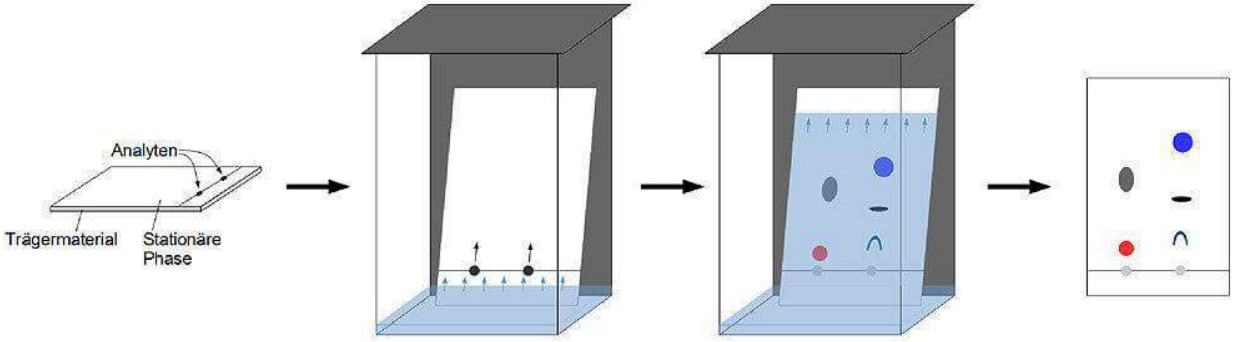


Figure: Thin-layer chromatography (TLC). Image Source: MZ-Analysentechnik GmbH. Steps of Thin-layer chromatography (TLC)

- The stationary phase is uniformly applied on the solid support (glass, thin plate or aluminum foil) and dried.
- The sample is injected as spots on the stationary phase about 1 cm above the edge of the plate.
- The sample loaded plate is then carefully dipped into the mobile phase not more than the height of 1 cm.
- After the mobile phase reaches near the edge of the plate, the plate is taken out.
- The retention factor is calculated as in paper chromatography, and the separated components are detected by different techniques.

Uses of Thin-layer chromatography (TLC)

- Thin-layer chromatography is routinely performed in laboratories to identify different substances present in a mixture.
- This technique helps in the analysis of fibers in forensics.
- TLC also allows the assay of various pharmaceutical products.
- It aids in the identification of medicinal plants and their composition.

Applications of Chromatography Pharmaceutical sector

- To identify and analyze samples for the presence of trace elements or chemicals.
- Separation of compounds based on their molecular weight and element composition.
- Detects the unknown compounds and purity of mixture.
- In drug development.

Chemical industry

- In testing water samples and also checks air quality.

- HPLC and GC are very much used for detecting various contaminants such as polychlorinated biphenyl (PCBs) in pesticides and oils.
- In various life sciences applications

Food Industry

- In food spoilage and additive detection
- Determining the nutritional quality of food

Forensic Science

- In forensic pathology and crime scene testing like analyzing blood and hair samples of crime place.

Molecular Biology Studies

- Various hyphenated techniques in chromatography such as EC-LC-MS are applied in the study of metabolomics and proteomics along with nucleic acid research.
- HPLC is used in Protein Separation like Insulin Purification, Plasma Fractionation, and Enzyme Purification and also in various departments like Fuel Industry, biotechnology, and biochemical processes.

TSWRAFPDCW – BHONGIR TEACHING MODULE

CLASS: BZC / MZC (BSC) DEPARTMENT: ZOOLOGY

Year ll (lV sem); Paper: IV (CELL BIOLOGY AND GENETICS)

Topic: CO DOMINANCE & IN COMPLETE DOMINANCE

Lecturer Name: V.JYOTHI No. of Teaching Hours: 4

1. **Objectives of the Topic:**

➢Student can able to understand the concept of Co dominance and In complete dominance

➢Understand the overall idea of co dominance & in complete dominance

➢Understand the Importance of Co dominance &In complete dominance.

➢Student can identify the differences between Co & In complete dominance

➢**PRE-TEST**:

➢**Understanding the student's basic knowledge by asking orally on the following**

i.Tell me why some flowers are in pink colour?

ii.How the mixed coloured flowers are created?

iii.How the 2 genes are in dominance?

iv.What is co dominance and in complete dominance?

v.How the characters ae expressed?

2. **Module Content:**

INTRODUCTION: After the discovery of Mendal's work in 1900 Devreis, Correns and Tshermak, the laws of segregation and independent assortment were confirmed. However, in certain instances they appeared to be deviating. Deviations to the Mendelian inheritance are attributed to linkage and crossing over, gene interaction.

From mendelian laws of inheritance, it is noted each trait is determined by a single pair of genes or factors. It might also be possible that several pairs of genes located at different places of same chromosomes or different chromosomes interact

IN COMPLETE DOMINANCE:

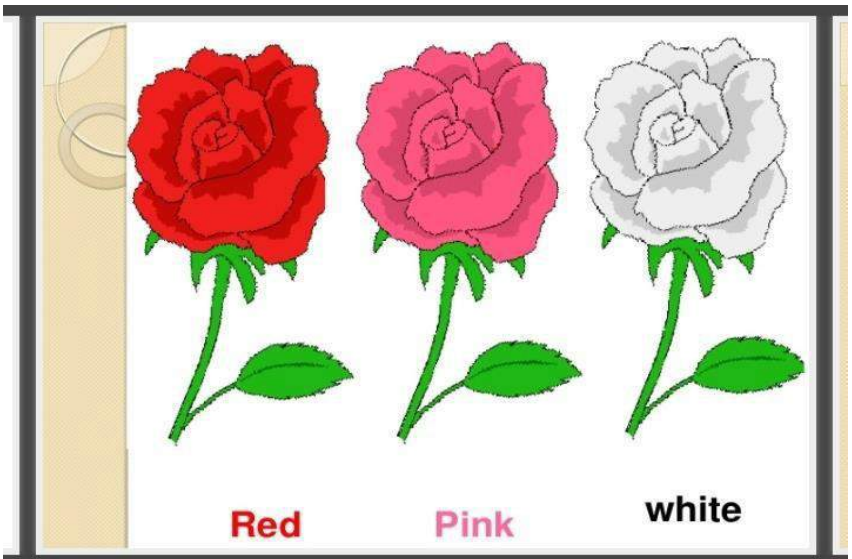
So far, we know about that phenotypes are indistinguishable from homozygous and heterozygous dominant individuals in recessiveness, complete dominance. A number of dominant alleles in plants and animals do not follow this pattern.

In four ‘o’ clock plant, 2 pink coloured flowers. Mirabeles jalapa, if a red flowered plant is crossed with a white flowered plant, both are homozygous, the F1 heterozygotes would bear pink flowers. When these F1 plants are crossed, they produced 1 red coloured. When these F1 plants are crossed , they produce 1 red, 2 pink and 1 white coloured progeny in F2.

Similarly, if the black Andalusian fowl is crossed with splashed white Andalusian fowl, F1 fowls are blue in colour and in F2, 1 black, 2 blue and 1 splashed white fowl are obtained,

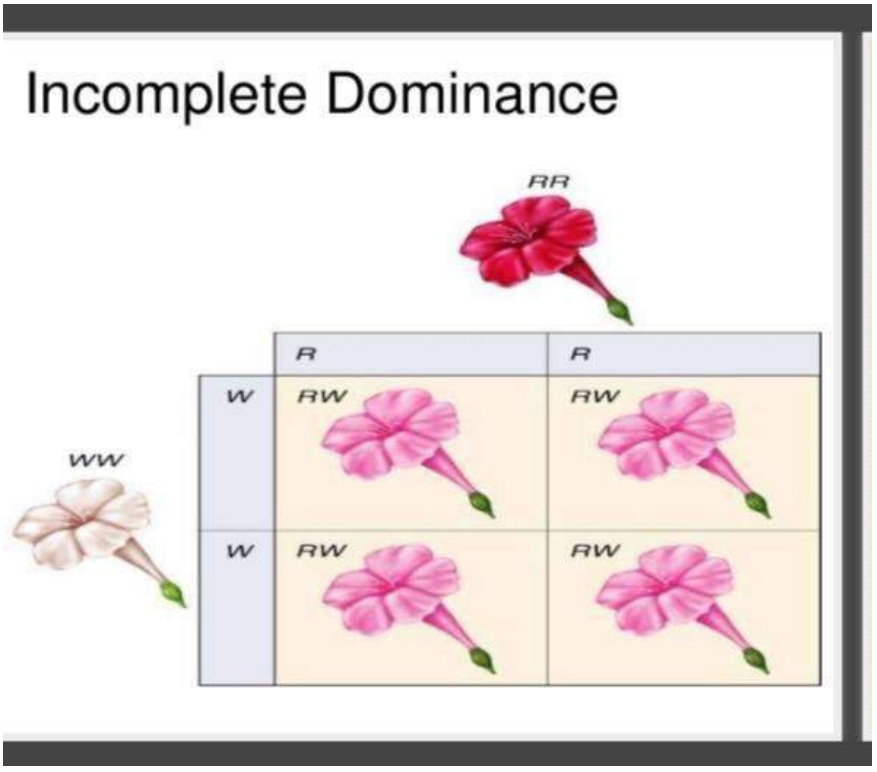
Here the red colour of Mirabiles jalapa and Black Andalusian fowls are in completely dominant, If the genes are not completely dominant the phenotypic and genotypic ratios would would remain the same, i.e.,1:2:1.

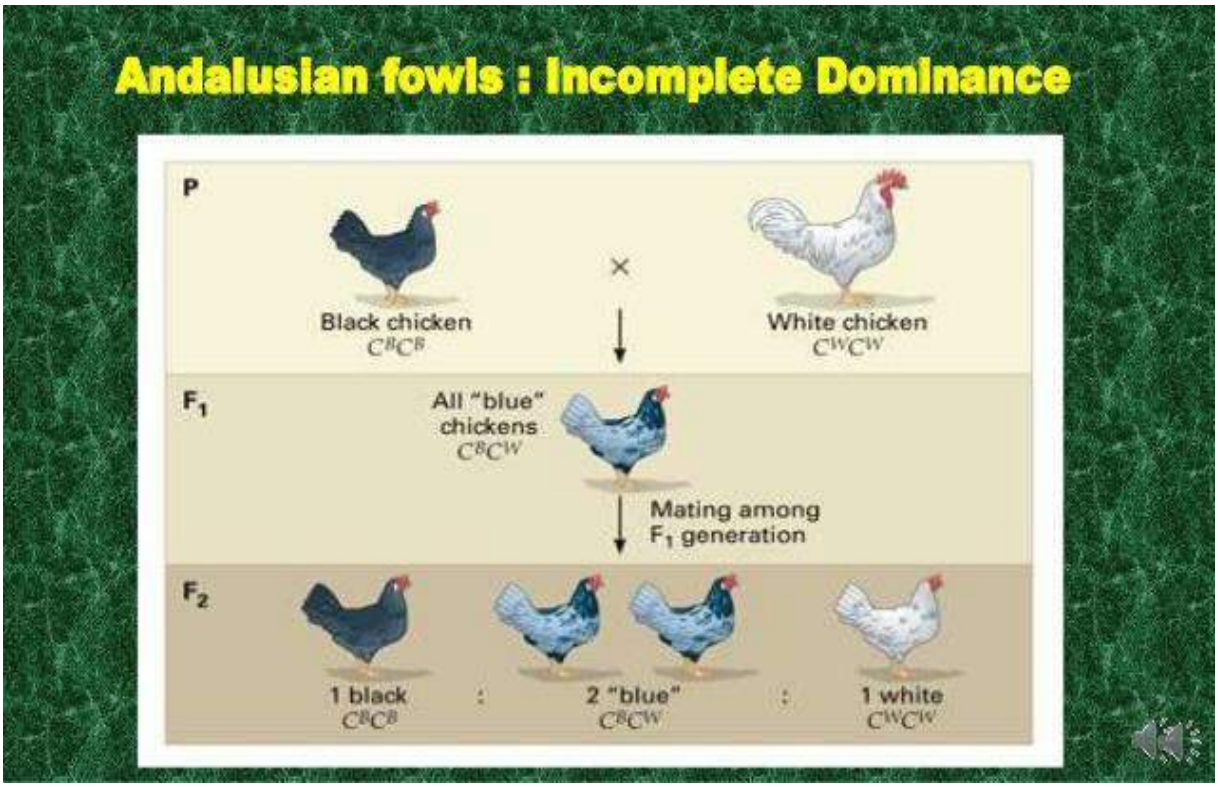
A similar situation occurs in humans regarding the type of hair. Curly hair is incompletely dominant over straight hair, which is a homozygous recessive trait. Heterozygotes in this case will carry wavy hair.



Incomplete dominance

14,526 views



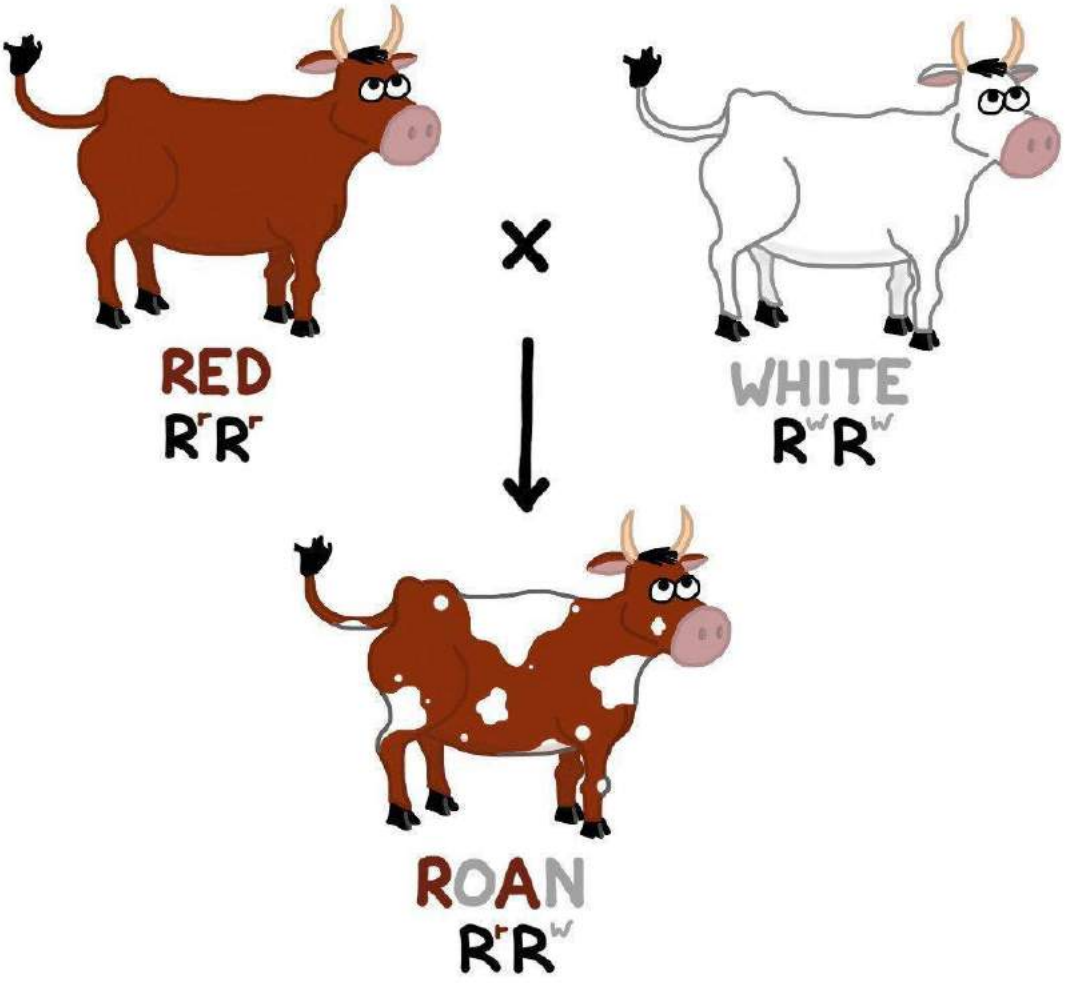


CO DOMINANCE:

In the inheritance of A B O blood groups, the type of antigen present on the RBC of person will decide his blood group. If he carries “A” Antigen he is “A” group person and if there is “B” antigen on the RBC, he is “B” group person.If a person carries both these antigens he is “AB” group man.The genotype of “A” group person is IA IA or IA I, that of “B” group person is IB IB or IB i. IA IB person will have AB group of blood. The expression of these alleles is known as Codominanace in which heterozygotes for two codominant alleles express both the traits determined by the alleles. So the IA IA and IB IB are codominanat alleles.

Genotype	Red blood cell appearance	Phenotype (blood group)
$I^A I^A$ or $I^A i$		A
$I^B I^B$ or $I^B i$		B
$I^A I^B$		AB
ii		O

CO-DOMINANCE:



Student Activities:

*Motivate the students to collect more information on genes which dominant other.

*they should understand gene interactions

*conduct group discussion on the genes express their character

*describe and illustrate the different flowers and animals.

*describe inter relations between genes.

3. **Assessment:**
   - Assessing the Student after completion of the Topic by conducting Oral Test or write exam

i. Explain the gene interactions.
ii. Describe different types of flowers Describe different colours of Animals
iii. Write the importance of codominance and in complete dominance
iv.

4. **Reference Books:**

*Encyclopedia of Genetics

By Sidney Brenner.

*The selfish gene

By Dawkins first published in 1976.

*Cell biology, Genetics and Evolution.

By C. Gopal and Dr. Kondaiah.

ज्ञान दीक्षा

జ్ఞానదీక్ష

GNAANA DEEKSHA

CONCEPT BASED DIGITAL CLASSES FOR SCHOOLS & UG COLLEGES

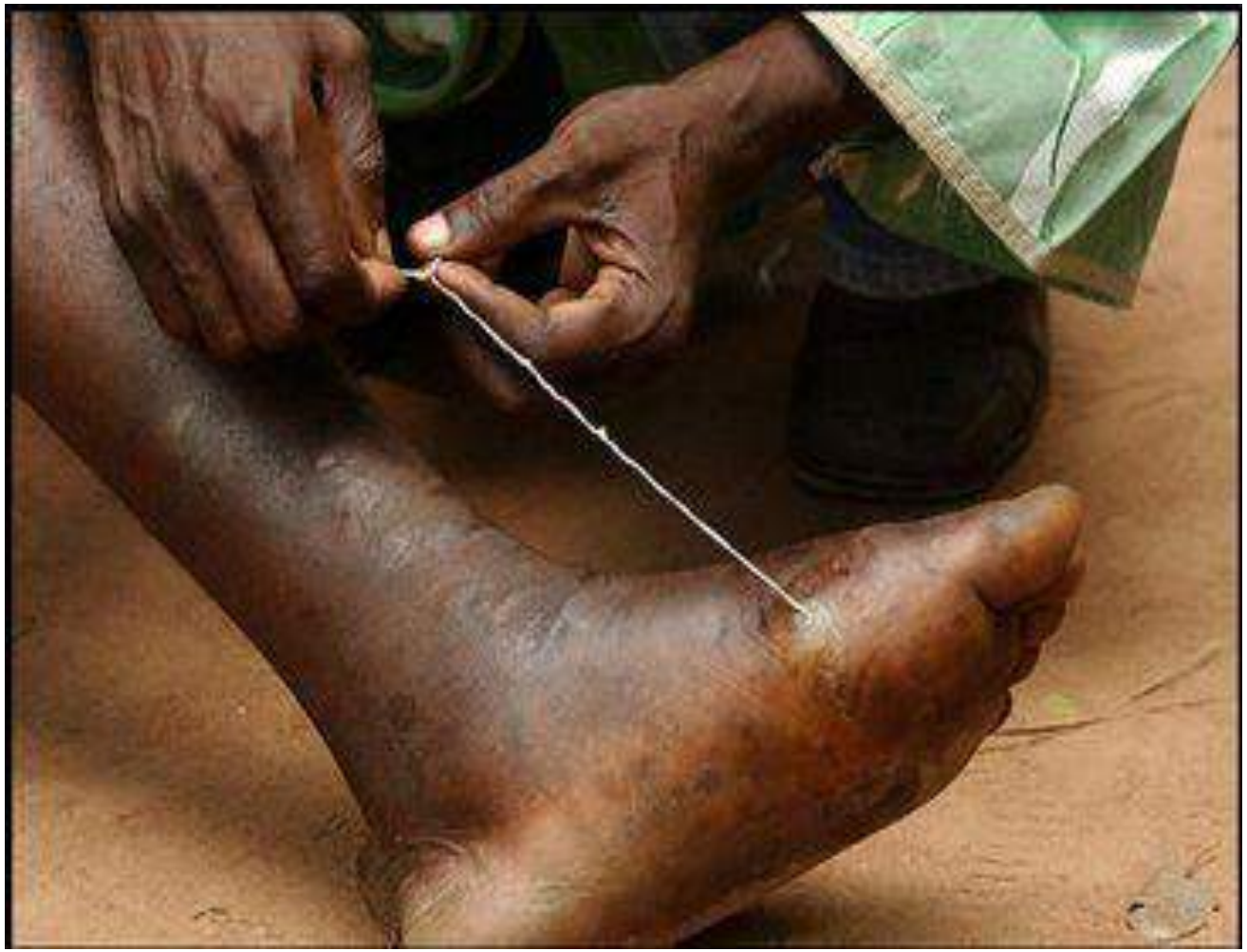
Dr. K. Srilatha
DL in Zoology
TSWRAFPDCW Bhongir

Subject : ZOOLOGY

Concept : ***Dracunculus medinensis*** **– Type study**

Class : BSc First Year

Dracunculus Medinensis – Type study



Learning objectives

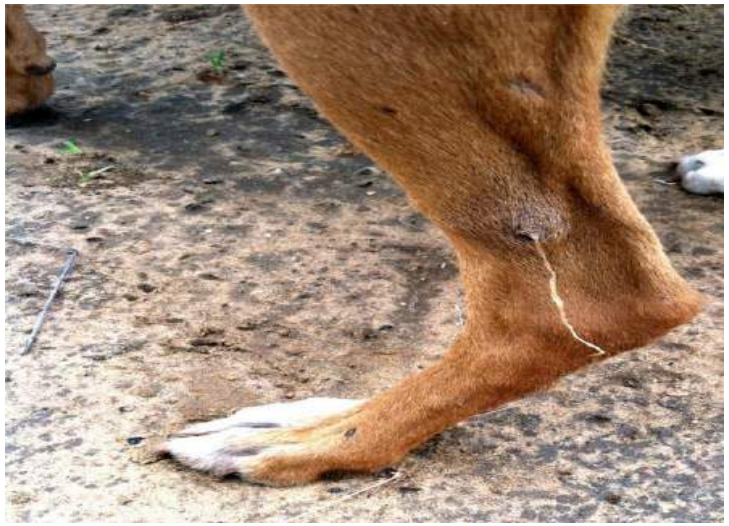
- Understand the medical importance of *Dracunculus medinensis*
- Identification of female worm
- Understand the Life cycle *of Dracunculus medinensis*
- Know the Symptoms of the disease caused by the parasite
- Methods for the prevention and control of the parasite

Introduction

- Fiery serpent
- Little snakes
- Guinea worm
- Medina worm
- Dragon worm and serpent worm
- *Dracunculus* have also been reported in the Egyptian mummies.

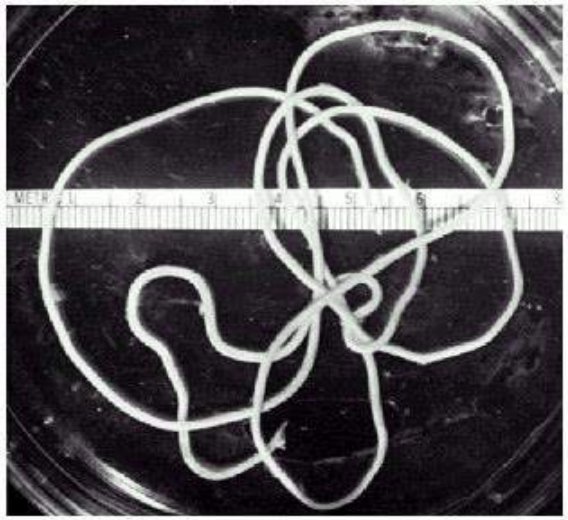
Introduction Cont..

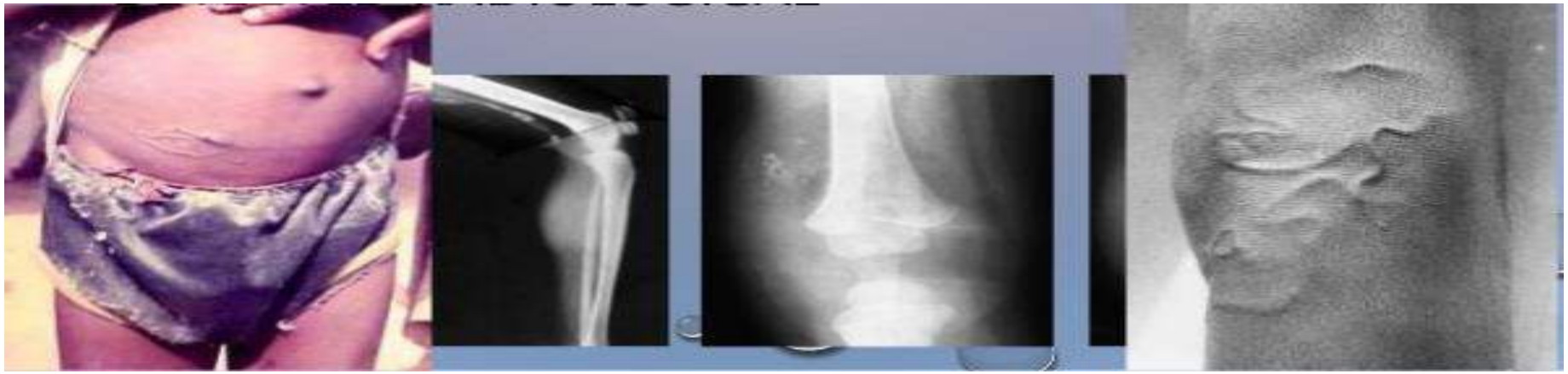
- ***Dracunculus medinensis (*man, dogs, cattle, horses and foxes)**
- ***Dracunculus insignis (*dogs and cats).**



Introduction Cont..

- Largest and Somatic nematode
- Lives in the subcutaneous tissues



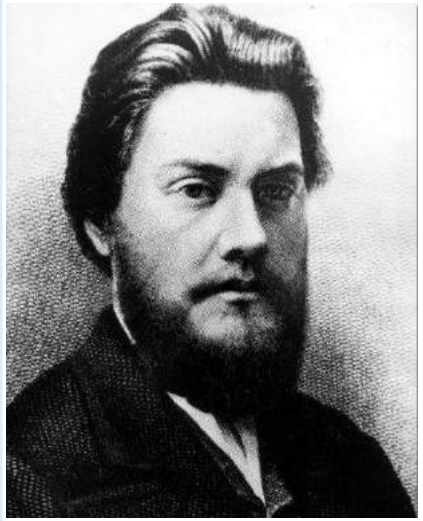


Historical Accounts

- ✓ Egyptian mythology pertaining to the Serpent of Isis.
- ✓ The "plague of the fiery serpents“.
- ✓ 1530 B.C.
- ✓ The symbol of a Physician is the "Caduceus“ represent the Guinea worm.

Discovery

- Calcified Guinea worm – Egyptian Mummy
- Carlus Linnaeus in 1758 suggested that *Dracunculus* were worms.
- Russian naturalist, Alexei Fedchenko in 1870 studied the life cycle - Digenetic
- Dyneshvar Atmaran Turkhud, solidified Fedchenkos knowledge in 1913
- The name dracontiasis was given by Galen (in Greek: draco means dragon or serpent)
- The disease is caused by the female worm.

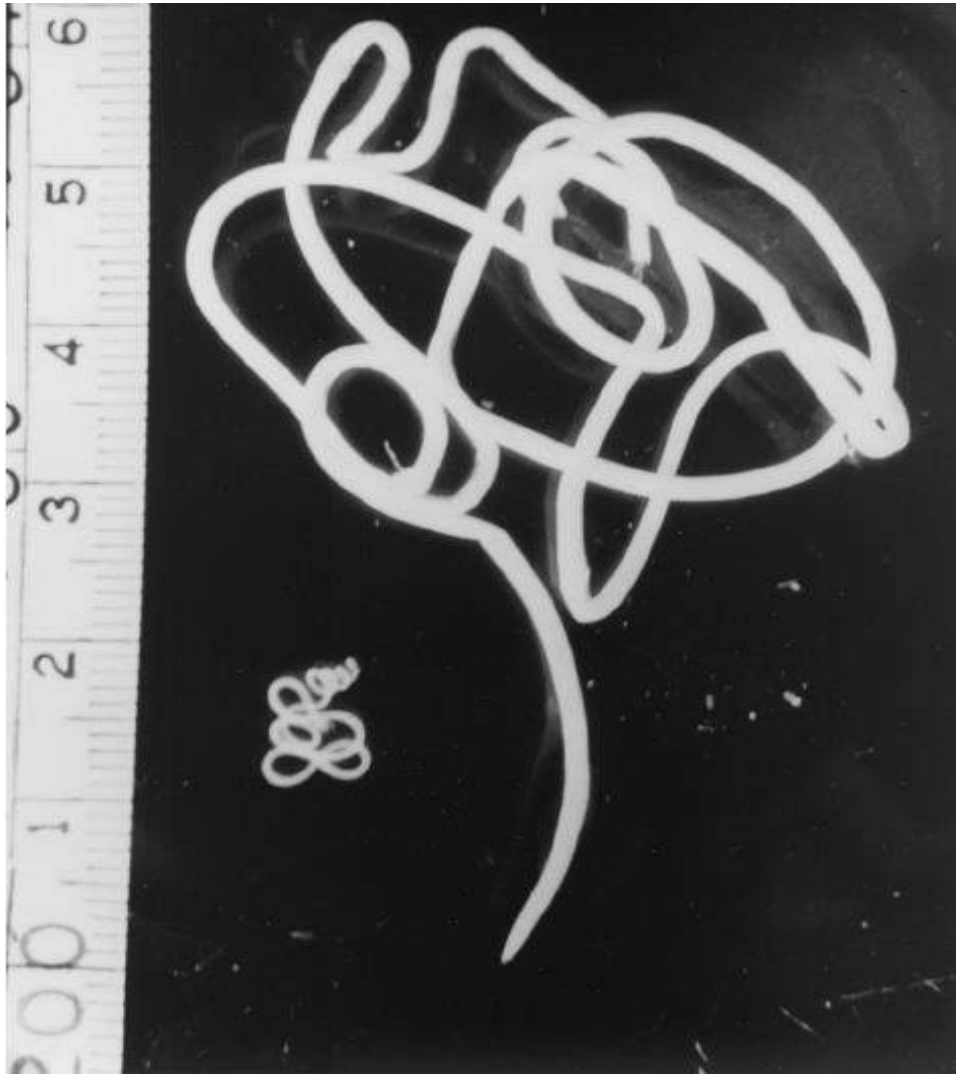


Alexei Fedchenko

Claudius Galen

Persian physicians removing the *Dracunculus medinensis* parasite from the leg of a patient

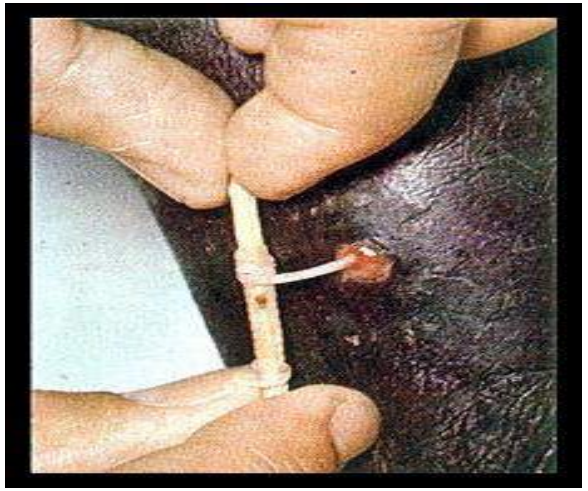
Classification



Kingdom:	**Animalia**
Phylum:	**Nematoda**
Class:	**Secernentea/Phasmidia**
Order:	**Camallanida/Spirurida**
Superfamily:	**Dracunculoidea**
Family:	**Dracunculidae**
Genus:	***Dracunculus***

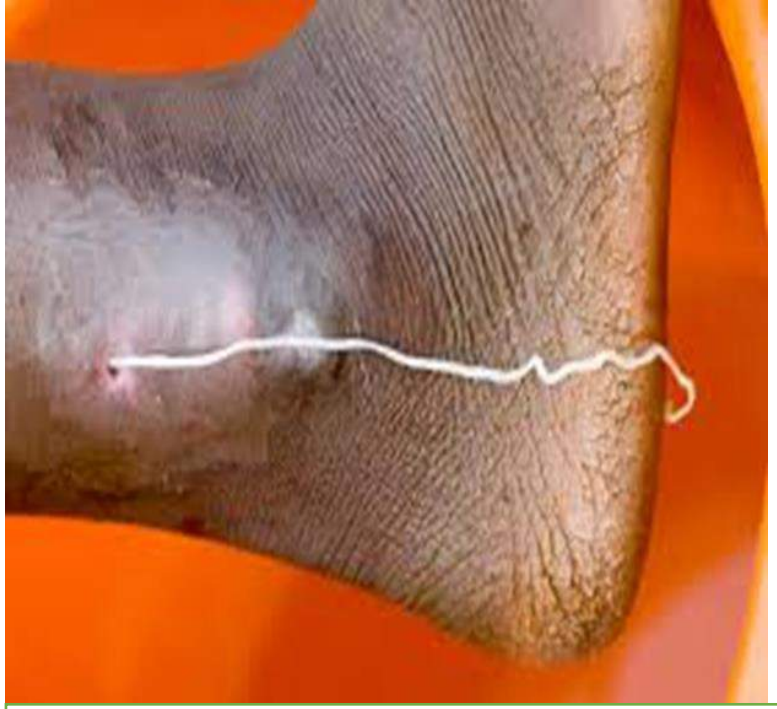
Cultural Practices

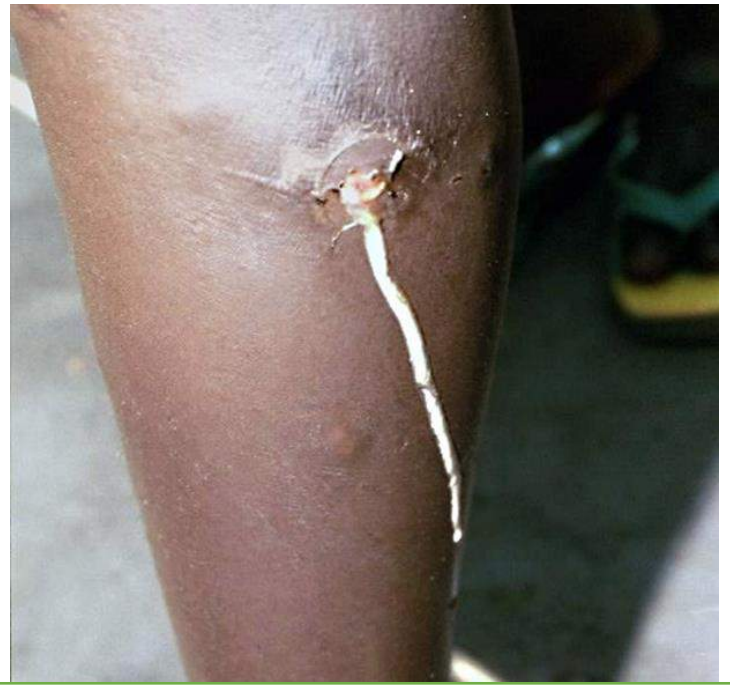
- **Transmission of the disease - drinking contaminated water.**

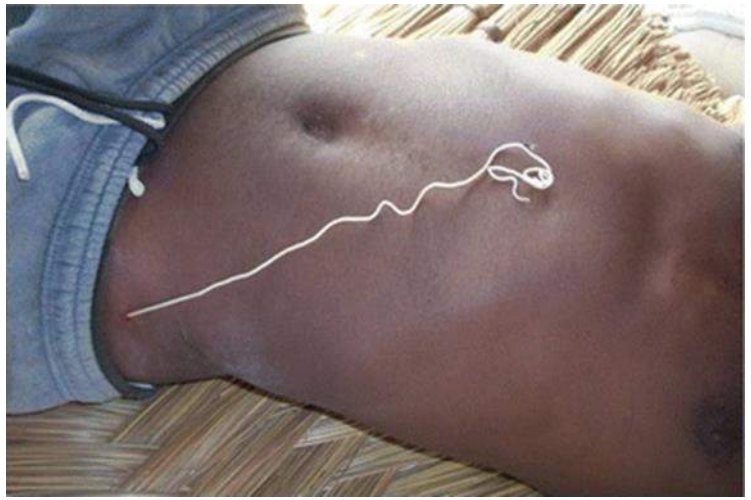


Geographical distribution

- Approximately 10 million people per year.
- India, Pakistan, Burma, Saudi Arabia, Iraq, Iran, Soviet Union, Africa to West Indies.
- In India - Punjab, Rajasthan, Gujarat, Maharashtra, Tamil Nadu, Andhra Pradesh, Karnataka and Madhya Pradesh.
- *Dracunculus medinensis* has been eradicated from several countries in Africa, Asia and Middle East.
- Now found only in some of the African states.





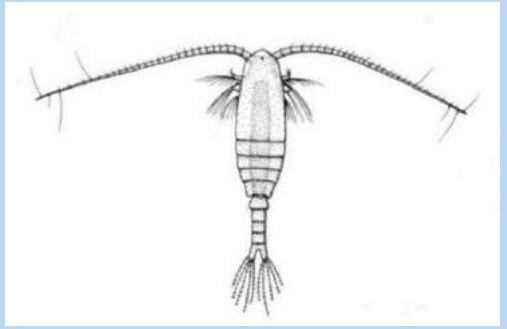


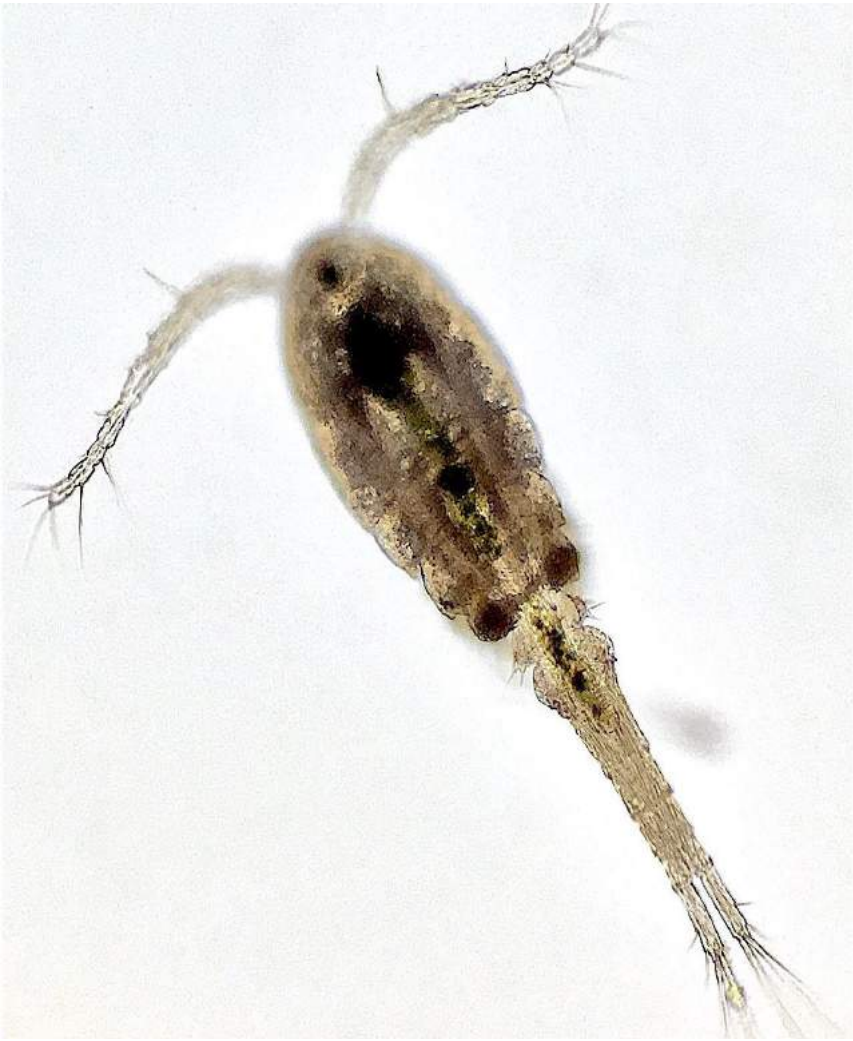
Dracunculus medinensis **(Guinea worm) a nematode parasite.**

Last case in India was reported in the year 1996. Zero incidence for the next three years Hence India was declared free of Guinea worm.

Host: Digenetic

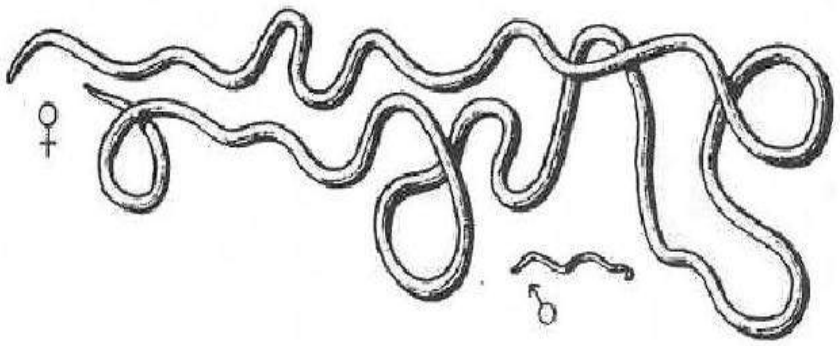
- Definitive host - Humans, dogs, cats and other wild animals
- Intermediate host - *Cyclops.*
  - Small crustacean
  - Commonly called Water fleas
  - Most benthic
  - Some planktonic
- Humans - only reservoir of infection.
- Dogs, cats, foxes naturally infected with *Dracunculus.*

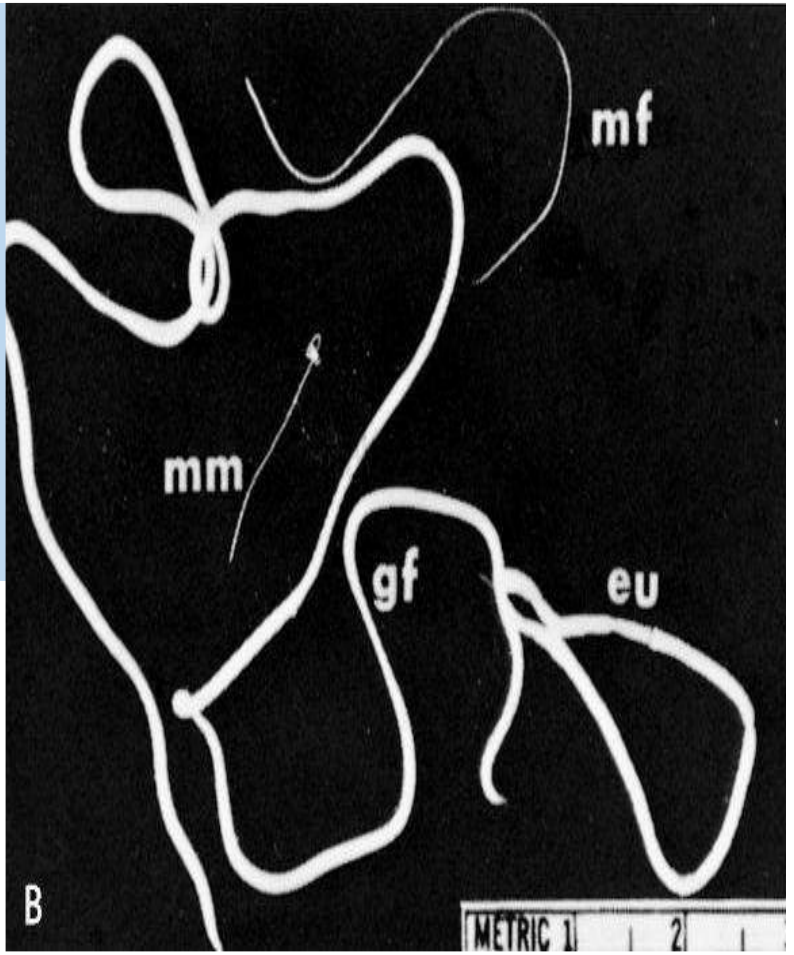




Morphology

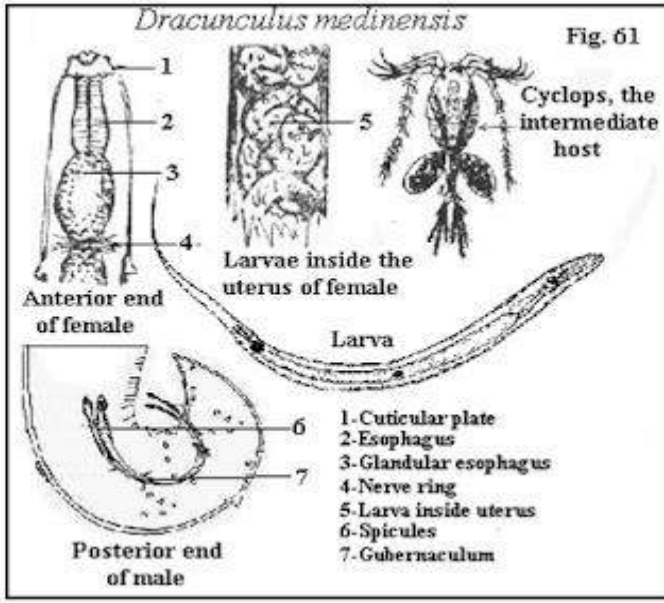
- Sexual dimorphism.
- Cylindrical, smooth and milky white in color.
- Papillae - sensory structures.
- Female is didelphic
- 3 million embryos found in the uterus
- Worm is viviparous

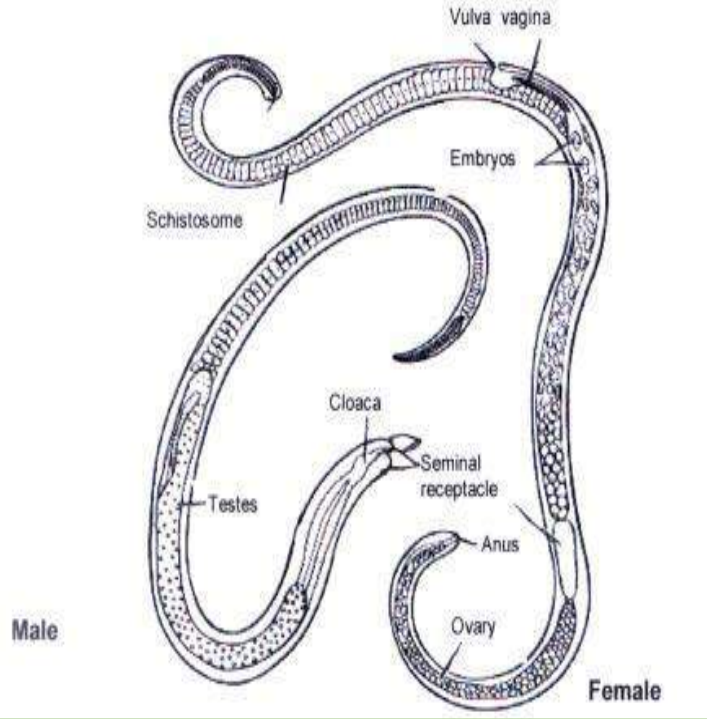




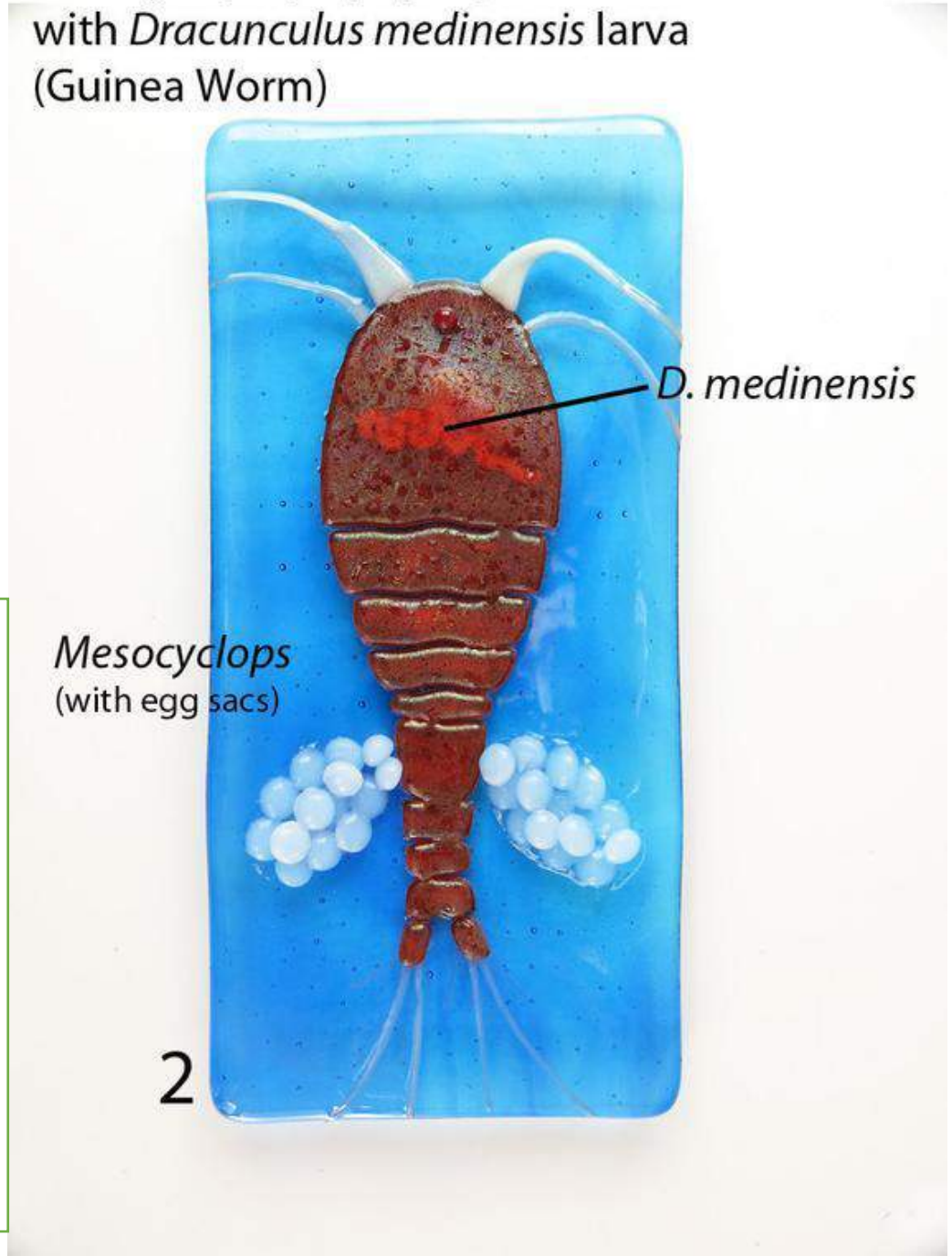
Difference between Male and Female *Dracunculus medinensis*

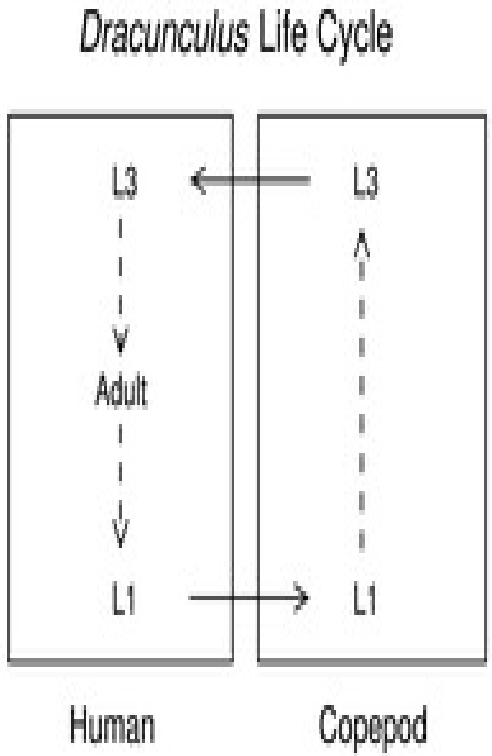
Characteristic Features	Male Dracunculus	Female Dracunculus
Shape	Adult male is smaller than the female	Longer and stouter than adult male
Length	12-30 mm	60cm -120cm (about a meter)
Width	Approximately 0.4mm	1.5-1.7mm
Posterior End	Sharply curved	Has a blunt anterior end and a tapering posterior end which is bent to form a chitinous hook
Opening at posterior end	Cloaca (anus and the male genital aperture both open into the cloaca)	The digestive system opening—anus is present at the posterior end, a little in front of the tail end
Reproductive opening (Gonopore)	Opens in cloaca	Vulva opens ventrally at anterior one-third of the body
Spicules	One pair of unequal length	Absent
Gubernaculum	Present	Absent
Genital Papillae	10 pairs of genital papillae: 4 Pairs of preanal and 6 pairs of post anal	Absent



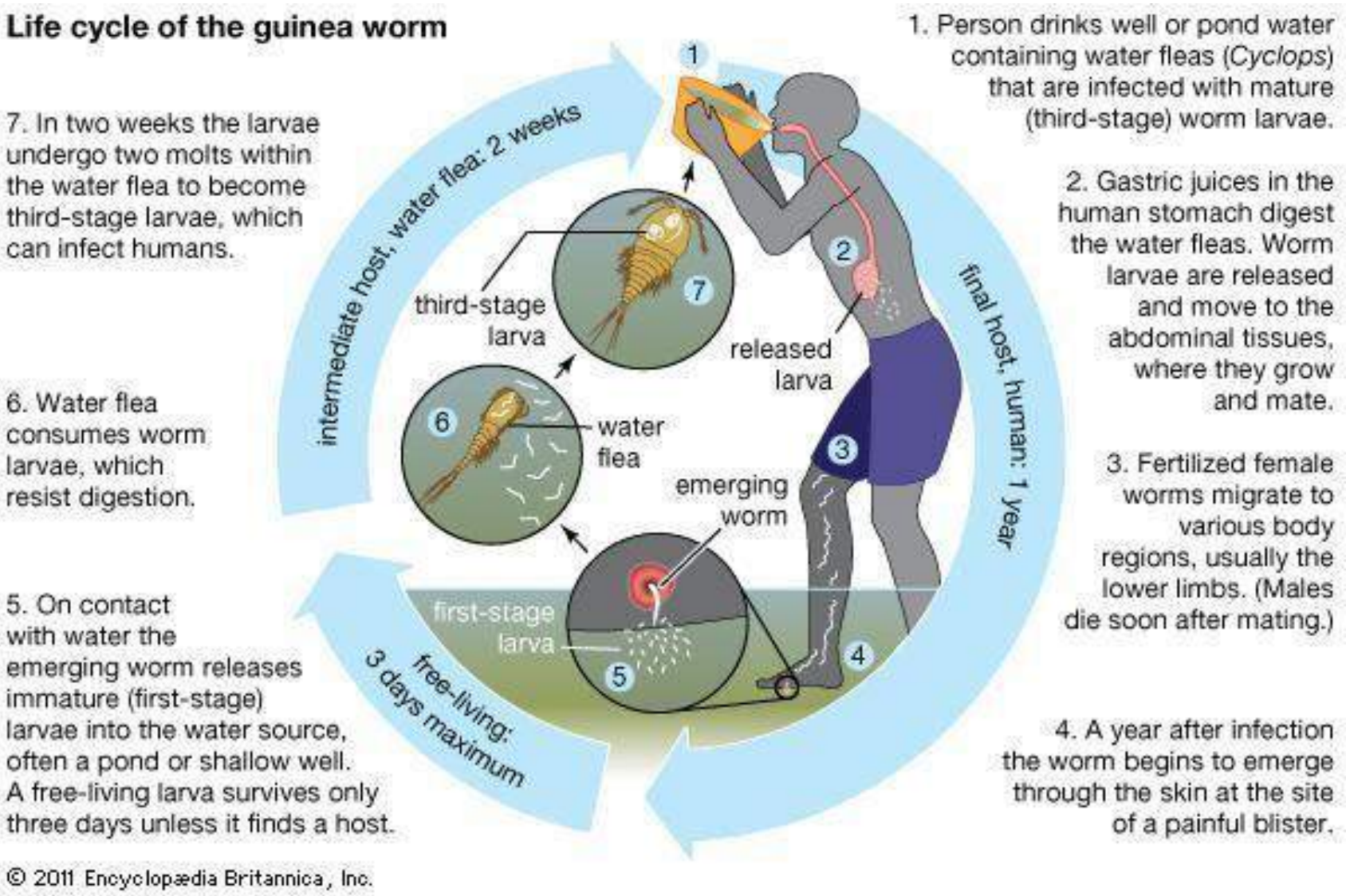


Man is the definitive host. Cyclops (M*esocyclops leuekarti,* A fresh water crustacean) is Intermediate host. Life span of female is 1 year and that of male is 6 months. Males are short lived. They die soon after copulation. Female *Dracunculus* is viviparous

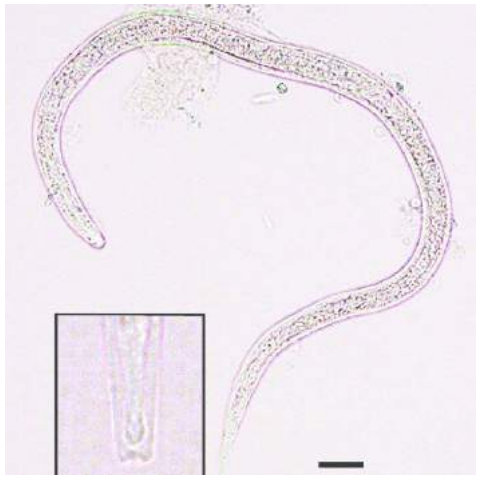


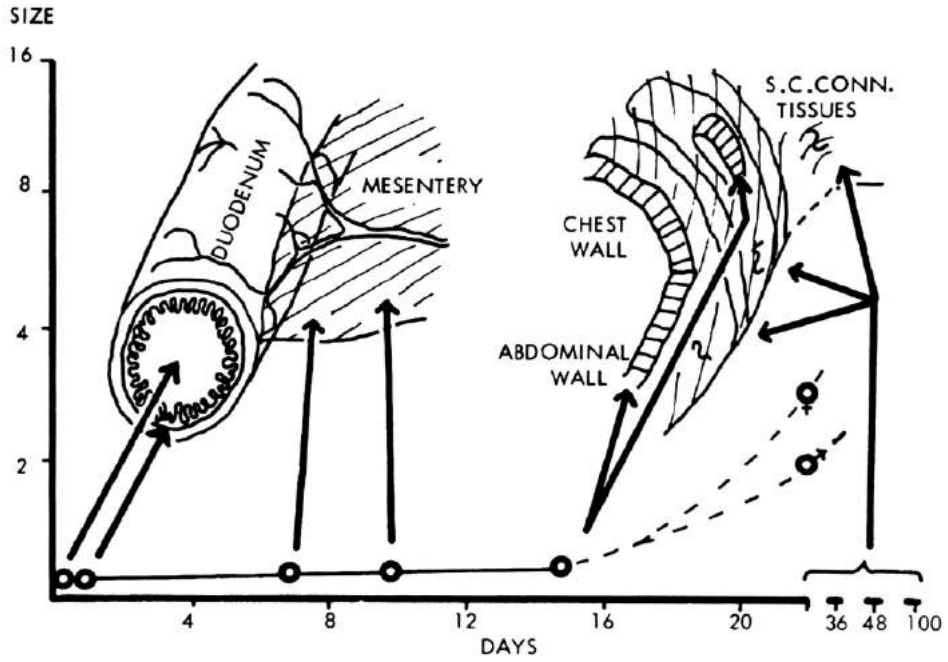


Life Cycle of *Dracunculus medinensis*

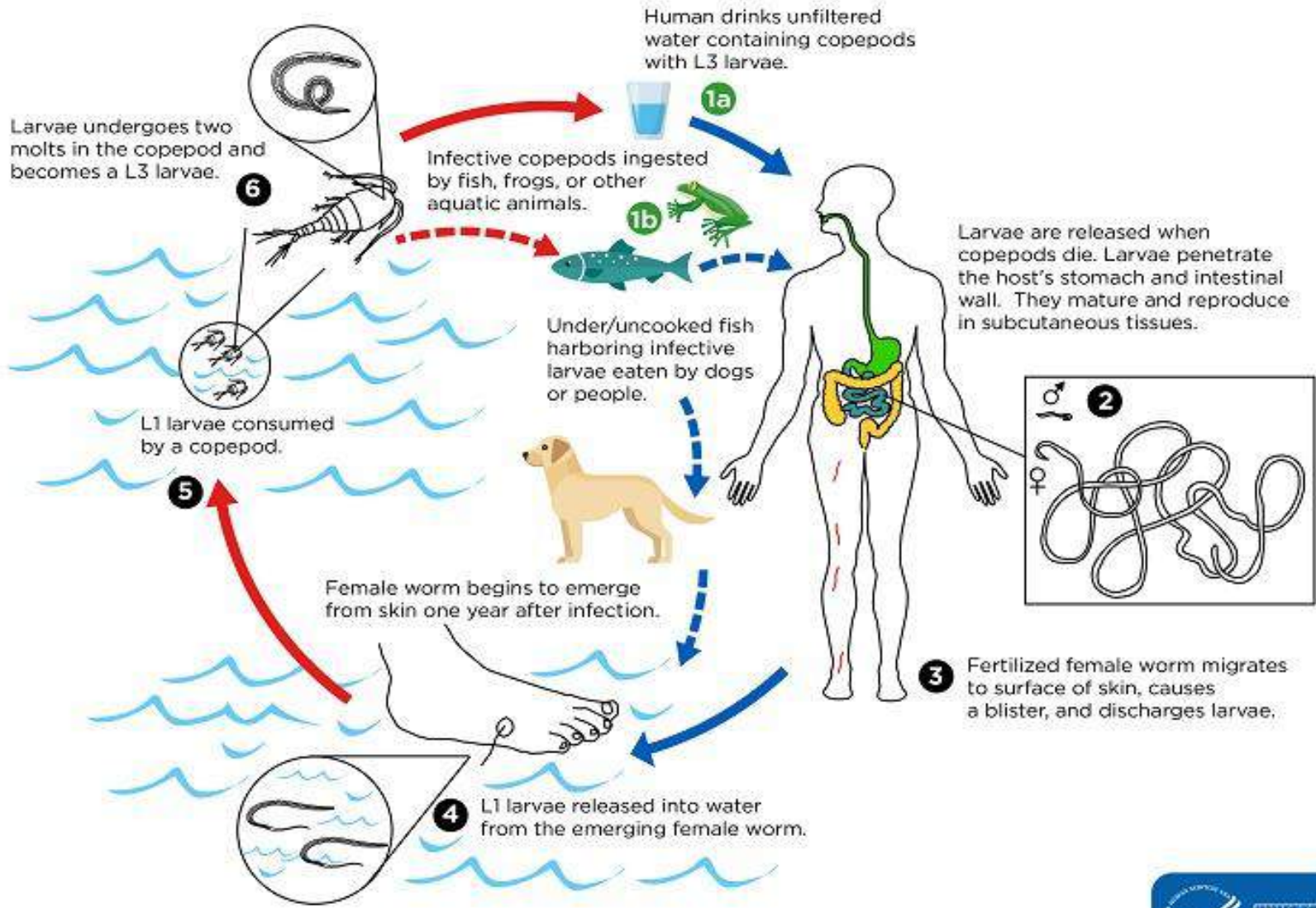


L3 larva



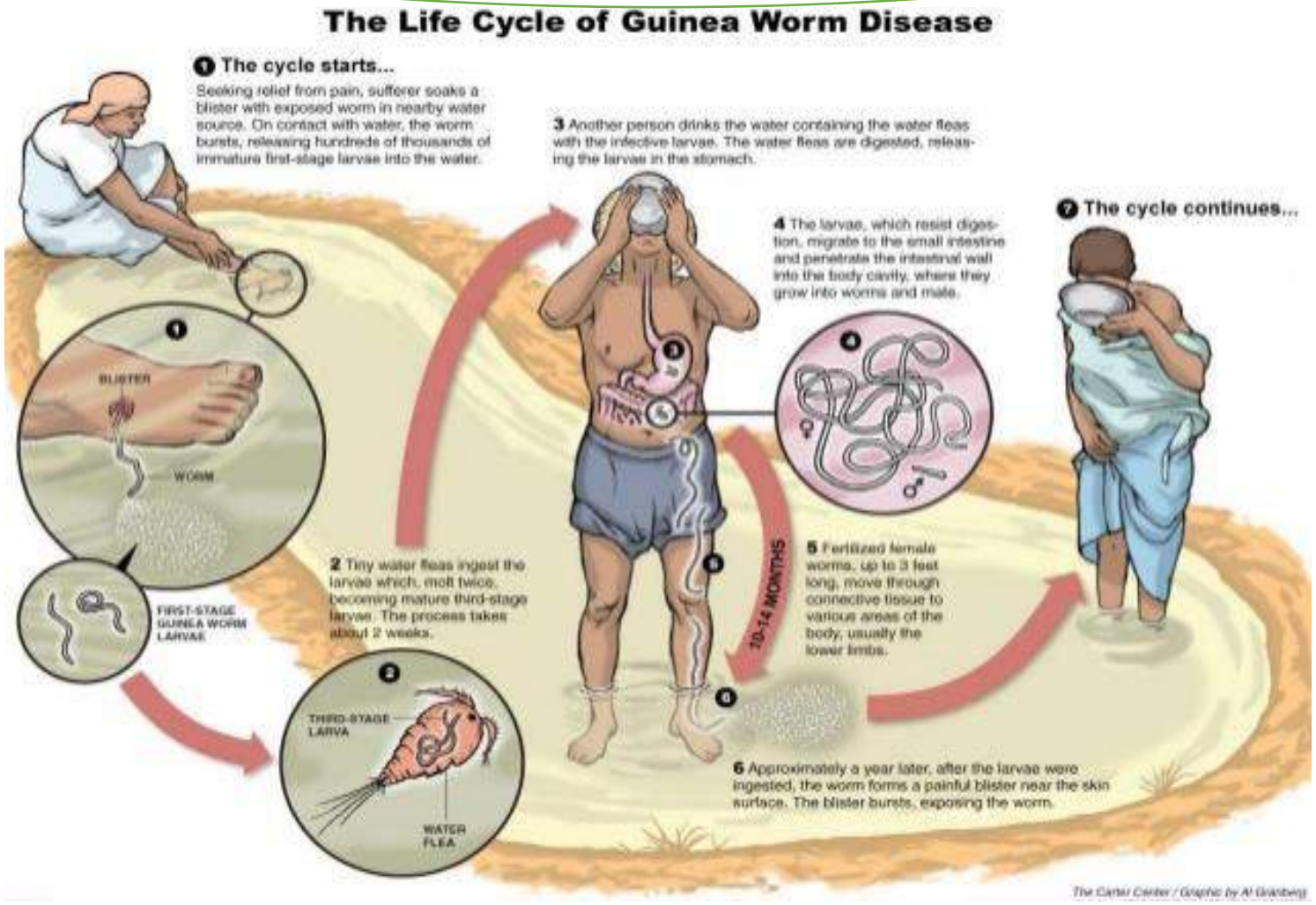


- **Infective form to Man**
- **Molt twice to attain maturity in 3 – 4 months and converted into adult worms.**


Human drinks unfiltered water containing copepods with L3 larvae.
1a
Infective copepods ingested by fish, frogs, or other aquatic animals.
1b
Larvae undergoes two molts in the copepod and becomes a L3 larvae.
6
Larvae are released when copepods die. Larvae penetrate the host's stomach and intestinal wall. They mature and reproduce in subcutaneous tissues.
Under/uncooked fish harboring infective larvae eaten by dogs or people.
L1 larvae consumed by a copepod.
5
2
Female worm begins to emerge from skin one year after infection.
3
Fertilized female worm migrates to surface of skin, causes a blister, and discharges larvae.
4
L1 larvae released into water from the emerging female worm.

CS289084-AB

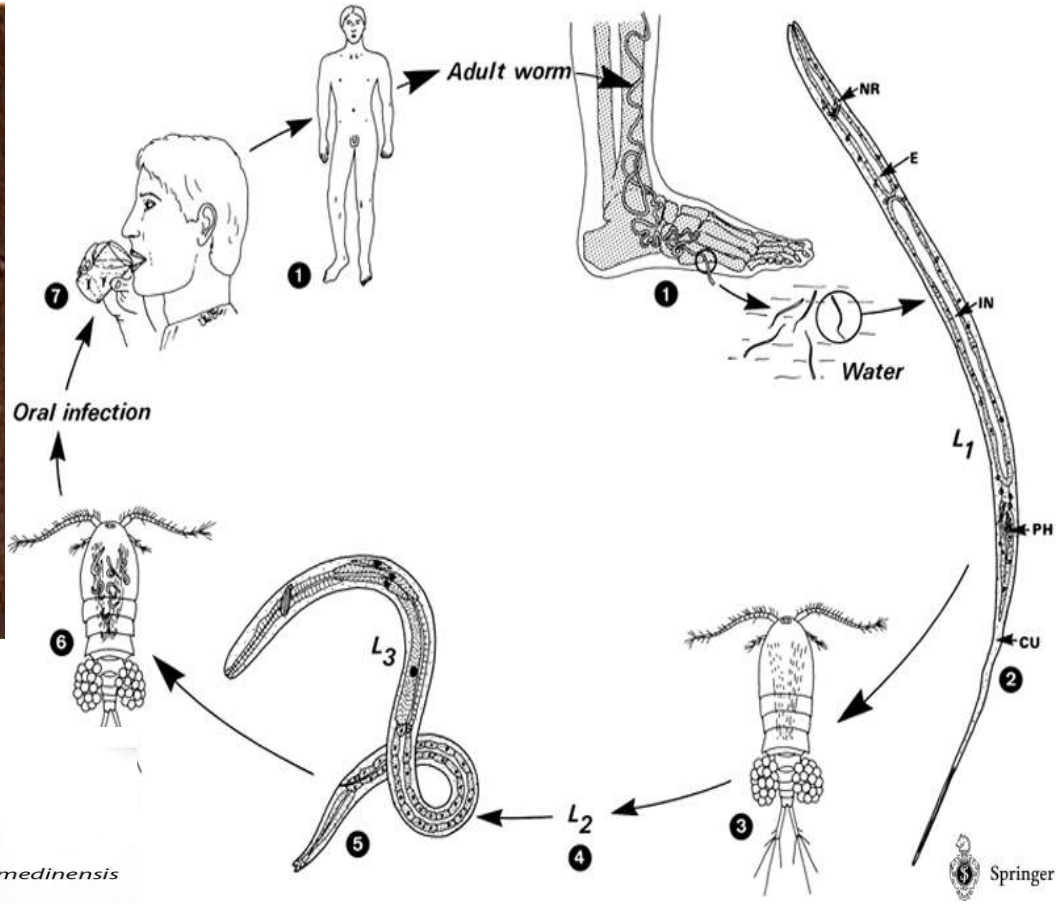
Gravid female goes down to lower limb, penetrates dermis and induces an inflammation and blister formation. **Upon contact with water it releases 1M. Larvae that swim in water for 3-6 days**



Rhabditoid larva/ L1 Larva

- The R**habditoid larva** - 650 – 750 um in length and 17 -20 um in width.
- Further development in ***Cyclops*** species.
- Each *Cyclops* can ingest 15 – 20 larvae of the guinea worm.

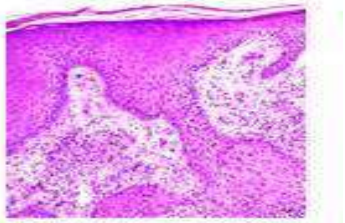
Rhabditoid larva



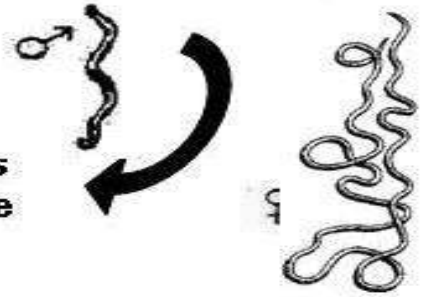
L1 larvae change to L3 larvae in 3-6 weeks

Cyclops containing L3 larvae taken up by man with contaminated water

L3 larvae release in intestine and reach subcutaneous tissues

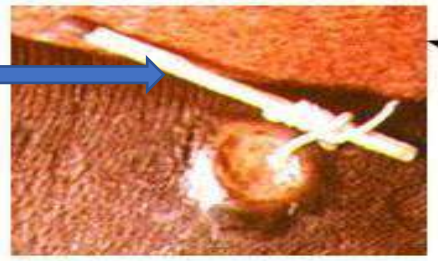


Worms mature in 3-4 months, reproduce, male worms die after copulation



After copulation, uterus gets filled with L1 larvae

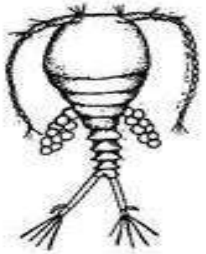
Female worms reach body surface especially on legs and cause irritation leading to papule and ulcer formation



Rod of Asclepius.

Ulcer ruptures on immersion in water and releases L1 larvae

Cyclops ingest L1 larvae released in water



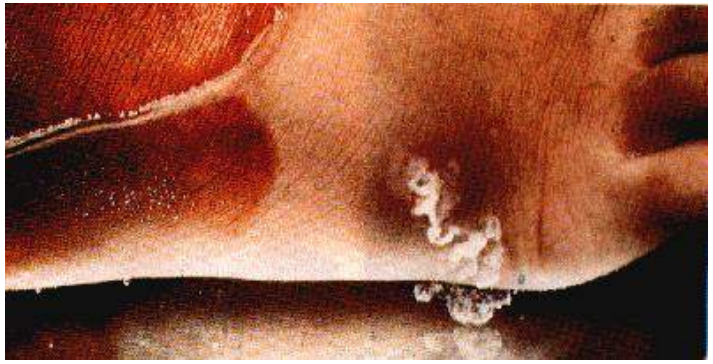
15 days in cyclops

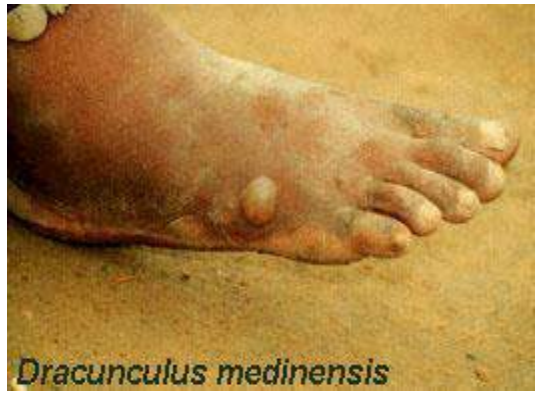
In cyclops (intermediate host)

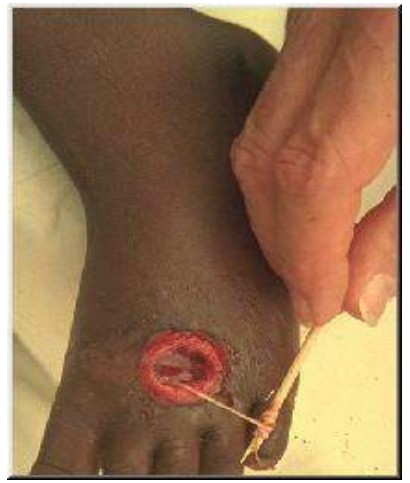
In man (definitive host)

Symptoms:
Intense pain in the path of travel of larva.
Fever,
Nausea,
Vomiting
and Allergy.
Skin blisters
Arthritis

Life cycle





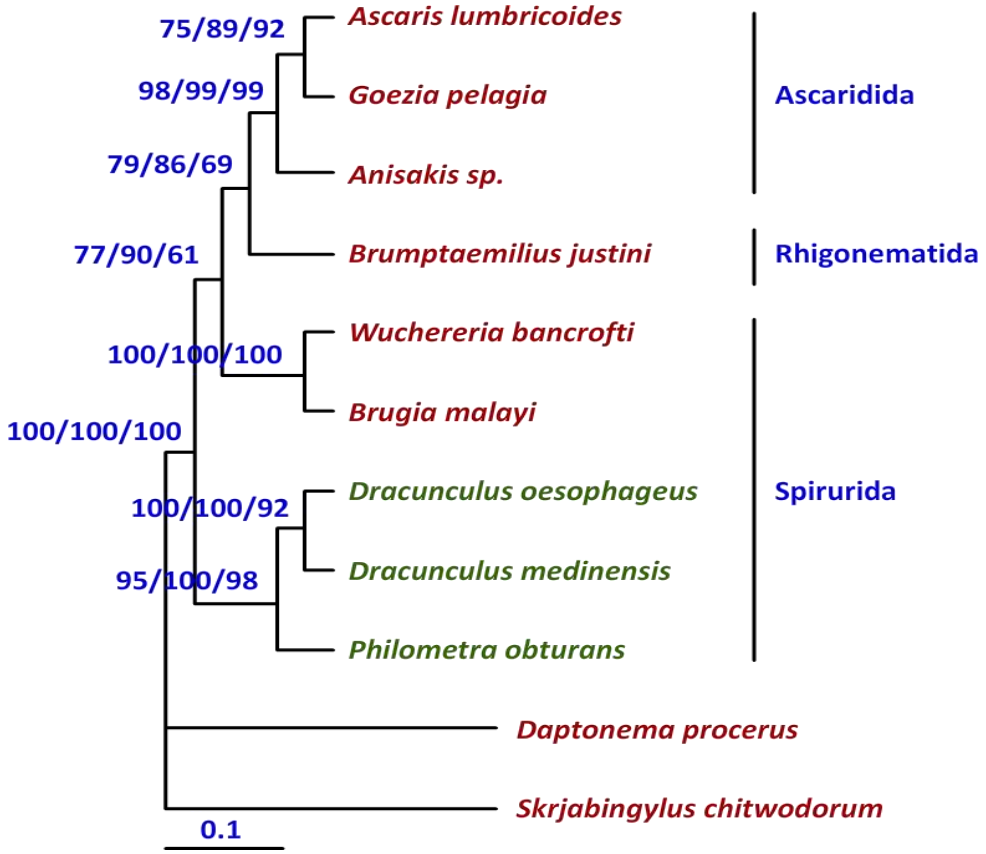


Genomics of *Dracunculus medinensis*

D. medinensis **18S-rRNA sequences are 1819 bases long.**

D.insignis **18S-rRNA sequences are 1821 bases long**

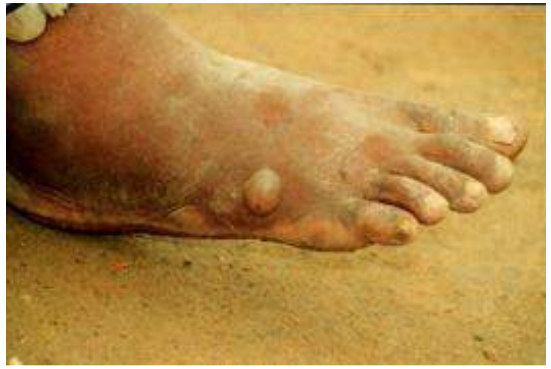
Dracunculus **and** ***Philometra*** **morphologically shared a close resemblance.**



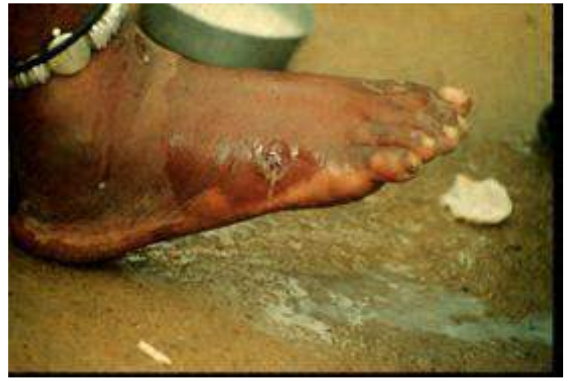
Phylogenetic position of *Dracunculus*

Pathogenicity of *Dracunculus medinensis*

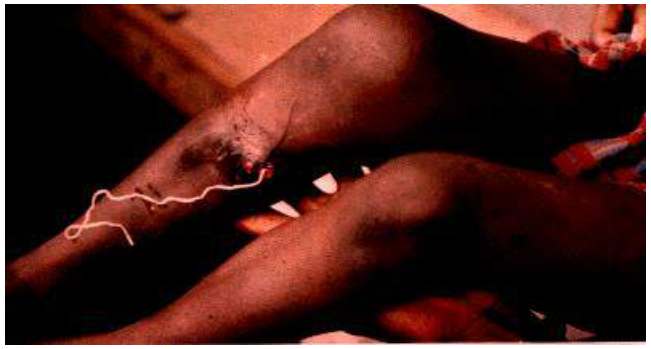
- Guinea worm disease is known as dracunculosis or dracunculiasis or dracontiasis.
- Blister formation
- Nausea, diarrhea, giddiness, skin rash, itching, or asthma may occur.

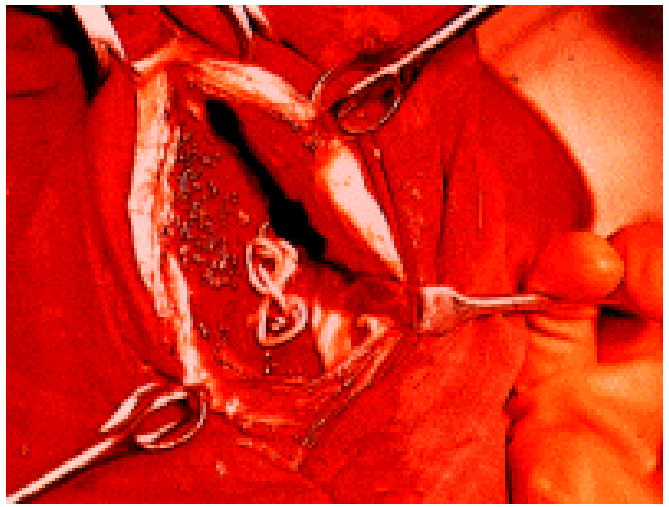


A mature blister

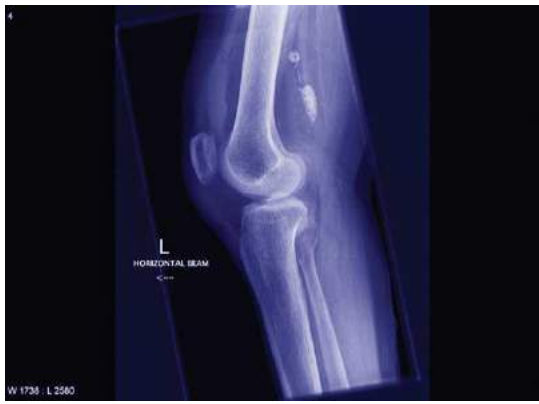


A ruptured blister—gravid female worm can be seen emerging out





Adult in joints



Calcified lesion in soft tissues

Host Parasite Relationship: Antibody Response

- Secretes opioids (Morphine-6-glucoronide) that suppresses the immune response of the body.
- Worm coats themselves with proteins - unnotice

Epidemiology

Latest situation as of 30 June 2019

- **Only Two countries reported a total of 19 human cases during January–June 2019 from 12 villages (1 village in Angola and 11 villages in Chad) from 3.5 million cases in 1986.**
- **The global burden of dracunculiasis has fallen significantly since the launch of eradication efforts in the 1980s when 20 countries were endemic for the disease.**
- **Globally, 3 countries reported - 1435 dogs and 6 baboons in 2018.**
- **No vaccine till day to prevent dracontiasis.**
- **Chances of re-infection as there is no protective immunity/acquired immunity against Guinea worm.**

Diagnosis

- Adult
- Embryo
- Blood examination - eosinophilia
- Skiagraphy: Skiagraphy reveals calcified worms.
- Intradermal test
- Falcon assay screening test-enzyme linked immunosorbent assay (FAST-ELISA) and
- Enzyme linked immune-electro transfer blot (EITB) TECHNIQUES are used to test human sera with *D. medinensis* adult worm antigen.

Prophylaxis

- **Eradicate the intermediate host, *Cyclops* by encouraging *Cyclops* eating fishes in ponds and streams used by people, or by chemical treatment of water.**
- **Drink clean, filtered, water. Boiled water is always safe to drink.**
- **Keep away people with sores from contaminating wells, laundry and bathing water.**

Treatment

- **Antihistaminics and steroids help in the initial stages of allergic reactions.**
- **Drug therapy has no lethal effect on the worm but helps to reduce the swelling and also in removing the worm easily by surgical method.**

PREVENTION:

Avoid drinking contaminated water with Cyclops seen as swimming specks on water

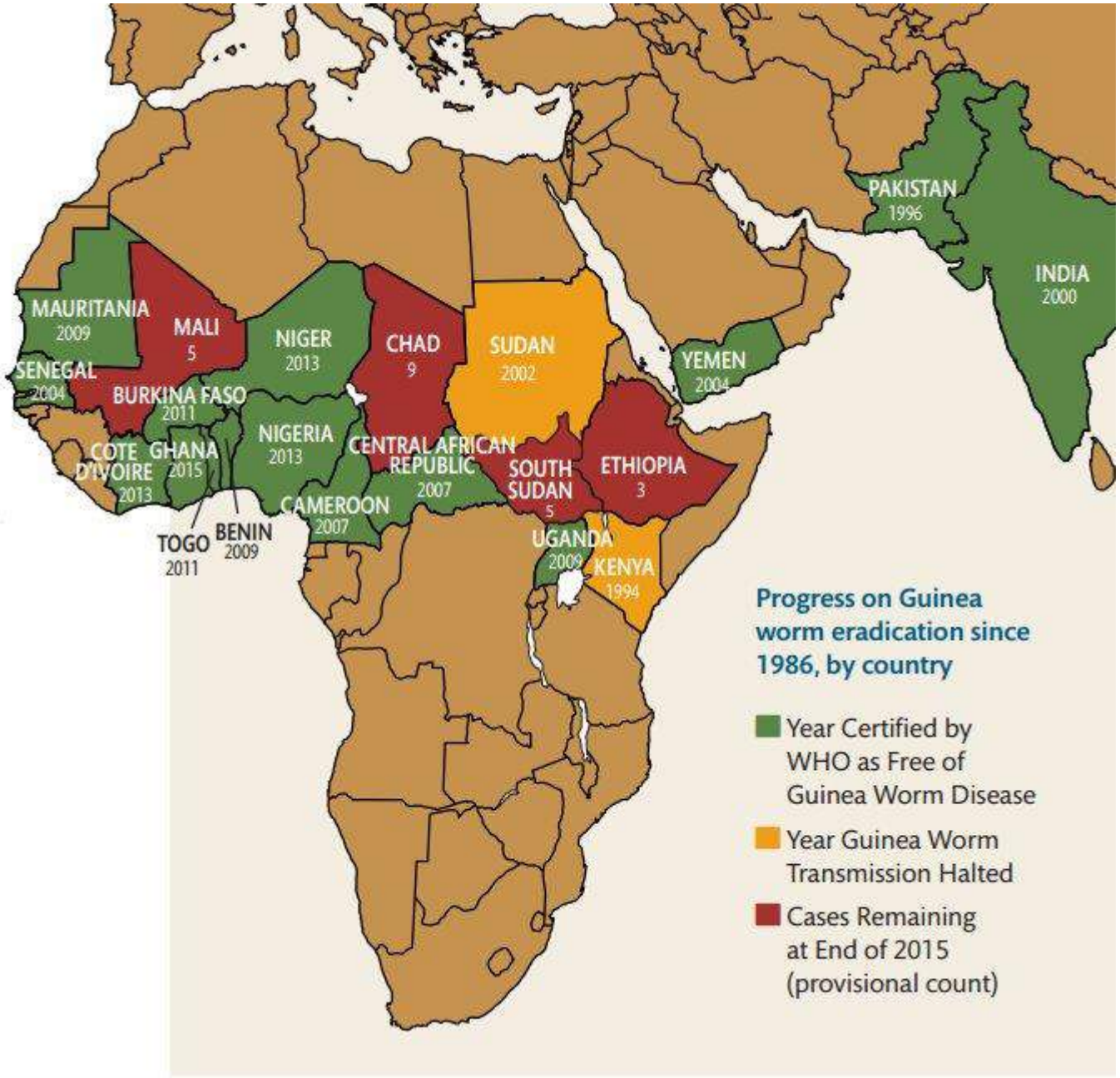
Drinking safe water.

Preventing the infected people entering into water sources.

Institutional steps.

DRUGS:

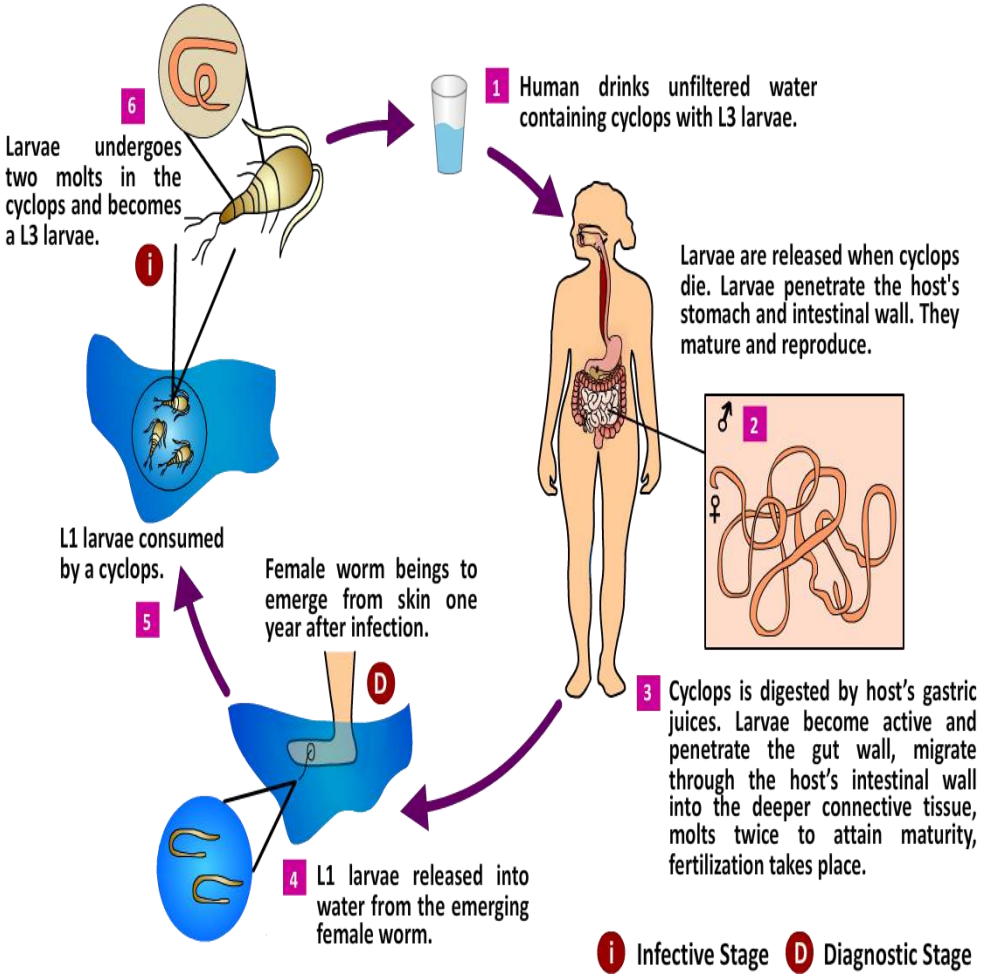
No drugs. Metronidazole/ Mebendazole to worm removal.

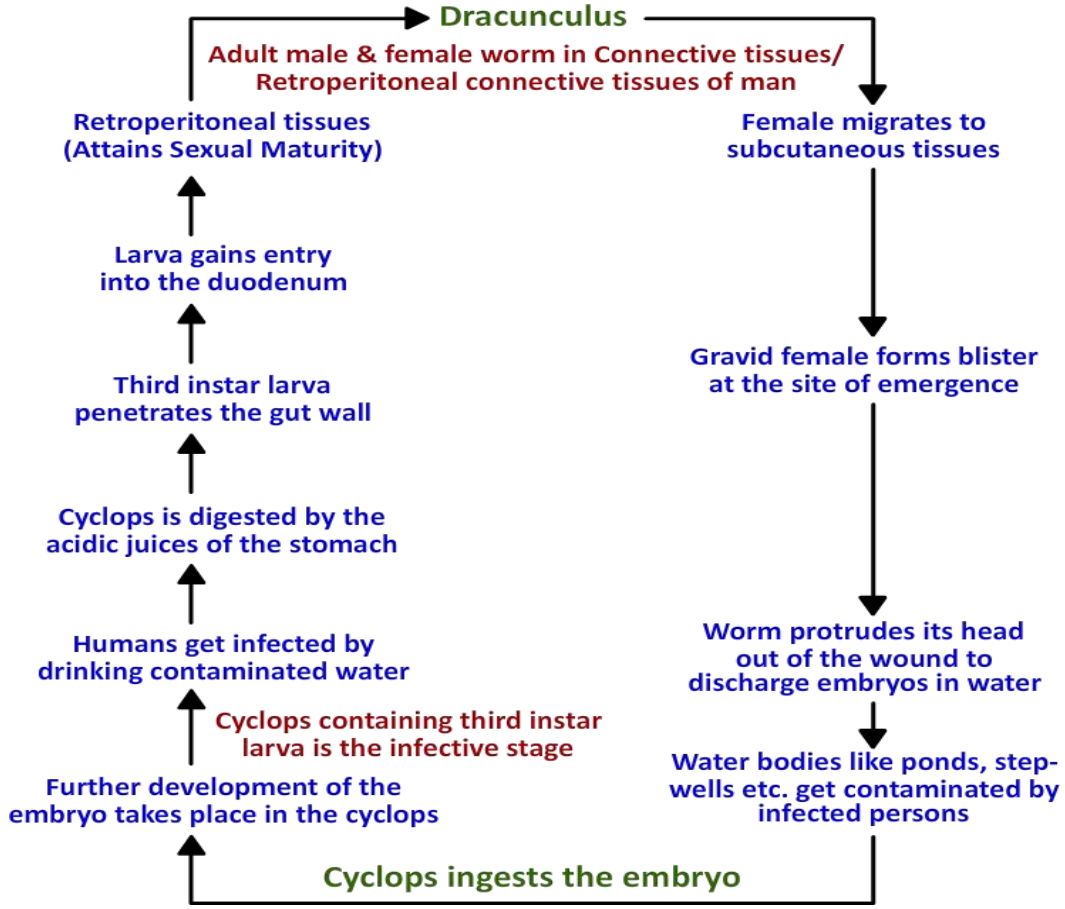


Summary

Feature	Characteristic
Mode of infection/ Port of entry	Oral, contaminated water
Source of infection	Infected Cyclops, MESOCYCLOPS LEUEKARTI (a fresh water crustacean)
Infective stage	Cyclops containing third instar larva
Digestion of Cyclops and migration of the 3rd instar larva in the body of definitive host	Cyclops is digested by host's gastric juices in the stomach. Under the influence of digestive juices the larvae become active and penetrate the gut wall migrate through the host's intestinal wall into the deeper connective tissue/retroperitoneal connective tissue attain maturity in 3 – 4 months
Site of localization	Subcutaneous tissues of exposed parts, like, legs, back, arms, and ankles
Pathogenic stage	Adult female Dracunculus
Incubation period	8-12 months
Pre-patent period	10-14 months (Period between infection and the emergence of the female parasite in the blister)
Pathogenesis	Disease caused: dracunculosis or dracunculiasis or dracontiasis Nausea, diarrhoea, guidiness, skin rash, itching, or asthma may occur. These symptoms are because of toxins produced by the parasite. Allergic reactions may also be caused by the toxins. Secondary infection by bacteria, sometimes leading to tetanus. The dead worms that get lodged in the joints or get calcified in the soft tissues may cause arthritis and paralysis of spinal cord
Pathogenic stage	Adult female Dracunculus

Summarized Life cycle of *Dracunculus medinensis*





Assessment Time!!

- What is the common name for *Dracunculus medinensis?* *Guinea worm*.
- What is the vector for *Dracunculus medinensis?* Cyclops/Copepod.
- What is the infective form for humans? L3 Larvae.
- How does Guinea worm disease spread? By drinking contaminated water containing copepods.
- What is the disease caused by *Dracunculus medinensis?* Dracunculosis or dracunculiasis or dracontiasis.

Student Assignment

- Understand the role of two hosts in the life cycle of Dracunculus medinensis and draw the life cycle of it.
- List out the sensitive parts of body that the worms migrate and emerge out.
- Go through the Global Eradication Campaign conducted for the eradication of disease by World Health Organization.

Conclusions

- Use only boiled or filtered water
- People with an open Guinea worm wound should not enter ponds or wells used for drinking water.
- The only treatment is to remove the worm
- “Prevention is better than cure”.

ELPHIDIUM OR POLYSTOMELLA

1. Q) Describe the structure and life history of Elphidium ?

(Or)

Describe the life history of a Dimorphic protozoan you have studied ?

(Or)

What is alternation of generations explain with reference to Elphidium life history ?

IMPORTANT POINTS

- Elphidium - Shelled Protozoan.
- Dimorphic - 1) Microspheric form
  2) Megalospheric form
- Shell shows pores,
- Pseudopodia are reticulopodia.
- Life history includes alternation of Generations

Introduction :

Phylum : Protozoa
Clase : Rhizopodia
Order : Foramini fera.

Elphidium is a marine form. It is found creeping on Seaweeds to a depth of 300 fathoms.

Structure :-

Elphidium is also called as 'Polystomella'. It is a '*dimorphic rhizopod*'. It is a unicellular microscopic protozoan, and "1mm" in diameter. It is pale yellow in colour. It lives in marine water. The body is covered by a shell. The shell is biconvex. The first formed chamber is *proloculum*. The shell contains spirally arranged 'V' shaped chambers. Hence it is "called polythalamus" or multilocular. They overlap one another. These chamber show *openings*, hence it is peforate these Through

openings cytoplasm will come out. The cytoplasm is produced into a number of network like reticulopodia. From hinder end of each chamber cytoplasmic processes develop. They are directed back wards. They are called 'retral processes'.

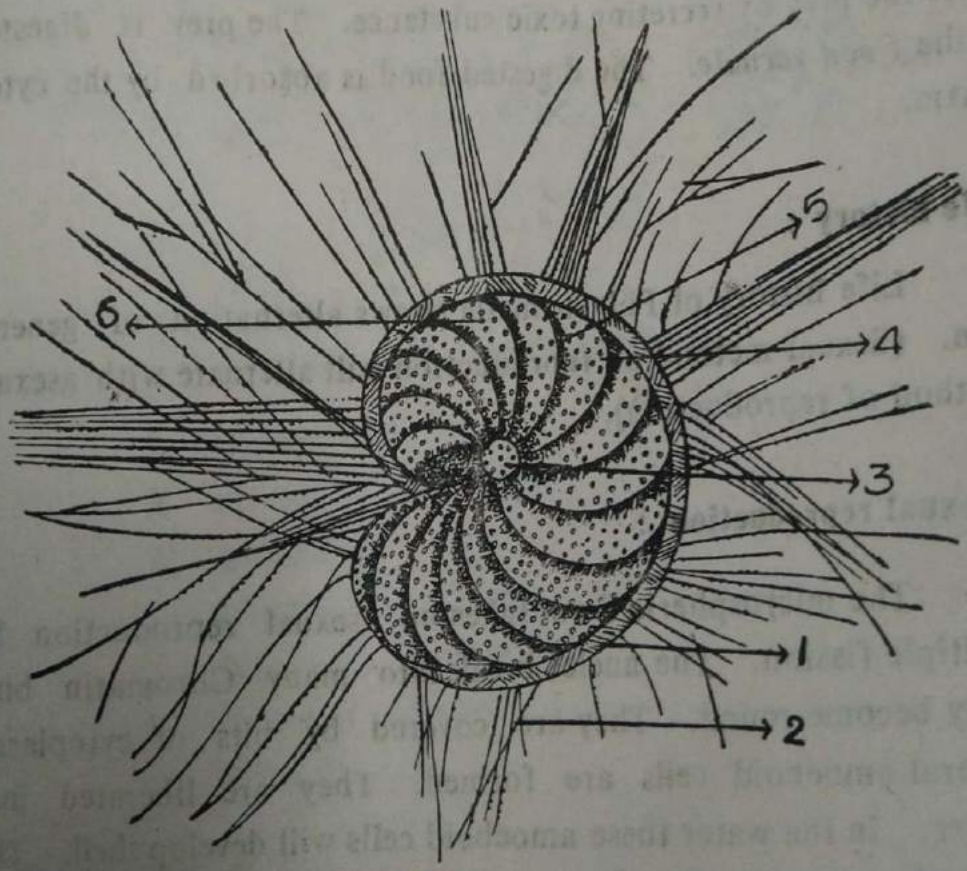
The chambers are filled with the cytoplasm.

1. The cytoplasm contains one or many nuclei.
2. Contractile vacuole is absent.
3. Mouth is absent.
4. Cytoplasm contains food vacuoles. They take up the process of digestion.

Dimorphism :-

Polystomella exhibits dimorphism. The individual occurs in two distinct forms.

1. Megalospheric form.
2. Microspheric form.



ELPHIDIUM

1. *Shell*
2. *Reticulopodia*
3. *Proloculum*
4. *Chamber*
5. *Retral Processes*
6. *Pores*

1. Megalospheric form :

Its proloculum is big in size. A singal large nucleus is present in one of the chambers. It takes up sexual reproduction.

2. Microspheric form :

Its proloculum is small in size. A large number of nuclie will be present in the cytoplasm. This form reproduces by Asexual reproduction.

Locomotion and Nutrition :

Polystomella perform slow creeping movements with the help of *reticulopodia*. It is a *holozoic feeder*. The reticulopodia will capture the prey. When it comes in contact with the prey it kills the prey by secreting toxic substance. The prey is digested in the *food vacuole*. The digested food is absorbed by the cytoplasm.

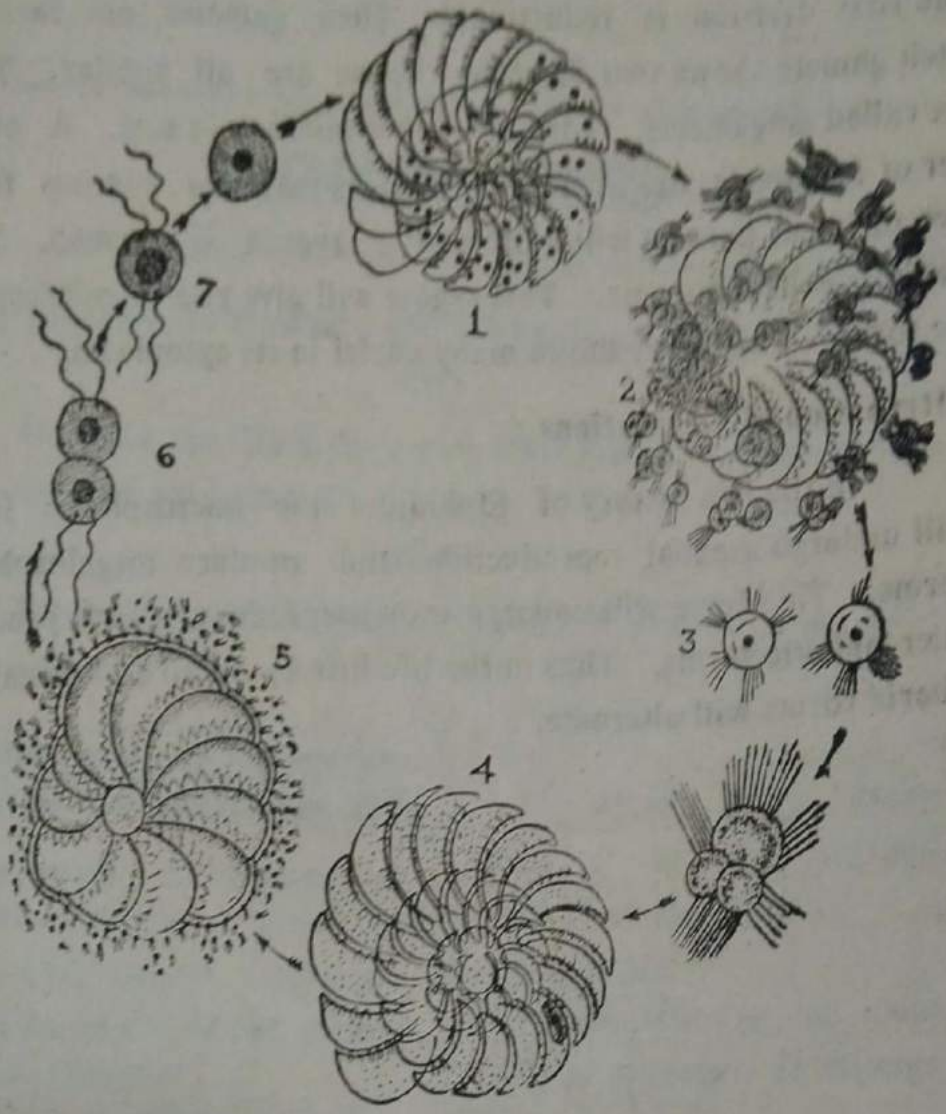
Life history :

Life history of Polystomella shows alternation of generation. (Sexual method of reproduction will alternate with asexual method of reproduction).

Asexual reproduction :

The microspheric form takes up asexual reproduction by multiple fission. The nuclei break into many Chromatin bits. They become round. They are covered by bits of cytoplasm. Several amoeboid cells are formed. They are liberated into water. In the water these amoeboid cells will develop shell. The first chamber is called 'Proloculum'.

This grows into megalospheric form. It contains only single nucleus. This takes up Sexual reproduction.



Life Cycle of Elphidium

1. *Microspheric form*
2. *Release of Amoebulae*
3. *Amoebulae*
4. *Megalospheric form*
5. *Release of gametes*
6. *Fusion of gametes*
7. *Zygote*

Sexual Reproduction :

Megalospheric form performs sexual reproduction. It has a single nucleus in its cytoplasm. It undergoes many divisions.

The first division is reductional. Then gametes are formed. Each gamete shows two flagella. These are all similar. They are called isogametes. They are 3 to 4 microns in size. A number of isogametes are liberated. Two isogametes coming from two different parents will unite and a zygote is formed. This union is called isogamy. This zygote will give rise to microspheric form. This form shows many nuclei in its cytoplasm.

Alternation of Generations :

In the life history of Elphidium the microspheric form will undergo asexual reproduction and produce megalospheric forms. This form will undergo sexual reproduction and produce microspheric forms. Thus in the life-history micro and megalospheric forms will alternate.

TSWRAFPDCW – BHONGIR TEACHING MODULE
CLASS: BZC/MZC (BSC) DEPARTMENT: ZOOLOGY
Year ll (lV sem); Paper: IV (CELL BIOLOGY AND GENETICS)

Topic: EPISTASIS

Lecturer Name: V. JYOTHI

1. **No. of Teaching Hours: 2**
2. **Objectives of the Topic:**

➢Student can able to understand the Epistasis

➢Understand the overall idea of Epistasis

➢Understand the Importance of Epistasis

➢Student can identify the differences between Mendelian and non-Mendelian inheritance

➢**PRE-TEST**:

➢**Understanding the student's basic knowledge by asking orally on the following**

i.Tell me the examples of different colours animals and plants?

ii.What is Epistasis?

iii.How the genes will interact?

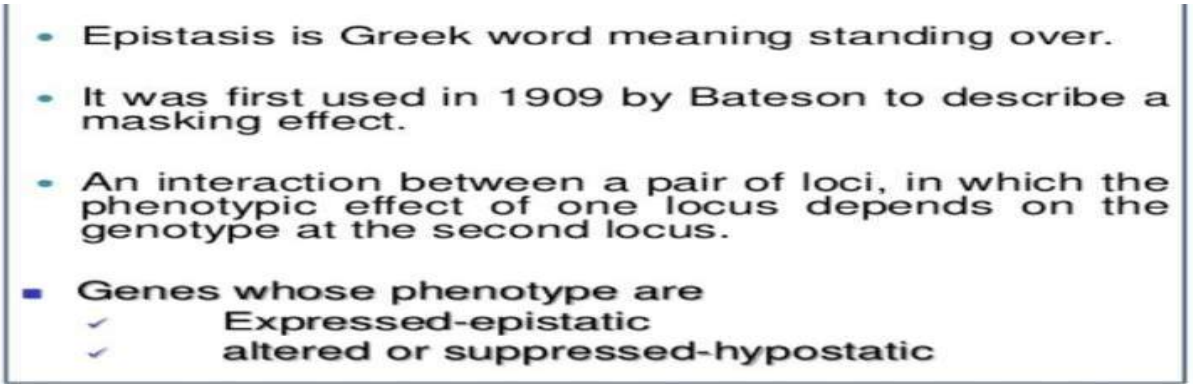
iv.How one gene suppressed other gene?

v.Types of epistasis?

Definition

- Epistasis is a form of gene interaction in which one gene masks the phenotypic expression of another.
- There are no new phenotypes produced by this type of gene interaction.

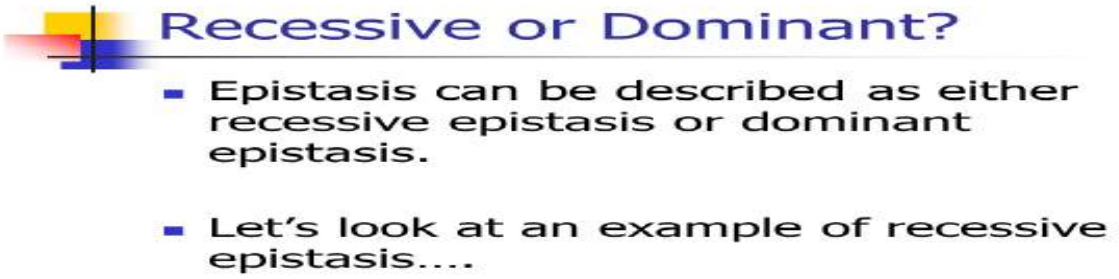
3. Module Content:



Two different genes which are not alleles and present on different chromosomes, both influencing the same part or trait of the organism. The expression of one gene conceals or hides the expression of the other gene. The which is suppressing is known as epistatic gene or inhibiter gene and the suppressed gene is called hypostatic gene. This phenomenon of masking is known as EPISTASIS.

■The alleles that are masking the effect are called epistatic alleles

Epistasis is a form on non-Mendelian inheritance in which one gene is capable of interfering with expression of another. This is often found associated with gene pathways where the expression of one gene is directly dependent on the presence or absence of another gene product within the



Dominant Epistasis. (12:3:1)

- Dominant allele (eg.,A) of one gene hides the effect of allele of another gene (eg., B) and expresses itself phenotypically.
- The B allele (hypostatic) will be expressed only when gene locus A contains two recessive (aa) alleles.
- Thus, the genotype AA BB or Aa Bb and AA bb or Aa bb produce the same phenotype
- genotype aa BB or aa Bb and aa bb produce two additional phenotype.
- This type of dominant epistasis modifies the classical ratio of 9:3:3:1 into 12:3:1

Example:

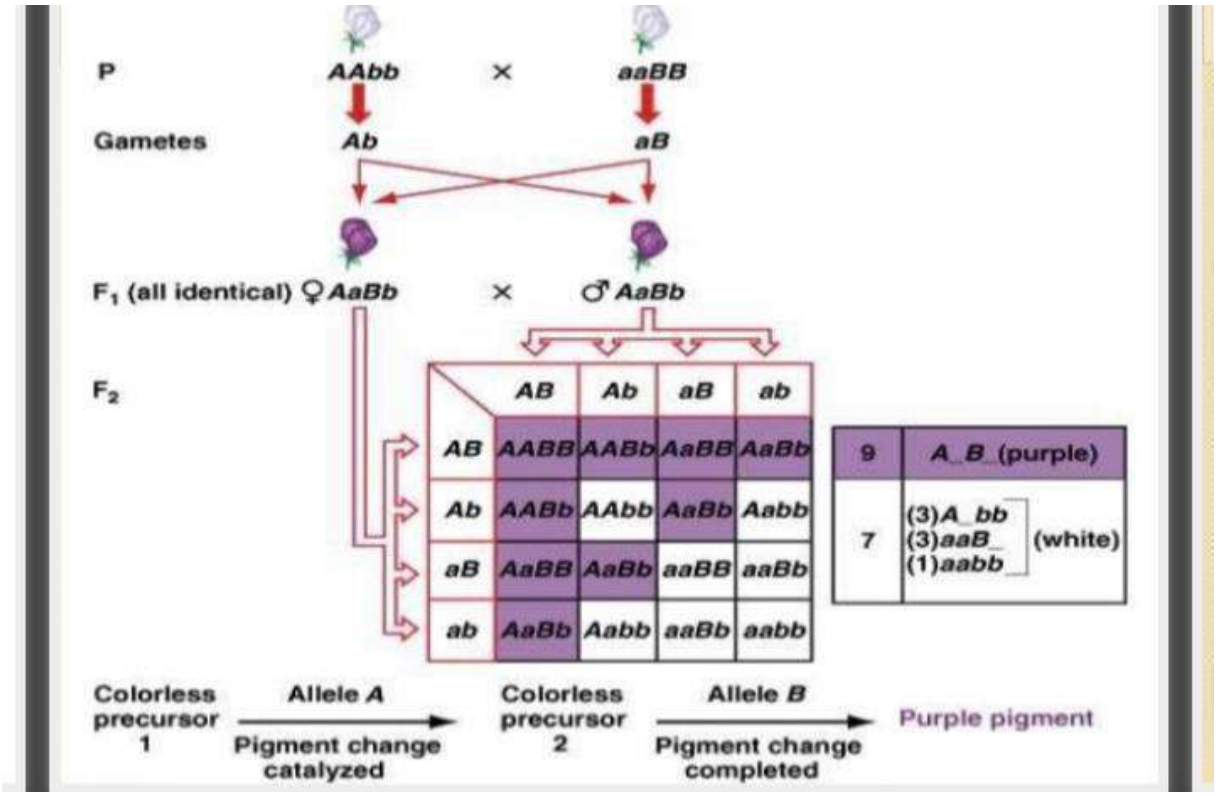
Studied in summer squash (*Cucurbita pepo*)

- Common fruit colors-white,yellow &green
- White (W) is dominant over colored squash
- Yellow (Y) is dominant over green squash
- Pure breeding white fruited variety is crossed with the double recessive green variety,F1 hybrids are all white
- When the hybrids are selfed-white, yellow &green fruited plants arise in the ratio of 12:3:1

Epistatic alleles	Hypostatic alleles	Phenotypic Expression
aa	BB, Bb, bb	No phenotype production
AA, Aa, aa	bb	
AA, Aa	BB, Bb	Phenotype due to dominant

- **Bateson** and **Punnett** observed that when two white flowered varieties of sweet pea, *Lathyrus odoratus* were crossed, F_1 progeny had coloured flowers. When F_1 was selfed, the F_2 ratio showed the presence of both coloured and white flowered varieties in the ratio 9:7.

- In man, **deaf mutism** is complementary gene dependent, depending upon two dominant genes A and B, the presence of both of them is responsible for normal hearing and speech.



- In this case dominant alleles on both locus are required hence wherever A and B both are present they result into purple effect masking the white.
- This is because A and B alleles modified the colorless precursor by showing their effects

RECESSIVE DOMINANT:

Labrador Retrievers

- If a Labrador retriever has a dominant B allele, they will have black fur.

- If they have two recessive alleles (bb) they will have brown fur.

Labrador Retrievers

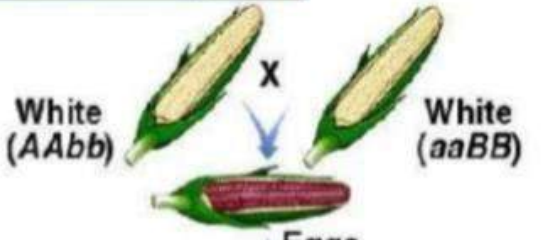
- If a retriever receives at least one dominant "E" allele, they will remain the color that the "B" allele coded for.
  - Either black of brown
- However, if a dog receives a pair of homozygous recessive "e" alleles, they will be golden regardless of their "B" alleles!

Duplicate Recessive Genes (9:7) (Complementary Genes)

- Both the genes loci have homozygous recessive alleles and both of them produce identical phenotype.
- Both dominant alleles are necessary to produce a different phenotype. e.g.: AABB, AaBB, AaBb, in all these combinations.
- Both the dominant alleles (A and B) are present and they will produce a different phenotype.
- Whereas aaBB or bbAA, in which the other dominant allele is absent, produces the normal phenotype.

Example

Epistasis

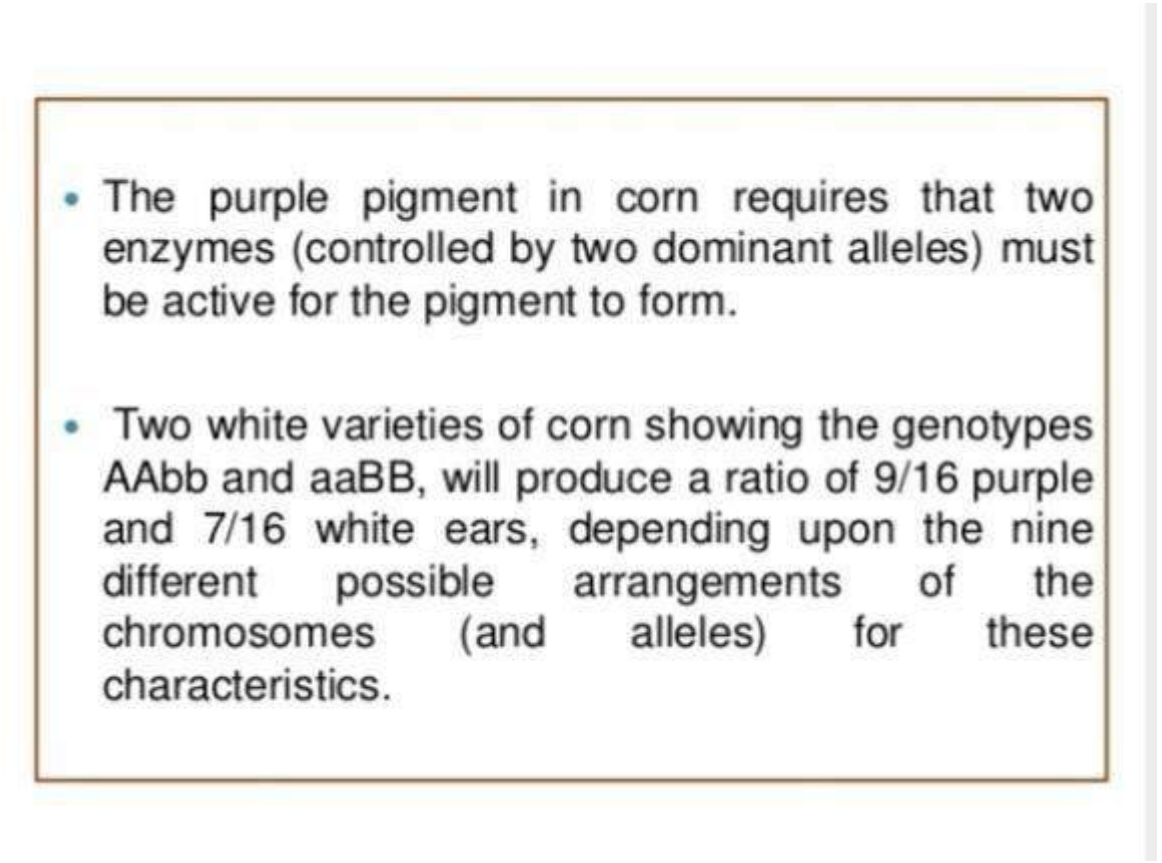


Eggs

Sperm	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

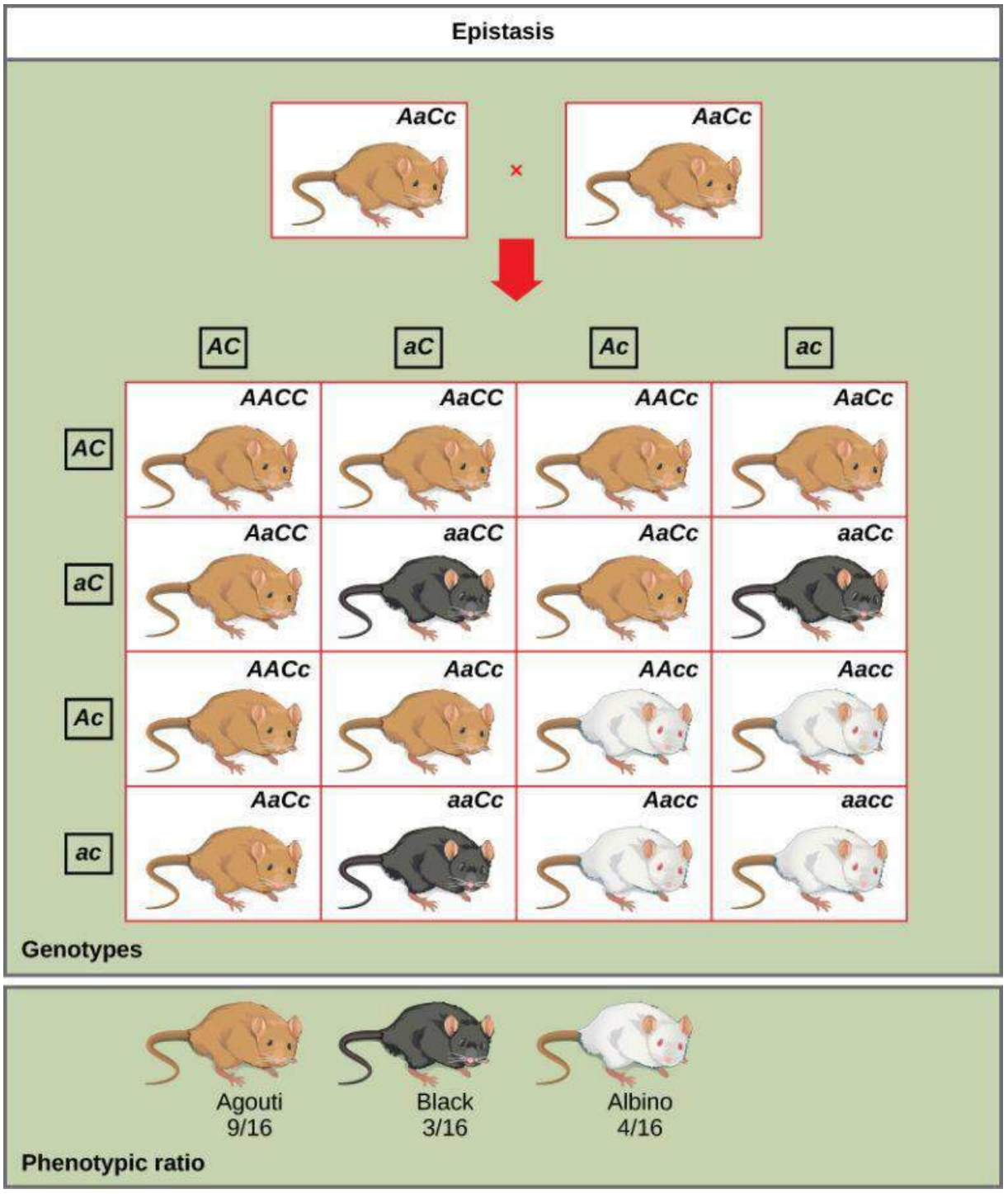
F_1 generation
All purple
(*AaBb*)

F_2 generation
9/16 purple
7/16 white



In epistasis, the interaction between genes is antagonistic, such that one gene masks or interferes with the expression of another. “Epistasis” is a word composed of Greek roots that mean “standing upon.” The alleles that are being masked or silenced are said to be hypostatic to the epistatic alleles that are doing the masking. Often the biochemical basis of epistasis is a gene pathway in which the expression of one gene is dependent on the function of a gene that precedes or follows it in the pathway.

An example of epistasis is pigmentation in mice. The wild-type coat color, agouti (*AA*), is dominant to solid-colored fur (*aa*). However, a separate gene (*C*) is necessary for pigment production. A mouse with a recessive *c* allele at this locus is unable to produce pigment and is albino regardless of the allele present at locus *A* (Figure 1). Therefore, the genotypes *AAcc*, *Aacc*, and *aacc* all produce the same albino phenotype. A cross between heterozygotes for both genes (*AaCc* x *AaCc*) would generate offspring with a phenotypic ratio of 9 agouti:3 solid color:4 albino. In this case, the *C* gene is epistatic to the *A* gene.


Epistasis
AaCc
×
AaCc
AC
aC
Ac
ac
AACC
AaCC
AACc
AaCc
AaCC
aaCC
AaCc
aaCc
AACc
AaCc
AAcc
Aacc
AaCc
aaCc
Aacc
aacc
Genotypes
Agouti
9/16
Black
3/16
Albino
4/16
Phenotypic ratio

Epistasis is a form on non-Mendelian inheritance in which one gene is capable of interfering with expression of another. This is often found associated with gene pathways where the expression of one gene is directly dependent on the presence or absence of another gene product with a pathway

4.Student Activities:

- Group discussion on interaction of genes
- Seminars by Students
- Quiz will be conducted to the students
- Preparing the punnet chart with some examples of Epistasis

5.Assessment:

- Assessing the Student after completion of the Topic by conducting Oral Test or written test.

i. Explain about epistasis
ii. Draw the punnet chart with examples Types of epistasis?
iii. Write the importance of Epistasis?
iv.

6. Reference Books:

- Molecular genetics -Sanfoundry
- Human genetics -Lewis,R.
- Genetics and Evolution -Saras publication
- Concept of Genetics -10 th edition by Michael

Electron Transport Chain Physiology and Biochemistry

Dr. K. Srilatha, HOD

Department of Zoology

TSWRAFPDCW, Bhongir

Electron Transport Chain (ETC)- Components and Steps

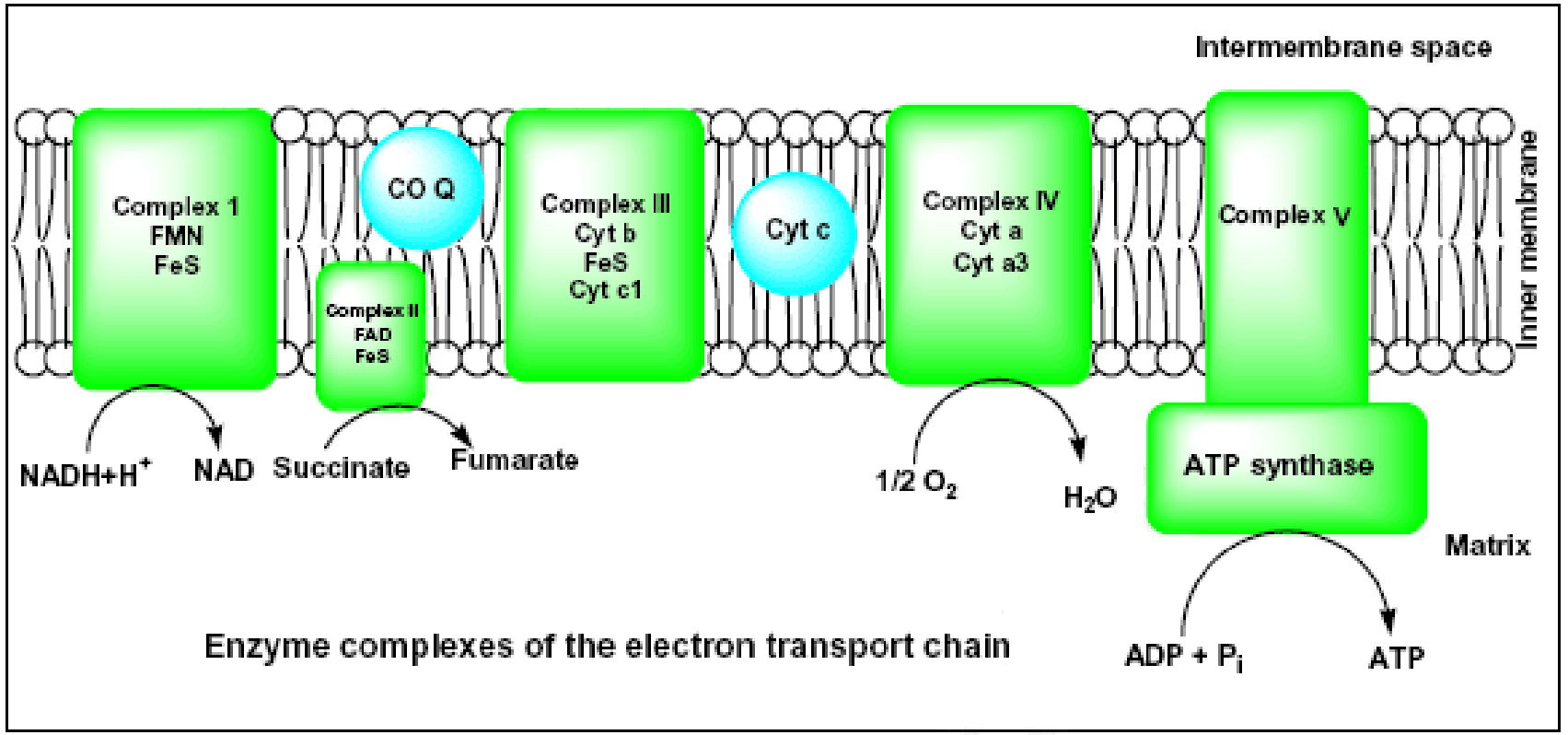
- The NADH and $FADH_2$ formed in **glycolysis**, **TCA cycle** and fatty acid oxidation are energy-rich molecules because they contain a pair of electrons that have high transfer potential.
- ATP is generated as a result of the energy produced when electrons from NADH and $FADH_2$ are passed to molecular oxygen by a series of electron carriers, collectively known as the electron transport chain (ETC).
- The electron transport chain is also called the Cytochrome oxidase system or as the Respiratory chain.
- The components of the chain include FMN, Fe–S centers, coenzyme Q, and a series of cytochromes (b, c1, c, and aa3).

- The energy derived from the transfer of electrons through the electron transport chain is used to pump protons across the inner mitochondrial membrane from the matrix to the cytosolic side.
- As a result, an electrochemical gradient is generated, consisting of a proton gradient and a membrane potential.
- The energy created by the formation of this gradient is then harnessed to form ATP as the protons travel down their gradient into the matrix through the ATP synthase channel.
- The oxidation of 1 mole of NADH generates approximately 2.5 moles of ATP, whereas the oxidation of 1 mole of $FADH_2$ generates approximately 1.5 moles of ATP.
- Because energy generated by the transfer of electrons through the electron transport chain to O_2 is used in the production of ATP, the overall process is known as **oxidative phosphorylation.**
- Thus, the electron transport and ATP production occur simultaneously and are tightly coupled.

Location of ETC

- The respiratory chain is located in the cytoplasmic membrane of bacteria but in case of eukaryotic cells it is located on the membrane of mitochondria.

Components of ETC:



1. **Complex I** (NADH dehydrogenase)

- It contains FMN, which accepts 2 electrons and H + from 2 NADH to become the reduced form of $FMNH_2$; also contains iron atoms, which assist in the transfer of the e − and H + to coenzyme Q.

2. **Complex II** (Succinate dehydrogenase)

- Contains iron and succinate, which oxidizes FAD to form $FADH_2$

3. **Coenzyme Q**

- Accepts electrons from $FMNH_2$ (complex I) and $FADH_2$ (complex II) and transfers electrons to complex III.

4. **Complex III** (cytochrome b)

- It contains heme group, in which the Fe 3+ accepts the electrons from coenzyme Q to become Fe 2+. Transfers electrons to cytochrome c.

5. **Cytochrome c**

- It contains the heme group, in which the Fe 3+ accepts the electrons from complex III to become Fe 2+. Transfers electrons to complex IV.

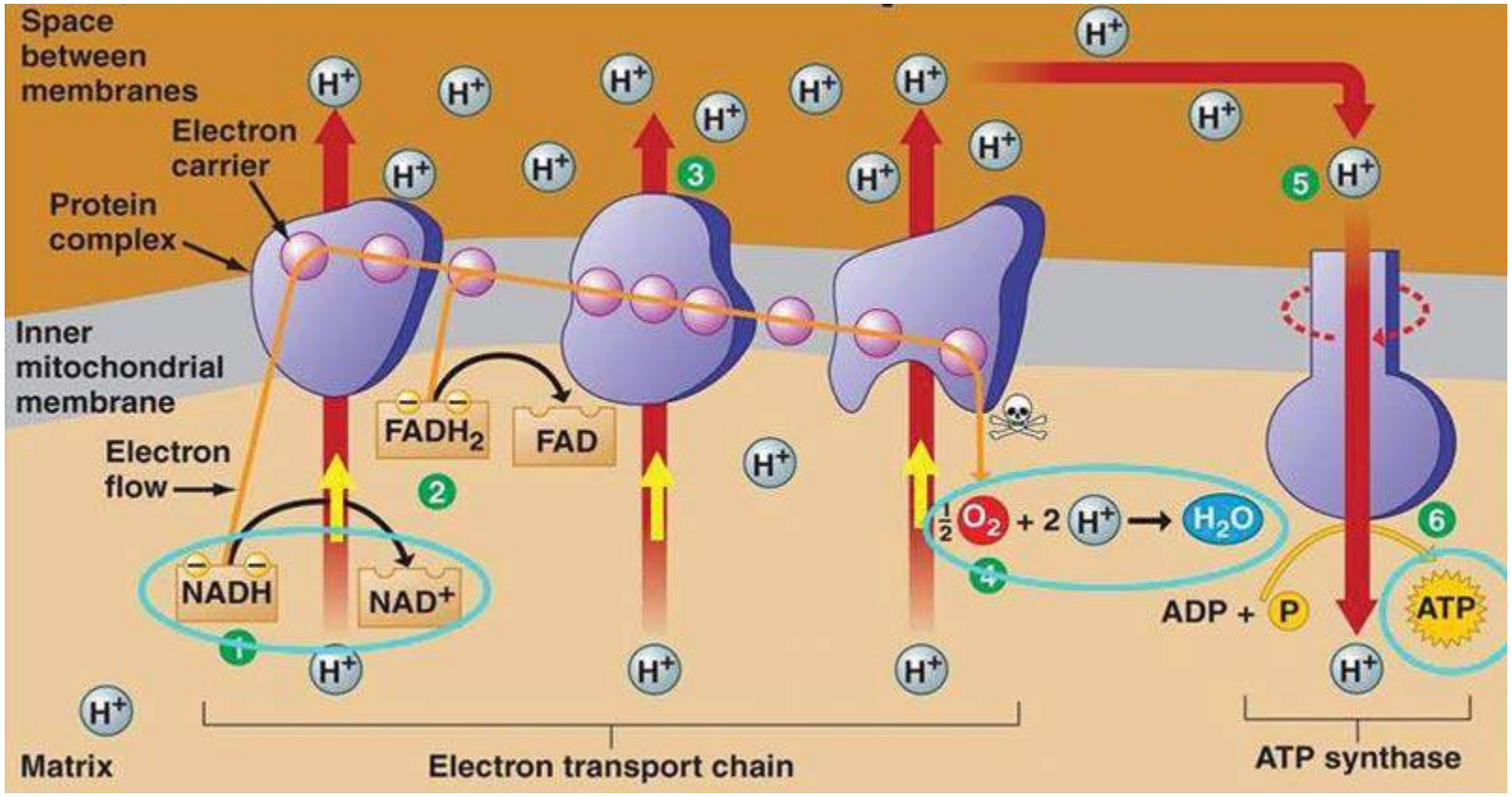
6. **Complex IV** (cytochrome a)

- It contains the heme group, in which the Fe 3+ accepts electrons from cytochrome c to become Fe 2+. Transfers electrons to O_2, which is combined with hydrogen to form H_2O.

7. **Complex V** (ATP synthase)

- It contains a proton channel that allows for protons to cross into the matrix, using the proton gradient energy to form ATP.

- Major steps in ETC:



1.Transfer of electrons from NADH to coenzyme Q

- NADH passes electrons via the NADH dehydrogenase complex (complex I) to FMN. The complex is also known as the NADH:CoQ oxidoreductase.
- NADH is produced by the α-ketoglutarate dehydrogenase, isocitrate dehydrogenase, and malate dehydrogenase reactions of the TCA cycle, by the pyruvate dehydrogenase reaction that converts pyruvate to acetyl-CoA, by β-oxidation of fatty acids, and by other oxidation reactions.
- NADH produced in the mitochondrial matrix diffuses to the inner mitochondrial membrane where it passes electrons to FMN, which is tightly bound to a protein.
- FMN passes the electrons through a series of iron–sulfur (Fe–S) complexes to coenzyme Q, which accepts electrons one at a time, forming first the semiquinone and then ubiquinol.
- The energy produced by these electron transfers is used to pump protons to the cytosolic side of the inner mitochondrial membrane.
- As the protons flow back into the matrix through the pores in the ATP synthase complex, ATP is generated.

2.Transfer of electrons from coenzyme Q to cytochrome c

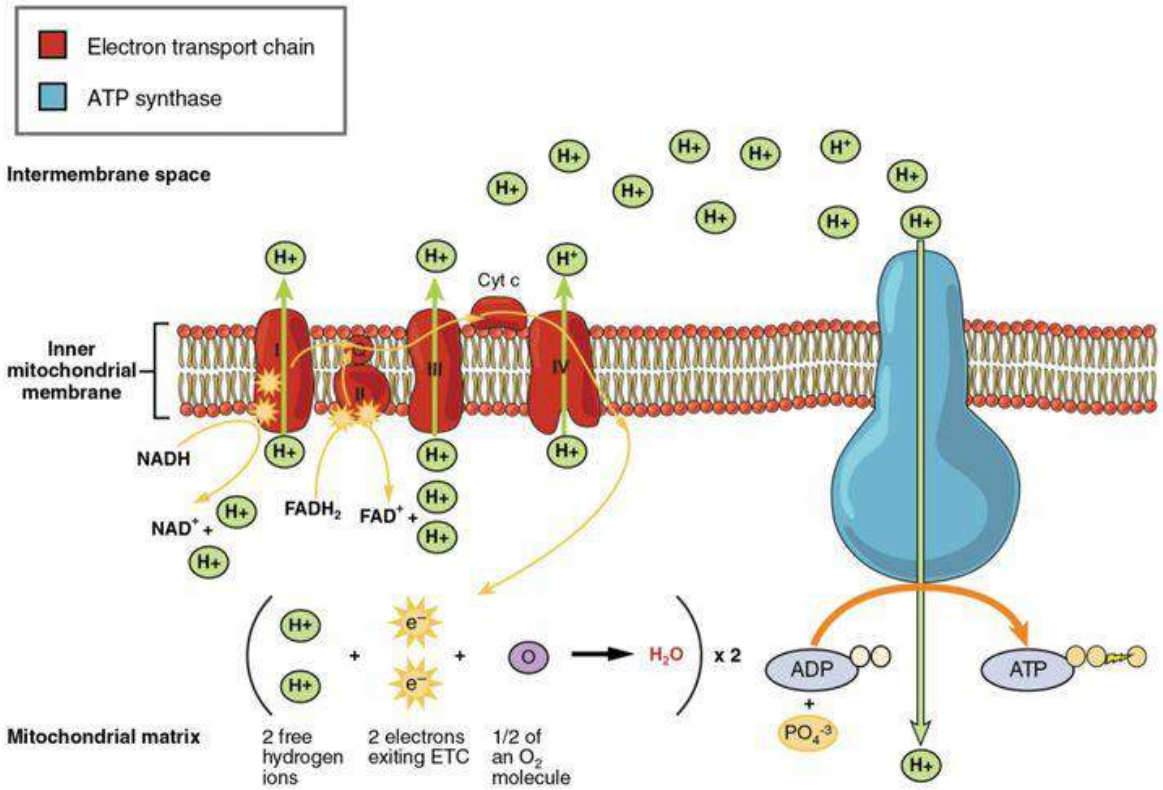
- Coenzyme Q passes electrons through Fe–S centers to cytochromes b and c1, which transfer the electrons to cytochrome c.
- The protein complex involved in these transfers is called complex III, or the cytochrome b-c1 complex. The complex is also known as CoQ:C1 oxidoreductase.
- These cytochromes each contain heme as a prosthetic group but have different apoproteins.
- In the ferric (Fe3+) state, the heme iron can accept one electron and be reduced to the ferrous (Fe2+) state.
- Because the cytochromes can only carry one electron at a time, two molecules in each cytochrome complex must be reduced for every molecule of NADH that is oxidized.
- The energy produced by the transfer of electrons from coenzyme Q to cytochrome c is used pump protons across the inner mitochondrial membrane.
- As the protons flow back into the matrix through the pores in the ATP synthase complex, ATP is generated.
- Electrons from $FADH_2$, produced by reactions such as the oxidation of succinate to fumarate, enter the electron transport chain at complex II, which contains succinate dehydrogenase.
- Complex II will transfer electrons to coenzyme Q, without the associated proton pumping across the inner mitochondrial membrane.
-

3.Transfer of electrons from cytochrome c to oxygen

- Cytochrome c transfers electrons to the cytochrome aa3 complex, which transfers the electrons to molecular oxygen, reducing it to water.
- Cytochrome oxidase (complex IV) catalyzes this transfer of electrons.
- Cytochromes a and a3 each contain a heme and two different proteins that each contain copper.
- Two electrons are required to reduce one atom of oxygen; therefore, for each NADH that is oxidized, one-half of O2 is converted to H2O.
- The energy produced by the transfer of electrons from cytochrome c to oxygen is used to pump protons across the inner mitochondrial membrane.
- As the protons flow back into the matrix, ATP is generated.
-

ATP generation in ETC

- The production of ATP is coupled to the transfer of electrons through the electron transport chain to O_2. The overall process is known as oxidative phosphorylation. Protons flow down their electrochemical gradient through the membrane-bound ATP synthase. The flow of protons through the ATPase allows the enzyme to synthesize ATP.



- The exact amount of ATP that is generated by this process has not been clearly established, but current thought indicates that for each pair of electrons that enters the chain from NADH, 10 protons are pumped out of the mitochondria. As it takes four protons to flow through the ATPase to synthesize one ATP, 2.5 moles (10 divided by 4) of ATP can be generated from 1 mole of NADH.
- For every mole of $FADH_2$ that is oxidized, approximately 1.5 moles of ATP are generated because the electrons from $FADH_2$ enter the chain via coenzyme Q, bypassing the NADH dehydrogenase step (lead to the extrusion of 6 protons per pair of electrons, instead of the 10 protons per pair of electrons).
- **Significance of ETC**
- The electron transport chain is the final and most important step of cellular respiration.
- While Glycolysis and the Citric Acid Cycle make the necessary precursors, the electron transport chain is where a majority of the ATP is created.
- It has an important role in both photosynthesis and cellular respiration.
-

TSWRAFPDCW, Bhongir

PAPER- GE (Fundamentals of Food and Nutrition), Final year

FACULTY: Dr. K. Srilatha

Topic: Functions of Food in Human body

- Food is important for life. To be healthy and active, we should certainly have enough food. The food we eat should be safe and rich in all the nutrients for our body needs. We should choose from a wide variety of foods and we should eat them regularly, every day. Do not forget that we should also enjoy the food that we eat; it should look, smell and taste good. Without good nutrition, children and young people cannot develop their potential to the full and adults will have difficulty in doing their best.
- Food provides our body with what they need to
- · Stay alive, be active, move and work;
- · Build new cells and tissues for growth;
- · Stay healthy and heal themselves;
- · Prevent and fight infections.
- Foods are classified according to their functions in the body. The functions of food can be broadly classified into three main categories.

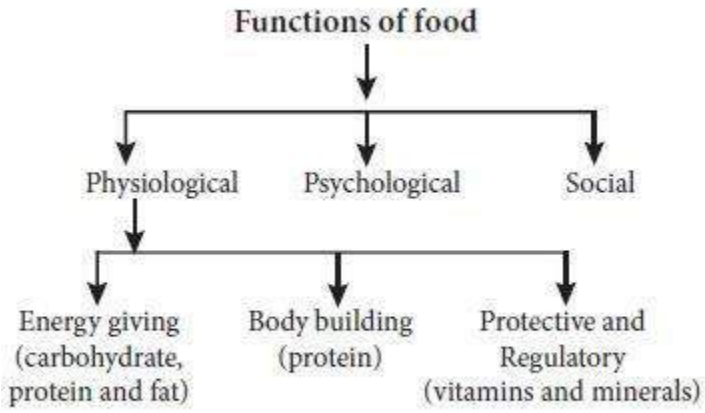


Fig 1.1: Functions of food

-
-
- **1. Physiological functions of food**
- The physiological functions of food can be further sub-divided as follows:
- a. Energy giving
- b. Body building
- c. Protective and Regulatory
-
- **a. Energy giving**
- This group includes foods rich in carbohydrate, fats and proteins. Energy is defined in terms of kilo calories and thus one gram of carbohydrate gives 4 kcal, one gram of protein gives 4 kcal, while one gram of fat gives 9 kcal. This group may be broadly divided into two groups:
- · Cereals, pulses, nuts and oilseeds, roots and tubers.
- · Simple carbohydrates like sugars, fats and oils.

- Cereals provide in addition to energy large amounts of protein, minerals and vitamins in the diet. Pulses also give protein and B vitamins besides giving energy to the body. Nuts and oilseeds are rich in energy yielding as they are good sources of fats and proteins. Roots and tubers though mainly provides energy, contribute to some extent to minerals and vitamins.
-
- **b. Body Building:**
- The foods we eat become us. Thus one of the most important functions of food is that of building the body. They are classified into two groups:
- · **Milk, egg, meat and fish:** They are rich in protein of high biological value. These proteins have all the essential amino acids in correct proportion for the synthesis of body tissues.
- · **Pulses, oilseeds and nuts:** They are rich in protein but may not contain all the essential amino acids required by the human body.
-
- **c. Protective and Regulatory function**
- Foods rich in protein, vitamins and minerals have regulatory functions in the body eg. maintaining the heart beat, water balance and body temperature. Protective foods are broadly classified into two groups.
- · Foods rich in vitamins, minerals and proteins of high biological value eg. milk, egg, fish and liver.
- · Foods rich in certain vitamins and minerals only eg. green leafy vegetables and fruits.
-
- **2. Psychological Functions of food**
- The second major function of food is the psychological function. Food must also satisfy certain emotional needs. These include sense of security, love and attention. Everyone of us belong to a particular culture with its own unique food habits characteristics of that culture and caste.
-

3. Social function of Food

Food and eating has significant social meaning. Food is also a symbol of our social life Sharing food with any other person implies social acceptance. When you share a meal with someone, you are expressing your acceptance of friendship and respect for that person. Food is a medium through which we express our happiness. For example, feasts are given at specific stages of life, such as birth, birthday, marriage etc.

Overnutrition and Undernutrition - Signs, Symptoms, Diagnosis, and Treatment

Introduction

Malnutrition in all forms includes undernutrition (wasting, stunting, and being underweight), insufficient vitamins and minerals, being overweight or obese, and developing diet-related noncommunicable diseases. Around 462 million adults worldwide were underweight in 2014, while 1.9 billion were overweight or obese. In 2020, it was estimated that 149 million children under five years were stunted (too short for their age), 45 million were wasted (too thin for their height), and 38.9 million were obese. Undernutrition is responsible for approximately 45 percent of deaths among

children under five. These are most common in low and middle-income countries. At the same time, childhood overweight and obesity rates are rising in these countries. The global burden of malnutrition has serious and long-term developmental, economic, social, and medical consequences for individuals and their families, communities, and countries.

What Is Undernutrition?

When most people think of malnutrition, they visualize undernutrition. Undernutrition is a nutrient deficiency. An individual may be malnourished if a balanced diet is not taken or the body has difficulty absorbing enough nutrients. Undernutrition can cause visible fat and muscle wasting, but it can also be invisible. Therefore, one can be both overweight and malnourished. Undernutrition, like overnutrition, is classified into energy (calories) and micronutrients.

- **Energy Undernutrition:** Most people associate the term "malnutrition" with energy undernutrition, also known as protein-energy malnutrition (PEM). Energy deficiency is more common in developing countries. Protein-energy malnutrition can occur in children who are malnourished and lose weight. They may also struggle in school and with learning. Pregnant women with PEM are more likely to have underweight babies. Certain diseases, such as certain types of cancer, can also result in malnutrition. PEM comes in two varieties:

    - **Starvation (Marasmus):** Marasmus is a type of malnutrition caused by a lack of total energy, which results in infertility, poor growth, and could even be fatal. The body breaks down its tissues to survive, resulting in an emaciated appearance.

    - **Protein Deficiency (Kwashiorkor):** Even if there is some carbohydrate or fat in the diet, a lack of protein can cause PEM. This is referred to as kwashiorkor. Kwashiorkor patients have thin arms and legs as well as bloated abdomens.

- **Micronutrient Undernutrition:** Micronutrient malnutrition is a lack of one or more vitamins or minerals. When the diet is out of balance, vitamin or mineral deficiency occurs, and it can occur whether or not calorie intake is adequate. Iron and calcium are frequently deficient in the average diet. Iron deficiency affects up to 25 percent of the world's population, particularly young children, women, and pregnant women. In addition, according to research, teenagers and older adults do not get enough calcium from their diet.

Nutrient deficiency can be caused by a chronic health condition such as pernicious anemia (which causes a lack of vitamin B12), Crohn's disease, celiac disease, or infection in some cases. Symptoms do not usually appear immediately but rather develop over time. Malabsorption occurs when the digestive system cannot break down nutrients for proper absorption. This can result in micronutrient deficiency. Malabsorption can occasionally be treated with dietary changes but may also require medical treatment.

What Is Overnutrition?

Overnutrition occurs when one consumes more nutrients than the body requires daily. While many people associate malnutrition with a lack of nutrients, overconsumption is also classified as malnutrition due to the negative health consequences.

It is of two types:

- **Energy Overnutrition:** Unless there is an increase in physical activity, eating too many calories (or energy) will cause weight gain over time. It makes no difference whether those extra calories come from macronutrients (carbohydrates, fat, or protein) because the body stores whatever it does not need as fat. In developed countries, energy overnutrition is common. People suffering from this type of overnutrition may also suffer from micronutrient undernutrition if their foods are high in calories but low in micronutrients.

- **Micronutrient Overnutrition:** It occurs when an excessive amount of a specific nutrient is consumed. Most vitamins and minerals can be consumed in excess. This usually occurs when extremely high doses of dietary supplements are taken. It is uncommon to consume an excessive amount of any micronutrient from food. Micronutrient overnutrition, such as taking too many iron pills at once, can result in acute poisoning. It can also become chronic if large amounts of a specific vitamin (such as vitamin B6) are taken over weeks or months.

What Are the Signs and Symptoms of Overnutrition and Undernutrition?

Undernutrition can manifest as:

Low body weight, prominent bones, and fat and muscle depletion. Thin arms and legs, with edema (fluid swelling) in the belly and face.
Children's growth and intellectual development are stunted.
Weakness, dizziness, and fatigue. Irritability, apathy, or inattention.
Rashes and lesions, and inelastic skin.
Brittle hair, hair loss, and pigment loss in the hair. Frequent and severe infections.
Low body temperature, inability to warm up. Low blood pressure and heart rate.
Overnutrition can manifest as: Obesity.
High blood pressure. Insulin resistance.
Cardiovascular disease.

How Is It Diagnosed?

A physician can detect malnutrition based on a patient's overall appearance, behavior, body fat distribution, and organ function. Patients may be asked to keep a food diary for a set period. X-rays detect bone density, gastrointestinal problems, and heart and lung damage. Blood and urine tests are used to assess the patient's vitamin, mineral, and waste product levels.

How Is It Treated?

Nutritional supplements are used to treat undernutrition. This could include individual micronutrients or refeed with a custom, highly nutritious formula to replenish everything the body lacks. Severe undernutrition may require weeks of refeeding to correct. However, refeeding can be hazardous, particularly in the first few days. To adapt to undernutrition, the body undergoes numerous changes. Refeeding forces it to revert to its previous mode of operation, which is sometimes more than it can bear. To avoid and manage the complications of refeeding syndrome, which can be serious and even life-threatening, it is best to begin refeeding under close medical supervision.

Overnutrition is typically treated with weight loss, dietary, and lifestyle modifications. Losing excess weight can reduce the likelihood of developing secondary conditions like diabetes and heart disease. Diet and exercise plans, medications, and medical procedures may all be used in weight loss treatment. An underlying condition, such as thyroid disease or a mental health disorder, may also necessitate treatment. Depending on the route taken, weight loss can be quick or slow and gradual. However, once they have lost weight, the lifestyle changes they maintain will help them keep it off. This could include long-term support systems like counseling, behavioral therapy, support groups, and nutrition education.

Conclusion

Malnutrition is a worldwide issue. Poverty and a lack of nutrition knowledge are the leading causes in both the developed and developing worlds. A properly-balanced diet is essential for good health. A healthy, wholesome diet can address the majority of causes of malnutrition. Medical help can be taken if one experiences malnutrition symptoms.

What Are Macronutrients and Micronutrients?

These essential elements from fats, protein, carbs, vitamins and minerals help your body function properly

What are macronutrients?

As the main nutrients found in food, macronutrients maintain your body's structure and functioning. You typically need a large amount of macronutrients to keep your body working properly. But don't stress: macronutrients come from proteins, fats and carbohydrates, which give your body energy in the form of calories.

Macros are typically measured in grams (g) and can be a useful way to track what you're consuming.

"Someone might want to count their macronutrients to be sure they're meeting their needs and not overconsuming or under consuming certain nutrients," says Zumpano.

Overall, counting macros is a way to focus on the variety of foods you're eating — and how much of each — instead of counting calories.

Even certain diets like the keto diet and the paleo diet use a macronutrient approach.

Examples of macronutrients

During digestion, foods that tend to fall into one of the three macronutrients are broken down to be used for different functions. Macronutrients include:

- **Carbohydrates.** As the main source of energy, carbs break down into glucose and aid digestion and fullness. Carbs include bread, rice, pasta, grains, fruits, starchy vegetables, beans, milk and yogurt. They provide 4 calories per gram.
- **Fat.** Fats are broken down into fatty acids and glycerol and provide fat-soluble vitamins A, D, E and K. Foods like nuts, seeds, oils, butter, sour cream, mayo and cream cheese provide 9 calories per gram.
- **Protein.** Protein helps build and repair muscle, tissues and organs, as well as aid in hormone regulation. Foods like meat, poultry, fish, eggs, cheese, cottage cheese, plain Greek yogurt and tofu provide 4 calories per gram.
-

- **What are micronutrients?**

Micronutrients consist of vitamins and minerals and are measured in either milligrams (mg), micrograms (mcg) or International Units (IU).

Compared to macronutrients, your body needs a smaller amount of micronutrients for optimal performance. Though micronutrients don't provide energy, they're essential for functions like digestion, hormone production and brain function.

And while it can be beneficial to track your macronutrients, it can be hard to measure and gauge how many micronutrients you consume each day.

Examples of micronutrients

Just like macronutrients, micronutrients can be found in the foods that you eat every day — think fruits and vegetables.

"Most vitamins are water-soluble," says Zumpano. "That means they get flushed out of your system when your body is done using what it needs."

Some vitamins that are examples of micronutrients include:

- **Vitamin B1.** Also known as thiamine, vitamin B1 aids in converting nutrients into energy. Foods include white rice, fortified breakfast cereals and black beans.
- **Vitamin B2.** Also known as riboflavin, this vitamin is good for energy production, cell function and fat metabolism. Foods include instant oats, fat-free yogurt and milk.
- **Vitamin B3.** Also known as niacin, vitamin B3 drives the production of energy from food. Foods include chicken breast, turkey breast, salmon and tuna.

- **Vitamin B5.** Also called pantothenic acid, this vitamin helps with fatty acid synthesis. Foods include shitake mushrooms, sunflower seeds and avocados.
- **Vitamin B6.** Also called pyridoxine, vitamin B6 helps your body release sugar from stored carbohydrates for energy, and creates red blood cells. Foods include chickpeas, tuna and potatoes.
- **Vitamin B7.** Also known as biotin, it aids the metabolism of fatty acids, amino acids and glucose. Foods include eggs, salmon, pork chops and sweet potatoes.
- **Vitamin B9.** Also known as folate. Vitamin B9 is important for proper cell division. Foods include spinach, fortified breakfast cereals, white rice and asparagus.
- **Vitamin B12.** Also called cobalamin, vitamin B12 helps with red blood cell formation and proper nervous system and brain function. Foods include beef liver, salmon, milk and yogurt.
- **Vitamin C.** Also known as ascorbic acid, vitamin C is required for the creation of neurotransmitters and collagen. Foods include red peppers, oranges, grapefruits and kiwis.

Minerals that are good examples of micronutrients include:

- **Calcium.** This mineral helps build strong bones and teeth and helps with muscle function. Foods include yogurt, orange juice, cheese and milk.
- **Magnesium.** Found in foods like pumpkin seeds, almonds and spinach, this mineral aids in the regulation of blood pressure.
- **Sodium.** You need sodium for optimal fluid balance and to maintain your blood pressure.
- **Potassium.** Potassium helps with muscle function and nerve transmission. You can find potassium in foods like apricots, lentils, prunes and raisins.

Unit 2

Regulation of Digestion

The entire digestion process involves six main steps: ingestion, propulsion (swallowing and movement of food through the alimentary canal), mechanical or physical digestion, chemical digestion, absorption, and excretion.

Each step of digestion is under neural and hormonal regulation. The regulation allows communication between different parts of the digestive tract, and it ensures sufficient secretions of enzymes in the presence of food. Regulation helps avoid over or under secretions of digestive juice. Neural and Hormonal are two kinds of digestive regulations.

Neural Control of Digestion

For proper coordination of different parts and the action of the gastrointestinal tract requires neurological control. The GI tract is regulated by two sets of nervous systems: extrinsic and intrinsic.

1. The **intrinsic or enteric nervous system** consists of the Meissner's plexus, located in the submucosa, and the Auerbach's plexus, located in the muscular layer. Most gastrointestinal activities, such as secretion and motility, are controlled by the enteric nervous system.

2. The **extrinsic innervation** of the GI tract comprises parasympathetic and sympathetic nerves. They can alter the activity of the intrinsic nervous system in response to reflex activity originating in the GIT or elsewhere in the body.

Apart from these, the stimulation of the vagus nerve causes saliva production in response to the sight, taste and smell of food.

Hormonal Control of Digestion

Hormonal control is crucial in the digestive process. These hormones are released by specialised epithelial cells, called endocrinocytes, present in the mucosal epithelium of the stomach and small intestine. The digestive hormones are released in the bloodstream, which reaches their target site and acts on the target organ.

These hormones play a crucial role in controlling food intake and energy expenditure. The GI tract is the largest endocrine organ in the human body. These hormones regulate the process of digestion along with appetite and influence the pleasure of eating.The release of hormones is regulated by stimulus and the nervous system. The list of digestive hormones and their functions are given below:

S.No	Hormone	Stimulus	Site	Action
1	Gastrin	Distention of stomach, Presence of Peptide in food	Produced in G-cells of the lining of the stomach.	Stimulates the release of HCl and Pepsin. Stimulates gastric motility.
2	Cholecystokinin (CCK-PZ)	Presence of Peptide and Fatty acid in food	Produced in I cell of the lining of the duodenum	Stimulates secretion of pancreatic juice, Inhibits secretion of gastric juice Increase motility of the colon
3	Secretin	Presence of Acid and peptides in food	Produced in S cells in the duodenum	Stimulate secretion of bile juice, Inhibits secretion of gastric juice, Augments action of CCK
4	GIP	Presence of Peptide, Fatty acid or Glucose in food	Produced in K cell of the intestine	Stimulates secretion of insulin from pancreases.

Prebiotics

Prebiotics are compounds in food that foster growth or activity of beneficial microorganisms such as bacteria and fungi. The most common environment considered is the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome.

Dietary prebiotics are typically nondigestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and help growth or activity of advantageous bacteria in the colon by acting as substrates for them. They were first identified and named by Marcel Roberfroid in 1995. Depending on the jurisdiction, they may have regulatory scrutiny as food additives for the health claims made for marketing purposes. Common prebiotics used in food manufacturing include beta-glucan from oats, resistant starch from grains and beans, and inulin from chicory root.

have been identified. Specifically, fructans and galactans are two oligosaccharide sources which have been found to stimulate the activity and growth of beneficial bacterial colonies in the gut. Fructans are a category of carbohydrate consisting of fructooligosaccharides (FOS) and inulins, while galactans consist of galactooligosaccharides.[3] Resistant starch has been shown to shift the intestinal bacteria, as well as improve biomarkers for numerous health conditions. Other dietary fibers also fit the definition of prebiotics, such as pectin,[14] beta-glucans, and xylooligosaccharides.

The European Food Safety Authority (EFSA), the regulatory agency for product labeling, differentiates between "prebiotic" and "dietary fiber", stating that "a cause and effect relationship has not been established between the consumption of the food constituents which are the subject of the health claims and a beneficial physiological effect related to increasing numbers of gastrointestinal microbiota".Consequently, under EFSA rules individual ingredients cannot be labeled as prebiotics, but only as dietary fiber and with no implication of health benefits.

Function

When the prebiotic concept was first introduced in 1995, the primary focus was on the effects that prebiotics confer on Bifidobacteria and Lactobacillus. With improved mechanistic techniques in recent years, the current prebiotic targets have expanded to a wider range of microbes, including Roseburia spp., Eubacterium spp., Akkermansia spp., Christensenella spp., Propionibacterium spp. and Faecalibacterium spp. These bacteria have been highlighted as key probiotics and beneficial gut bacteria as they may have several beneficial effects on the host in terms of improving digestion (including but not limited to enhancing mineral absorption) and the effectiveness and intrinsic strength of the immune system. Both Bifidobacteria and Lactobacillus have been shown to have differing prebiotic specificity and to selectively ferment prebiotic fiber based on the enzymes characteristic of the bacterial population. Thus, Lactobacilli prefer inulin and fructooligosaccharides, while Bifidobacteria display specificity for inulin, fructooligosaccharides, xylooligosaccharides and galactooligosaccharides. Studies have also shown that prebiotics, besides helping growth of beneficial gut bacteria, can also inhibit detrimental and potentially pathogenic microbes in the gut, such as clostridia.

Mechanism of action

Fermentation is the main mechanism of action by which prebiotics are used by beneficial bacteria in the colon. Both Bifidobacteria and Lactobacillus are bacterial populations which use saccharolytic metabolism to break down substrates. The bifidobacterial genome contains many genes that encode for carbohydrate-modifying enzymes as well as genes that encode for carbohydrate uptake proteins. The presence of these genes indicates that Bifidobacteria contain specific metabolic pathways specialized for the fermentation and metabolism of plant-derived oligosaccharides, or prebiotics. These pathways in Bifidobacteria ultimately produce short chain fatty acids, which have diverse physiological roles in body functions.

Sources

Prebiotic sources must be proven to confer a benefit to the host in order to be classified as a prebiotic. Fermentable carbohydrates derived from fructans and xylans are one well documented example of prebiotics. Resistant starch from starchy foods are also well documented prebiotics and have historically been the highest source of prebiotics in the diet, as 4-10% of starch in mixed diets has been shown to reach the large intestine. One study reported that individuals consuming a traditional diet in Africa consumed 38 grams of resistant starch/day.

GENERAL CHARACTERS AND CLASSIFICATION

7. Describe the General charocters of Protozoa and Classify it to the order level.

IMPORTANT POINTS

* Leewenhouk – first observed Protozoans.
* Von Siebold – Protozoans are unicellular.
* Gold fuss proposed the name Protozoa.
* Locomotor organelles are
  i) Pseudopodia
  ii) Flagella
  iii) Cilia
* Holozoic, Holophytic, Saprozoic nutrition common
* Binary fission, Multiple fission, conjugation etc, Reproductive methods.

Introduction :

Leeuwenhock first described the protozoan animals under a microscope he called them as animalcules. In 1871 Godfuss gave the name protozoa. The term protozoa means primitive animals. Protozoans are present from precambrian period to recent times 40,000 species are included in this Phylum.

General Characters :

1. All protozoans are unicellular, hyman called them as acellular.
2. They will show radial symmetry in some organisms, bilateral kymmetry in some organisms. Some organisms are irregular.

3. Each animal is covered by cell membrane which contain proteins, and lipids.
4. Some animals may be covered by pellicle. Some animals covered by shell Ex : Elphidium.
5. In fresh water animals contractile vacuole is present. It is useful for osmoregulation. In marine and parasitic protozoans contractile vacuole is absent. Food vacuoles will work as temperory stomach. These protozoans will show locomotary organells like flagella, pseudopodia, cilia etc., some are parasites.
6. Nutrition in protozoans is by holozoic, holophytic, saprophytic and parasitic etc.
7. Respiration is by diffusion.
8. Exerction is by diffusion.
9. Asexual reproduction is by binary fission, multiple fission, and budding etc.
10. Sexual reproduction is by syngamy, conjugation.
11. During unfavourable conditions the protozoans will develop cyst around themselves.

CLASSIFICATION :

Introduction :-

Protoza is classified by many but the classification of Protozoa is complicated and difficult. The classification followed here is based on Hyman's classification.

Phylum Proto zoa is divided into two subphyla :

Subphylum I : Plasmodroma :-

The locomtory organs are pseudopodia and flagella. Nucleus is single or many but all are one kind. Aexual reproduction is by binary and multiple fissions. Sexual reproduction by complete fusion of gametes The subphylum is classified into three classes and the classification is based on the locomotor organelles.

Class-I : Mastigophora :-

Locomotory organ is flagellum. Body is covered by pellicle or cuticle. Asexual reproduction is by longitudinal binary fission. The class Mastigophora is distinguished into 10 orders.

Order-I : Chrysomonadina :-

The mouth and gullet are absent. Flagella one or two Chromatophores either one or two,and the body is in thin pellicle.

Ex :- Chrysamoeba

Order-II : Cryptomonadina :-

The gullet is present and reaches upto the middle of body, Flagella one or two,chromatophores two,or absent pellicle is firm.

Ex. Cryptamonas

Order–III : Dinoflagellata :-

Two flagella almost of equal size. Chromatophores green, brown or yellow. Body enclosed in a thick and rigid shell of cellulose. A few forms are luminescent.

Ex :- Noctiluca.

Order IV : Chloromonadina :-

Gullet present but transverse groove absent. Flagella two Chromatophores numerous, body covering is think cuticle and usually amoeboid.

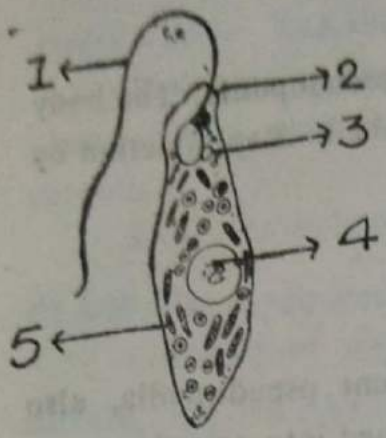
Ex :- Coelomonas.

Order–V : Euglenoidea :-

Cytostome and cytopharynx are present, Flagellum single. Chromatophores are numerous or absent.

Ex :- Euglena.

Euglena



1) *Flagellum*
2) *Cytostome*
3) *Contractile vacuole*
4) *Nucleus*
5) *Chromatophore.*

Order - VI : Phytomonadina :-

Gullet absent Flagella one, two, four or rarely more, body enclosed in the rigid cellulose covering, Chromatophores green or brown.

Ex :- Chlamydomonas & Volvox.

Order - VII : Protomonadian :-

Flagella one, two or rarely more, Mouth absent and nutrition is holozoic in free living and saprozoic or saprophytic in parasitic forms.

Ex :- Trypanosoma & Leishmania.

Order - VIII : Polymastigina :-

Flagella 3 to 8 or more, Mouth present Harmless intestinal paraites.

Ex :- Trichomonas, Giardia.

Order - IX : Hypermastigina :-

Numerous flagella, aranged in one or more tufts, Mouth absent food ingestion by pseudopodia Symbionts in inset and parasites in the intestine.

Ex :- Lophomonas, Trichonympha.

Order - X : Rhizomastigina (or) Pantostomatida :-

Locomotory organs both flagella and pseudopodia.

Ex :- Mastigamoeba.

CLASS - II : Rhizopodia :-

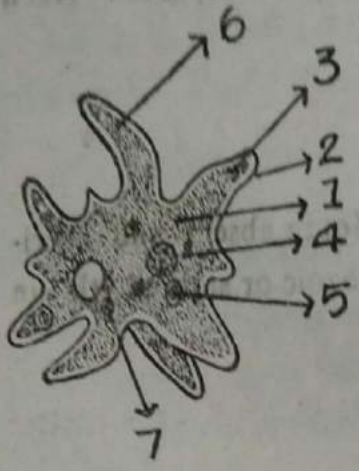
In Rhizopodia ingestion of food by pseudopodia. The body either naked or with internal or external shell. Reproduction by binary fission.

It is divided into,

Order - I : Lobosa or Amoebozoa :-

Locomotory organs are short and blunt pseudopodia, also known as lobopodia, Cytoplasm distinguished into ectoplasm and endoplasm.

Ex :- Amoeba.



Amoeba

1) *Endoplasm*
2) *Plasmalemma*
3) *Pseudopodium*
4) *Nucleus*
5) *Food vacuole*
6) *Ectoplasm*
7) *Contractive vacuole*

Order - II : Foraminifera :-

Pseudopodia are fine and branched and from a network, Ectoplasm vacuolated and body enclosed in a chambered shell.

Ex :- Polystomella.

Order - III : Heliozoa :-

Pseudopodia fine, stiff and raylike being supported by an axial filament these are also known as axopodia.

Cytoplasm is distinguished into outer vacuolated cortex and inner dense medulla, fresh water forms.

Ex :- Actinosphaerium.

Order - IV :- Radiolaria :-

Pseudopodia are axopodia but lack axial filament, Ectoplasm highly vacuolated and endoplasm encloses a central capsule.

Ex :- Actinomma.

CLASS : III :- Sporozoa :-

Locomotory organs are absent, Nutrition through general surface, Asexual reproduction by multiple fission; parasitic protozoans. This class is distinguished into 4 subclasses.

Subclass I & Telosporidia :-

Trophozoite with single nucleus spore case devoid of polar capsules and filaments and contains numorous spores.

Order - I :- Gregarinida :-

Trophozoite is extracellular, free and gametocytes are is ogamous or anisogamous.

Ex :- Monocystis.

Order - II :- Coccidia :-

Trophozoite is intracellular and gametogony is anisogamy. Zygote is non-motile.

Eg : Eimeria.

Order - III :- Haemosporidia :-

Trophozoite intracellular, small amoeboid and zygote is motile. Sporozoites naked, gametogony anisogamous.

Subclass II : Conidosporidia :-

Trophozoites multinucleate, spore cases complex and a number of spores are present, which are large in size.

Order - I : Myosporidia :-

Large spores with a bivalved membrane and 1, 2 or 4 polar capsules.

Ex :- Myxidium.

Order - II :- Actinomyxidia :-
Spores large with a trivalved shell with three polar capsules.

Ex :- Triactinomyon.

Order : III :- Microsporidia :-
Spores small with single shell having single polar capsule

Ex :- Nosema.

Subclass - III : Sarcosporidia :-
Tropozoites multinucleate, Spore cases simple, without polar capsules.

Order - I :- Sarcosporidia :-
Muscle parasites of higher vertebrates, and spores are without spore cases.

Ex :- Sarcocystis.

Order - 2 :- Globidier :-
Spore case is formed from host.

Ex :- Globidium.

Subclass - 4 :- Haplosporidia :-
Muscle parasites of invertebrates and spores with spore cases.

Ex :- Haplosporidium.

Subphylum - II :- Ciliophora or (Infusoria) :-
The locomotory organs are cilia. There are 2 types of nuclei, mega and micronuclens. Sexual reproduction by conjugation, Asexual reproduction by binary fission and by budding.

Class - I : Ciliata :- Cilia persist throughout life.

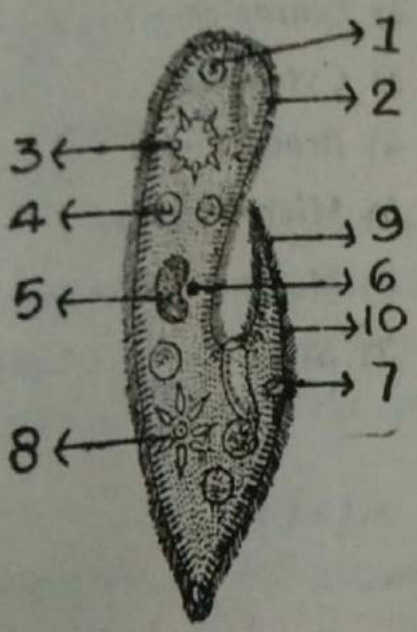
Sub class I :- Protociliata :- Cilia are all equal and uniform, cytostome absent 2 nuclei are present, reproduction sexually by syngamy.

Ex :- Opalina.

Subclass - 2 :- Euciliata :- Cilia of different sizes and cytostome usually present. 2 nuclei and sexual reproduction by conjugation.

Order - I :- Holotricha :- Ciliation of body uniform.

Eg :- Paramecium.



Paramecium

1) *Food Vacuole*
2) *Pellicle*
3) *Contractile Vacuole*
4) *Trichocyst*
5) *Meganucleus*
6) *Micronucleus*
7) *Cytopyge*
8) *Radiating canal*
9) *Oral groove*
10) *cilium.*

Order - II :- Spirotricha :- Ciliation is not uniform, adoral zone of membranelle are present around cytostome.

Ex :- Balantidium.

Order - III :- Peritricha :- Ciliation reduced to one or more girdles, gullet with undulating membrane.

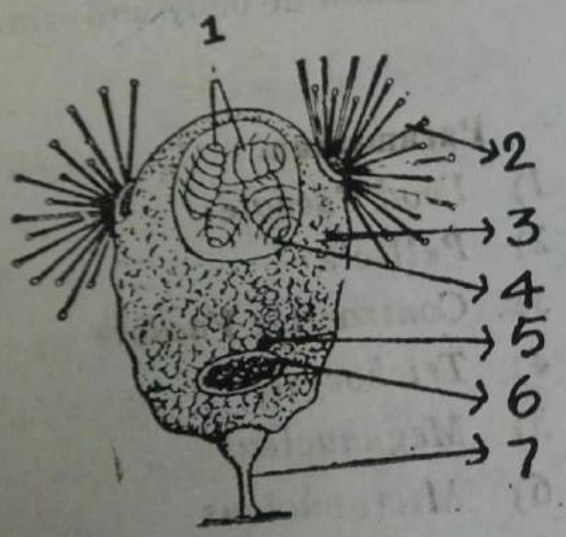
Eg : Vorticella.

Order - IV :- Chonotricha :- Adoral zone of membranellae arranged clock wise.

Ex :- Spirochona.

Class: II :- Suctoria :- Adults sedentary and without cilia which are present only in young stages.

Ex :- Epelota and Acineta.



Acineta

1) *Buds*
2) *Tentacles*
3) *Cytoplasm*
4) *Brood Pouch*
5) *Micronucleus*
6) *Meganucleus*
7) *Stalk*

TSWRAFPDCW – BHONGIR TEACHING MODULE
CLASS: BZC/ MZC (BSC) DEPARTMENT: ZOOLOGY
Year ll (lV sem); Paper: V (Physiology and Biochemistry)

Topic: GLYCOLYSIS

Lecturer Name: E. JYOTHI

1. **No. of Teaching Hours: 2**
2. **Objectives of the Topic:**

- Student can able to understand the concept of glycolysis
- Understand the overall idea of Glycolysis path way
- Understand the Importance of Glycolysis
- Student can identify the differences between Glycolysis and Krebs cycle

PRE-TEST:

- **Understanding the student's basic knowledge by asking orally on the following**

i.Tell me how the energy will be produced?

ii.What is the importance of Glycolysis?

iii.What is the structure of the Glucose?

iv.Where the Glycolysis occur?

v. Definition of glycolysis?

3. **Module Content:**

GLYCOLYSIS: DEFINITION:

Glycolysis is the central pathway for the glucose catabolism in which glucose (6-carbon compound) is converted into pyruvate (3-carbon compound) through a sequence of 10 steps.

- Glycolysis takes place in both aerobic and anaerobic organisms and is the first step towards the metabolism of glucose.
- The glycolytic sequence of reactions differs from one species to the other in the mechanism of its regulation and the subsequent metabolic fate of the pyruvate formed at the end of the process.

- In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain, which together release most of the energy contained in glucose.
- It is also referred to as Embden-Meyerhof-Parnas or EMP pathway, in honor of the pioneer workers in the field.

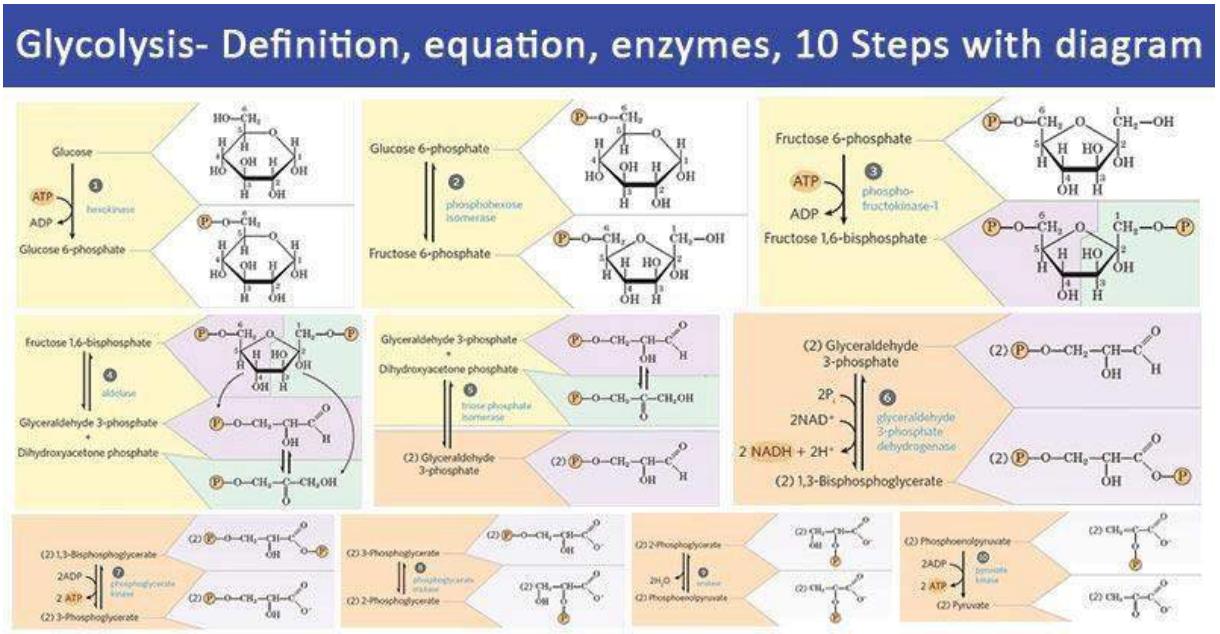


Image Source: Lehninger Principles of Biochemistry.

Glycolysis equation

A summary of the process of glycolysis cab be written as follows:

$C_6H_{12}O_6 + 2ADP + 2Pi + 2NAD^+ \rightarrow 2C_3H_4O_3 + 2H_2O + 2ATP + 2NADH + 2H^+$

In words, the equation is written as:

Glucose + Adenosine diphosphate + Phosphate + Nicotinamide adenine dinucleotide

↓

Pyruvate + Water + Adenosine triphosphate + Nicotinamide adenine dinucleotide + Hydrogen ions

Glycolysis enzymes

In most kinds of cells, the enzymes that catalyze glycolytic reactions are present in the extra-mitochondrial fraction of the cell in the cytosol. One common characteristic in all the enzymes involved in glycolysis is that nearly all of them require Mg^{2+}. The following are the enzymes that catalyze different steps throughout the process of glycolysis:

1. Hexokinase
2. Phosphoglucoisomerase
3. Phosphofructokinase
4. Aldolase
5. Phosphotriose isomerase
6. Glyceraldehyde 3-phosphate dehydrogenase
7. Phosphoglycerate kinase
8. Phosphoglycerate mutase
9. Enolase
10. Pyruvate kinase

Glycolysis steps

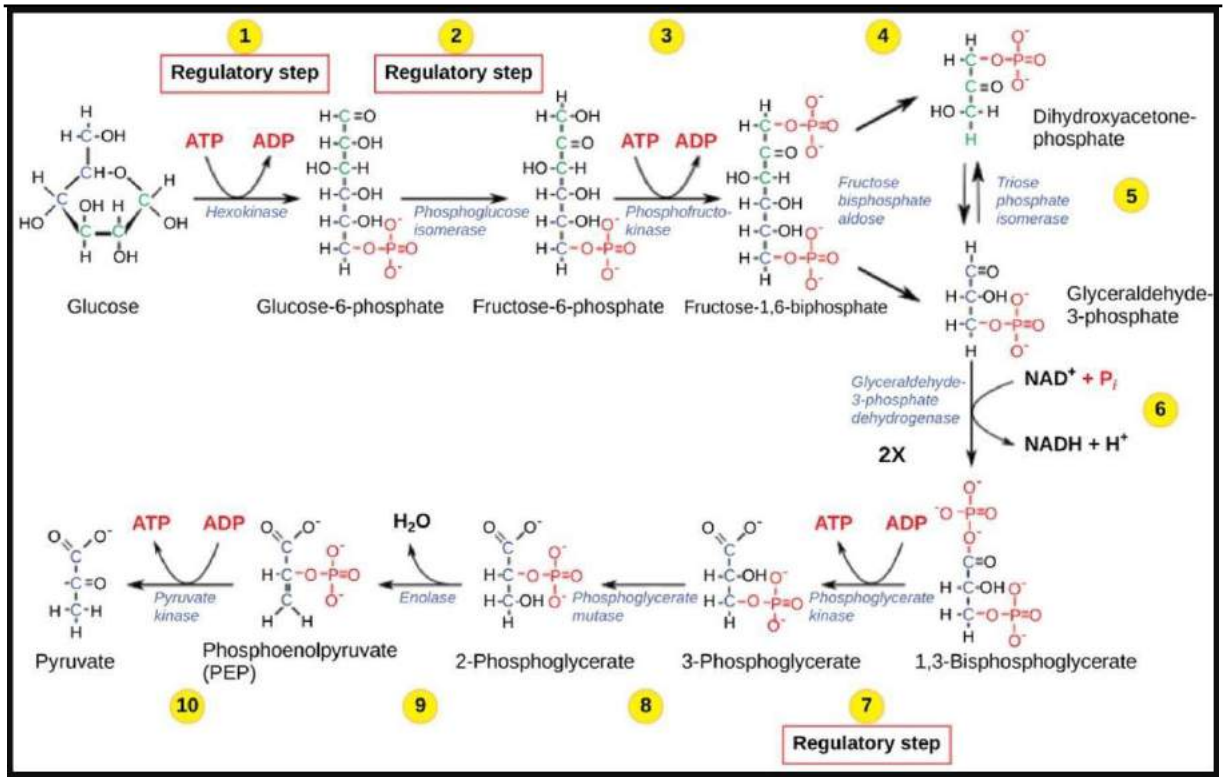


Figure: Glycolysis 10 steps. Image Source: Quizlet Inc.

•During glycolysis, a single mole of 6-carbon glucose is broken down into two moles of 3-carbon pyruvate by a sequence of 10 enzyme-catalyzed sequential reactions.

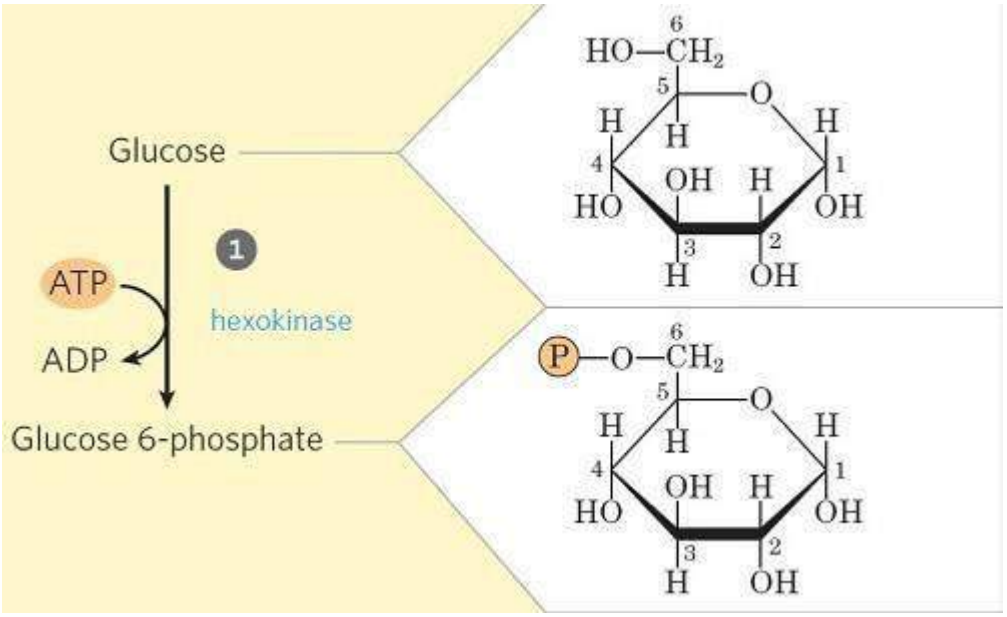
These reactions are grouped under 2 phases, phase I and II.

•Stage I comprises "preparatory" reactions which are not redox reactions and do not release energy but instead lead to the production of a critical intermediate of the pathway.

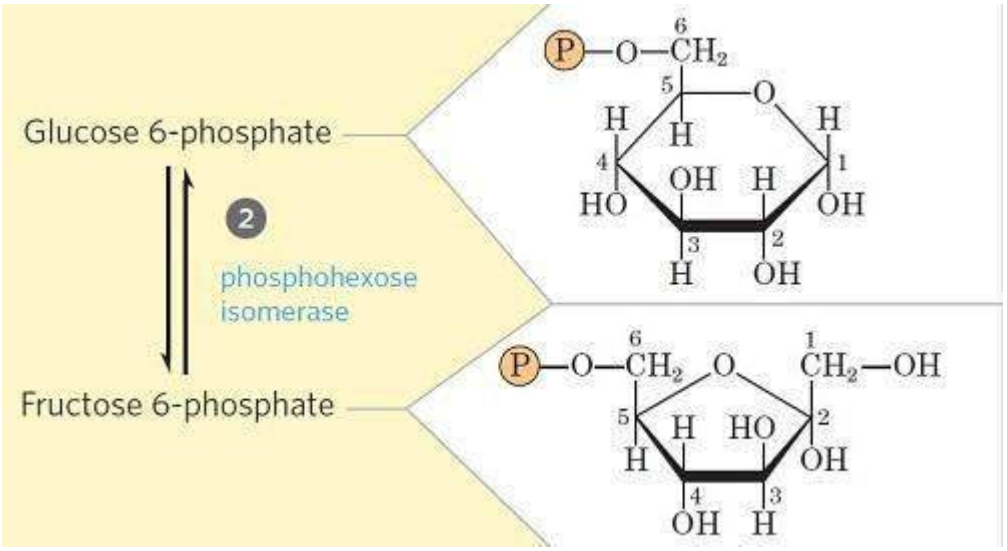
- Stage I consists of the first five steps of the glycolysis process.
- Similarly, in Stage II, redox reactions occur, energy is conserved in the form of ATP, and two molecules of pyruvate are formed.
- The last five reactions of glycolysis constitute phase II.

The ten steps of glycolysis occur in the following sequence:

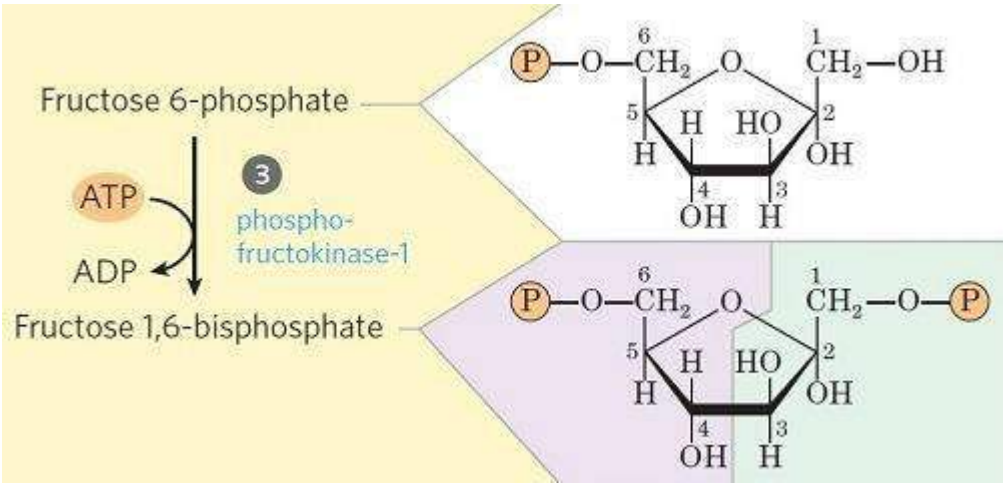
Step 1- Phosphorylation of glucose



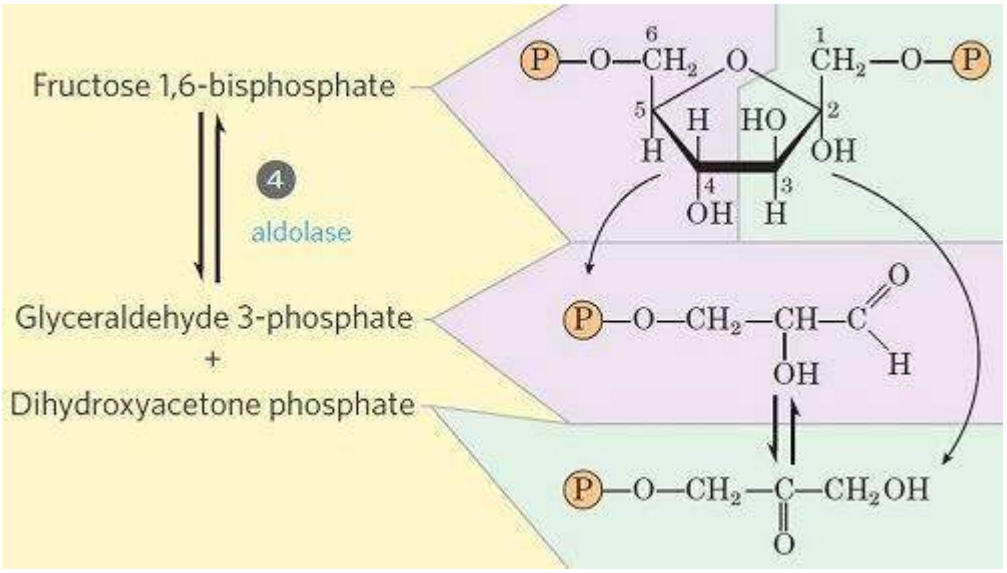
Step 2- Isomerization of Glucose-6-phosphate



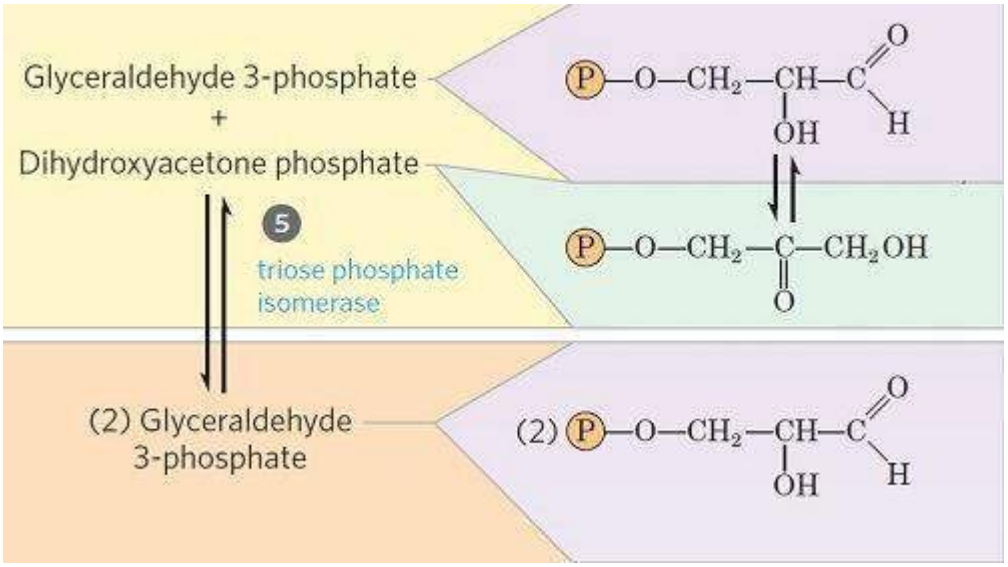
Step 3- Phosphorylation of fructose-6-phosphate



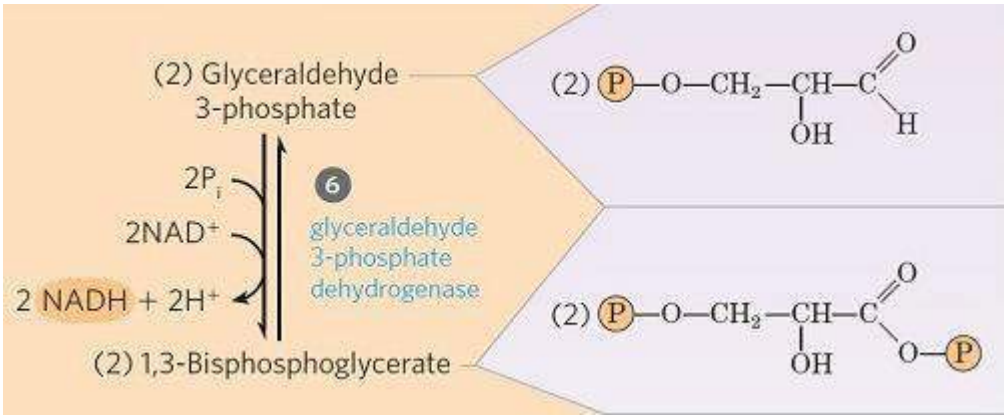
Step 4- Cleavage of fructose 1, 6-diphosphate



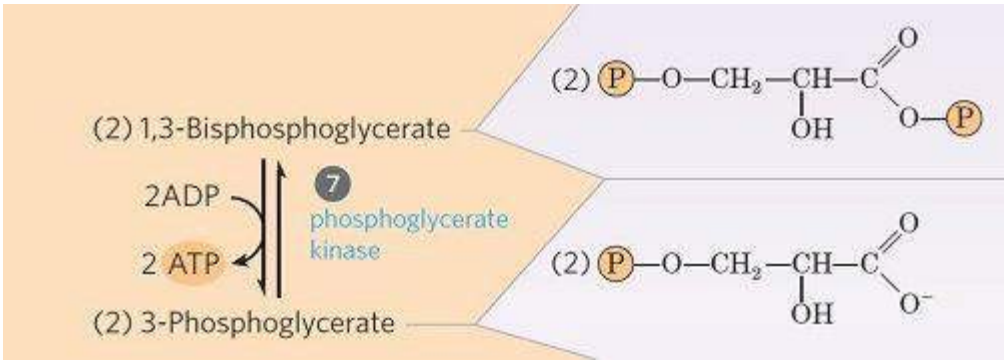
Step 5- Isomerization of dihydroxyacetone phosphate



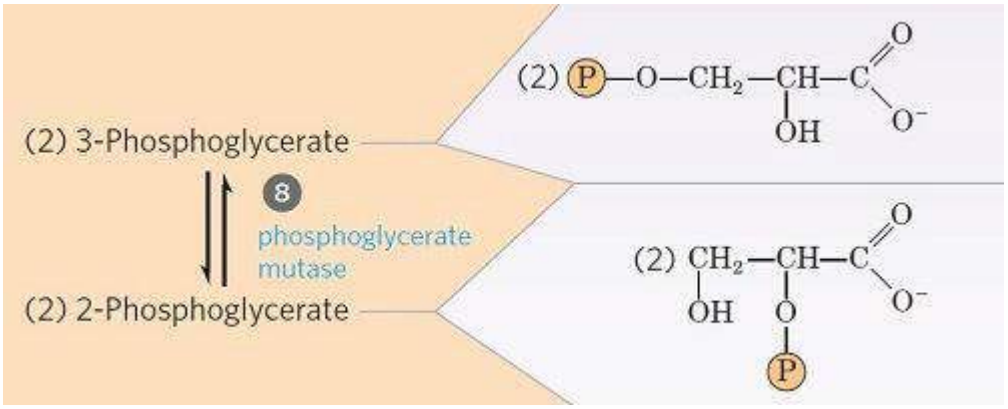
Step 6- Oxidative Phosphorylation of Glyceraldehyde 3-phosphate



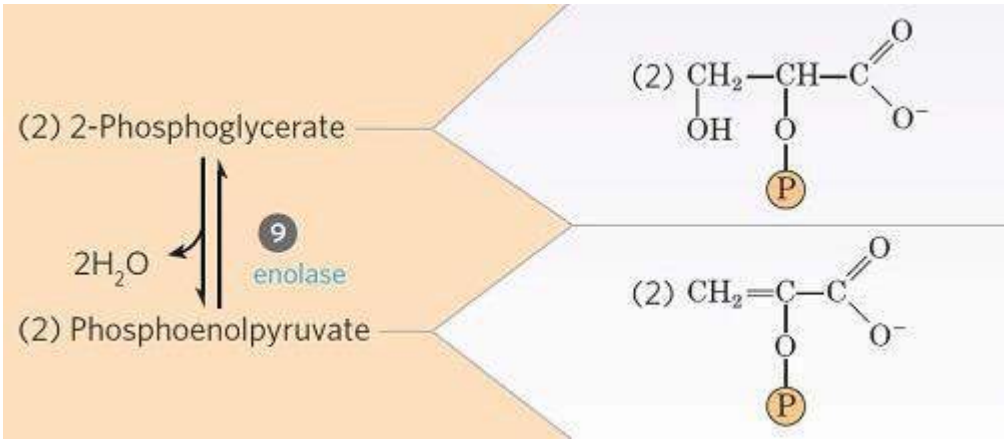
Step 7- Transfer of phosphate from 1, 3-diphosphoglycerate to ADP



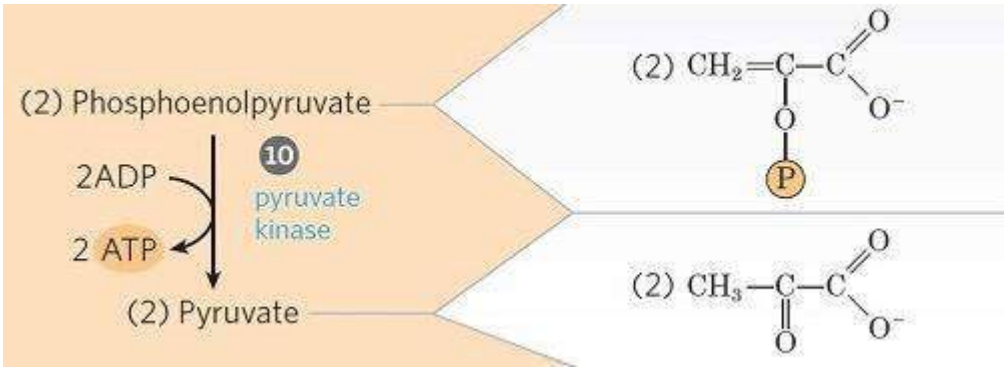
Step 8- Isomerization of 3-phosphoglycerate



Step 9- Dehydration 2-phosphoglycerate



Step 10- Transfer of phosphate from phosphoenolpyruvate



4. **<u>Student Activities:</u>**
   - Group discussion on cycle of Glycolysis
   - Seminars by Students
   - Quiz will be conducted to the students
   - Preparing the model of glycolysis pathway

5. Assessment:

- Assessing the Student after completion of the Topic by conducting Oral Test or written test.

  i. Explain the pathway of glycolysis?
  ii. Draw the cycle?
  iii. Steps involved in cycle?
  iv. Write the importance of the Glycolysis?

6. Reference Books:

- Human physiology - Dr.N. Arumugam -SARAS PUBLICATION
- Human physiology - Jaypee brother’s medical publishers
- Cell biology - C. Gopal & Dr. Kondaiah & David H Kaye

B.Sc III Year V Semester
Paper V:
Animal Physiology & Biochemistry

" Homeostasis "

By

Dr. K. Srilatha

DL in Zoology

TSWRAFPDCW, Bhongir


“ Homeostasis ”
WASH
YOUR HANDS

Concept of Homeostasis

- According to **Claude Bernard**, Homeostasis is defined as "***The constancy of the internal environment is the essential condition of free life***".
- Homeo = same; stasis = standing

- Homeostasis is a **self-regulating dynamic process by which the living organisms maintain a constant internal milieu**.

Concept of Homeostasis

- If the steady state is maintained, the life is sustained, otherwise, if it is disturbed, it results in death.
- Growth and development of organisms depend on the balanced state.
- **Walter Cannon**(American physiologist) coined the term Homeostasis in 1932.

Examples of Homeostasis

- Eg1: **Aves and Mammals** (endotherms) maintain **constant body temperature** in a continuously changing external environment.
- Eg2: Maintenance of constant levels of **water and salt concentrations** in variable osmotic environments.
- Eg3: Our blood maintains **constant levels of glucose**
- Eg4**:** Our blood maintains **constant levels of ions (Ca2^{+}, PO4^{-})**.

Examples of Homeostasis

- Eg5: Our blood maintains **constant levels of** pH.
- Eg6: Our blood maintains **constant levels of** Urea.
- Eg7: Maintenance of constant blood volume, blood pressure, heart rate.
- Eg8: Maintenance of constant O_2 & CO_2 levels.

- In order to achieve stable internal conditions, the activities of organisms must be regulated at all levels of organization (molecular, cellular, tissue, organ, system and organism).

Mechanism of Homeostasis

- When any system is disturbed, **in-built regulatory devices** respond to the changes to establish new balance. Such a process is called **feedback control**.
- The homeostatic control mechanism of living organisms include three components-the **detectors**, **regulators** (**coordinators**) and **effectors**.

Mechanism of Homeostasis

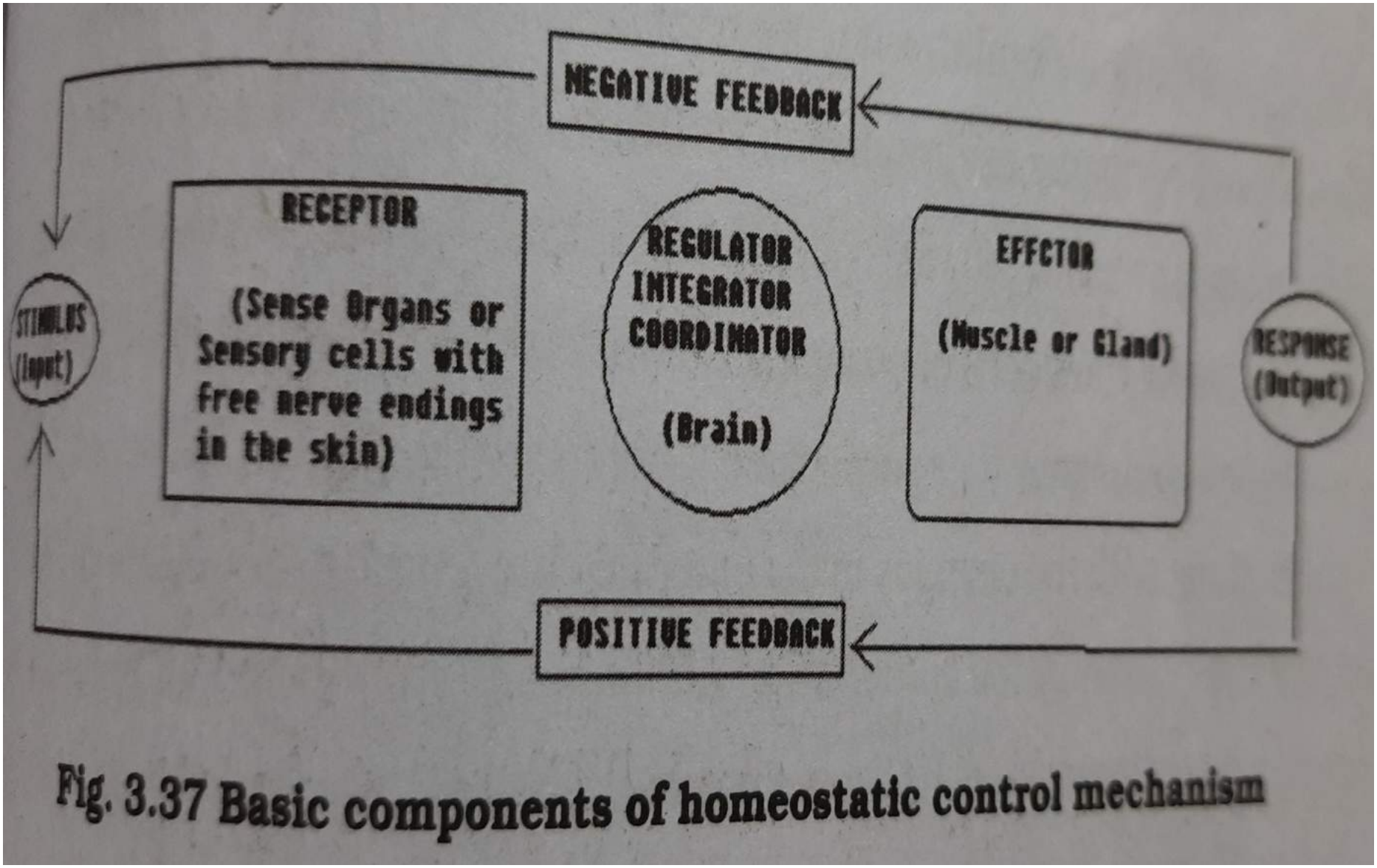


Fig. 3.37 Basic components of homeostatic control mechanism

Mechanism of Homeostasis

- The **detectors** include **sense organs**, **receptors** etc., that help in detecting the changes in the environment.

- **CNS** acts as the **regulator**. It **receives inputs** from the detectors and **gives instructions** to the **effectors** (**muscles or endocrine glands**) to show responses by their **actions** or **secretions**.

Mechanism of Homeostasis-Positive and Negative Feedback loops

- The **responses** are **automatic** and involve **two feedback loops**.
- In a feedback mechanism, the **output** of a process **alters** (**Inhibits** or **activates**) the **course** of events(feedback may be +ve or -ve).
- Eg1: **Negative feedback** is analogy of **thermostat** and **air conditioner**.
- Eg2: Availability of thyroxine in the blood inhibits further production of thyroxine by the thyroid.

Mechanism of Homeostasis-Positive and Negative Feedback loops

- In a **positive feedback loop**, the product further activates the stimulus and evokes same response again and again.
- Eg1: Release of **Oxytocin** from neurohypophysis causes **contraction** of **uterine muscles** and **labour pains**. The labour pains in turn will enhance the secretion of oxytocin.

Hormonal control of Blood Glucose level

- The control of blood glucose level is an important example of homeostasis in the body.
- **Glucose** is the **main source of energy** for organisms.
- In **mammals**, some **tissues** (human **brain(**120 g/day**)**, **rbcs**, **testes**, **renal medulla** & **embryonic tissues**) depend **exclusively on glucose** for their energy needs.

Hormonal control of Blood Glucose level

- After vigorous exercise, blood glucose is depleted.
- **Brain cannot work properly** under **low** blood glucose levels (headache, sweating, trembling, fainting & coma).
- Under **normal** conditions, blood maintains about **80 to 120 mg of glucose / 100 ml**.

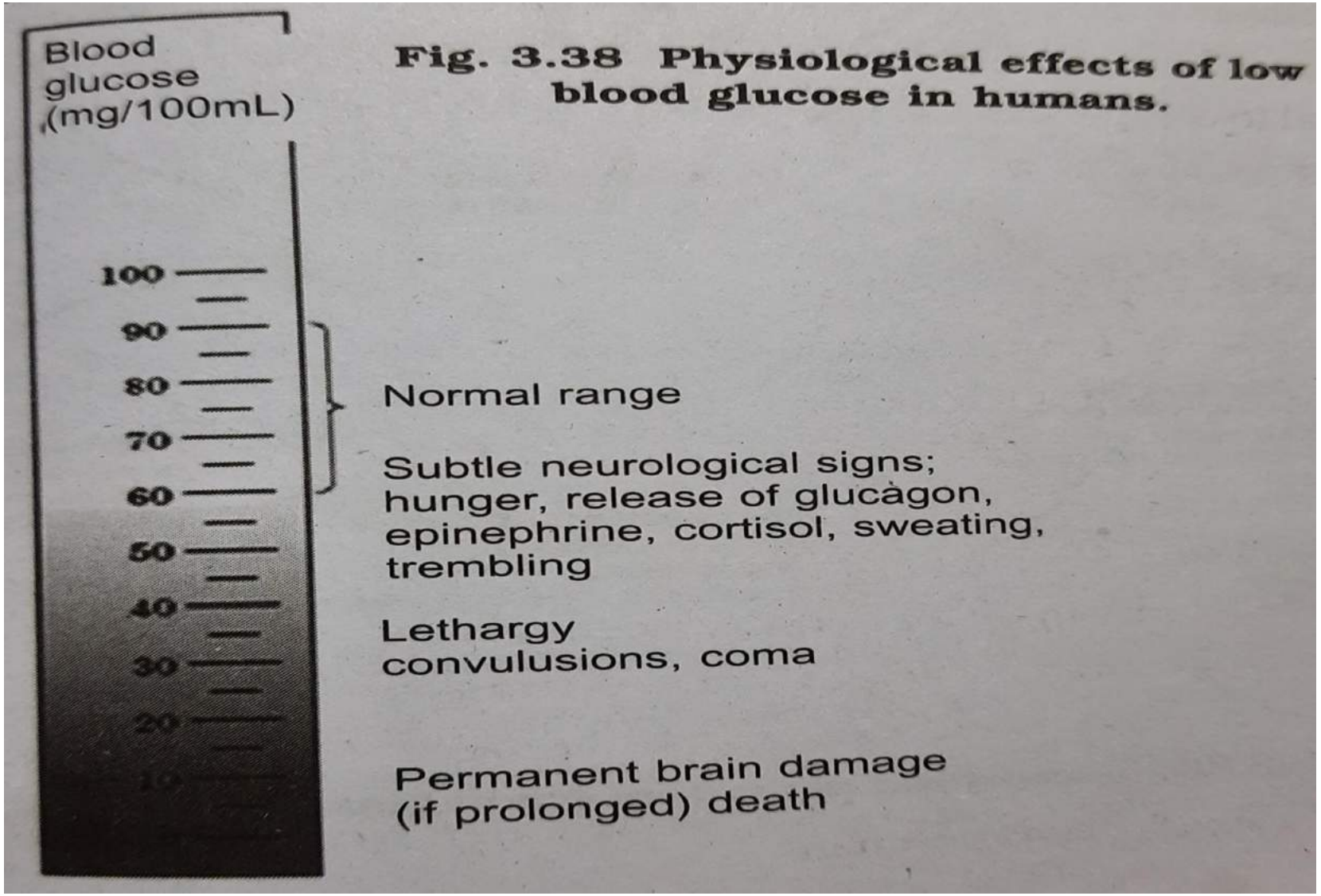


Fig. 3.38 Physiological effects of low blood glucose in humans.

Hormonal control of Blood Glucose level

- An **increase** in glucose level from this set point is called **hyperglycemia(>120 mg%)**.
- It results in **diabetes mellitus** in which excess sugar is eliminated in urine **(glycosuria**).

- A **decrease** in glucose level from this set point is known as **hypoglycemia(<80 mg%)**.

Hormonal control of Blood Glucose level

- When **glucose** level is **high**, **Insulin** (β cells of Pancreas) and **Thyroxine** (Thyroid gland) are released to decrease the glucose to set-point.
- When glucose level is **low**, **Glucagon(**α cells of Pancreas), **Cortisol** (adrenal cortex), **Epinephrine** (adrenal medulla) are released to increase the glucose to set-point.

Hormonal control of Blood Glucose level

- The homeostatic control of blood glucose level involves **two negative feedback inhibitions**.
- The first one is **glucose** (High glucose switch on insulin secretion and switch off glucagon and vice versa).
- The second one is of **hormones** (both insulin and glucagon are inhibited by their own higher concentrations by –ve feedback loop).

S

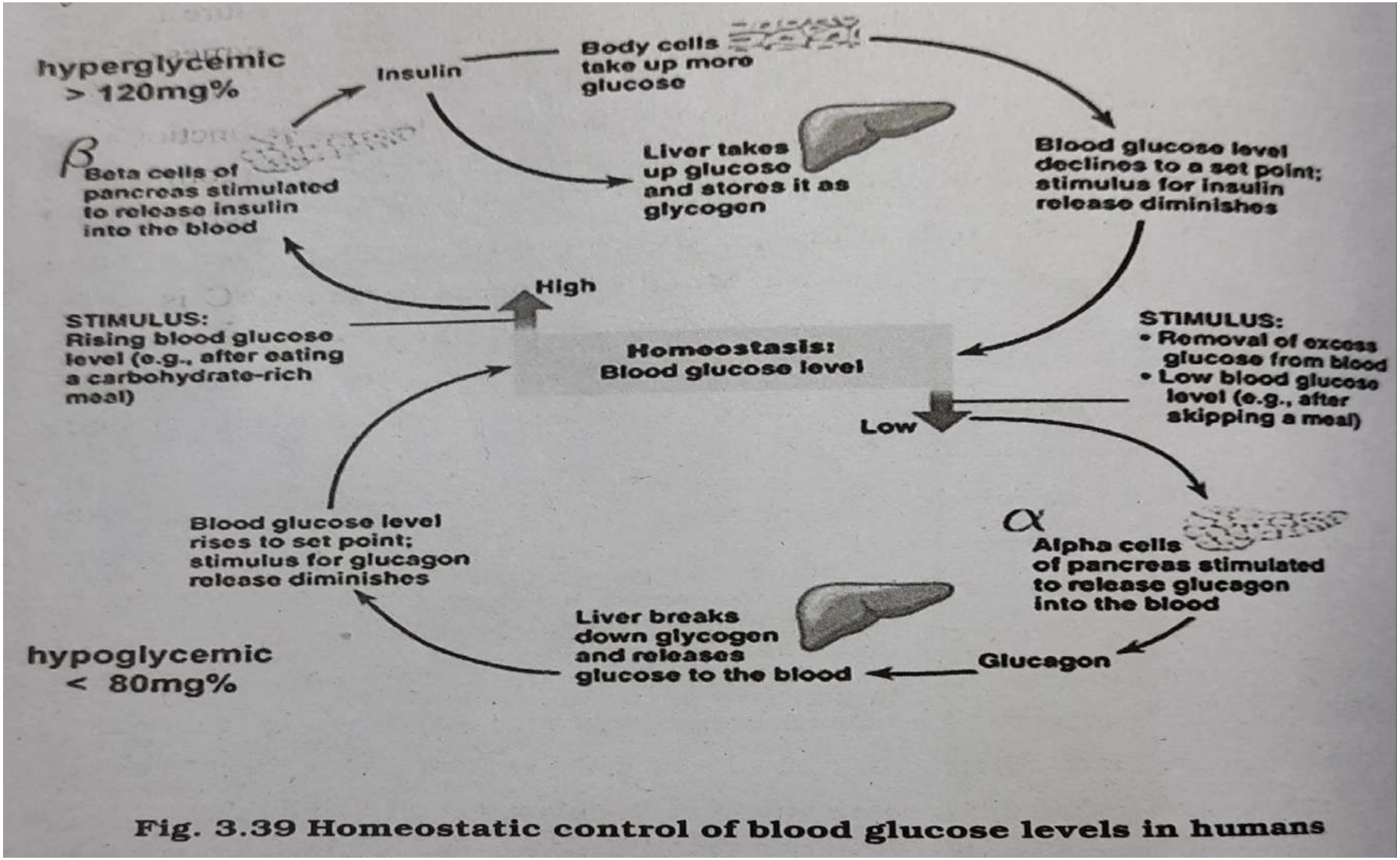


Fig. 3.39 Homeostatic control of blood glucose levels in humans

Video on Blood glucose control

Thermoregulation in Man

- Thermoregulation refers to maintenance of constant internal body temperature within a tolerable range.
- Eg: Endotherms like Mammals and Aves
- This ability is crucial for the **survival** of the animals because most of the **biochemical** and **physiological processes** take place **optimally** in a **narrow range** of **temperature**.
- The rates of enzyme-mediated reactions increase two fold for an increment of 10^0 C.

Thermoregulation in Man

- At **high** temperature, **enzymes denature** and **lose** their **function**.
- At **low** temperatures also, enzymes **cannot work**.
- Majority of living organisms survive within **10-45^{0}C** range of temperature which is known as **bio-kinetic zone**.
- **Endotherms** have a **thermoneutral zone** from **28^{0}C to 37^{0}C (Lower & Higher critical temp's.,)**.

Thermoregulation in Man

- In this range (28^0C - 37^0C), the body temperature is maintained by BMR.
- Outside the thermoneutral zone, maintaining a constant body temperature requires the expenditure of energy.
- Outside (0^0C – 40^0C), the animal cannot maintain its body temperature and dies.

Thermoregulation in Man

- All endotherms are able to balance heat gain and heat loss by generating heat internally.
- The internal control mechanism involves thermoreceptors (detector), **hypothalamus** (regulator) and muscles and glands(effectors).
- Hypothalamus is the **major control centre** of **thermoregulation**.
- The sensation of heat or cold is experienced according to the intensity & duration of stimulus and number of receptors involved.

Thermoregulation in Man

- Hypothalamus contains two distinct regions namely, the **heat gain centre** and **heat loss centre**.
- The **heat loss centre** is activated by decrease in temperature (Cold climate). It causes:

❖ Vasoconstriction

❖ Shivering (heat production)

❖ Increasing the metabolic rate

❖ Nonshivering thermogenesis

- **Brown fat** (abundant mitochondria and rich blood supply)
- **Thermogenin** (protein in brown fat cells that allows protons to leak across the mitochondrial membrane, releasing heat without producing ATP i.e. uncouples proton movement)

Thermoregulation in Man

- The heat gain centre is activated by increase in temperature (hot climate). It causes:

❖ Vasodilation

❖ Sweating (1g of water absorbs 580 calories of energy)

❖ Pilorelaxation

❖ Stretching out

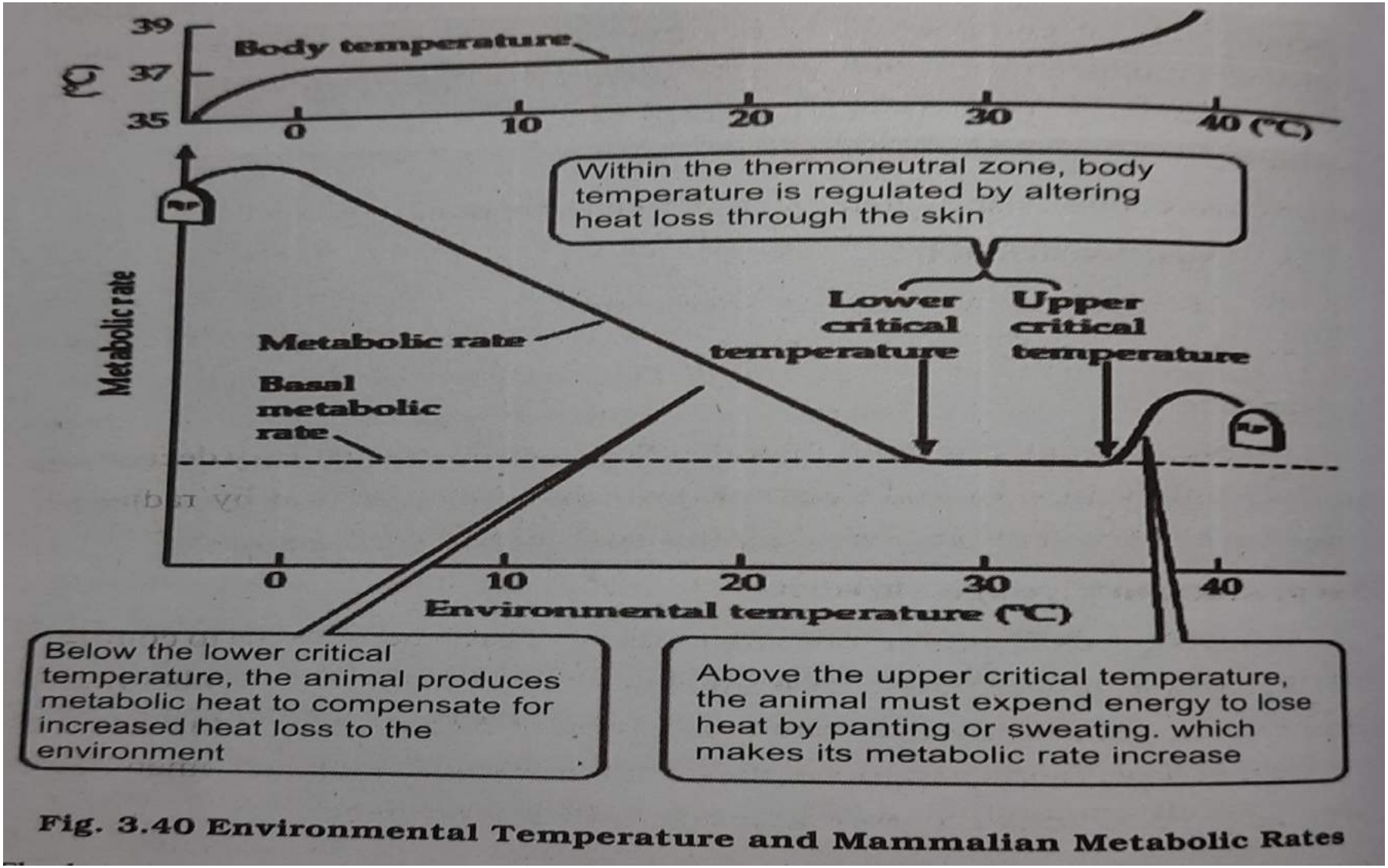


Fig. 3.40 Environmental Temperature and Mammalian Metabolic Rates

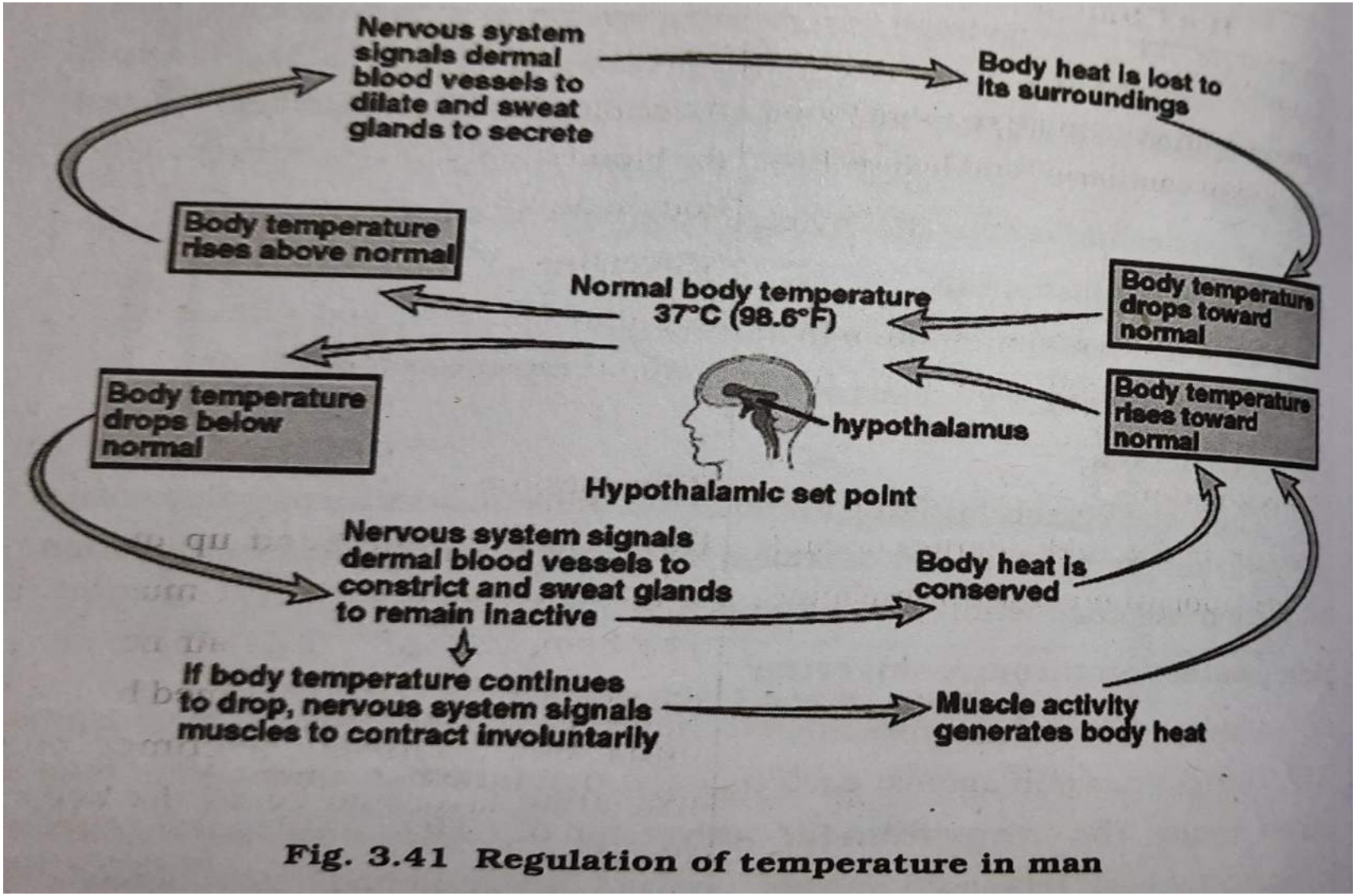


Fig. 3.41 Regulation of temperature in man

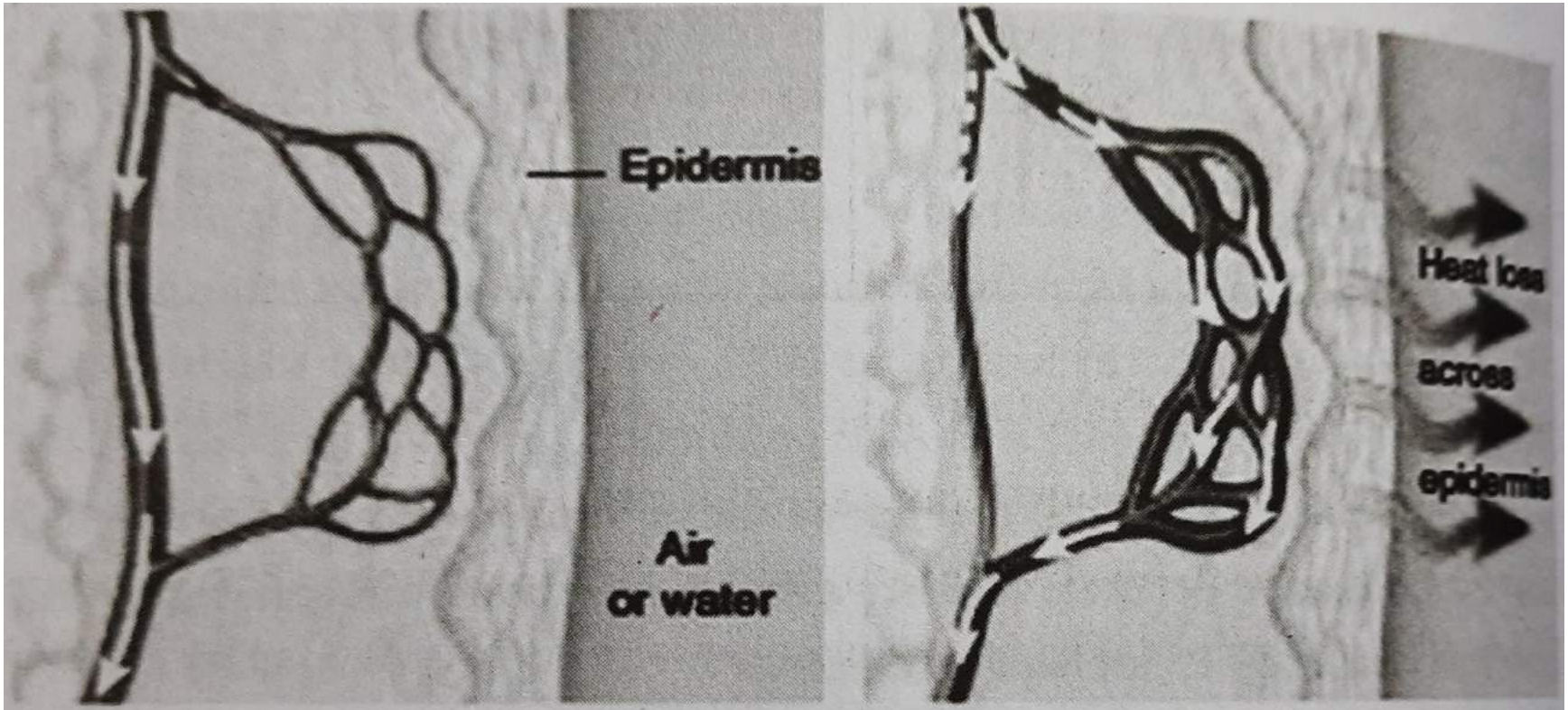


Fig. 3.42 Role of blood capillaries of skin in temperature regulation

Events of thermoregulation in man

Table 3.5 Events of thermoregulation in man

Hot Climate	Cold climate
Vasodilatation Arterioles in skin dilate so more blood enters skin capillaries and heat is lost.	**Vasoconstriction** Arterioles in skin constrict to reduce the blood supply to skin: keeping core body worm.
Sweating Sweat glands secrete sweat which evaporates by taking heat away from body and cooling it.	**Shivering** Rapid contraction and relaxation of skeletal muscles produce heat.
Pilorelaxation The hair in the body flatten because of relaxation of erector pili muscles.	**Piloerection** Hairs on the skin stand up due to contraction of erector pili muscles. The hairs trap a layer of air next to the skin which is then warmed by the body heat. The air becomes an insulating layer and keeps the body warm.
Stretching out By opening up the body offers larger surface area so that the body loses more heat.	**Curling up** By curling up the body offers less surface area so that heat is conserved.

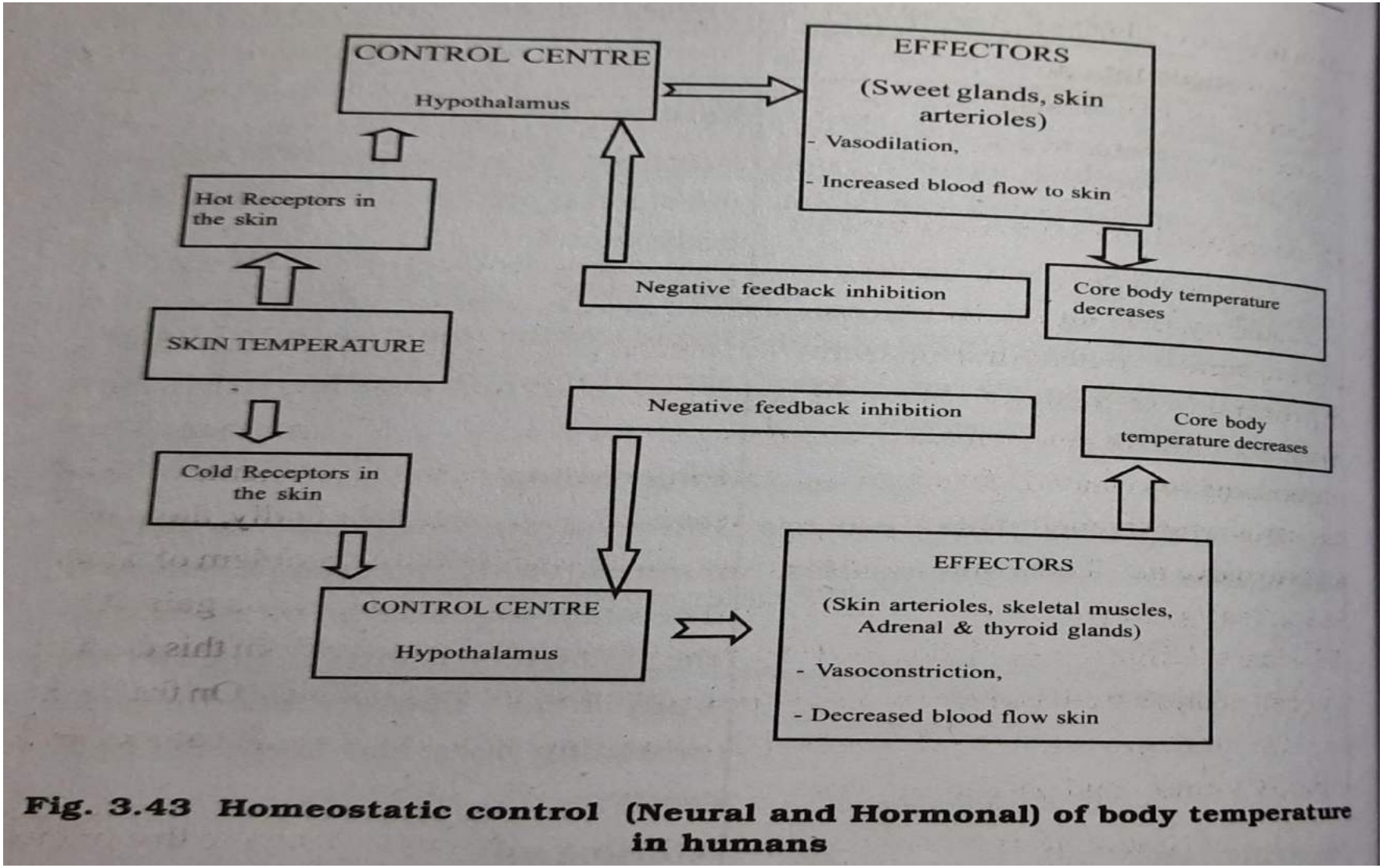


Fig. 3.43 Homeostatic control (Neural and Hormonal) of body temperature in humans

Thermoregulation video

Osmoregulation in Animals

- The maintenance of constant osmotic pressure in the fluids of an organism by the control of water and salt concentrations is known as **osmoregulation**.
- The physiological systems of cells, tissues, organs and organ-systems of all organisms operate in the **fluid environment**.
- They **work well** when the proportion of water and solutes is maintained in the **tolerable narrow limits**.

Osmoregulation in Animals

- Aquatic animals are subjected to life-threatening osmotic problems.

- The freshwater (salt concentration between 1 to 10 mOsm/l) animals face the problem of entry of water and loss of salts from the body.

- The marine (salt concentration: 1070 mOsm/l) and desert animals face problems of water loss and salt gain.

Regulators and Conformers

- **Regulating** and **conforming** are two **extreme conditions** of homeostasis.
- **Fresh water fish (*Catla catla*-Regulator) and Paramecium are** able to maintain a stable internal concentration of solutes(**variable**) in its **blood and interstitial fluid** even under changing external solute concentrations**.**

Regulators and Conformers

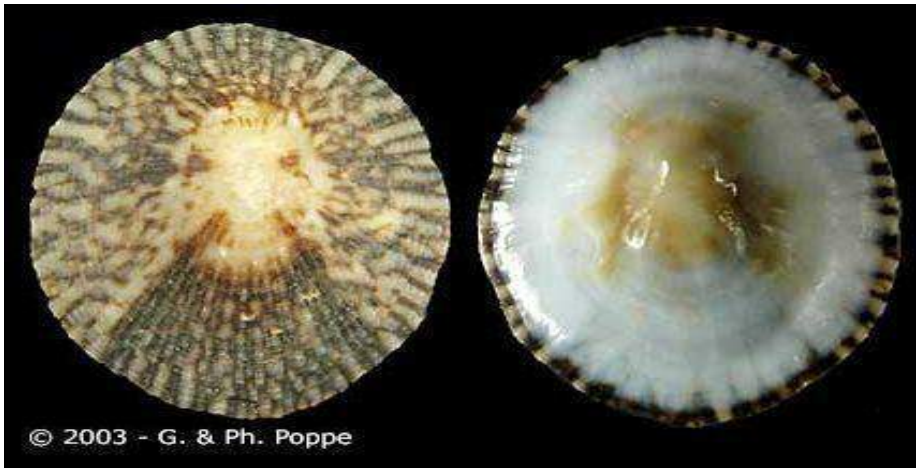
- Some invertebrates viz., *Libinia emarginata* (**spider crab-Conformer**), sea anemone, jelly fish, star fish cannot regulate its internal solute concentration. It **changes** its internal solute concentration in **accordance** with that of **exte**

Osmoregulation in marine animals

- The sea water is hyperosmotic while body fluids are hypoosmotic.
- **Salt** comes **in** through diffusion and food they eat while **water** tends to move **out** (exosmosis).
- Marine animals have developed adaptive strategies and are classified into two categories:
- **Osmoconformers** and **Osmoregulators**

Osmoregulation in marine animals

- **Osmoconformers** maintain isotonicity with sea water.
- It changes with changes outside and requires a little energy.
- Eg1: ***Acmaea limatula*** (sea snail)



Osmoregulation in marine animals

- Eg2: Sharks and Rays(Chondrichthyes-osmoconformers)

- They maintain a high concentration of urea and TMAO(Trimethylamine oxide) in their blood.

Osmoregulation in marine animals

- Due to the presence of hyperosmotic blood, water slowly enters by endosmosis.
- The **water** is disposed off by the **kidneys** through urine.
- The **kidneys** also remove some **salt** from the body and the rest is expelled by **rectal glands** and some in **faeces**.

Osmoregulation in marine animals

- **Osmoregulators** maintain the osmolarity of their body fluids at a constant level as the environment changes.
- Eg1: ***Artemia salina*** (Brine shrimp-crustacean) that lives in salt ponds.
- **Gill** actively transports excess salt from the body.

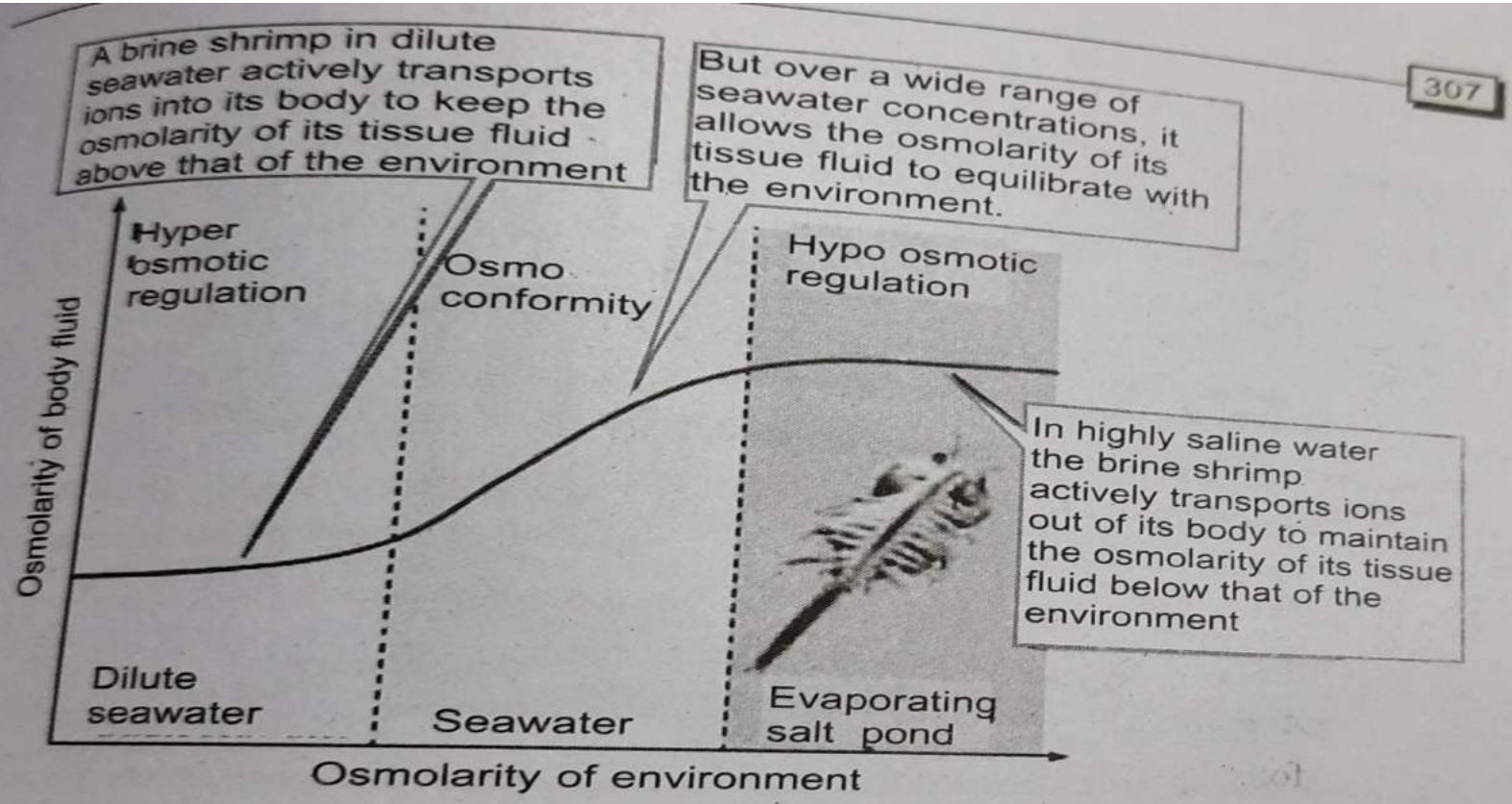


Fig. 3.44 .Brine shrimp that live at the extremes of environmental osmolarities display flexible osmoregulatory abilities.They become hyperosmotic regulators in very dilute water and hypoosmotic regulators in very saline water.

Osmoregulation in marine animals

- Eg2: **Marine bony fishes** balance the water loss by **drinking** large amounts of sea water.
- **Chloride cells** on the **gills** actively transport Cl^- followed by Na^+ ions passively.
- Other ions($Ca2^{+,}$ $Mg2^{+,}$ SO_4^{2-}) are excreted by kidneys or sent out through

  gut unabsorbed.

Osmoregulation in marine animals

- **Marine birds and reptiles** also take sea water through food.
- Kidneys are not efficient salt removers.
- Salt glands near the eyes actively excrete excess salt into nose through ducts.
- Eg: ***Macronectus gigantus***

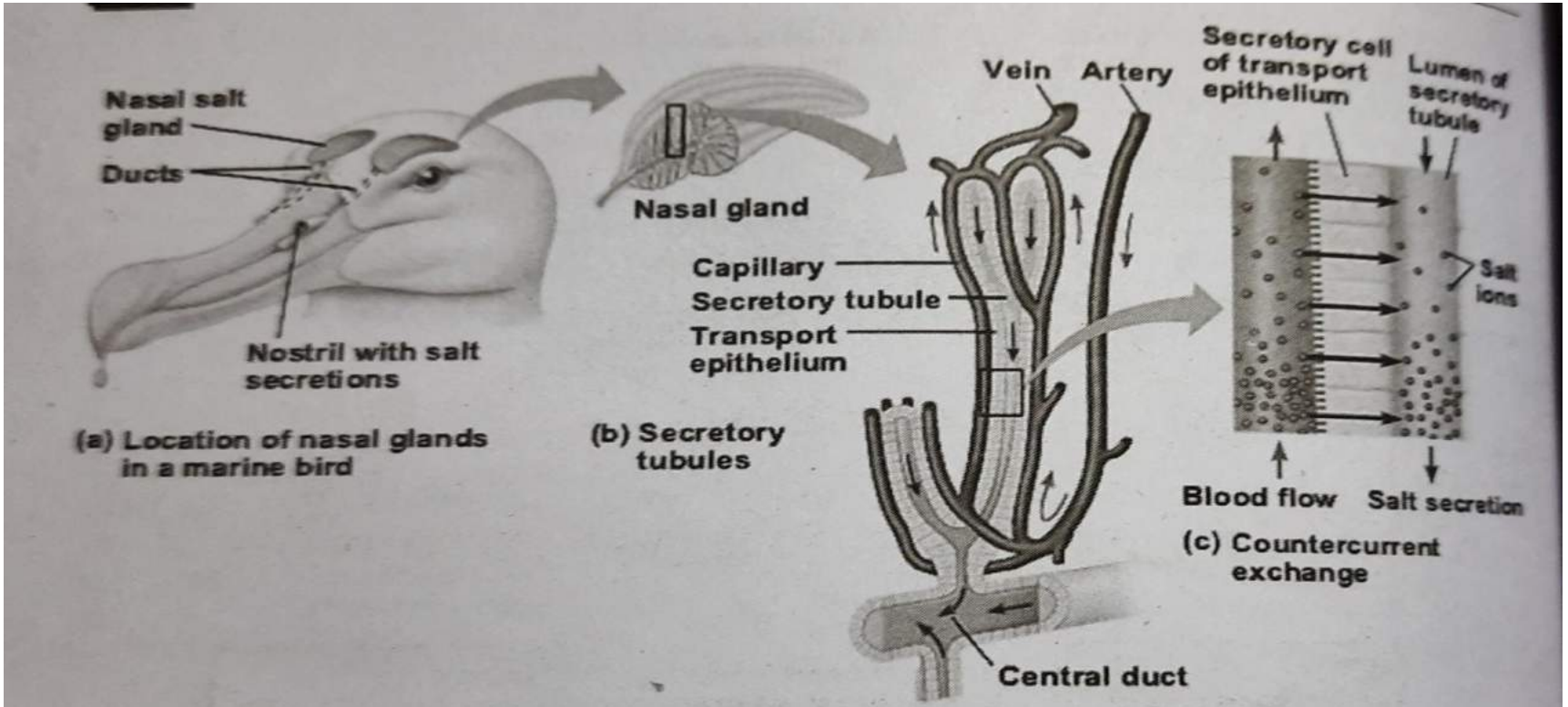


Fig. 3.45 Nasal Salt Glands Excrete Excess Salt. Marine birds havenasal salt glands adapted to excrete the excess salt they consume with their food *E.g. Macronectus gigantus*

Osmoregulation in marine animals

- **Marine mammals** have efficient kidneys which act as salt-excreting glands & produce hypertonic urine.
- Most of the marine mammals **avoid drinking sea water**, but take low-solute **body fluids of fish** they eat.

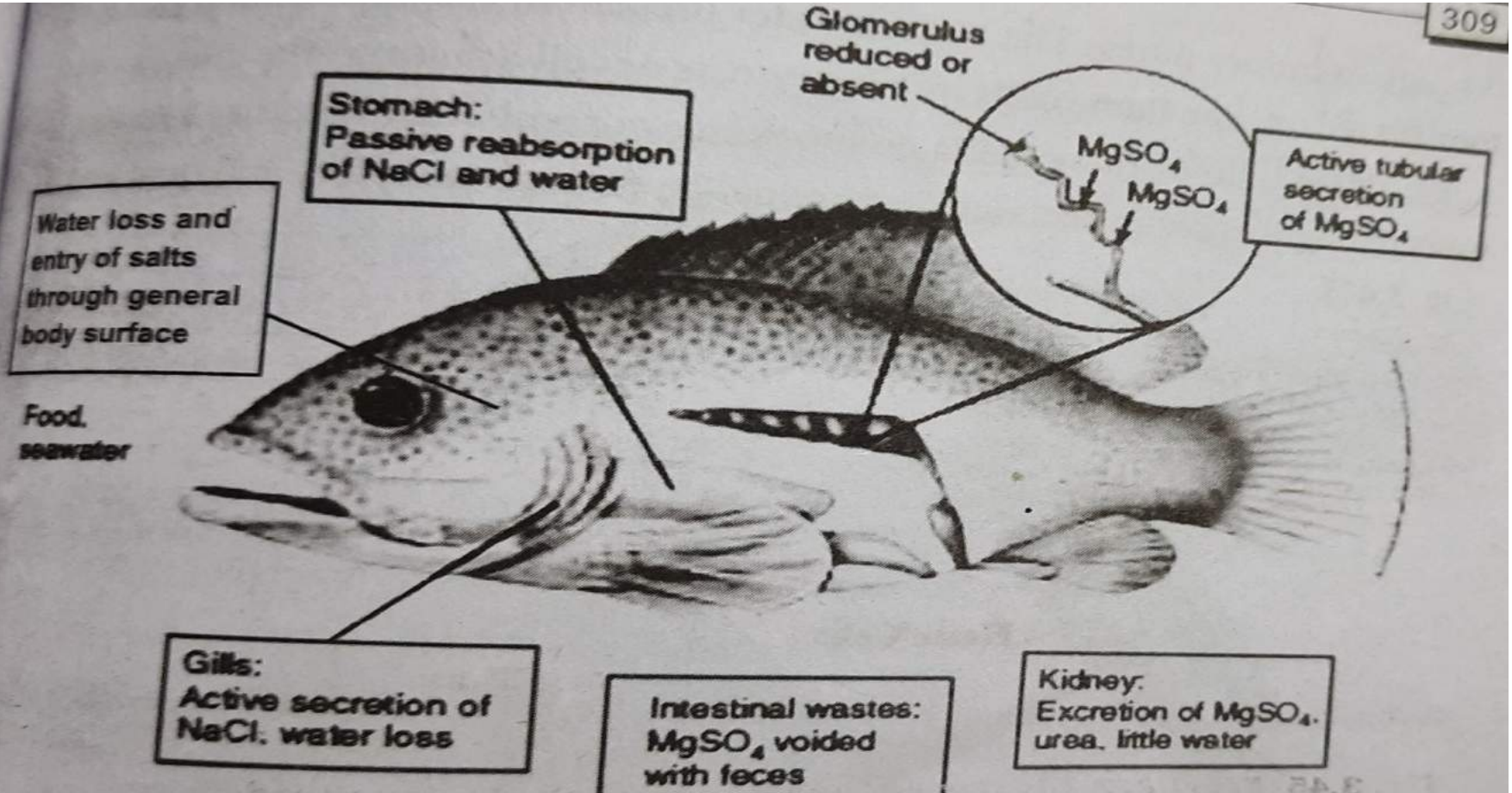


Fig. 3.46 Osmoregulation in marine fish – problem: water loss and salt gain

Osmoregulation in freshwater animals

- The problem faced by **freshwater** animals is **water gain** by endosmosis and **loss of salt** by diffusion.
- Salt loss can be balanced by **spending energy** for active transport from water through **skin**, **gills** and **kidney**.
- In **Amoeba** and **Paramoecium**, the **contractile vacuoles** remove the excess water.

Osmoregulation in freshwater animals

- In **Planaria** (fw flatworm), the **flame cells** or **protonephridia** act as osmoregulatory organs. They expel the **excess water** through flame cell system.
- **Freshwater fishes** eliminate excess water by excreting **dilute urine**.
- The salt lost is compensated by the **food** and **uptake of Na^{+}, Cl^{-}** through **chloride cells** present in the **gills**.

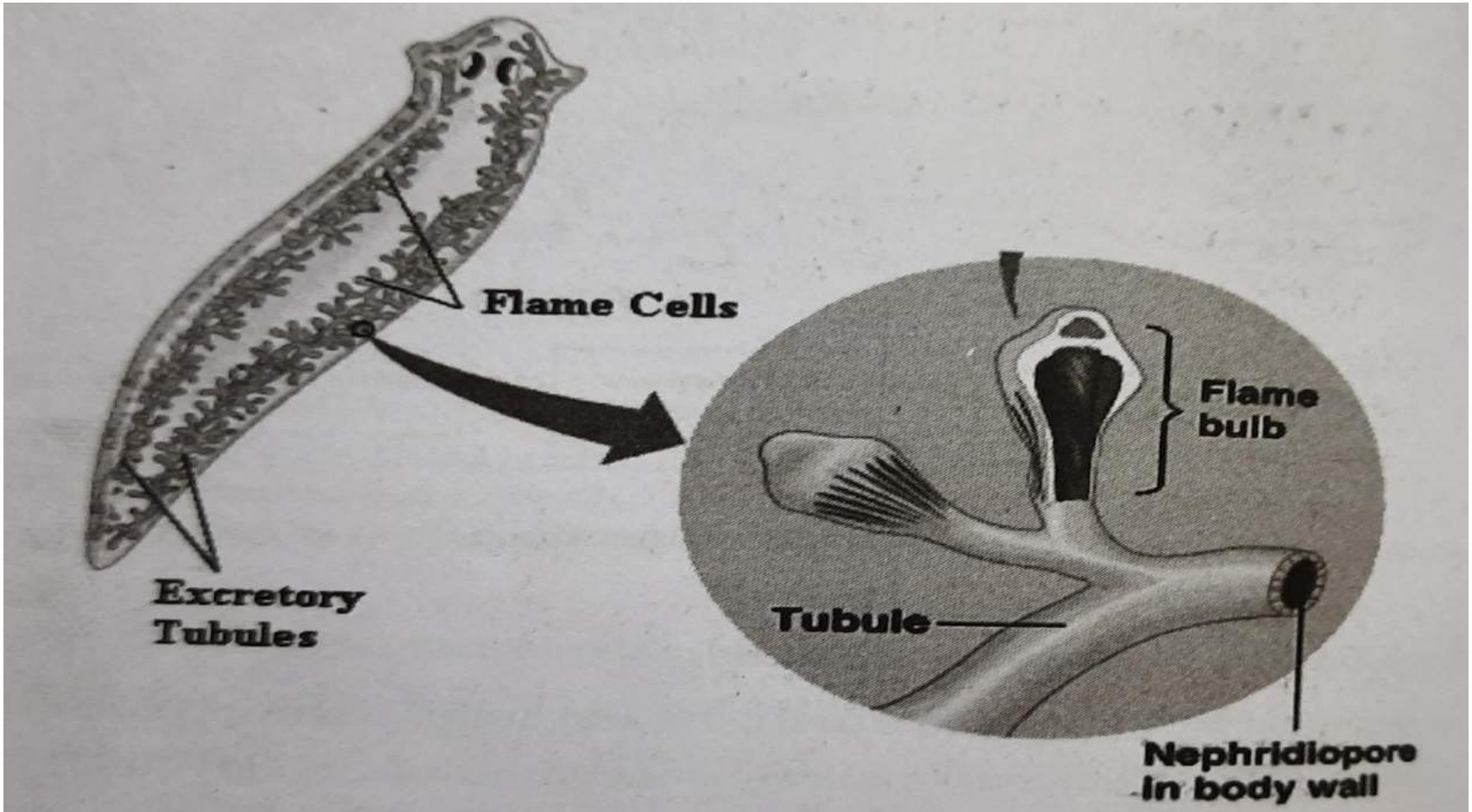


Fig. 3.47 Flame cell system in a flatworm (e.g. *Planaria*)

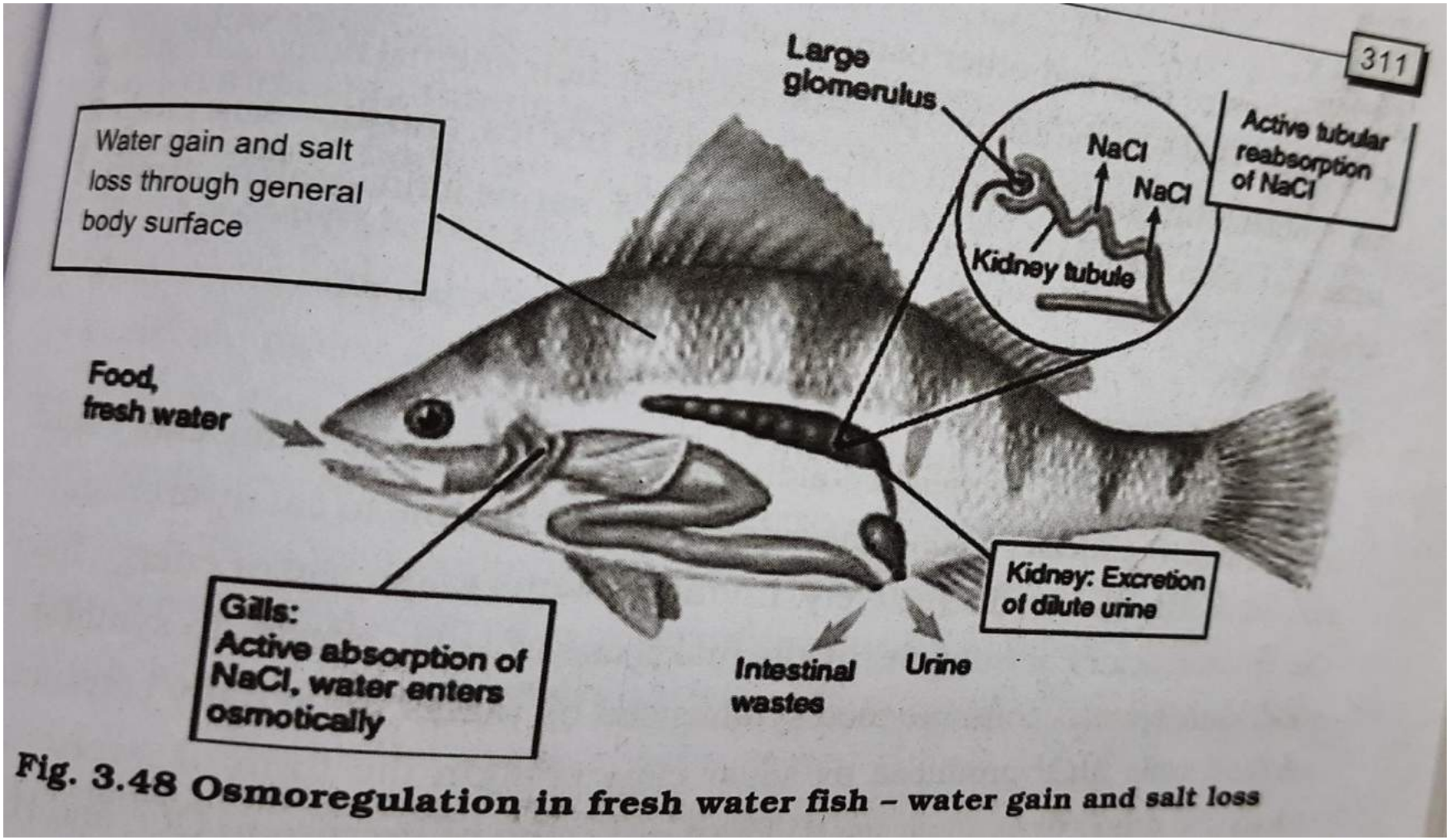
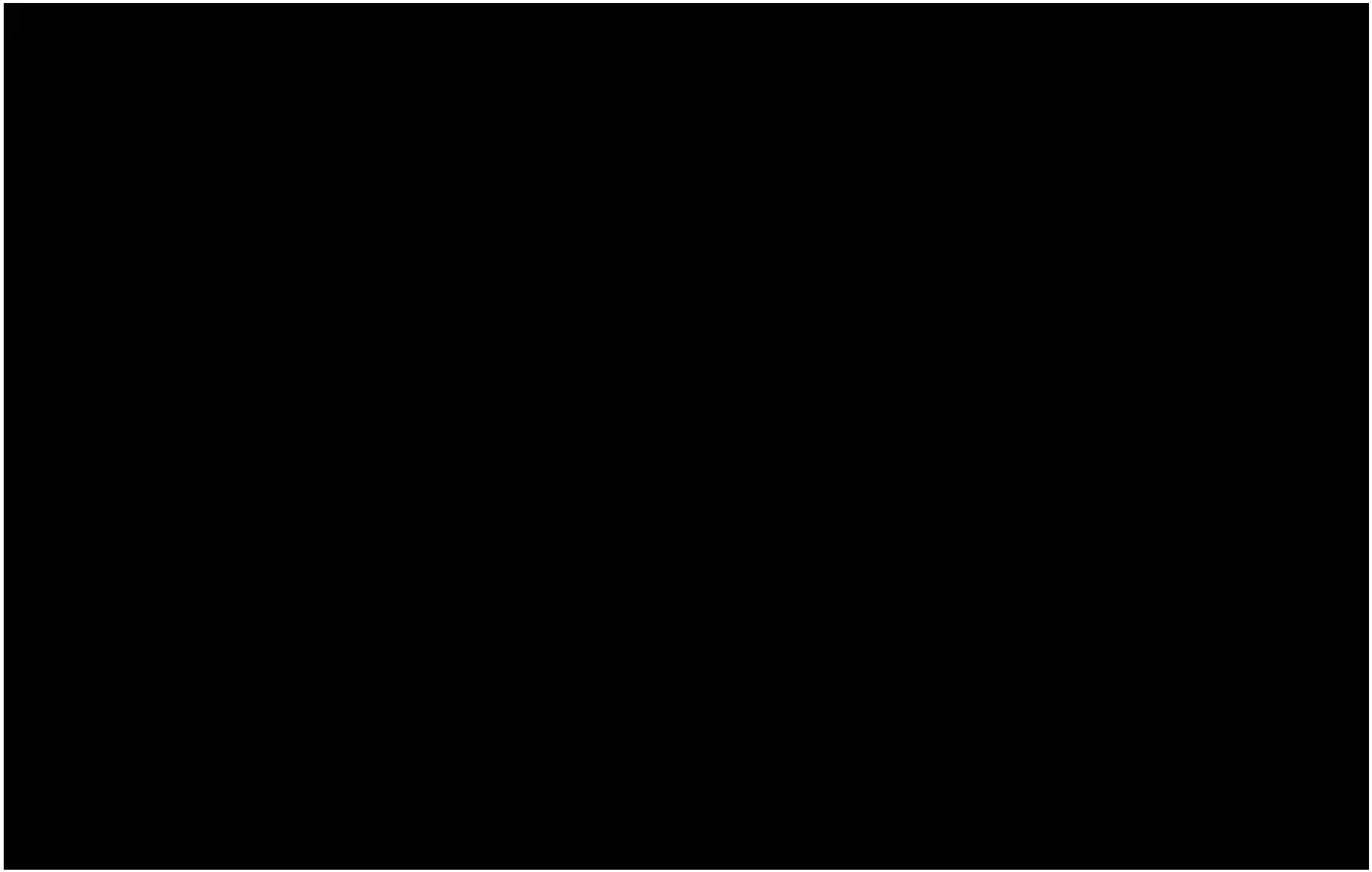


Fig. 3.48 Osmoregulation in fresh water fish – water gain and salt loss

Osmoregulation in brackish water animals

- The organisms living in brackish water are **euryhaline** that are able to adapt to **wide range of salinities**.
- Eg1: **Salmon fish** behaves like marine fish when in sea water and osmoregulates like fresh water fish in fresh water environment.
- Eg2: ***Poecilias phenops,*** euryhaline fish
- Eg3: ***Carcinus maenas*** (green crab), euryhaline

Osmoregulation video



Additional References

- https://www.khanacademy.org/science/biology/principles-of-physiology/metabolism-and-thermoregulation/a/endotherms-ectotherms

THANK YOU FOR YOUR ATTENTION

TSWRAFPDCW, Bhongir SUBJECT: Zoology GENETICS, PAPER-IV

FACULTY: K. Srilatha

Topic: INBORN ERRORS OF METABOLISM

Definition:

The term metabolism refers to all biochemical processes and pathways in the body. Enzymes play an important role in many of the catabolism and anabolism processes. Most commonly an enzyme involved in the synthesis of intermediate or breakdown of intermediates in the metabolic process. In the metabolic disorder the enzymes required for metabolic processes of carbohydrates, fats or proteins are deficient. The field of metabolic diseases now also includes defects in the function of subcellular organelles mitochondria, peroxisomes and lysosomes and defects in the metabolism of additional molecules, such as neurotransmitters. As a result of genetic mutation cell is unable to synthesize some enzymes required in the metabolic processes.

A genetic defect in any part of the major metabolic pathways is known as aninborn or congenital (if present from birth) error of metabolism.

Phenylketonuria

Phenylketonuria is caused by lack of the enzyme phenylalanine hydroxylase needed to convert amino acid phenylalanine to tyrosine in the liver. It is due to a recessively inherited defect in the enzyme. It increases the concentration of the amino acid phenylalanine, and is toxic to the brain, causing intellectual disability. Phenylalanine is an essential amino acid that cannot be synthesized in the body.

Incidence:

The incidence of PKU is about 1:6,000-10,000.

- Nausea.
- Vomiting.
- An eczema-like rash.

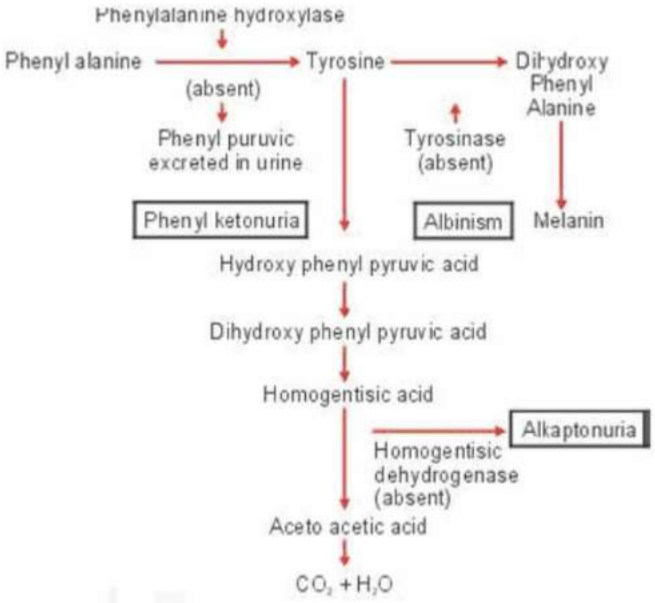
It give-off a mousy body and urine odour as a result of a by-product, phenyl acetic acid in their urine and sweat a mousy body odour.

Other symptoms include aggressive or self-injurious behaviour, hyperactivity, and sometimes psychiatric symptoms. New-borns with PKU rarely have symptoms right away, although sometimes they are sleepy or eat poorly. If not treated, affected infants progressively develop intellectual disability over the first few years of life, eventually becoming severe. The diagnosis is based on a blood test.

Treatment:

A strict phenylalanine-restricted diet allows for normal growth and development. A phenylalanine-restricted diet should continue for life, or intelligence may decrease and neurologic and mental problems may ensue. PKU occurs in most ethnic groups. If PKU runs in the family and DNA is available from an affected family member. DNA analysis can be done to determine whether a foetus has the disorder or not.

Parents and siblings of children with PKU can be tested to find out whether they carry the gene that causes the disease. If two carriers conceive a child, that child has a 1 in 4 chance of being born with the disease. Because all-natural sources of protein contain too much phenylalanine for children with PKU, affected children cannot have meat, milk, or other common foods that contain protein. Instead, they must eat a variety of processed foods, which are specially manufactured to be phenylalanine-free. Low-protein natural foods, such as fruits, vegetables, and restricted amounts of certain grain cereals, can be eaten. Special nutritional products, including infant formula without phenylalanine, are also available. Future treatments may include cell transplantation and gene therapy.



Sites of biochemical block in Phenyl ketonuria, Alkaptonuria and Albinism

A boy of Albinism

Siblings born with Phenylketonuria

Alkaptonuria

Alkaptonuria (AKU) is a rare disease, painful and degenerative disease. Other names for AKU include black urine disease.

Alkaptonuria is caused by a genetic mutation. It is a recessive disorder, meaning that the AKU gene must be passed on by both of the patient's parents. Alkaptonuria is caused by the lack of an enzyme called homogentisic dioxygenase (HGD) in which patients cannot fully break down a toxic acid called homogentisic acid, or HGA. A large amount builds up in the body. This accumulates at 2,000 times the normal rate, leading to severely debilitating health problems. Some HGA is eliminated in urine and gives Black colour to

the urine is a common symptom of AKU.

Incidence:

AKU is a rare disease with an estimated frequency of one in 250,000 to one in 500,000.

Sign and symptoms:

In this condition the accumulation of HGA causes discolouration of bone and cartilage in a process called ochronosis. Black and brittle cartilage is more susceptible to the normal

wear and tear that occurs in the body, quickly leading to painful movements in the joints. As the cartilage degrades over time, bones rub against one another, causing painful

osteoarthritis. AKU heart disease and related heart complica l valves to harden, making them less eff managing the flow of b

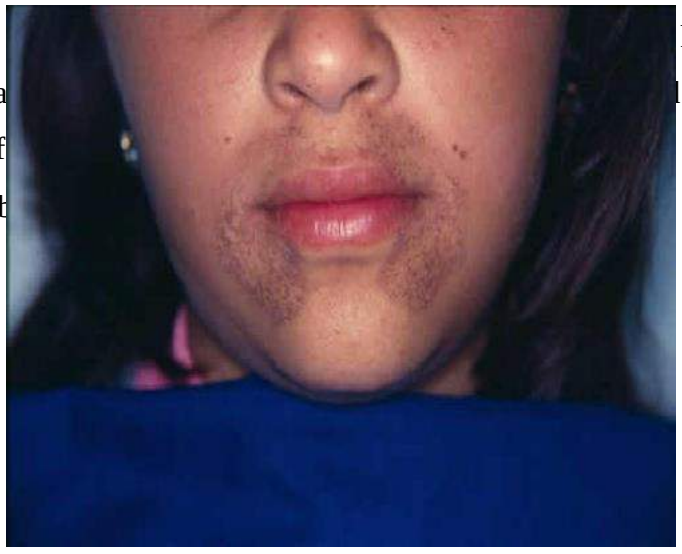


Fig: Pigmentation of the face in Alkaptonuria.

Treatment:

Apart from treatment of the complications is pain relief and joint replacement for the cartilage damage. Vitamin C has been used to reduce the ochronosis. Low-protein diet may be used to lower the homogentisic acid level. Recently the drug nitisinone has been found to suppresses homogentisic acid production.

▪Albinism is an inherited disorder that's present at birth. Children are at risk of being born with albinism if they have parents with albinism, or parents who carry the gene for albinism.

▪Albinism is an inherited disease characterized by a substantially lower rate of melanin production.

▪Melanin is the pigment responsible for the color of the skin, hair, and eyes.

▪In general, but not always, people with albinism have lighter colored skin and hair than the other members of their family or ethnic group.

▪Regardless of skin or hair tone, people with albinism always have some level of dysfunction with their vision.

▪Because melanin normally protects the skin from UV (ultraviolet) damage, people with the disorder are more sensitive to sun exposure and have an increased risk of skin cancer.

Types of albinism

Albinism is split into a number of subgroups depending on the specific genes that are affected. These subgroups include the following:

Oculocutaneous albinism (OCA): caused by a mutation in 1 of 4 genes, OCA is further split into seven types depending on the mutations. These subdivisions include:

OCA type 1: individuals tend to have milky skin, white hair, and blue eyes. With age, some individuals' skin and hair may darken.

OCA type 2: similar to type 1 and occurs most often in sub-Saharan Africans, African-Americans, and Native Americans.

OCA type 3: occurs mostly in black South

Africans. OCA type 4: occurs most often in East Asian populations. **Inheritance of albinism**

Most types of albinism are inherited in an autosomal recessive inheritance pattern, the exception being X-linked ocular albinism which is passed on in an X-linked inheritance pattern.

Autosomal recessive inheritance

▪With autosomal recessive inheritance, an individual must receive faulty copies of a gene from the mother and father to develop albinism.
▪If both parents carry the gene, there is a 1 in 4 chance that their offspring will have albinism and a 1 in 2 chance that the offspring will become a carrier (without symptoms).
▪An estimated 1 in 70 people carry the genes associated with albinism but are not affected by the mutations.

X.linked inheritance

- X-linked recessive conditions predominantly affect males.
- Because females carry two X chromosomes, if one gene damaged, the other can often make up the shortfall.
- Females can still carry and pass on the gene.
- Men, however, have one X and one Y chromosome, so any albino mutations in their singular X chromosome will generate the condition.
- If the mother has an X-linked mutation, each daughter will have a 1 in 2 chance of becoming a carrier and each son will have a 1 in 2 chance of developing albinism.Symptoms

People with albinism will have the following symptoms:

- an absence of color in the hair, skin, or eyes

- lighter than normal coloring of the hair, skin, or eyes
- patches of skin that have an absence of color

Albinism occurs with vision problems, which may include:

- strabismus (crossed eyes)
- photophobia (sensitivity to light)
- nystagmus (involuntary rapid eye movements)
- impaired vision or blindness
- astigmatism

Diagnosis

The most accurate way to diagnose albinism is through genetic testing to detect defective genes related to albinism. Less accurate ways of detecting albinism include an evaluation of symptoms by your doctor or an electroretinogram test. This test measures the response of the light-sensitive cells in the eyes to reveal eye problems associated with albinism.

Treatment

There is no cure for albinism. Treatment for albinism can relieve symptoms and prevent sun damage. Treatment may include:

- sunglasses to protect the eyes from UV rays
- protective clothing and sunscreen to protect the skin from UV rays
- prescription eyeglasses to correct vision problems
- surgery on the muscles of the eyes to correct abnormal eye movements

TSWRAFPDCW – BHONGIR TEACHING MODULE
CLASS: BZC/MZC (BSC)
(V sem); Paper: V (HUMAN PHYSIOLOGY AND BIOCHEMISTRY)

Lecturer Name: V. JYOTHI TOPIC: KREB'S CYCLE

1. **No. of Teaching Hours: 2**
2. **Objectives of the Topic:**

- Student can able to understand the concept of energy production
- Understand the overall idea of energy pathway
- Understand the Importance of Krebs cycle
- Student can identify the differences between Glycolysis and Krebs cycle
- **PRE-TEST**:
- **Understanding the student's basic knowledge by asking orally on the following**

i.Tell me the different names of Krebs cycle?

ii.What is the Full form TCA cycle?

iii.What are the steps involved in TCA cycle?

iv.Tell me where the energy required?

v. Who was described TCA CYCLE?

3. **Module Content:**

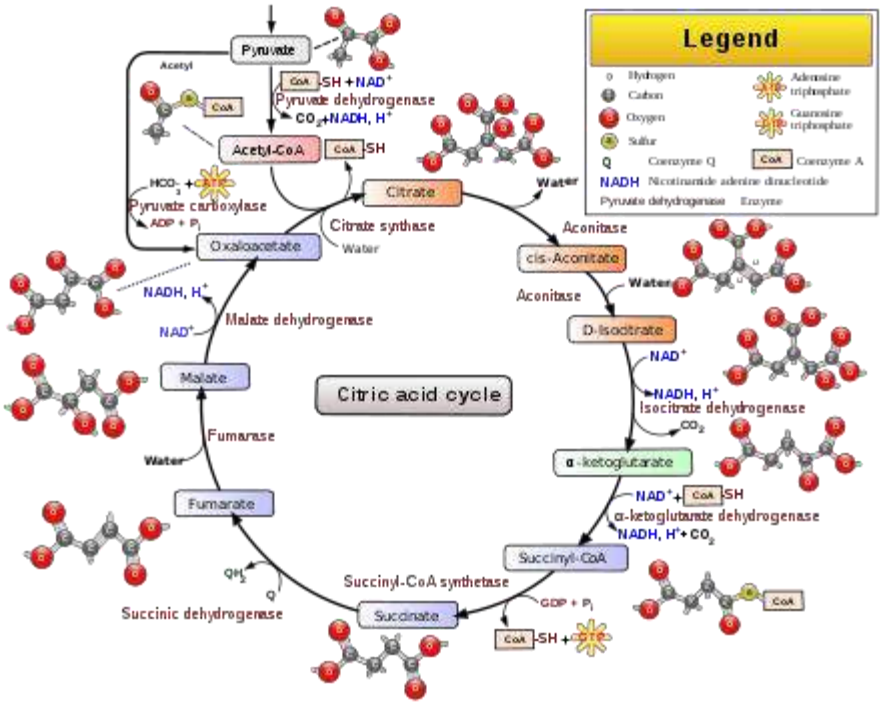
KREBS CYCLE:

Krebs cycle also called as Citric acid cycle OR Tri carboxylic acid. The acetic acid so formed enters Mitochondria and is broken down in to CO_2 and water in Krebs cycle.

Several of the components and reactions of the citric acid cycle were established in the 1930s by the research of Albert Szent-Györgyi, who received the Nobel Prize in Physiology or Medicine in 1937 specifically for his discoveries pertaining to fumaric acid, a key component of the cycle. He was able to make this discovery successful with the help of pigeon breast muscle. Because this tissue maintains its oxidative capacity well after breaking down in the "Latapie" mill and releasing in aqueous solutions, breast muscle of the pigeon was very well qualified for the study of oxidative reactions. The citric acid cycle itself was finally identified in 1937 by Hans Adolf Krebs and William Arthur Johnson, while at the University

Sheffield,[10] for which the former received the Nobel Prize for Physiology or Medicine in 1953, and for whom the cycle is sometimes named (Krebs cycle)

The **citric acid cycle** (**CAC**) – also known as the **TCA cycle (tricarboxylic acid cycle)** or the **Krebs cycle**– is a series of chemical reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins. In addition, the cycle provides precursors of certain amino acids, as well as the reducing agent NADH, that are used in numerous other reactions. Its central importance to many biochemical pathways suggests that it was one of the earliest components of metabolism and may have originated abiogenically. Even though it is branded as a 'cycle', it is not necessary for metabolites to follow only one specific route; at least three segments of the citric acid cycle have been recognized.



Overview of the citric acid cycle

The name of this metabolic pathway is derived from the citric acid (a tricarboxylic acid, often called citrate, as the ionized form predominates at biological pH) that is consumed and then regenerated by this sequence of reactions to complete the cycle. The cycle consumes acetate (in the form of acetyl-CoA) and water, reduces NAD^+ to NADH, releasing carbon dioxide. The NADH generated by the citric acid cycle is fed into the oxidative phosphorylation (electron transport) pathway. The net result of these two closely linked pathways is the oxidation of nutrients to produce usable chemical energy in the form of ATP.

In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion. In prokaryotic cells, such as bacteria, which lack mitochondria, the citric acid cycle reaction sequence is performed in the cytosol with the proton gradient for ATP production being across the cell's surface (plasma membrane) rather than the inner membrane of the mitochondrion. The overall yield of energy-containing compounds from the TCA cycle is three NADH, one $FADH_2$, and one GTP.

The citric acid cycle is a key metabolic pathway that connects carbohydrate, fat, and protein metabolism. The reactions of the cycle are carried out by eight enzymes that completely oxidize acetate (a two carbon molecule), in the form of acetyl-CoA, into two molecules each of carbon dioxide and water. Through catabolism of sugars, fats, and proteins, the two-carbon organic product acetyl-CoA (a form of acetate) is produced which enters the citric acid cycle. The reactions of the cycle also convert three equivalents of nicotinamide adenine dinucleotide (NAD^+) into three equivalents of reduced NAD^+ (NADH), one equivalent of flavin adenine dinucleotide (FAD) into one equivalent of $FADH_2$, and one equivalent each of guanosine diphosphate (GDP) and inorganic phosphate (P_i) into one equivalent of guanosine triphosphate (GTP). The NADH and $FADH_2$ generated by the citric acid cycle are, in turn, used by the oxidative phosphorylation pathway to generate energy-rich ATP.

One of the primary sources of acetyl-CoA is from the breakdown of sugars by glycolysis which yield pyruvate that in turn is decarboxylated by the pyruvate dehydrogenase complex generating acetyl-CoA according to the following reaction scheme:

$CH_3C(=O)C(=O)O^-$pyruvate + HSCoA + $NAD^+ \rightarrow CH_3C(=O)SCoA$acetyl-CoA + NADH + CO_2

The product of this reaction, acetyl-CoA, is the starting point for the citric acid cycle. Acetyl-CoA may also be obtained from the oxidation of fatty acids. Below is a schematic outline of the cycle:

- The citric acid cycle begins with the transfer of a two-carbon acetyl group from acetyl-CoA to the four-carbon acceptor compound (oxaloacetate) to form a six-carbon compound (citrate).
- The citrate then goes through a series of chemical transformations, losing two carboxyl groups as CO_2. The carbons lost as CO_2 originate from what was oxaloacetate, not directly from acetyl-CoA. The carbons donated by acetyl-CoA become part of the oxaloacetate carbon backbone after the first turn of the citric acid cycle. Loss of the acetyl-CoA-donated carbons as CO_2 requires several turns of the citric acid cycle. However, because of the role of the citric

acid cycle in anabolism, they might not be lost, since many citric acid cycle intermediates are also used as precursors for the biosynthesis of other molecules.

- Most of the electrons made available by the oxidative steps of the cycle are transferred to NAD^+, forming NADH. For each acetyl group that enters the citric acid cycle, three molecules of NADH are produced. The citric acid cycle includes a series of oxidation reduction reaction in mitochondria .[*clarification needed*]
- In addition, electrons from the succinate oxidation step are transferred first to the FAD cofactor of succinate dehydrogenase, reducing it to $FADH_2$, and eventually to ubiquinone (Q) in the mitochondrial membrane, reducing it to ubiquinol (QH_2) which is a substrate of the electron transfer chain at the level of Complex III.
- For every NADH and $FADH_2$ that are produced in the citric acid cycle, 2.5 and 1.5 ATP molecules are generated in oxidative phosphorylation, respectively.
- At the end of each cycle, the four-carbon oxaloacetate has been regenerated, and the cycle continues.

Steps:

There are ten basic steps in the citric acid cycle, as outlined below. The cycle is continuously supplied with new carbon in the form of acetyl-CoA, entering at step 0 in the table.[14]

	Substrates	Products	Enzyme	Reaction type	Comment
0/10	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	irreversible, extends the 4C oxaloacetate to a 6C molecule
1	Citrate	*cis*-Aconitate + H_2O	Aconitase	Dehydration	reversible isomerisation
2	*cis*-Aconitate + H_2O	Isocitrate		Hydration	
3	Isocitrate + NAD^+	Oxalosuccinate + NADH + H^+	Isocitrate dehydrog	Oxidation	generates NADH (equivalent of 2.5 ATP)

4	Oxalosuccinate	α-Ketoglutarate + CO_2	enase	Decarboxylation	rate-limiting, irreversible stage, generates a 5C molecule
5	α-Ketoglutarate + NAD^+ + CoA-SH	Succinyl-CoA + NADH + H^+ + CO_2	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	irreversible stage, generates NADH (equivalent of 2.5 ATP), regenerates the 4C chain (CoA excluded)
6	Succinyl-CoA + GDP + P_i	Succinate + CoA-SH + GTP	Succinyl-CoA synthetase	substrate-level phosphorylation	or ADP→ATP instead of GDP→GTP,[15] generates 1 ATP or equivalent. Condensation reaction of GDP + P_i and hydrolysis of succinyl-CoA involve the H_2O needed for balanced equation.
7	Succinate + ubiquinone (Q)	Fumarate + ubiquinol (QH_2)	Succinate dehydrogenase	Oxidation	uses FAD as a prosthetic group (FAD→$FADH_2$ in the first step of the reaction) in the enzyme.[15] These two electrons are later transferred to QH_2 during Complex II of the ETC, where they generate the equivalent of 1.5 ATP
8	Fumarate + H_2O	*L*-Malate	Fumarase	Hydration	Hydration of C-C double bond
9	L-Malate + NAD^+	Oxaloacetate + NADH + H^+	Malate dehydrogenase	Oxidation	reversible (in fact, equilibrium favors malate), generates NADH (equivalent of 2.5 ATP)
1	Oxaloacetate + A	Citrate + CoA-SH	Citrate	Aldol	This is the same as step 0 and

0 / 0	cetyl CoA + H_2O	synthase condensation	restarts the cycle. The reaction is irreversible and extends the 4C oxaloacetate to a 6C molecule

Two carbon atoms are oxidized to CO_2, the energy from these reactions is transferred to other metabolic processes through GTP (or ATP), and as electrons in NADH and QH_2. The NADH generated in the citric acid cycle may later be oxidized (donate its electrons) to drive ATP synthesis in a type of process called oxidative phosphorylation.[6] $FADH_2$ is covalently attached to succinate dehydrogenase, an enzyme which functions both in the CAC and the mitochondrial electron transport chain in oxidative phosphorylation. $FADH_2$, therefore, facilitates transfer of electrons to coenzyme Q, which is the final electron acceptor of the reaction catalyzed by the succinate:ubiquinone oxidoreductase complex, also acting as an intermediate in the electron transport chain.

Mitochondria in animals, including humans, possess two succinyl-CoA synthetases: one that produces GTP from GDP, and another that produces ATP from ADP. Plants have the type that produces ATP (ADP-forming succinyl-CoA synthetase). Several of the enzymes in the cycle may be loosely associated in a multienzyme protein complex within the mitochondrial matrix.

The GTP that is formed by GDP-forming succinyl-CoA synthetase may be utilized by nucleoside-diphosphate kinase to form ATP (the catalyzed reaction is GTP + ADP → GDP
+ ATP).

Products of the first turn of the cycle are one GTP (or ATP), three NADH, one QH_2 and two CO_2.

Because two acetyl-CoA molecules are produced from each glucose molecule, two cycles are required per glucose molecule. Therefore, at the end of two cycles, the products are: two GTP, six NADH, two QH_2, and four CO_2.

Description	**Reactants**	**Products**
The sum of all reactions in the citric acid cycle is:	Acetyl-CoA + 3 NAD^+ + UQ + GDP + P_i + 2 H_2O	→ CoA-SH + 3 NADH + UQH_2 + 3 H^+ + GTP + 2 CO_2

Combining the reactions occurring during the pyruvate oxidation with those occurring during the citric acid cycle, the following overall pyruvate oxidation reaction is obtained:

$$\text{Pyruvate ion} + 4\ \text{NAD}^+ + \text{UQ} + \text{GDP} + \text{UQH}_2 + 4\ \text{H}^+ + \text{GTP} + \text{P}_\text{i} + 2\ \text{H}_2\text{O} + 3\ \text{CO}_2$$

Combining the above reaction with the ones occurring in the course of glycolysis, the following overall glucose oxidation reaction (excluding reactions in the respiratory chain) is obtained:

$$\text{Glucose} + 10\ \text{NAD}^+ + 2\text{UQ} + 2\ \text{ADP} + 2\ \text{GDP} + 4\ \text{P}_\text{i} + 2\ \text{H}_2\text{O} \rightarrow 10\ \text{NADH} + 2\text{UQH}_2 + 10\ \text{H}^+ + 2\ \text{ATP} + 2\ \text{GTP} + 6\ \text{CO}_2$$

The above reactions are balanced if P_i represents the H_2PO_4^- ion, ADP and GDP the ADP^{2-} and GDP^{2-} ions, respectively, and ATP and GTP the ATP^{3-} and GTP^{3-} ions, respectively.

The total number of ATP molecules obtained after complete oxidation of one glucose in glycolysis, citric acid cycle, and oxidative phosphorylation is estimated to be between 30 and 38.

The theoretical maximum yield of ATP through oxidation of one molecule of glucose in glycolysis, citric acid cycle, and oxidative phosphorylation is 38 (assuming 3 molar equivalents of ATP per equivalent NADH and 2 ATP per UQH_2). In eukaryotes, two equivalents of NADH and four equivalents of ATP are generated in glycolysis, which takes place in the cytoplasm. Transport of two of these equivalents of NADH into the mitochondria consumes two equivalents of ATP, thus reducing the net production of ATP to 36. Furthermore, inefficiencies in oxidative phosphorylation due to leakage of protons across the mitochondrial membrane and slippage of the ATP synthase/proton pump commonly reduces the ATP yield from NADH and UQH_2 to less than the theoretical maximum yield. The observed yields are, therefore, closer to ~2.5 ATP per NADH and ~1.5 ATP per UQH_2, further reducing the total net production of ATP to approximately 30. An assessment of the total ATP yield with newly revised proton-to-ATP ratios provides an estimate of 29.85 ATP per glucose molecule.

While the citric acid cycle is in general highly conserved, there is significant variability in the enzymes found in different taxa (note that the diagrams on this page are specific to the mammalian pathway variant).

Some differences exist between eukaryotes and prokaryotes. The conversion of D-*threo*-isocitrate to 2-oxoglutarate is catalyzed in eukaryotes by the NAD^+-dependent EC 1.1.1.41, while prokaryotes employ the $NADP^+$-dependent EC 1.1.1.42. Similarly, the conversion of (*S*)-malate to oxaloacetate is catalyzed in eukaryotes by the NAD^+-dependent EC 1.1.1.37, while most prokaryotes utilize a quinone-dependent enzyme, EC 1.1.5.4.

A step with significant variability is the conversion of succinyl-CoA to succinate. Most organisms utilize EC 6.2.1.5, succinate–CoA ligase (ADP-forming) (despite its name, the enzyme operates in the pathway in the direction of ATP formation). In mammals a GTP- forming enzyme, succinate–CoA ligase (GDP-forming) (EC 6.2.1.4) also operates. The level of utilization of each isoform is tissue dependent. In some acetate-producing bacteria, such as *Acetobacter aceti*, an entirely different enzyme catalyzes this conversion – EC 2.8.3.18, succinyl-CoA:acetate CoA-transferase. This specialized enzyme links the TCA cycle with acetate metabolism in these organisms. Some bacteria, such as *Helicobacter pylori*, employ yet another enzyme for this conversion – succinyl-CoA:acetoacetate CoA-transferase (EC 2.8.3.5).

Some variability also exists at the previous step – the conversion of 2-oxoglutarate to succinyl-CoA. While most organisms utilize the ubiquitous NAD^+-dependent 2-oxoglutarate dehydrogenase, some bacteria utilize a ferredoxin-dependent 2-oxoglutarate synthase (EC 1.2.7.3). Other organisms, including obligately autotrophic and methanotrophic bacteria and archaea, bypass succinyl-CoA entirely, and convert 2-oxoglutarate to succinate via succinate semialdehyde, using EC 4.1.1.71, 2-oxoglutarate decarboxylase, and EC 1.2.1.79, succinate- semialdehyde dehydrogenase.

In cancer, there are substantial metabolic derangements that occur to ensure the proliferation of tumor cells, and consequently metabolites can accumulate which serve to facilitate tumorigenesis, dubbed oncometabolites. Among the best characterized oncometabolites is 2-hydroxyglutarate which is produced through a heterozygous gain-of- function mutation (specifically a neomorphic one) in isocitrate dehydrogenase (IDH) (which under normal circumstances catalyzes the oxidation of isocitrate to oxalosuccinate, which then spontaneously decarboxylates to alpha-ketoglutarate, as discussed above; in this case an additional reduction step occurs after the formation of alpha-ketoglutarate via NADPH to

yield 2-hydroxyglutarate), and hence IDH is considered an oncogene. Under physiological conditions, 2-hydroxyglutarate is a minor product of several metabolic pathways as an error but readily converted to alpha-ketoglutarate via hydroxyglutarate dehydrogenase enzymes (L2HGDH and D2HGDH) but does not have a known physiologic role in mammalian cells; of note, in cancer, 2-hydroxyglutarate is likely a terminal metabolite as isotope labelling experiments of colorectal cancer cell lines show that its conversion back to alpha- ketoglutarate is too low to measure. In cancer, 2-hydroxyglutarate serves as a competitive inhibitor for a number of enzymes that facilitate reactions via alpha-ketoglutarate in alpha- ketoglutarate-dependent dioxygenases. This mutation results in several important changes to the metabolism of the cell. For one thing, because there is an extra NADPH-catalyzed reduction, this can contribute to depletion of cellular stores of NADPH and also reduce levels of alpha-ketoglutarate available to the cell. In particular, the depletion of NADPH is problematic because NADPH is highly compartmentalized and cannot freely diffuse between the organelles in the cell. It is produced largely via the pentose phosphate pathway in the cytoplasm. The depletion of NADPH results in increased oxidative stress within the cell as it is a required cofactor in the production of GSH, and this oxidative stress can result in DNA damage. There are also changes on the genetic and epigenetic level through the function of histone lysine demethylases (KDMs) and ten-eleven translocation (TET) enzymes; ordinarily TETs hydroxylate 5-methylcytosines to prime them for demethylation. However, in the absence of alpha-ketoglutarate this cannot be done and there is hence hypermethylation of the cell's DNA, serving to promote epithelial-mesenchymal transition (EMT) and inhibit cellular differentiation. A similar phenomenon is observed for the Jumonji C family of KDMs which require a hydroxylation to perform demethylation at the epsilon-amino methyl group.[32] Additionally, the inability of prolyl hydroxylases to catalyze reactions results in stabilization of hypoxia-inducible factor alpha, which is necessary to promote degradation of the latter (as under conditions of low oxygen there will not be adequate substrate for hydroxylation). This results in a pseudohypoxic phenotype in the cancer cell that promotes angiogenesis, metabolic reprogramming, cell growth, and migration.

Allosteric regulation by metabolites. The regulation of the citric acid cycle is largely determined by product inhibition and substrate availability. If the cycle were permitted to run unchecked, large amounts of metabolic energy could be wasted in overproduction of reduced coenzyme such as NADH and ATP. The major eventual substrate of the cycle is ADP which

gets converted to ATP. A reduced amount of ADP causes accumulation of precursor NADH which in turn can inhibit a number of enzymes. NADH, a product of all dehydrogenases in the citric acid cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase. Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits alpha- ketoglutarate dehydrogenase and citrate synthase. When tested in vitro with TCA enzymes, **ATP** inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.

Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate, a precursor of pyruvate. This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.

Regulation by calcium. Calcium is also used as a regulator in the citric acid cycle. Calcium levels in the mitochondrial matrix can reach up to the tens of micromolar levels during cellular activation. It activates pyruvate dehydrogenase phosphatase which in turn activates the pyruvate dehydrogenase complex. Calcium also activates isocitrate dehydrogenase and α- ketoglutarate dehydrogenase.[34] This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.

Transcriptional regulation. Recent work has demonstrated an important link between intermediates of the citric acid cycle and the regulation of hypoxia-inducible factors (HIF). HIF plays a role in the regulation of oxygen homeostasis, and is a transcription factor that targets angiogenesis, vascular remodeling, glucose utilization, iron transport and apoptosis. HIF is synthesized constitutively, and hydroxylation of at least one of two critical proline residues mediates their interaction with the von Hippel Lindau E3 ubiquitin ligase complex, which targets them for rapid degradation. This reaction is catalysed by prolyl 4-hydroxylases. Fumarate and succinate have been identified as potent inhibitors of prolyl hydroxylases, thus leading to the stabilisation.

4.<u>Student Activities:</u>

- Group discussion on Krebs pathway
- Seminars by Students
- Quiz will be conducted to the students

- Preparing the chart of Krebs cycle

5. **Assessment:**

   - Assessing the Student after completion of the Topic by conducting Oral Test or written test.

   i. Explain the pathway of Krebs cycle? Draw the chart of Krebs cycle ?

   ii. Tell me the different names of Krebs cycle? Write the importance of Krebs cycle?

   iii.

   iv.

6. **Reference Books:**

   - HUMAN PHYSIOLOGY
     - Dr. N. Arumugam - SARAS PUBLICATION
   - HUMAN PHYSIOLOGY
     -JAYPEE BROTHERS MEDICAL PUBLISHERS
   - CELL BIOLOGY - C. GOPAL & Dr KONDAIAH

ज्ञान दीक्षा

జ్ఞానదీక్ష

GNAANA DEEKSHA

CONCEPT BASED DIGITAL CLASSES FOR SCHOOLS & UG COLLEGES

Dr. K. Srilatha
DL in Zoology
TSWRAFPDCW Bhongir

Subject : **ZOOLOGY**

Concept : **Mechanism of Hormonal Action**

Class : **BSc**

HORMONE: MECHANISM & ACTION

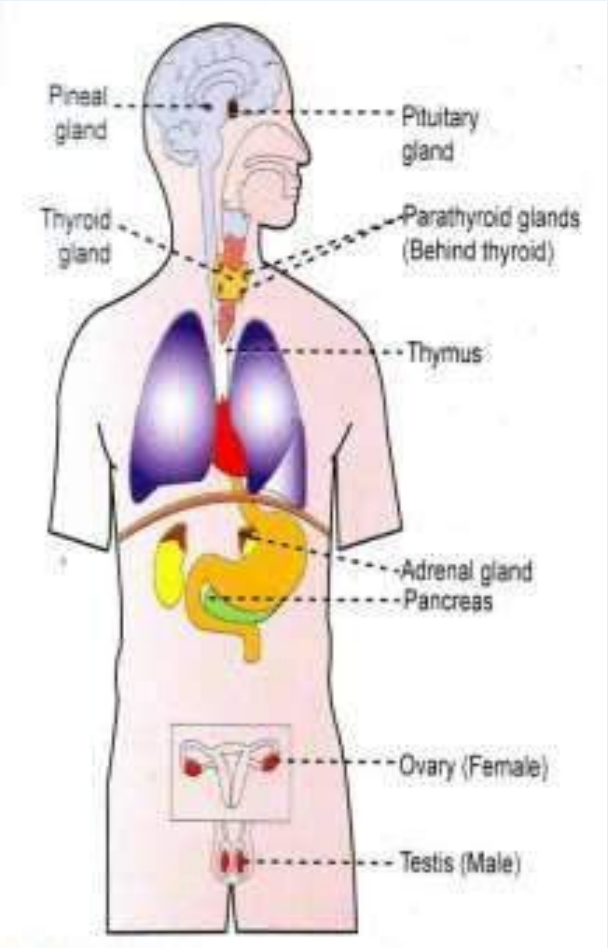
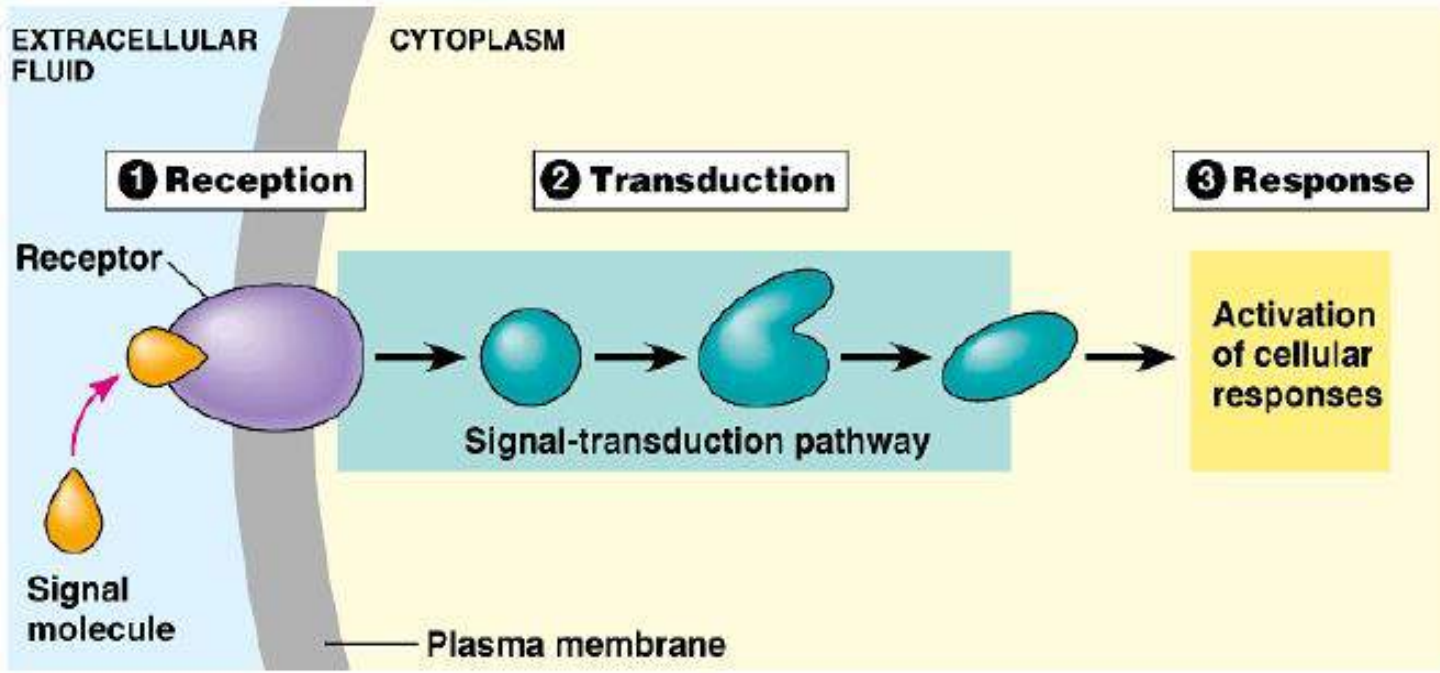


Diagram showing major Endocrine glands

Learning objectives

- Learn about various receptors.
- Understand the mode of action of Hydrophilic Hormones.
- Understand the mechanism of Lipophilic hormones.
- Know the concept of Secondary messengers
- Understand how hormones induce changes in cellular metabolism

Three Stages of Signal Transduction



Types of signals

TABLE 12–1

Some Signals to Which Cells Respond

Antigens	Light
Cell surface glycoproteins/ oligosaccharides	Mechanical touch
Developmental signals	Neurotransmitters
Extracellular matrix components	Nutrients
Growth factors	Odorants
Hormones	Pheromones
	Tastants

Hormone

- A hormone is a chemical regulatory substance, secreted by ductless glands (Endocrine glands).
- Natural organic substances - regulate growth, metabolism
- Biochemical messengers
- It passes through blood stream to reach the tissues on which it acts. These tissues are called “target tissues”.

General functions of hormones

- Hormones regulate different metabolic pathways.
- Hormones co-ordinate activities of different organs of the body
- Some hormones control the rate and type of growth of the body.

General Characteristics of Hormones

- Not secreted at a uniform rate
- Exert their effects in biocatalytic amounts
- Turnover is varied and usually rapid
- Exert multiple actions
- Exhibit high degree of specificity
- Different tissues may respond differently to a given hormone

GENERAL PRINCIPLES OF HORMONE ACTION

- ✓ Trophic hormone
- ✓ Synergism
- ✓ Permissiveness
- ✓ Antagonism - one hormone produces the opposite effect of the other

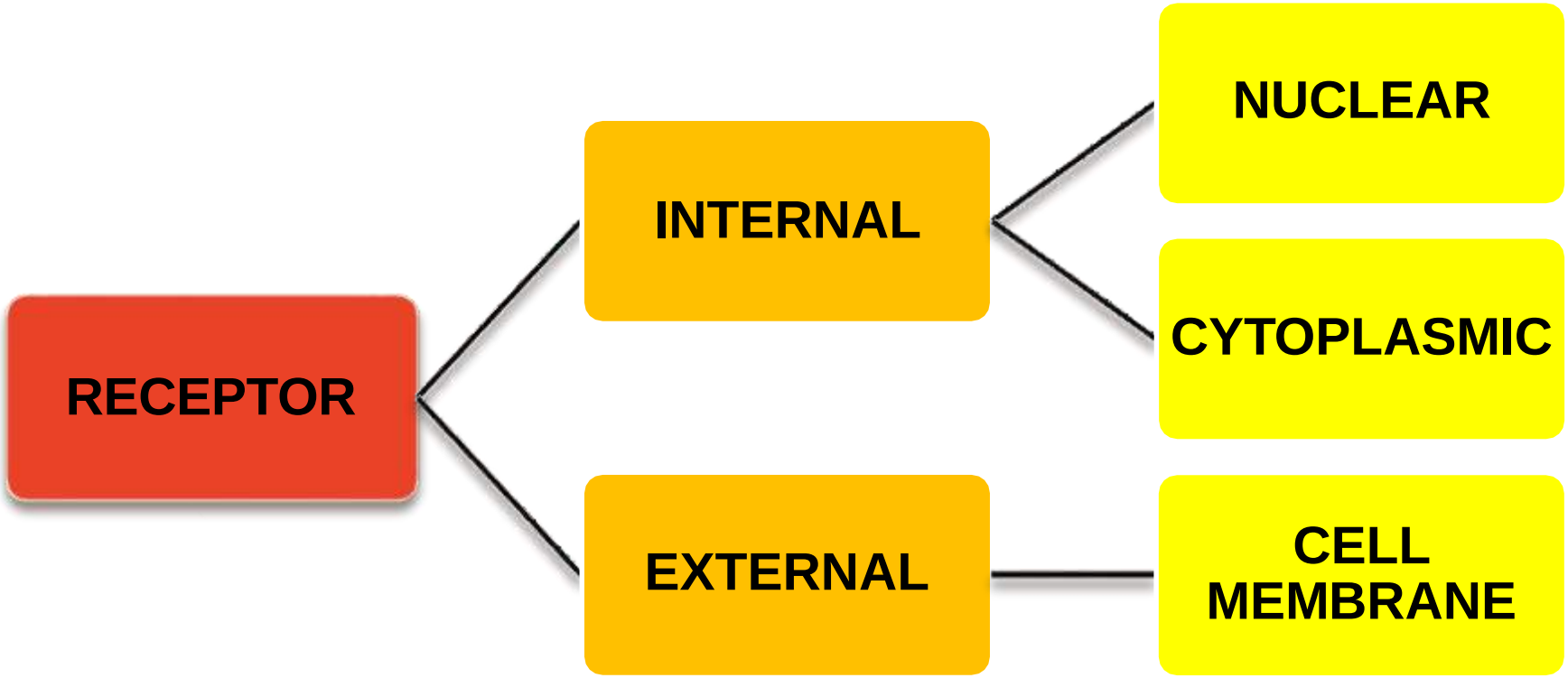
The processes involve in both negative and positive feedback. Ex. A>B>C>D

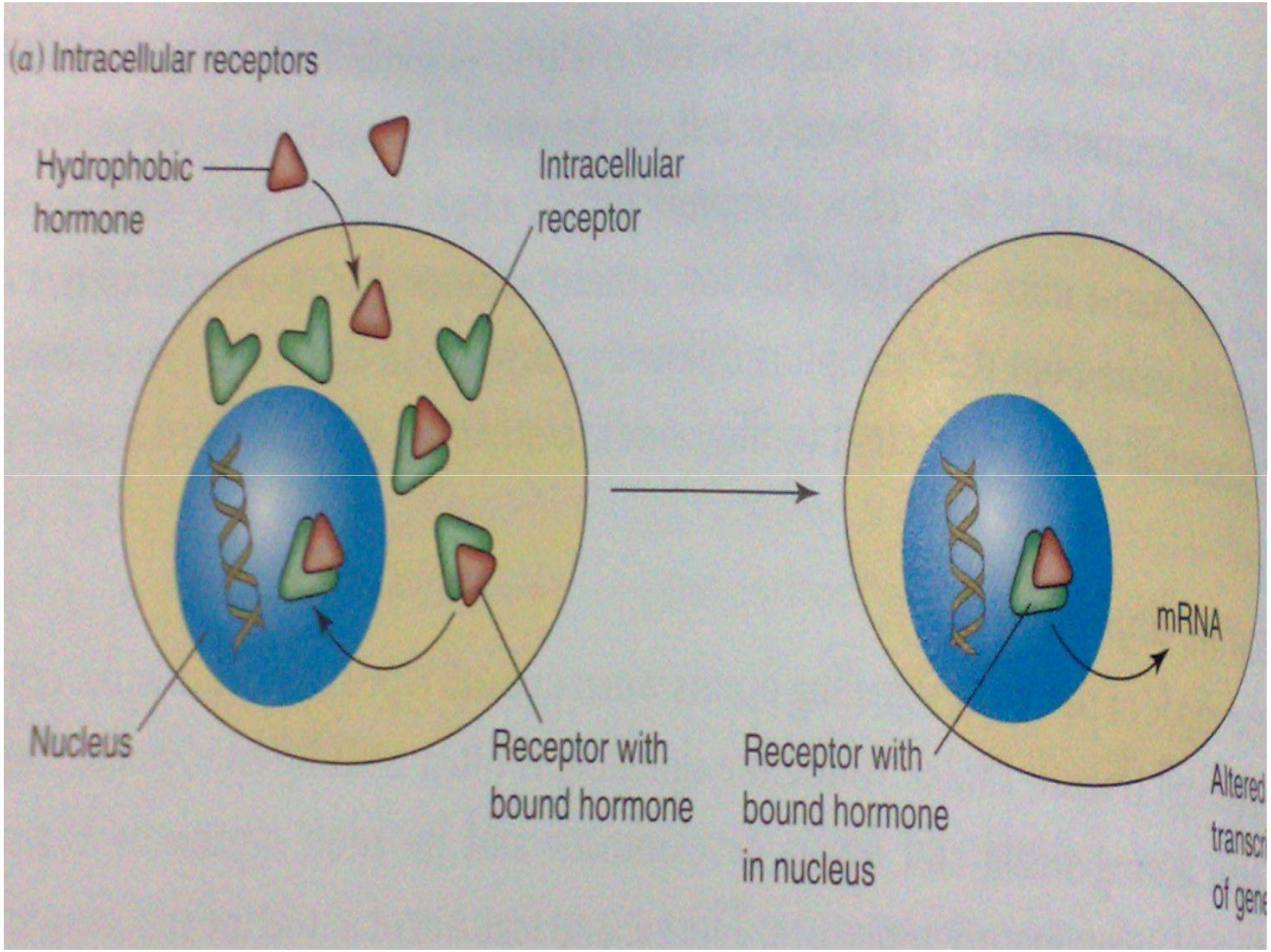
Definition of Hormone Receptors:

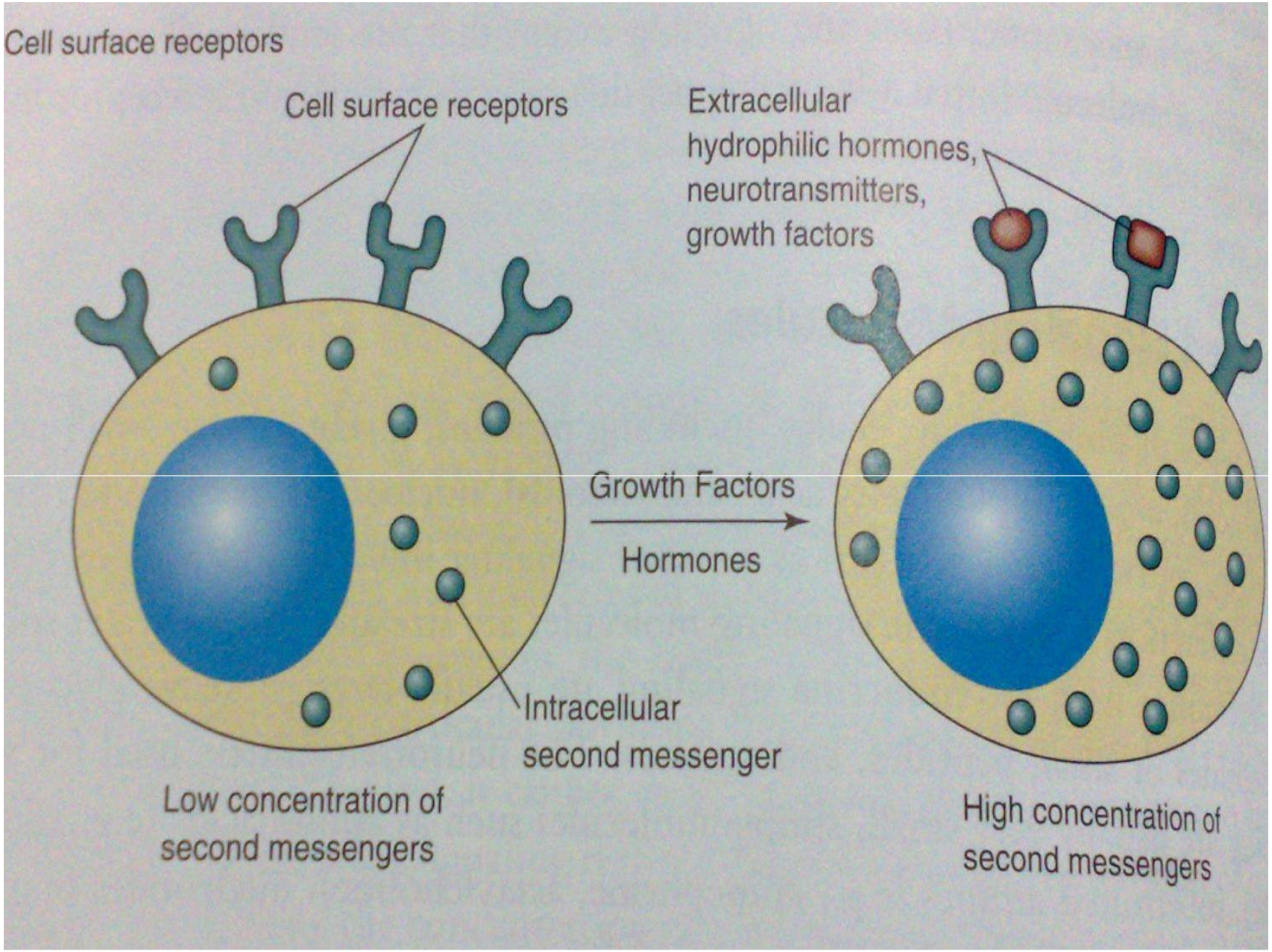
- Cell-associated recognition molecules which are protein in nature.

Functional sites: Two functional sites:

- Recognition site: It binds the hormone specifically.
- Signaling site: It couples hormone binding to intracellular effect.




(a) Intracellular receptors
Hydrophobic hormone
Intracellular receptor
Nucleus
Receptor with bound hormone
Receptor with bound hormone in nucleus
mRNA
Altered
transcr
of gene


Cell surface receptors
Cell surface receptors
Extracellular
hydrophilic hormones,
neurotransmitters,
growth factors
Growth Factors
Hormones
Intracellular
second messenger
Low concentration of
second messengers
High concentration of
second messengers

Classification of Hormones based on their solubility

Based on their solubility in water, Hormones are classified into

1. Hydrophilic Hormone: The water soluble hormone.

Examples: the catecholamines (epinephrine and norepinephrine) and peptide/protein hormones.

2. Lipophilic Hormone: They are poorly soluble in water.

Examples: The lipid soluble hormones include thyroid hormone, steroid hormones and Vitamin D3

Types of Hormones

Feature	Group I	Group II
Types	Steroids	Polypeptides
	Iodothyronines	Proteins
	Calcitriol	Glycoproteins
		Catecholamines
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma half-life	Long (hours, days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediators	Receptor-hormone complex	Cyclic adenosine monophosphate, cyclic guanosine monophosphate, Ca^{2+}, diacylglycerol, kinase cascades, phosphatidyl inositides, others

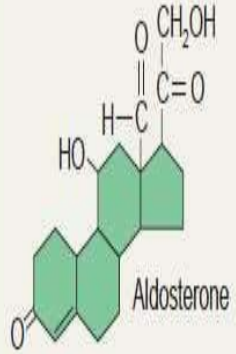
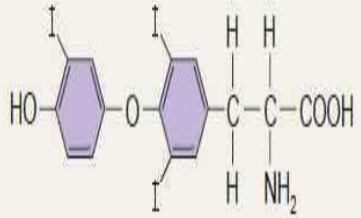
Group I: Steroid hormones: These hormones are derived from cholesterol.

- **Glucocorticoids.**
- **Mineralocorticoids.**
- **Sex hormones.**
- **Thyroid hormones**
- **Retinoic acid**

The last two those are Thyroid hormones (T3 & T4) and Retinoic acid act on Intranuclear receptors.

TABLE 18.2

Summary of Hormones by Chemical Class

CHEMICAL CLASS	HORMONES	SITE OF SECRETION
LIPID-SOLUBLE		
Steroid hormones	Aldosterone, cortisol, and androgens.	Adrenal cortex.
	Calcitriol.	Kidneys.
	Testosterone.	Testes.
	Estrogens and progesterone.	Ovaries.
Thyroid hormones	T_3 (triiodothyronine) and T_4 (thyroxine).	Thyroid gland (follicular cells).
 Triiodothyronine (T_3)		
Gas	Nitric oxide (NO).	Endothelial cells lining blood vessels.

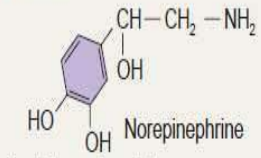
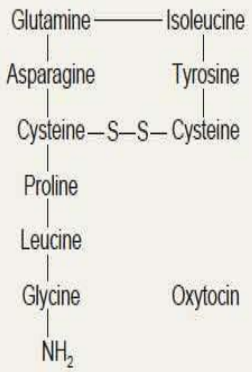
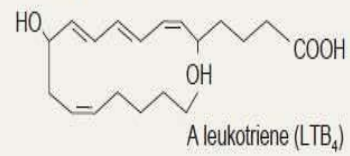
Group II:

1) cAMP: Adrinalin, Somatostatin, parathyroid hormone, Pituitary hormones such as ACTH, FSH, LH, TSH, HCG, α2-adrenergic catecholamines and β- adrenergic catecholamines.

2)cGMP: NO, ANF (atrial natriuretic factor)

3) Ca++/PI: Oxytocin, GnRH (Gonadotropin releasing hormone), Gastrin, Cholecystokinin, TRH (Thyrotropin releasing hormone), Substance P, PDGF (Platelet derived growth factor), Muscuranic ACh, α1-adrenergic Catecholamines

4) Kinase/Phosphatase: GH, EGF (Epidermal growth factor), Prolactin, Insulin

WATER-SOLUBLE		
Amines		
 Norepinephrine	Epinephrine and norepinephrine (catecholamines).	Adrenal medulla.
	Melatonin.	Pineal gland.
	Histamine.	Mast cells in connective tissues.
	Serotonin.	Platelets in blood.
Peptides and proteins		
 Oxytocin	All hypothalamic releasing and inhibiting hormones.	Hypothalamus.
	Oxytocin, antidiuretic hormone.	Posterior pituitary.
	Human growth hormone, thyroid-stimulating hormone, adrenocorticotropic hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, melanocyte-stimulating hormone.	Anterior pituitary.
	Insulin, glucagon, somatostatin, pancreatic polypeptide.	Pancreas.
	Parathyroid hormone.	Parathyroid glands.
	Calcitonin.	Thyroid gland (parafollicular cells).
	Gastrin, secretin, cholecystokinin, GIP (glucose-dependent insulinotropic peptide).	Stomach and small intestine (enteroendocrine cells).
	Erythropoietin.	Kidneys.
	Leptin.	Adipose tissue.
Eicosanoids		
 A leukotriene (LTB_4)	Prostaglandins, leukotrienes.	All cells except red blood cells.

Sequence of Events

Group I Sequence of events

1) Formation of Hormone-receptor Complex

2) Binding of Hormone – Receptor complex to Hormone receptor element (HRE).

3) Activation, Transcription and synthesis of protein.

4) Expression and Biochemical response.

Group-II

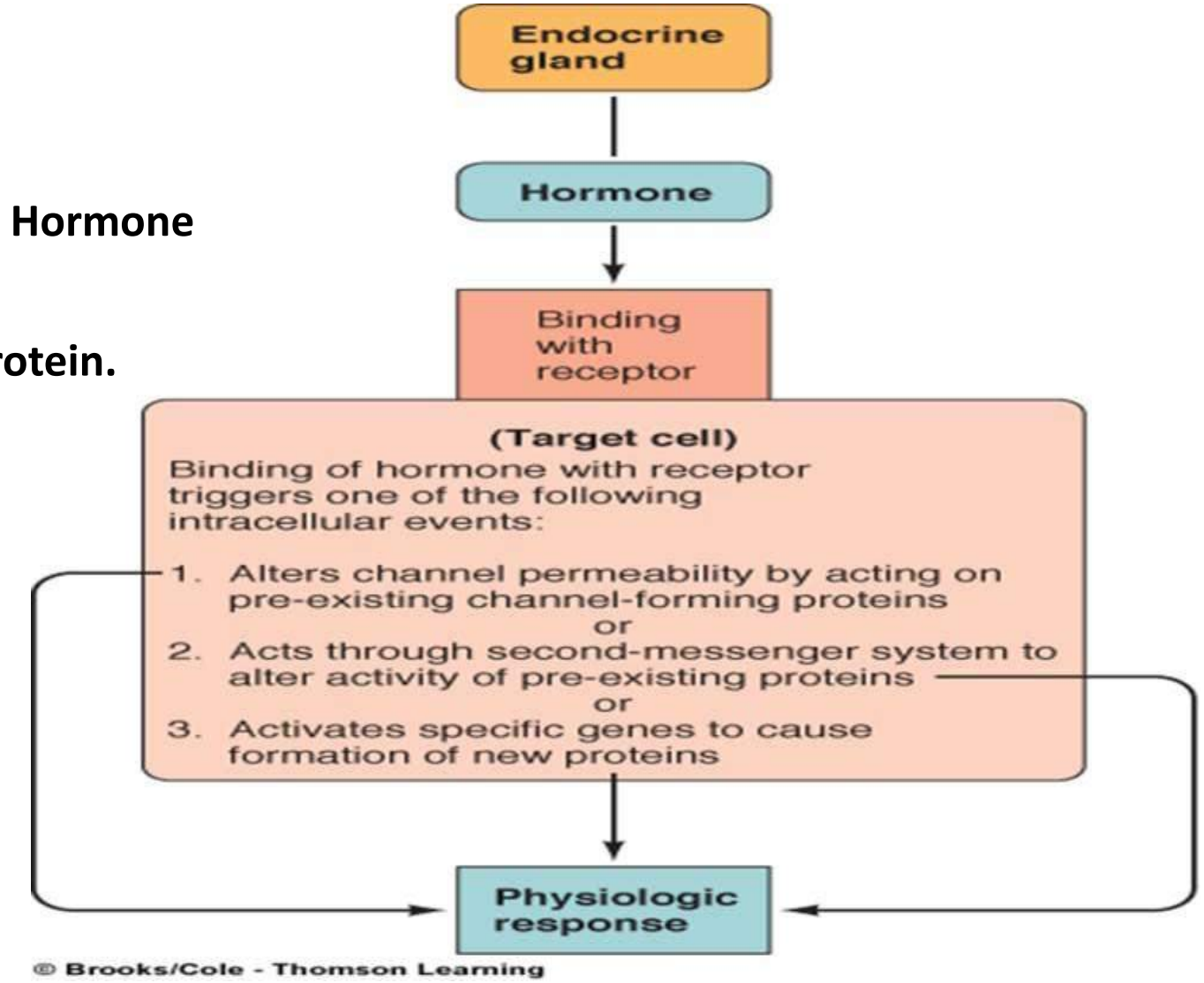
1) Bind to cell surface receptors

2) Activation of G-protein

3) Activation Protein Kinases

4) Phospho protein formation

5) Biochemical response



Secondary messengers

Hormones and Secondary messengers:.
Second messengers are the chemical molecules that relay signals received at receptors on the cell surface — such as the arrival of protein hormones, growth factors, etc. — to target molecules in the cytosol and/or nucleus.

amplify the strength of the signal causing some kind of change in the activity of the cell.

Earl Wilbur Sutherland Jr. discovered these secondary messengers for which he won the 1971 Nobel prize in medicine.

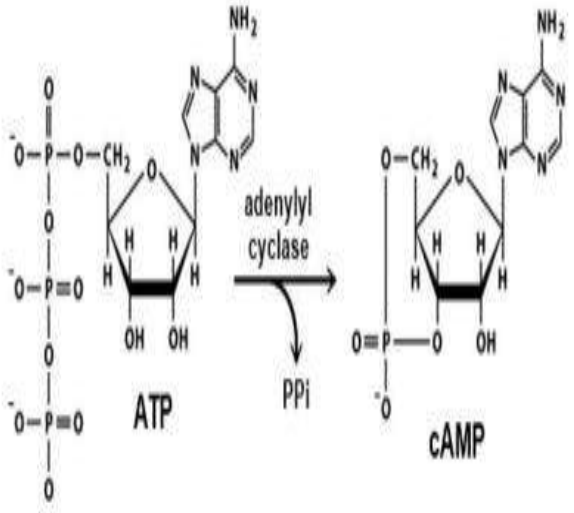
Earl Wilbur Sutherland Jr.

Secondary messengers

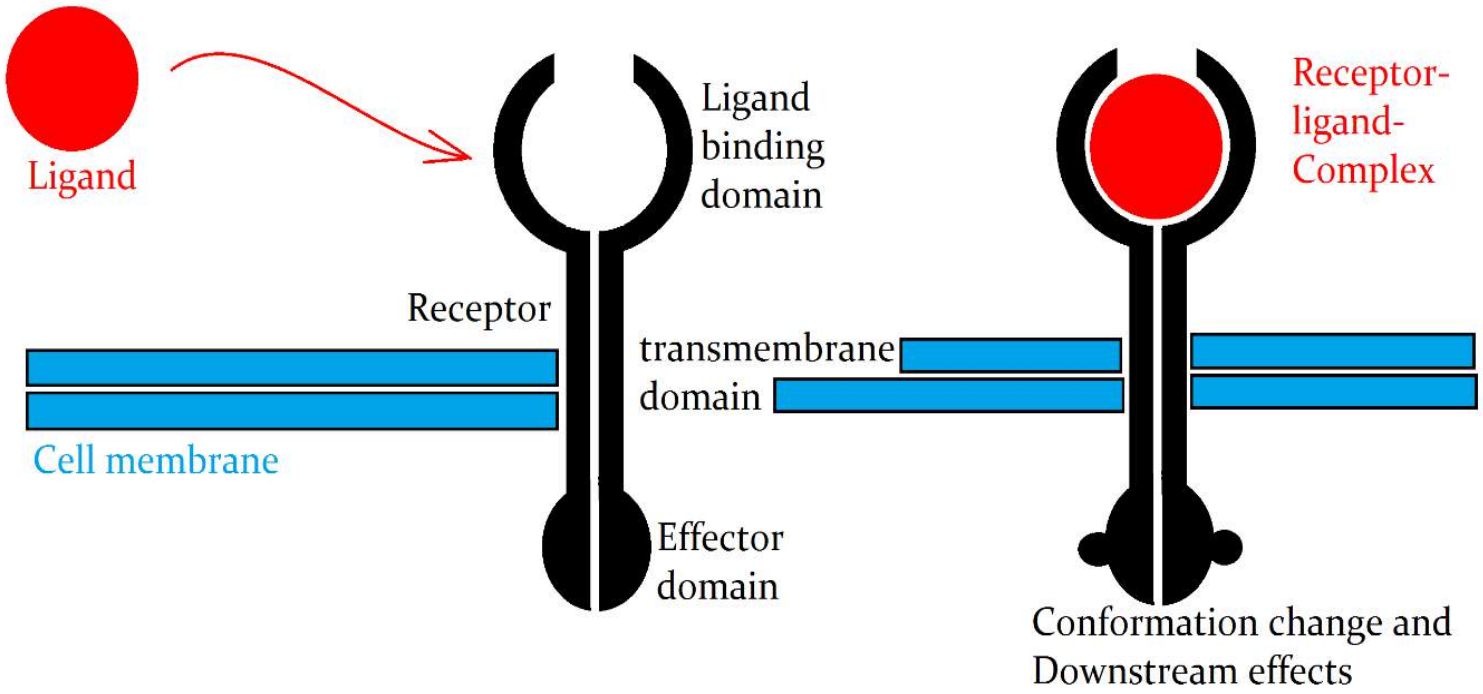
The second messenger may be:

1. Cyclic Adenosine Monophosphate (cAMP).
2. Cyclic Guanosine Monophosphate (cGMP).
3. Calcium or phosphatidyl inositol or both.
4. Protein kinase cascade.

The hormone is considered to be the first messenger

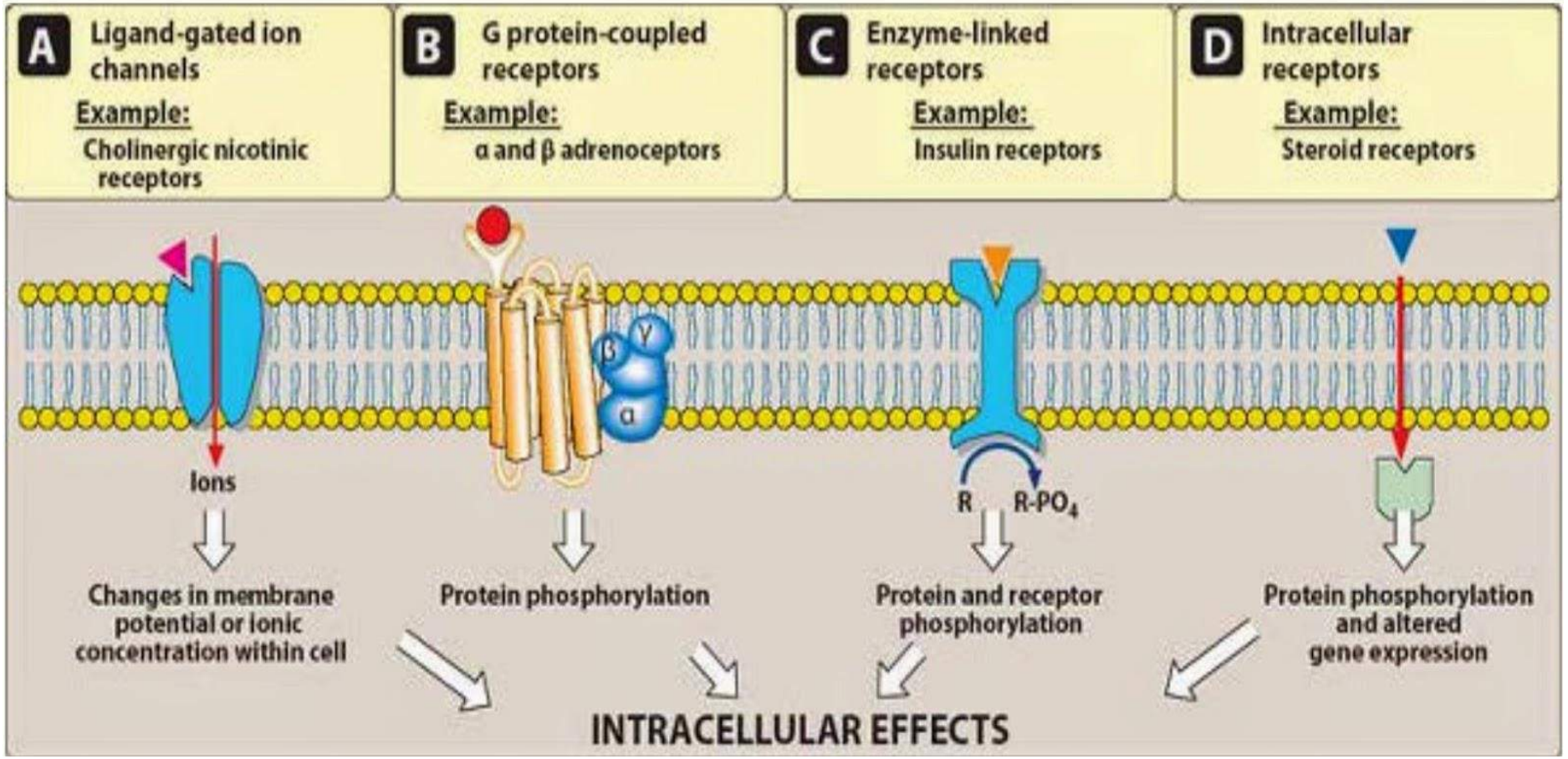


Hormone-Receptor Interaction



Specificity of Receptors

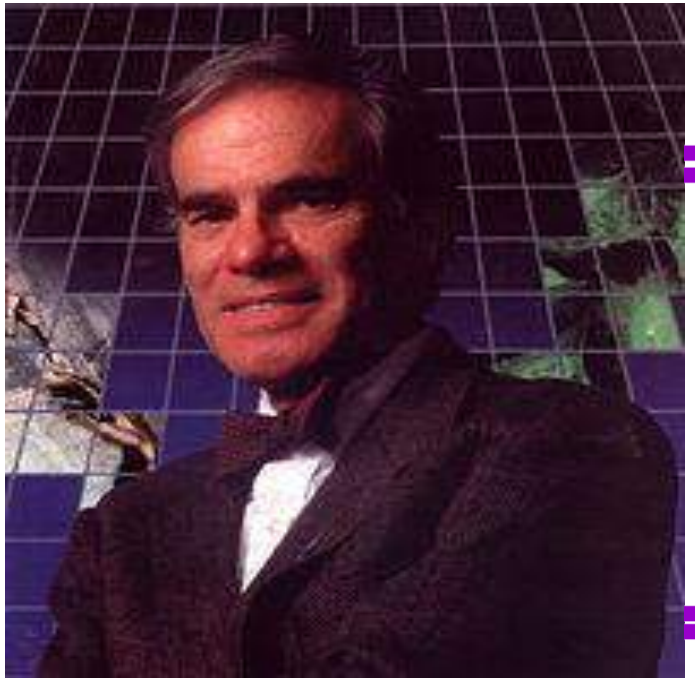
Receptor Families



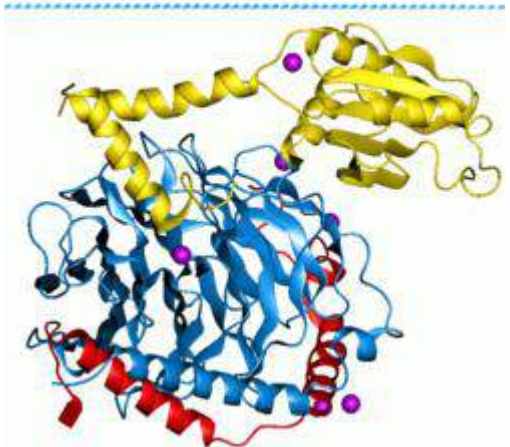
Transmembrane signaling mechanisms

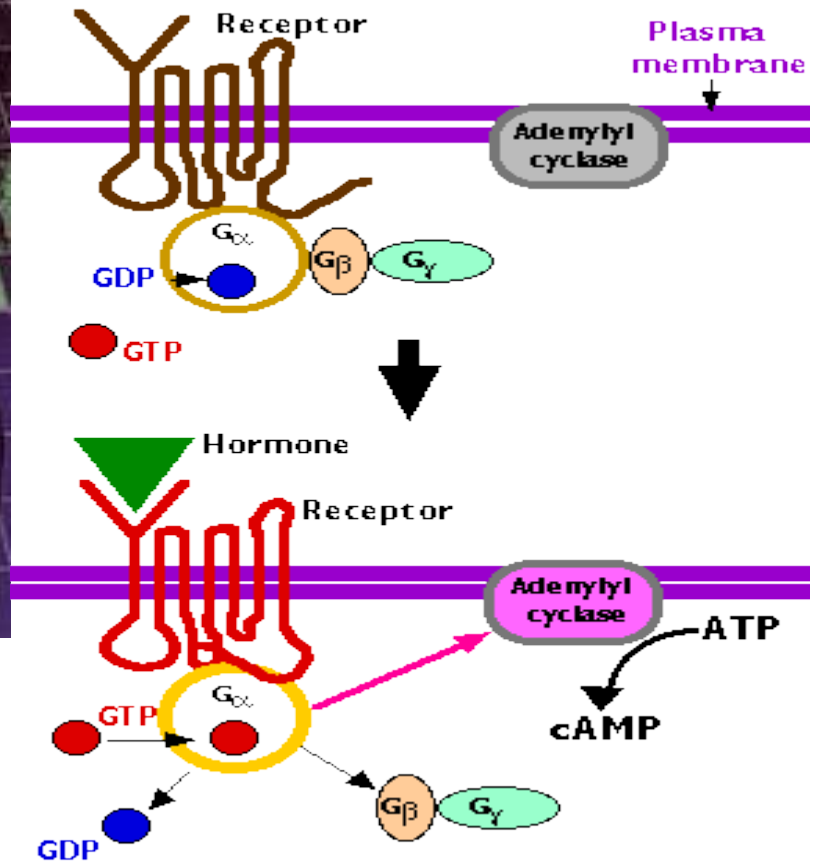
G protein

Alfred Gilman



Martin Rodbell

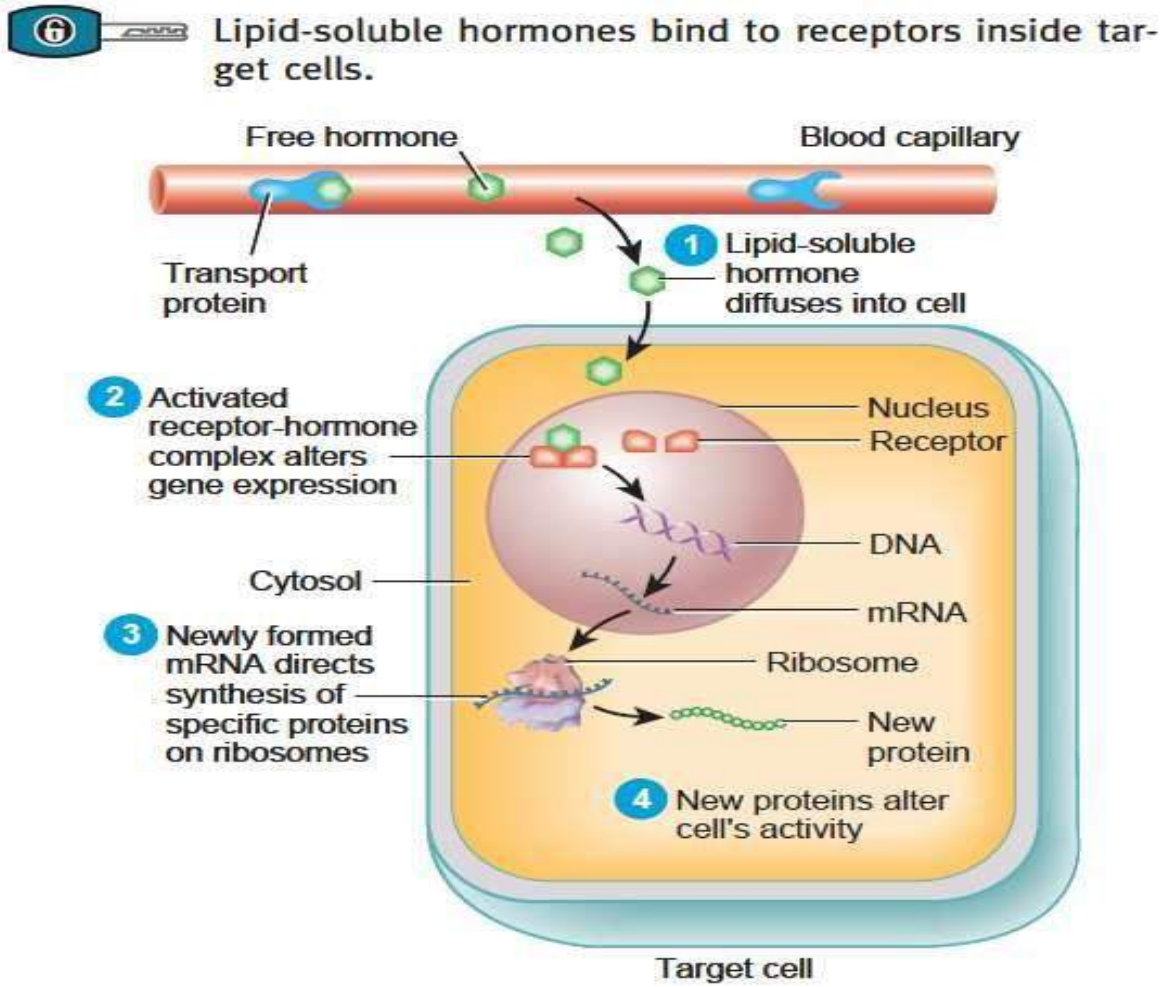




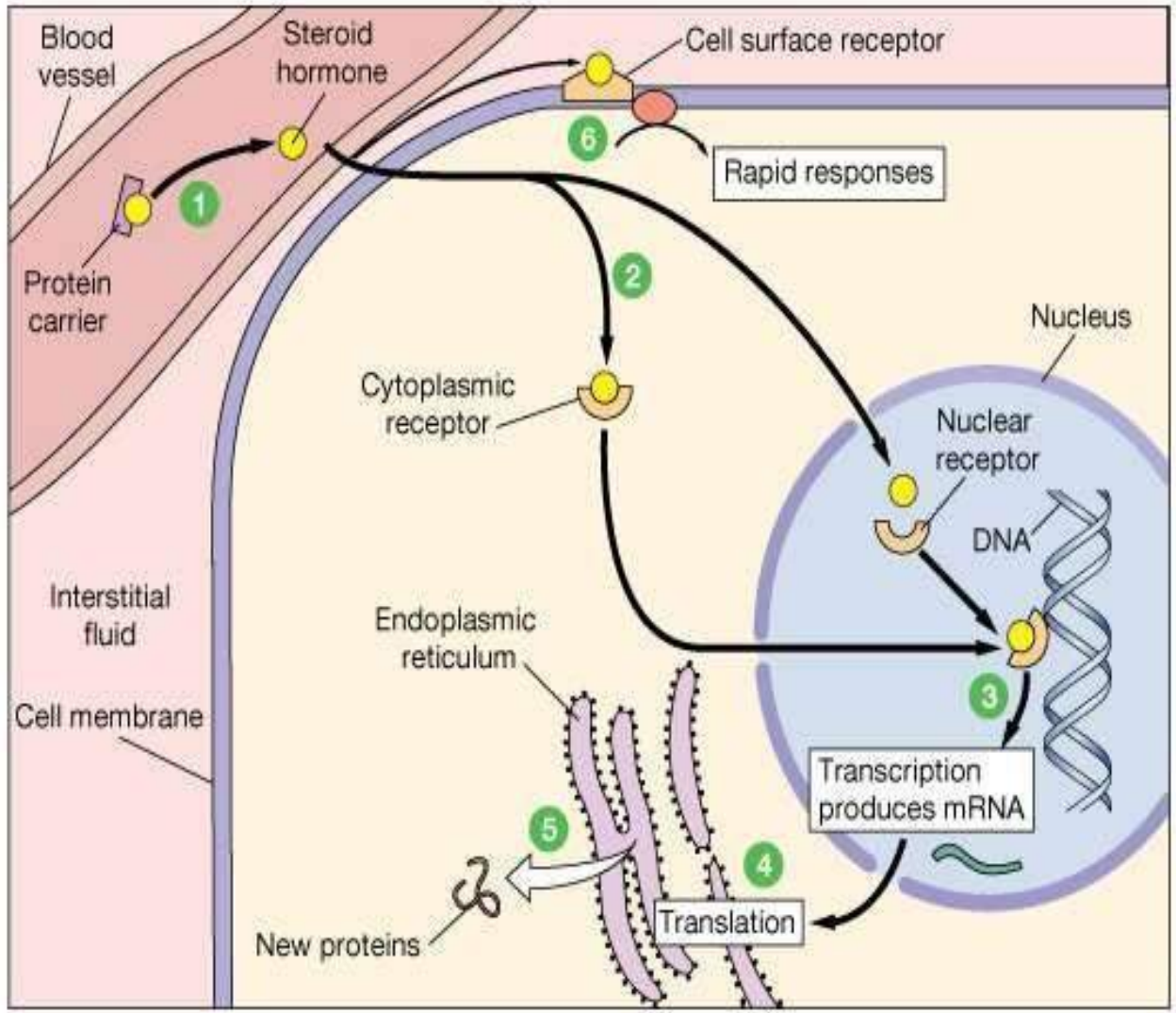
G Protein

How Lipid-soluble Hormones Work?

- **Binding to specific cell receptor in the cell membrane and form hormone-cell receptor complex, which diffuses to nucleus**
- **The receptor is eventually released for re-use**
- **Steroid activates a specific gene to produce mRNA**
- **mRNA pass out into the cytoplasm and initiates protein [enzyme] synthesis**

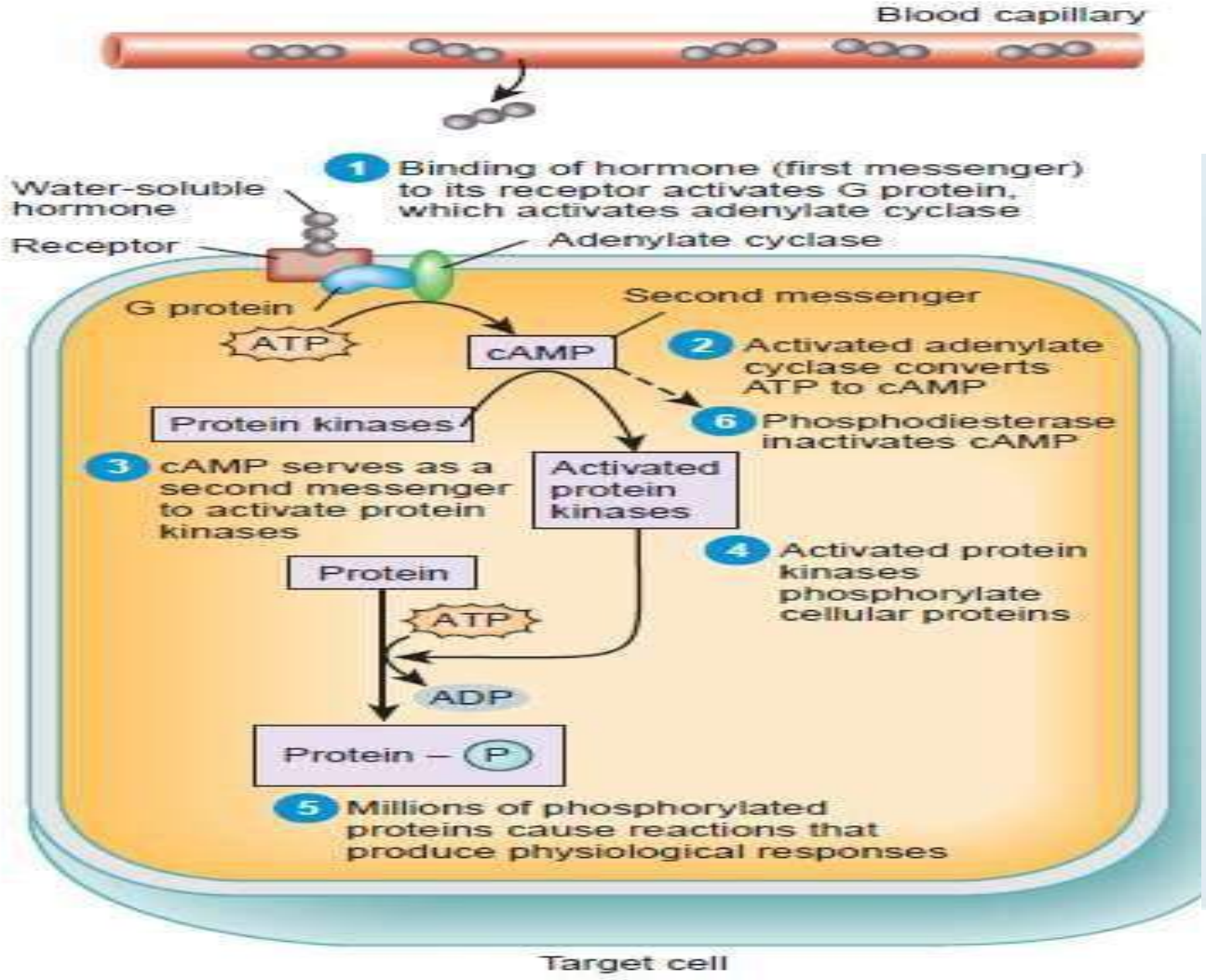


Steroid Hormones: Molecular Action



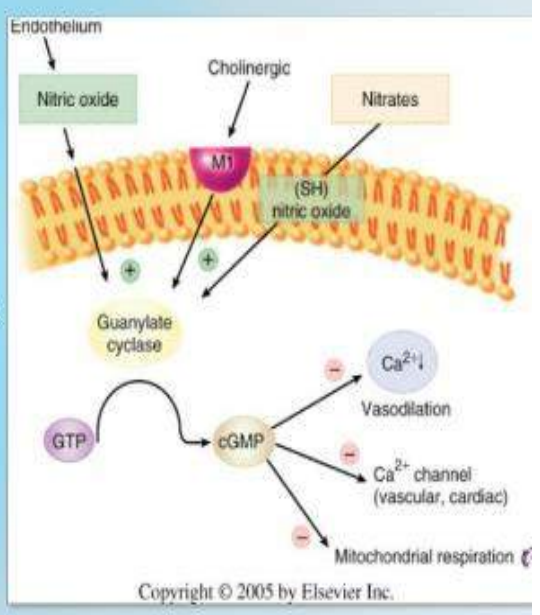
1. Most hydrophobic steroids are bound to plasma protein carriers. Only unbound hormones can diffuse into the target cell.
2. Steroid hormone receptors are in the cytoplasm or nucleus.
3. The receptor-hormone complex binds to DNA and activates or represses one or more genes.
4. Activated genes create new mRNA that moves back to the cytoplasm.
5. Translation produces new proteins for cell processes.
6. Some steroid hormones also bind to membrane receptors that use second messenger systems to create rapid cellular responses.

How Water-soluble Hormones Work?

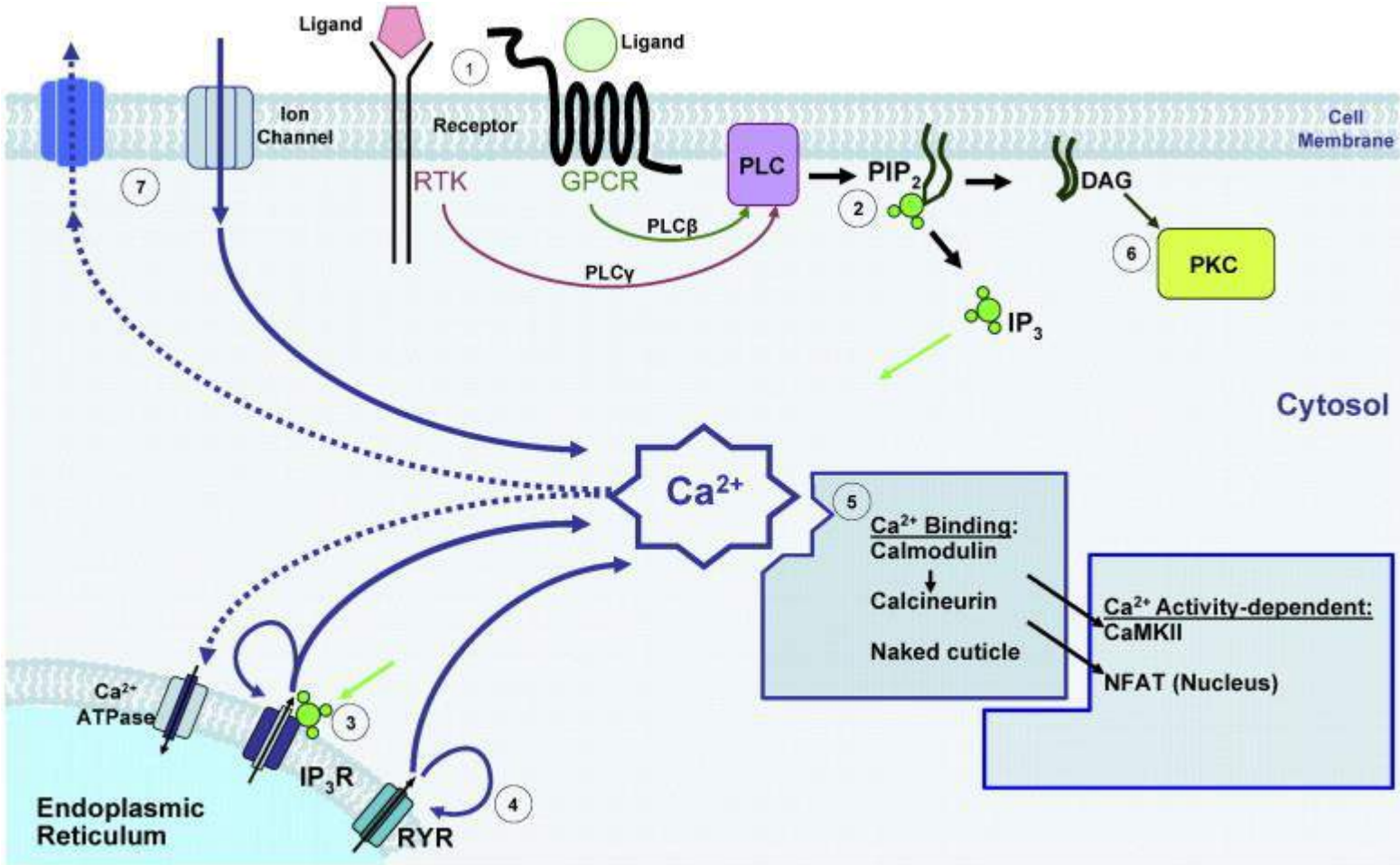


Cyclic GMP (cGMP)

- Cyclic GMP is synthesized from the nucleotide GTP using the enzyme **guanylyl cyclase**. Cyclic GMP serves as the second messenger for :
- atrial natriuretic peptide (ANP)
- nitric oxide (NO)
- the response of the rods of the retina to light.
- Some of the effects of cGMP are mediated through **P**rotein **K**inase **G** (**PKG**) — a c**G**MP-dependent protein kinase that phosphorylates target proteins in the cell

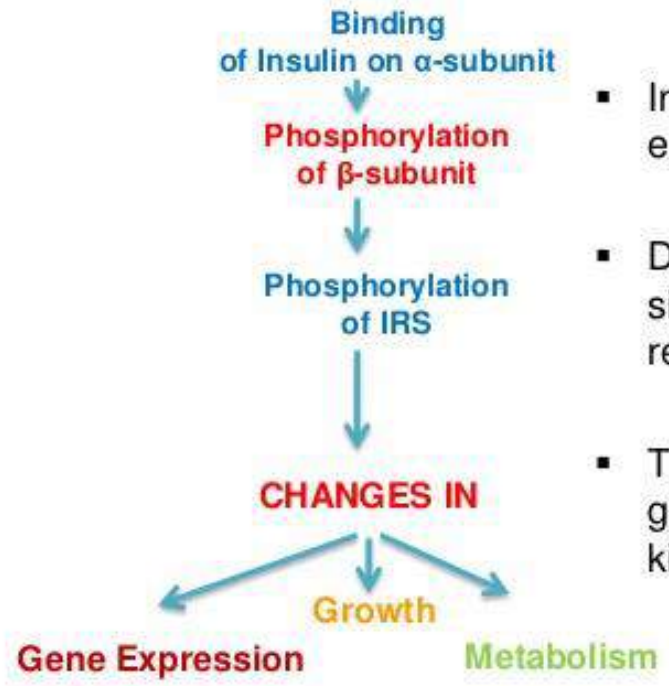


Mechanism of Ca++/PI



Kinases/Phosphatases cascade

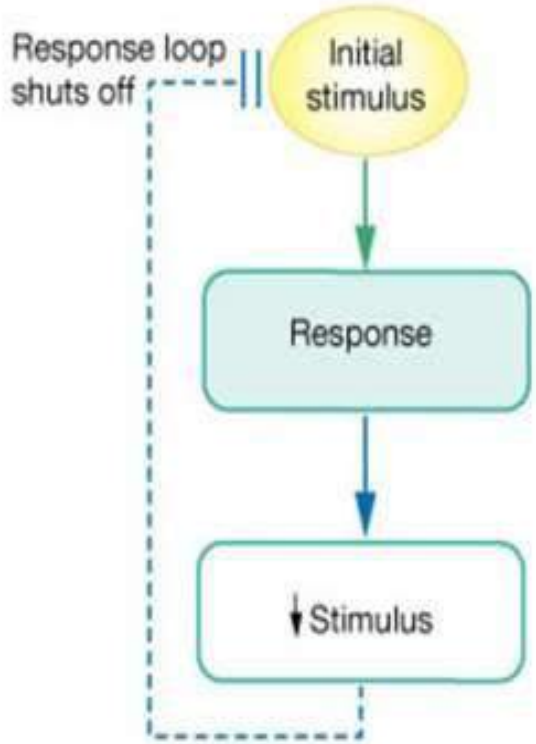
Mechanism of action of insulin



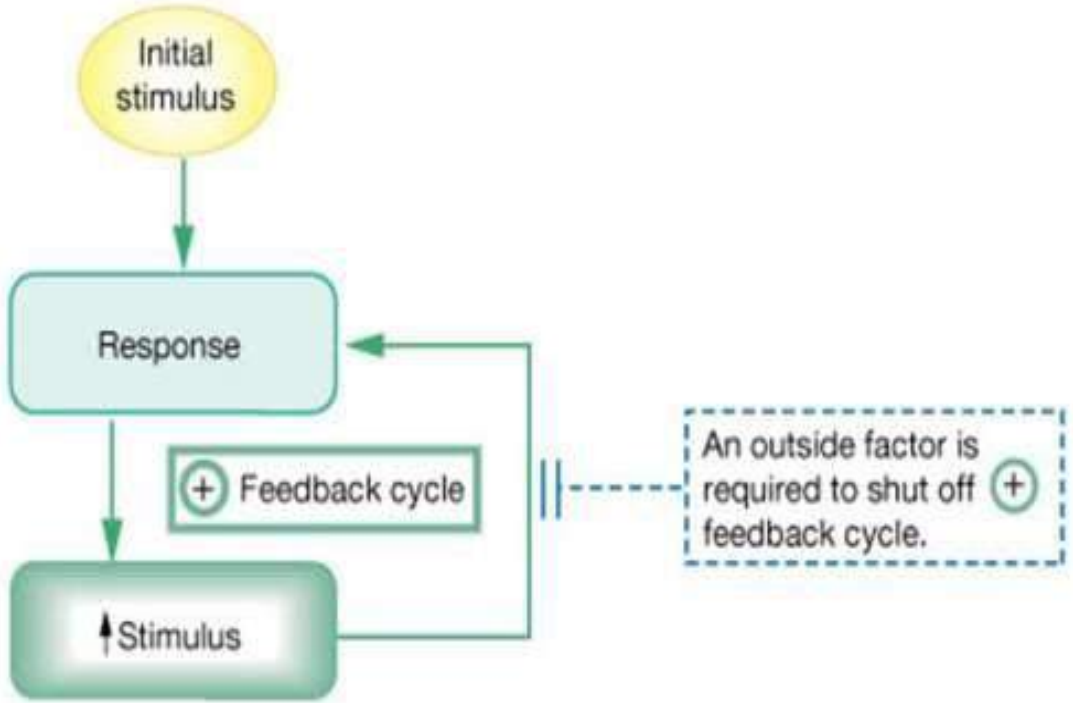
- Insulin regulates both metabolic enzymes and gene expression.
- Does not enter cells, but initiates a signal that travels from the cell surface receptor to -cytosol and to the nucleus.
- The insulin receptor (INS-R) is a glycoprotein receptor with tyrosine kinase activity.

FEEDBACK CONTROL

(a) Negative feedback: the response counteracts the stimulus shutting off the response loop.



(b) Positive feedback: the response reinforces the stimulus sending the parameter farther from the setpoint.



Summarize!!

- hormones bind with specific receptors on target cells which further proceed to trigger intracellular signals either by altering membrane permeability, acting through second messenger system or activating specific genes to form new proteins.
- Starting with receptors, they are specific for every hormone.
- Present in or on the surface of the cell membrane. g. protein or peptide hormones and catecholamines.
- In the cytoplasm. E.g. steroid hormones; Or
- In the cell nucleus. E.g. thyroxine.
- Furthermore, hormones can be divided into groups on the basis of their chemical nature:
- Peptide, polypeptide, protein hormones.
- Steroids
- Iodothyronines (thyroid hormone).

Assessment Time!!

- Steroid hormones increase cellular activities by binding to DNA and forming gene-hormone complex
- The hormone response element is located on DNA
- Binding of hydrophilic hormones lead to the activation of secondary messengers
- Water soluble hormones exhibit the shortest Half life
- Calmodulin is a Calcium binding protein

Student Assignment

- Understand and list out the various mechanisms of Hormones action.
- Write the sequence of steps involved in the mechanism of protein hormones.
- Prepare a chart on Hormones classification.

Topic: Migration of Birds and Scientific Reasons

Presented by

Mrs. K. Srilatha

TSWRAFPDCW, Bhongir

OBJECTIVES

- Migration Facts
- Why they migrate?
- How they migrate?
- Factors affecting the Birds Migration
- Threats during migration
- Birds migrate to India
- Preventive measures
- Summary

- Migration – Latin ‘Migrara’.
  Cahn – Periodic passage of animals from one place to another.
  William Rowan – Movement of Birds - Lamarckism.

Resident birds

Passer domesticus (Sparrow)

Colinus virginianus (Bobwhites)

Pteroclidae (Sandgrouse)

Migratory birds

Tyto alba (Barn owl)

Branta Canadensis (Geese)

Sialia (Blue birds)

Migration Facts:

- The Arctic tern - 20,000 miles/year - Arctic to Antarctic and back.
- The Sandhill and Whopping cranes - 2,500 miles.

How did they keep going?

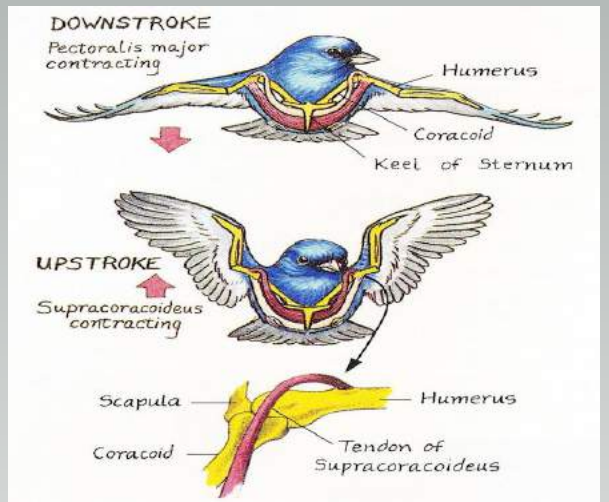
- Special high energy fat beneath the skin - Soaring raptors.
- Cranes eat along their migration route (Stopover).

How high they can fly?

- Higher than Mount Everest.
- Bar headed Gees - across the Himalayas at 29,000 feet.

WMBD – Second Saturday of May or October

Flight Adaptations in Birds:



✓ **Morphological Adaptations:**

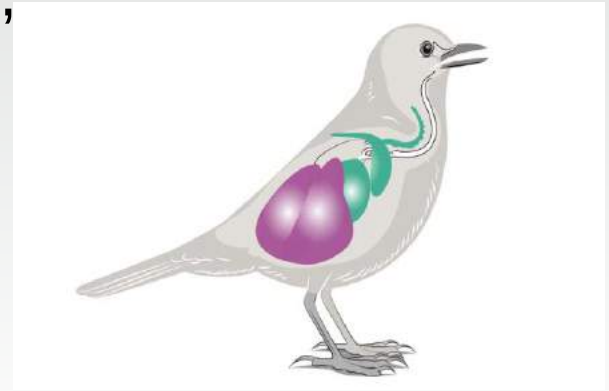
- Body contour, Feathers, organs of Flight, Perching and Tail.

✓**Anatomical Adaptations:**

- Flight muscles, Lightness and rigidity of Endoskeleton

✓**Physiological Adaptations:**

- High fat metabolic rate, Double respiration, Homeothermy, Absence of Gall bladder, Sense organs and Homeostasis



Why do birds migrate?

How do they migrate?

Which factors effect the migration?

Why do Birds Migrate?

- Scarcity of food
- Climate change
- Natural selection
- Instinct and gonadal changes

Scarcity of Food

- Shortage of food and water
- It may happens at anytime in a year

Climate Change

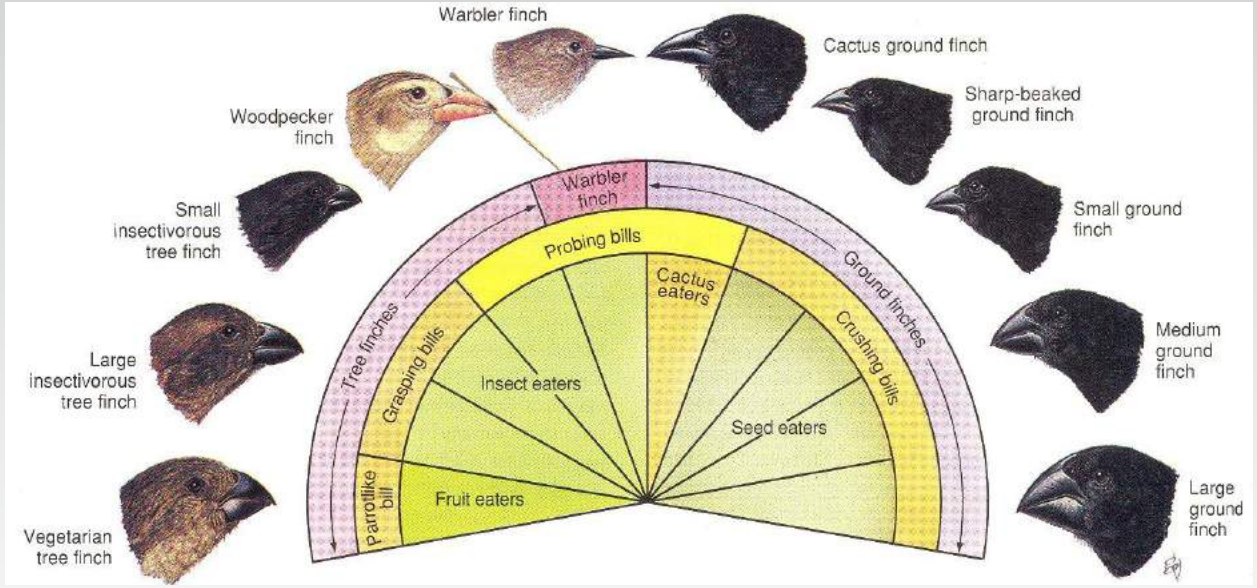
- Warming temperature/ Thermal currents.

(“Thermal soaring” used by birds to lift it high in the air)

- Shifting seasons
- Changing precipitation
- Rising sea level
- Sudden rise in barometric pressure

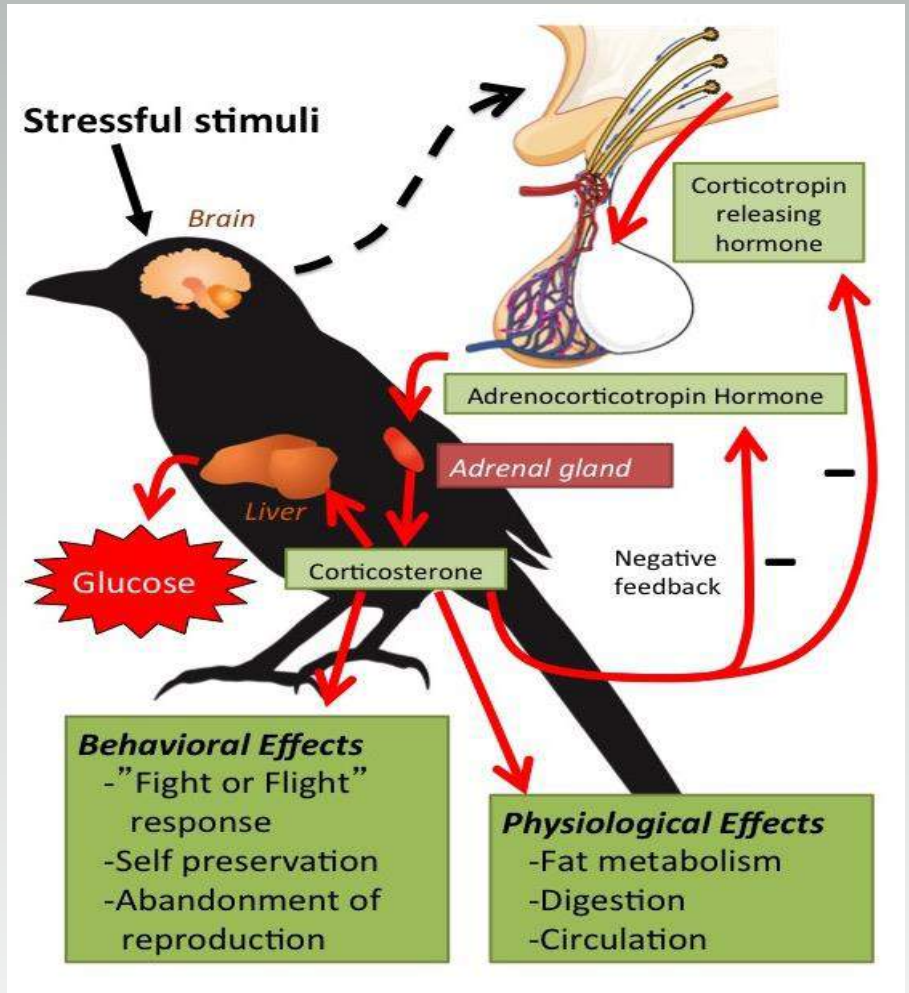
Rapid Natural Selection

- Study of Darwin finches and kind of natural selection causes Evolutionary change.



Instinct and Gonadal changes

- Maturation of sex organs triggers to reach the breeding area.
- Decrease of sunlight stimulates Pituitary gland (Prolactin) and Adrenal gland (Corticosterone).
- Thyroid-metabolism
- Circadian and Circannual rhythms -Pineal gland.

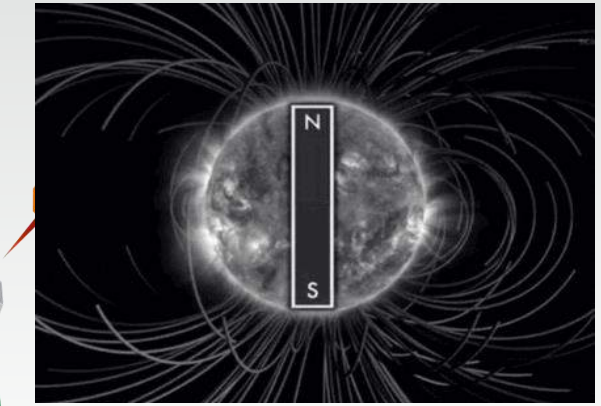


How do migratory birds find their way ?

Magnetic Sensing

Geographic mapping

Stars orientation



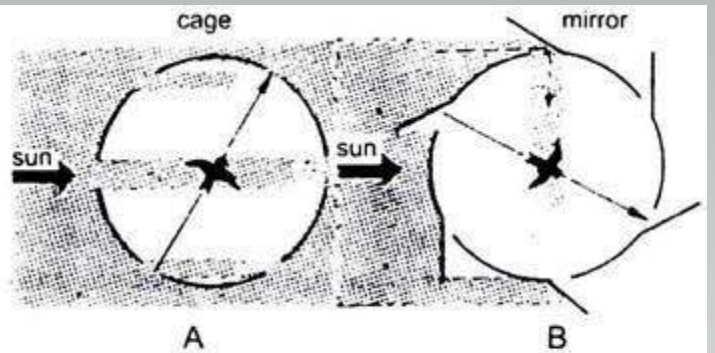


Fig. 28.9. Gustav Kramer's experiment with starlings to show that day migrants use sun as a compass.

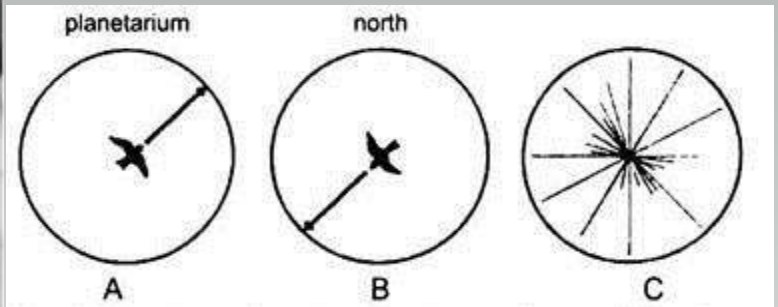
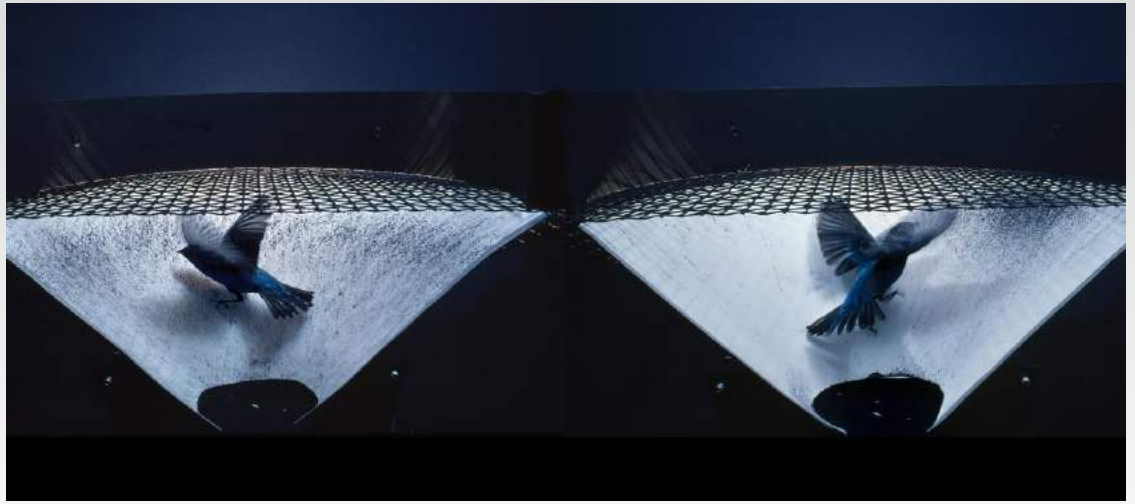


Fig. 28.10. Franz Sauer's experiment with warblers in a planetarium to show that night migrants use sun as a compass.

Vertebrates book, R.L.Kotpal



Emlen's experiment on European Robins

Peter friederici, Audubon magazine, 2015

Latitudinal

Pluvialis dominica (American golden plover)

Longitudinal

Charadrius falklandicus (Patagonial plover)

Altitudinal

Scolopax (Common wood cock)

Partial

Cyanocitta cristata (Blue jays)

Irregular

Cuculidae (Cuckoos)

Seasonal

Anser (Gees)

Diurnal

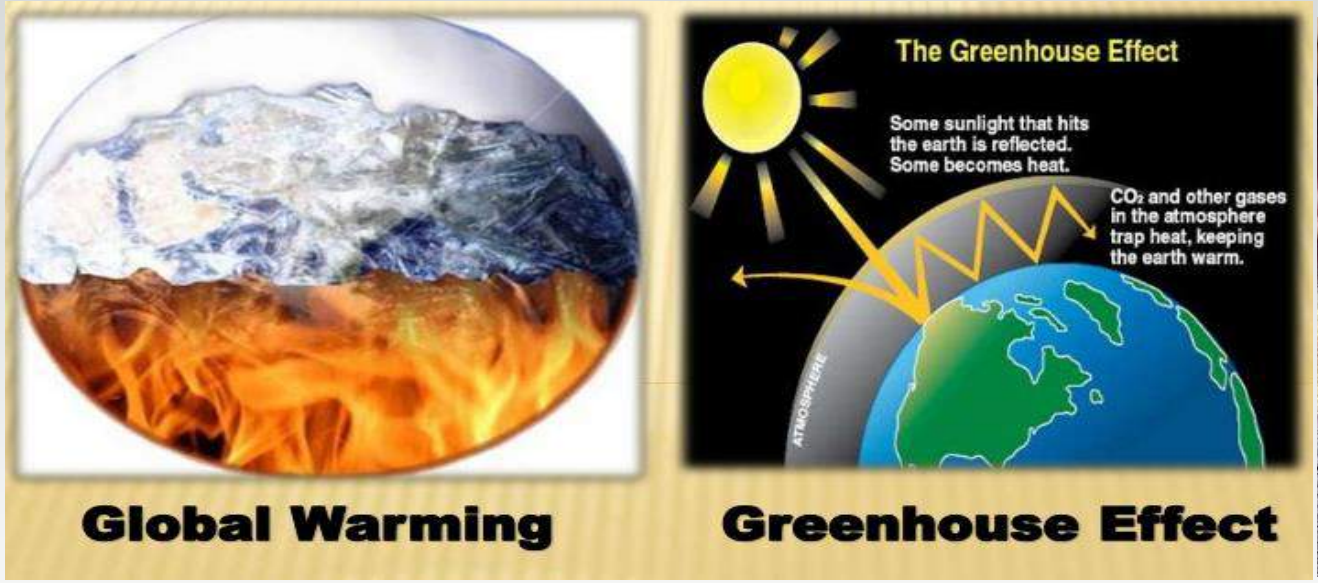
Gyps indicus(Vulture)

Nocturnal

Tyrannidae(Fly Catcher)

Factors that effect migration:

- Pollution
- Human intervention
- Invasive alien species
- Global warming
- Photoperiodism
- Diseases: Bird flu, SARS, Avian pox etc.,



Deadly Corona virus attack in china

Threats during migration:

- Light pollution in cities.
- Hunting as well as poaching.
- Collision with windows, buildings, power lines etc…
- Sudden change in climate may hurts.
- Inadequate food and lack of energy to travel.

A rare guest at Kolleru Lake

The lone great white pelican must have missed its flock, says expert

T. APPALA NAIDU
ATAPAKA

The lone great white pelican (Pelecanus onocrotalus), found mingling with thousands of winged guests at Kolleru Lake in Krishna district, Andhra Pradesh, offers a rare visual treat to bird watchers.

In the past week, the feathered guest has been found with the grey pelicans and painted storks at the Atapaka Bird Sanctuary in the Kolleru Lake, earning its prey in the water body. Forest department's boatman G. Suresh, who noticed its presence from at least 200 metres, has been involved in studying its movements.

"Those who see the white pelican at the sanctuary are being considered lucky. I have been fortunate to see it many times this winter. It is a distinctive beauty to watch compared with the other migratory birds," Mr. Suresh told *The Hindu*.

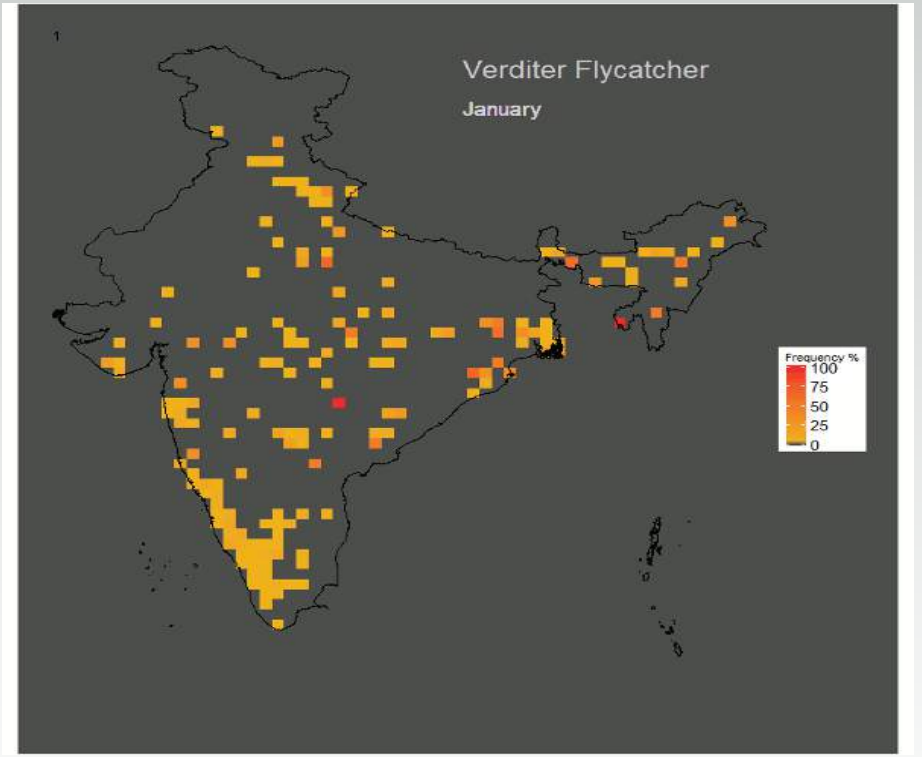
The white pelican which is also known as rosy pelican is almost double the size of the grey pelican. "The sighting of the white pelican is very rare in the Kolleru Lake. We documented it in 2008 and 2013. In 2013, a pair of white pelicans was sighted at the sanctuary which is very rare in the wetlands in South India," said P. Gracious, an authority on the Kolleru Lake. He said the bird could be treated as 'Passing Migrant' and must have missed its flock. "The lone white pelican has never been seen breeding or nesting in the Kolleru Lake till date," confirmed Mr. Gracious.

Seeking company: A Great White Pelican (at left) at Atapaka in Kolleru . ▪T. APPALA NAIDU

THE HINDU, 24/1/2017

T.Appalanaidu, 2017

Birds Migrate to India



Siberian crane

From : Russia
Locality: Rajasthan
Season: Winter
Reason: Breeding

Blue Throat

From : Europe
Locality : Rajasthan
Season : Winter
Reason : Breeding

Rosy Starling

From : Europe
Locality : Telangana, Kerala, Andhra Pradesh, Karnataka, Maharashtra andTamilnadu
Season : Winter
Reasons : Food starvation

Greater Flamingo

From : Europe and Africa
Locality: Gujarat
Season : Winter
Reason : Breeding

conserve

The vulture ambassador

Through an upcoming carnival, Raghunath Krishna hopes to spread the message about the disappearing raptor

COME December and children and elders alike, will get a chance to learn about the scavenger commonly known as the vulture. And no, there will be no classrooms or seminars or notes handed out. Raghunath Krishna, founder of Pencilsrock Academy, and his team of volunteers will teach through games, art, tattoos and more. An initiative by Pencilsrock Academy and the Madras Naturalists' Society, the carnival is being conducted for the Coimbatore-based NGO Arulagam, whose mission is to spread awareness about the bird.

"When you draw an elephant, do you know why you are drawing its legs straight instead of at an angle, like you would for other animals?" asks Krishna, hinting at how children tend to ask questions when they draw, thereby making art an effective means to teach. "You cannot 'tell' children something and expect their attention," he says, adding "you have to explain and satisfy their curiosity." The artist and animator found himself drawn to wildlife as a boy and joined wildlife census projects to learn about them.

But this carnival is not just about getting children to learn about the vulture and its role in the ecosystem. In addition to roping in aeronautical engineers to demonstrate how thermal columns (rising columns of warm air; vultures use these while gliding) work, day one of the three-day carnival will feature a drawing competition for different age groups. The winners of this competition will get to travel to the Niligiri biosphere and educate the tribal children there, while getting to learn about their way of life as well. After all, conservation is for the benefit of the next generations.

The carnival, which will be conducted in Coimbatore next, is tentatively scheduled for the end of December. Details: 8870643761

— RP

White Rumped Vulture Spotted in City after 20 Long Years

by Sonali Shenoy

Chennai: You may blink at the name. But for the birding community of Chennai that completed the 9th edition of the Chennai Bird Race on Tuesday - not even a new Rajinikanth release could have been more exciting.

"The last time we saw a White Rumped Vulture in Chennai was 20 years ago," says G Vijay Kumar, secretary of the Madras Naturalists' Society.

And the spotting at Pulicat lake certainly didn't come easy. Sixteen-year-old Vikas Madhav recalls, "We woke up at 3 am in order to be at Pulicat by 6 am. So it took quite a bit of planning and will power to do it on a day when all my classmates from school were fast asleep."

The rest of the three-member team comprised Vikas' mother Prasanna Sriya and fellow bird enthusiast, 18-year-old Kedar Baskar.

Other rare spottings as part of the competitive event held on Republic Day were the White Bellied Sea Eagle, Bar Headed Goose and Red Crested Pochard that were seen at the Kelambakkam backwaters and Pulicat lake.

While rejoicing was aplenty on Tuesday evening as the teams gathered together after their day out with the city's avian life, there was one dismal note.

Although 43 teams were registered initially, only 18 teams showed up, a member of the Madras Naturalists' Society told *Express*. "This can possibly be attributed to a lot of school and college students dropping out as they have been having extended sessions in class post the flood," Vijay explains.

However, given that 50 per cent of the 72 participants in the event were in their teens, it is safe to say that bird watching is 'trending' in Chennai.

Zai Whittaker, grand niece of famed ornithologist Salim Ali graced the function that concluded the event as special guest.

Association for the conservation of Indian raptors, 2015-16

The State of India's Birds 2020 Report - 13th COP Convention on the Conservation of Migratory Birds Gandhinagar, Gujarat

✓ Massive database (>10 million) observations uploaded by 15,000 birdwatchers on 'e-bird' platform.

✓ It is India's first of its kind report

Category	No. of Birds
High Concern	101
Moderate Concern	319
Low Concern	442

(project started from May 2018 & report was released on February 17, 2020 in 13th COP.

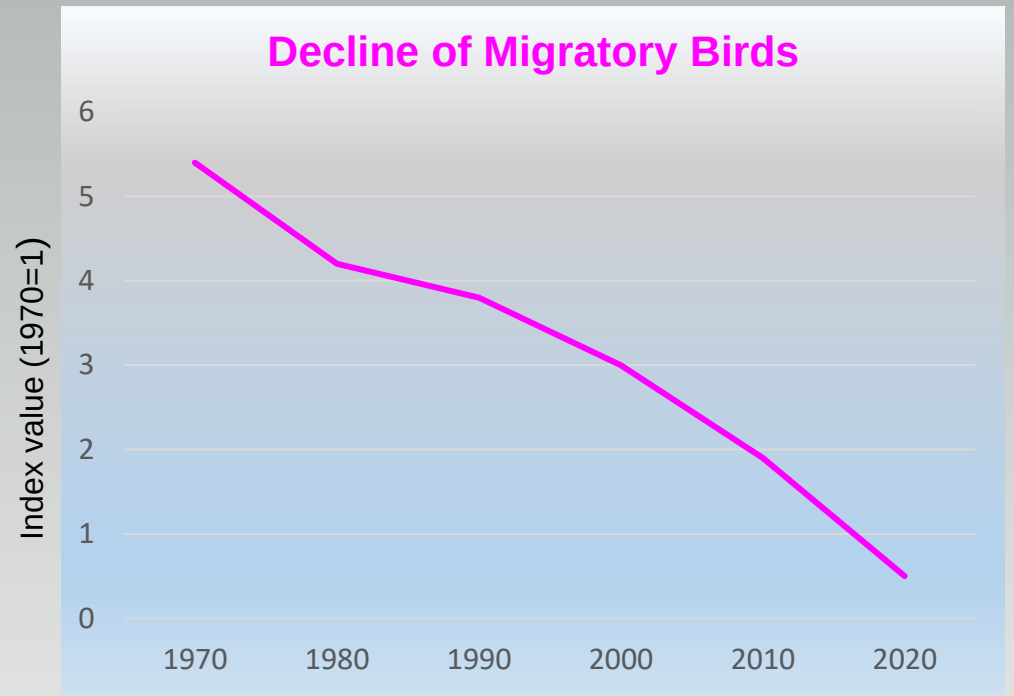
↓ Shorebirds, Raptors, Indian Vulture, Large-billed Lea Warbler, Curlew Sandpiper, Richard's Pipit and While-rumped Vulture.

Migratory Birds

↑ Glossy Ibis, Rosy Starling, Ashy Prinia, and Feral Pigeon

Preventive measures

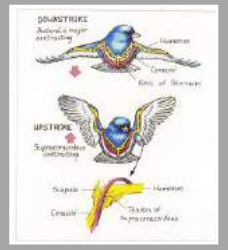
- Reduce the pollution.
- Avoid the chemical pesticides.
- Plantation.
- Protect birds from pets.
- Clean your bird feeders.
- Prevent collision with your windows.
- Slow down when you are driving.



Migration of birds

Scientific reasons

Why they migrate?



How they migrate?

Factors effect migration

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- Books

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- Articles

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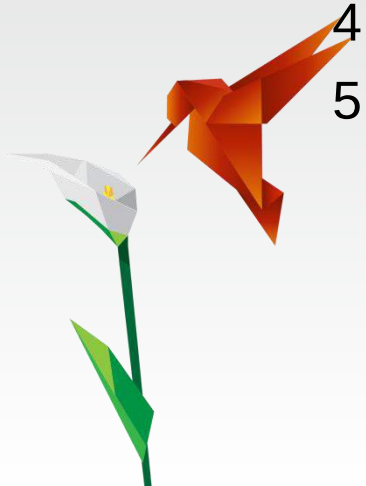


JAI HIND

Name: Dr. K. Srilatha, HOD
subject: Zoology
Topic: Parasites and Diseases

Topic

Parasites And Diseases

definition

- Para-on the others , sitos-site of from Greek language
- A Parasite is an organism that lives with in or on host . The host is another organism

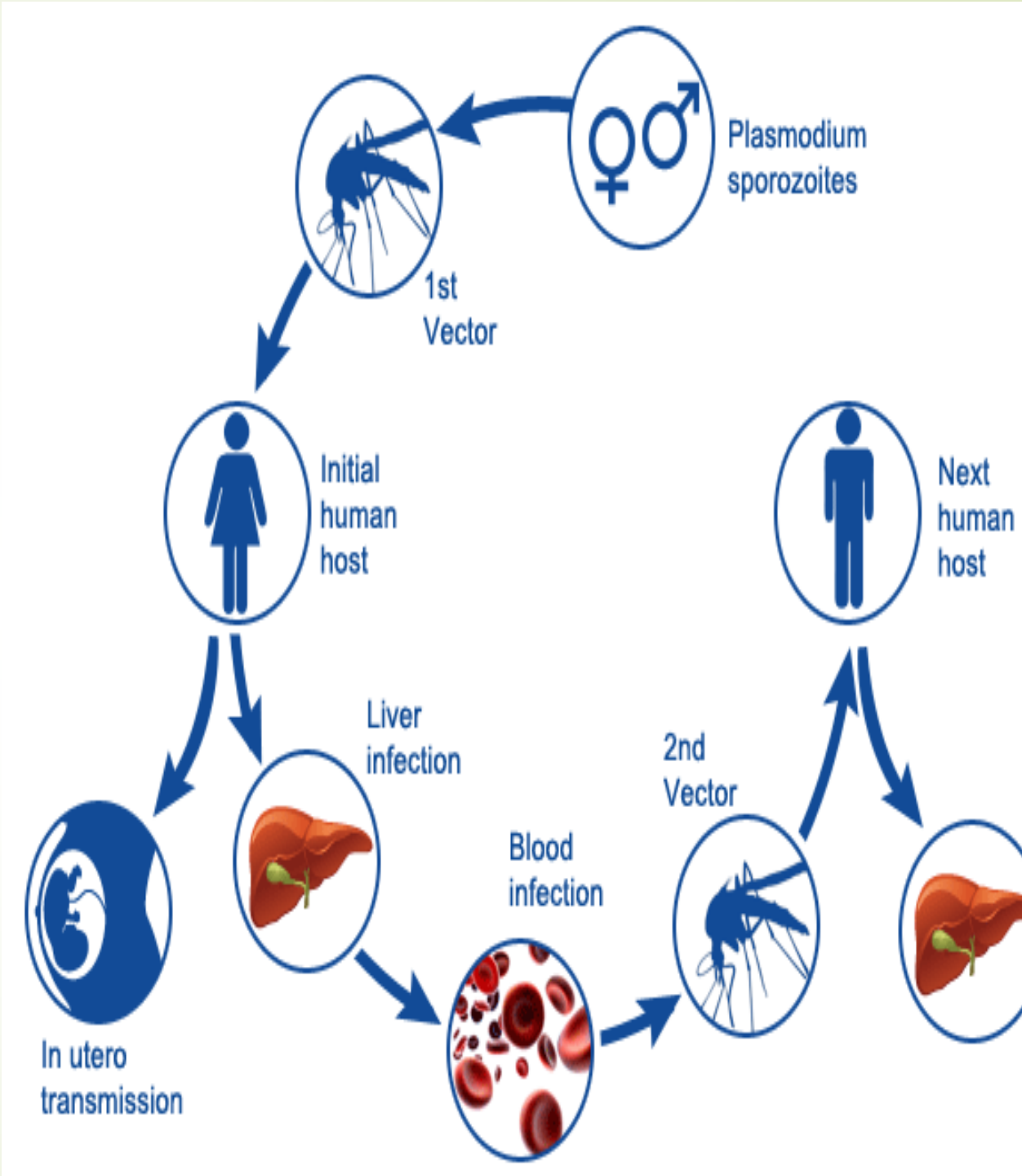
occurance

- Parasites vary widely.around 70% or not visible to us such as malarial parasite but some worms can

reach over 30m length

- Parasites are not a disease but they spread diseases different parasites have different effects

- They can spread through contaminated food, blood,water and some parasites by sexual contact



Types of parasites

Ectoparasites:

which lives on host body

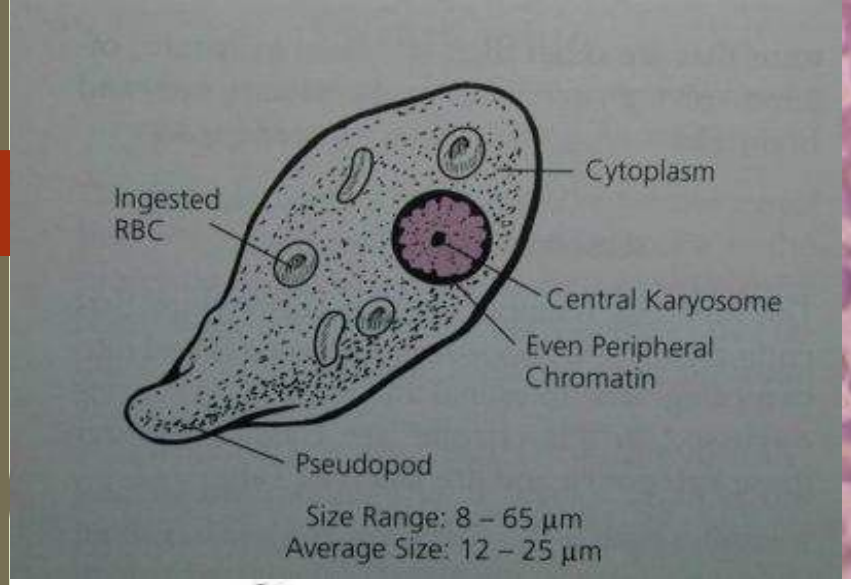
EX:lice on human,ticks on dogs

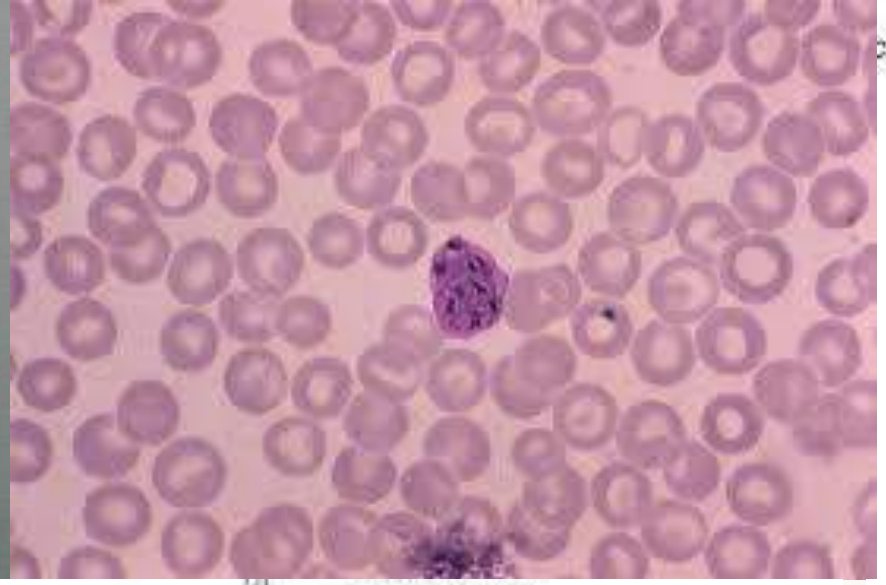
Endoparasites:

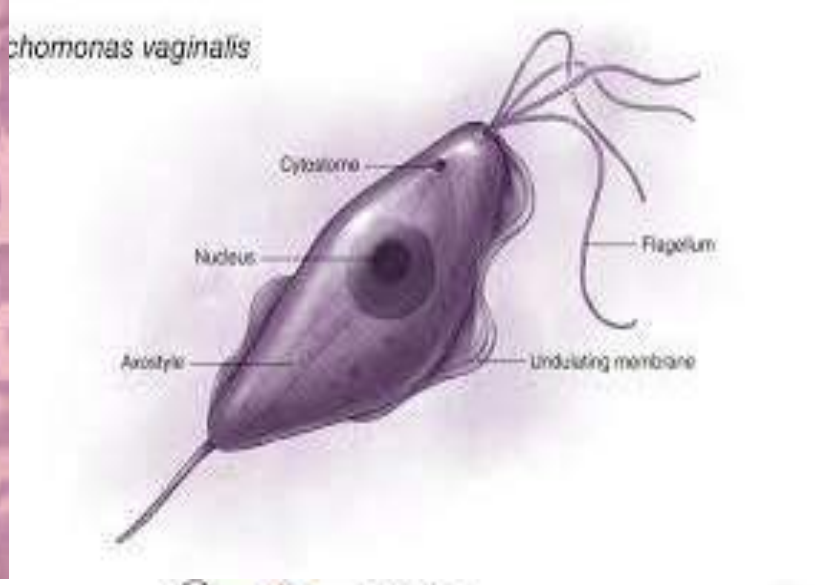
which lives inside the host body

EX:plasmodium

parasites	***Causing diseases***
Entamoeba histolytica	Amoebic dysentery
Plasmodium malariae	Malaria
Trichomonas vaginalis	Trichomoniasis
Trypanosoma gambiense	Sleeping sickness
Ascaris lumbricoides	Ascariasis
Wuchereria bronchofti	Filariasis







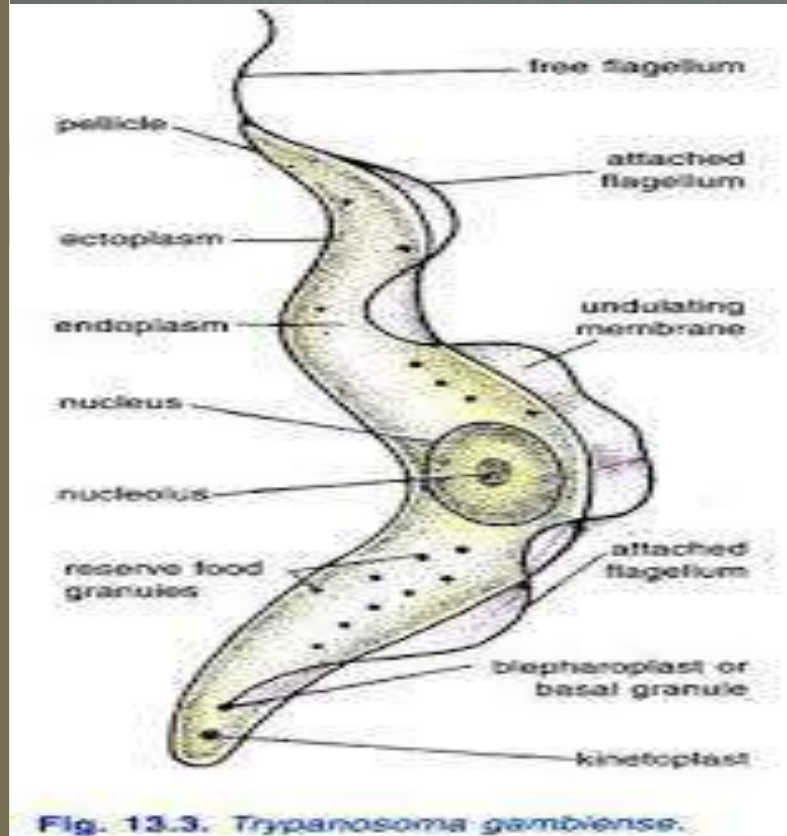
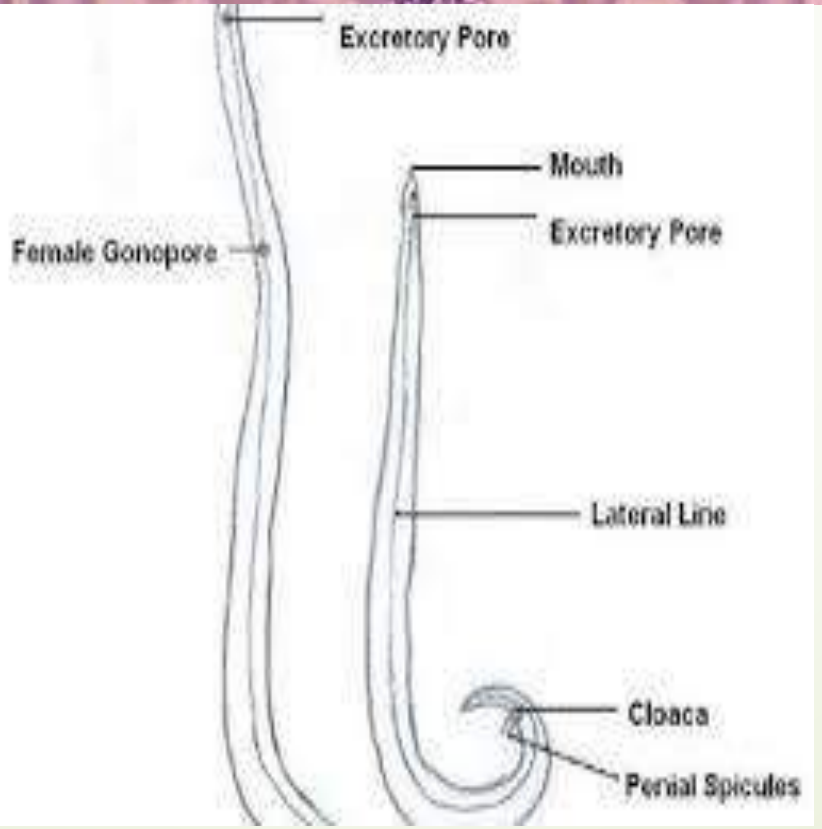
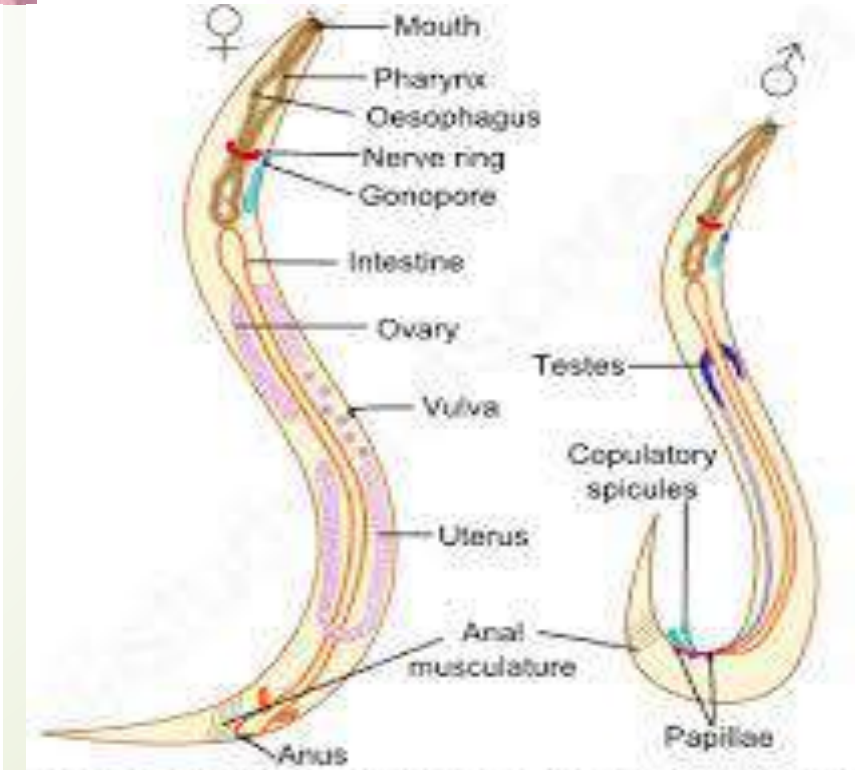


Fig. 13.3. *Trypanosoma gambiense.*





WUCHERERIA - ADULT FEMALE AND MALE

Signs and symptoms

- Not always be obvious they may be mimic anaemia or hormone deficiency
- Some may caused by several worm infestations include;
- Itching at the site of anus or vaginal area,abdominal pain, weight loss,increased appetite,bowel obstructions,diarrhoea,dehydration,worms in vomit or stools,allergies,may also be confused with pneumonia or food poisoning
- The effects caused by those range from mild discomfort to severe death

causes

- They normally enter through skin or mouth, close contact with pets can lead to parasite infestations as dogs and cats are host to many parasites.
- Walking with barefeet, inadequate disposal of faeces, lack of hygiene, close contact with someone carrying specific parasites, eating undercooked foods, unwashed fruits and vegetables or foods from contaminated regions.
- Parasites also transferred to their host by the bite of insect vector i.e., mosquito, bedbug, fleas.

Treatment

- Can be treated with anti-parasitic drugs.
- Albendazole and mebendazole are administered to control hookworm infection.
- Another medication used to kill those infections has been pyrantel pamoate.
- For some parasitic diseases,there is no treatment and in the case of serious symptoms, medication intended to kill the parasite administered where as in other cases symptoms relief options are used .

Recent papers proposed that "the use of viruses to treat infections" caused by protozoa .


Thank you!

Enhancing the teaching and learning approaches of some Zoological courses

11.09.2021

DEPARTMENT OF ZOOLOGY

TSWRAFPDCW, BHONGIR

Dr.K.Srilatha, HOD

E.Jyothi

B.Harshitha

Objectives

- To stimulate discussions on teaching approaches and methodologies for some zoological courses.
- To provide information about appropriate educational strategies for teaching zoology.
- To make most effective use of the internet to improve E-learning programs in Zoology department.
- Concern about the change from what has been a traditional emphasis on rote learning and shifting to creative thinking, problem solving, and the development of personal attributes.

Teaching Approaches

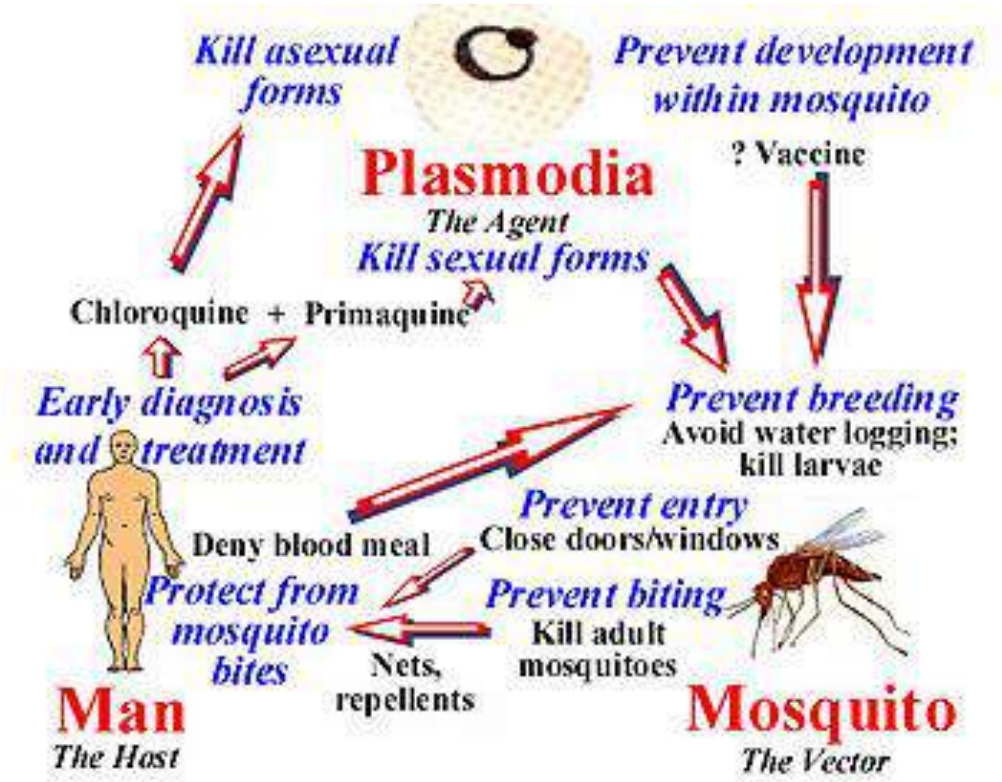
- Disciplinary approach
- Problem-oriented approaches focused on diseases of animal species, organ systems or other subjects
- Combined approaches

Disciplinary Approach

Characteristics:

- In depth conceptual knowledge
- Content based teaching
- Main purpose
- Mastery of the subject
- Source of knowledge or information

Disciplinary Approach (Adel Nabi et al., 2007).



Concept map

- Classification of invertebrate or vertebrate
- Parasite morphology & biology
- Molecular biology
- Epidemiology
- Pathology and immunology, together with clinical manifestations,
- Diagnosis
- Therapy
- Prevention of parasitic diseases
- Covering the structure of different system in the human body and compared with the animal system.

Advantages

Provides an effective and easy access for the students.

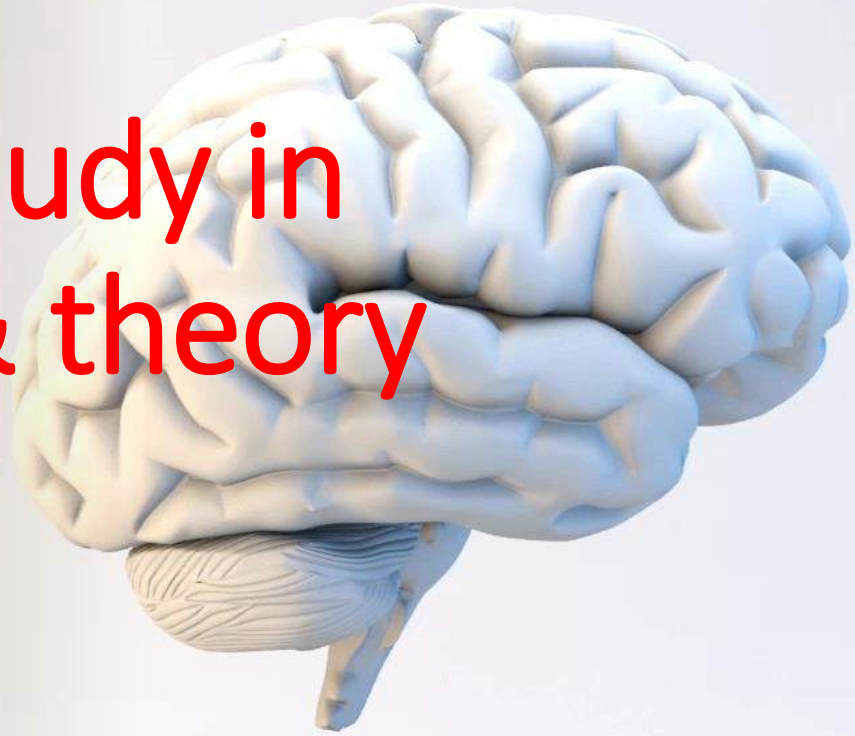
Provide an adequate basis of knowledge and skills of invertebrate and parasitology for professional life.

Disadvantages

Insufficient integration

Lack of training of the students for self-directed learning and for applying their knowledge to practical problems.

Group wise interactive specimen study in practical's & theory



Name _____ **Date** _____

Invertebrates

Words you need to know:

Asymmetrical:	no symmetry
Benthic:	lives on the floor of a body of water, especially the ocean
Biltateral symmetry:	one line of symmetry
Colonial:	animals that live in groups
Pelagic:	free swimming
Radial symmetry:	symmetry around a central radial, multiple lines of symmetry
Sessile:	immobile, permanently fixed

Directions: Observe the specimens at each invertebrate center and record your observations below.

	Name of Organism with Scientific Drawing	External observations: (body type, legs, body segments, tentacles, head, eyes, body shape)	Sessile and/or benthic and/or pelagic	Asymmetrical, radial symmetry or neither	Solitary o colonial
Porifera (sponges)					
Echinoderms (spiny skin)					
Mollusks (snails, slugs, squids and octopuses)					
Cnidarians (Jellyfish, corals, and sea anenomes)					

Problem Oriented Approach

Problem-Oriented Approaches

(Boud and Feletti, 1998).

- **Applied during teaching the developed courses.**
- **First introduced in medical schools, e.g. at Harvard Medical School in 1985 and at Bowman Gray School of Medicine (USA) in 1987/1988** (Philip and Camp, 1990).

- **The main objectives of the curriculum**
  - **Training of independent learning**
  - **Critical thinking**
  - **Learning of problem-solving skills**
  - **Enhancement of understanding of disease mechanisms, training of team-work and lifelong learning habits.**

Problem-Oriented Approaches Cont..

The basic principle supporting the concept of PBL is older than formal education itself; namely, **“learning is initiated by a posed problem”**, query, or puzzle that the learner wants to solve

2. Think critically and be able to analyze and solve complex, real-world problems.

3. Find, evaluate, and use appropriate learning resources.

4. Work cooperatively in teams and small groups.

5. Use content knowledge and intellectual skills acquired at the under-graduation level to become continual learners.

Calendar of Scientific Events

No.	Date	Scientific Day/Week/Event
1.	January 1st Week	Indian Science Congress
2.	January 30	National Anti Leprosy Day
3.	February 28	National Science Day
4.	March 16	Measles Vaccination Day
5	April 7	World Health Day
6	April 22	Earth Day
7.	May 1-7	Malaria Prevention Week
8.	May 11	Technology Day
9.	May 31	World No-Tobacco Day
10.	June 5	World Environment Day
11.	July 11	World Population Day
12.	August 1-7	World Breast-Feeding Week
13.	August 20	World mosquito day
14.	August 25 – September 8	National Eye Donation Fortnight
15.	September 1-7	National Nutrition Week
16.	September 8	Eye Donation Day
17.	September 16	World Ozone Day
18.	October 1	Voluntary Blood Donation Day
19.	October 1-7	Wildlife Week
20.	October, 1st Monday	World Habitat Day
21.	October, 1st Monday	Universal Children's Day
22.	October, 2nd Wednesday	International Day for Natural Disaster Reduction
23.	October 16	World Food Day
24.	November 10	International Science Day
25.	Nov. 19 – Dec. 18	National Environment Month
26.	November	International Week of Science and Peace
27.	December 1	World AIDS Day
28.	December 2	National Pollution Prevention Day
29.	December 14	National Energy Conservation Day
30.	December 29	International Day for Biological Diversity
31.	December 27-31	National Children's Science Congress

Preparation of Insect box as a mini project for students in vacation time

Argue with science – students can refine ideas with others and engage with open ended questions and restate observations in a more scientific language.

Live models

Mini projects


1. Identifying The Problem
2. Reviewing Literature
3. Setting Research Questions, Objectives, And Hypotheses
4. Choosing The Study Design
5. Deciding On The Sample Design
6. Collecting Data
7. Processing And Analyzing Data
8. Writing The Report
Research Process
iEduNote.com

Models

https://www.eiscolabs.com/collections/zoology-models-1

Projects

https://www.1000sciencefairprojects.com/Biology/Zoology-Projects.php
https://www.sciencebuddies.org/science-fair-projects/project-ideas/zoology

- http://virtualbiologylab.org

- free, online educational resource provided for educational purposes. The models generated are biologically realistic and are displayed numerically and graphically. Students will design experiments and conduct them using this website, collect and analyze their data in software.

Animal Album- real world-based learning experiences

Combined Approaches

- It is a combined disciplinary and problem-oriented approach (Eckert, 2000).
- Enough time has to be reserved for practical training and self-learning of the students
- In this period, some modest options for specialization may be offered as electives, e.g. tropical parasitology or parasitic diseases of wild and zoo animals.

Region-Specific Approaches

- There are certain parasitic diseases of animals which are specific for a particular area e.g. fascioliasis

- Similarly, the diagnosis and control practices used by the local farmers are also equally important and helpful in field conditions.

- The knowledge on these area specific parasites and control measures should be gathered and be included after scientific validation.

- **Practical Courses**
- Power point presentation and videos for some practical Courses.

- https://www.vlab.co.in/
- An initiative of Ministry of Education under the national mission on Education through ICT

- https://vlab.amrita.edu/index.php?sub=3&brch=188&sim=1102&cnt=1

Enhancing the Efficacy of the Administrators and faculty through workshops

To encourage the administrators to improve the practical class

For the faculty concerned about using the computer and how to work on the word program.

Training the faculty on all the equipment's and instruments in the department.

E-learning Services Approaches Bates and Poole (2003) and the OECD (2005)

Where computer-based activities are integrated with practical or classroom-based situations.

Blended & Hybrid learning

Technology enhanced learning (TEL)

Conclusion

- **Improving the contents, curricula, methods, structures of courses by improving the teaching methods.**
- **How to make the teaching more effective!!!**
- **Commitment to a majority of teaching staff and to ensure they are able and willing to use appropriate teaching strategies to develop these abilities.**
- **Introduce training in teaching and a lot of attention will need to be given in institutions to management of change strategies.**

THANK YOU

TEACHING MODULE STEPS FOR IMMUNOLOGY

BY

Dr. K. Srilatha Dept of Zoology

T.S.W.R.A.F.P.D.C.W. Bhongir

1.Aims and learning Outcomes. 2.Course content.

3.Teaching methods and resources. 4.Assesment.

5.Monitoring and reviews.

I .Aims and learning Outcomes ;

You should study this subject because it provides the practical skills and conceptual understanding required for managing in organisation. It provides a broad understanding of management under diverse operating conditions and builds for you a contextual and conceptual foundation to manage organisations understanding dramatic change. All the basic functions of Management that are deemed to comprise Planning, Organisation, Staffing, Directing, Controlling and other contemporary issues are covered and their relevance to the content of rapid contemporary change id covered in depth.

2.Learning Objectives:

A.Dear students after completing this chapter you will be able to; 1.Define the term Immunology.

2.Use immunological concept and principles to learn immunological activities.

3.Identify types of Immunity.

4.Know roles of Immunity.

B. Key points:

Immunity is Universal, Immunity process or practices are applied in all types of Institutions or health organisations. In the broadest sense, Immunological practices determine the development of a nation as development od nation greatly affected by how well the health organisations of the nation are performance.

KEY POINTS;

1.The term Immunology is defined in many different ways.

2.Good Immunological practices benefits nations, health organizations, Managers and Employees.

3.There are two types of Immunity;

1. Innate immunity 2. Acquire immunity.

4.Organizational performance can be measured by two interrelated terms called health effectiveness and efficiency.

II.COURSE CONTENT

The teaching material is organized into the fallowing chapters…….

- The first chapter presents the nature, concepts and principles of Immunology.
- The second chapter is about history of developmental immunity thoughts.
- Third chapter presents types of immunity.
- The fourth chapter is about organs of immunity
- The fifth chapter discuss the types of Antigens and Antibodies.
- The sixth chapter discuss about organizing function.
- The seventh chapter gives about Hypersensitivity.
- Chapter eight discuss about types of vaccines.

III.TEACHING METHODS AND RESOURCES

To facilitate learning, a number of activity and self-assessment discussion -review questions are provided across the module you are advised to read the teaching material carefully and other related books to have better knowledge.

Specifically;

1.Take 10-20 minutes to answer each activity questions.

2.As much as possible form a group consisting 3-5 members with your classmates and discuss your subject, if possible, by meeting physically or if not using internet.

IV.. ASSESMENT

Will cover only MCQS for student's degree level as we ate not intending other assessments as mentioned above under teaching methods.

V.MONITIRING AND REVIEW

* For monitoring their understanding and learning level we are applying Blooms educational objectives.

The following is the explanation of each level of learning by the students. So, questions will be formed to testify the knowledge acquired by the student in this course.

1.Knowledge: Involves the recall of fundamental subject. Ex: Definition, Memorising the concept.

2.Understanding: Refers to the type of understanding. EX: Able to recognize, discuss.

3.Application: Refers to the use of abstractions in particular and concrete situations. Ex: cause and effect

4.Analysis: Represents the establishing relationship between variables, situations.

Ex: Application of certain techniques.

5.Synthesis: Involves putting together of elements and parts so as to form a whole. Ex: Research.

6.Evaluation: Endangers judgement about the value of material and methods for given purposes.

Ex: Research outcomes and judgements.

CONTENT WRITING:

Immunology is a branch of biology that covers the study of immune systems in all organisms Immunology charts, measures, and contextualizes

the physiological functioning of the immune system in states of both health and diseases; malfunctions of the immune system in immunological disorders (such as autoimmune diseases, hypersensitivities, immune deficiency, and transplant rejection]); and the physical, chemical, and physiological characteristics of the components of the immune system *in vitro*, *in situ*, and *in vivo* Immunology has applications in numerous disciplines of medicine, particularly in the fields of organ transplantation, oncology, rheumatology, virology, bacteriology, parasitology, psychiatry, and dermatology.

Many components of the immune system are typically cellular in nature and not associated with any specific organ, but rather are embedded or circulating in various tissues located throughout the body.

Classical immunology ties in with the fields of epidemiology and medicine. It studies the relationship between the body systems, pathogens, and immunity. The earliest written mention of immunity can be traced back to the plague of Athens in 430 BCE. Thucydides noted that people who had recovered from a previous bout of the disease could nurse the sick without contracting the illness a second time.[14] Many other ancient societies have references to this phenomenon, but it was not until the 19th and 20th centuries before the concept developed into scientific theory.

Clinical immunology is the study of diseases caused by disorders of the immune system (failure, aberrant action, and malignant growth of the cellular elements of the system). It also involves diseases of other systems, where immune reactions play a part in the pathology and clinical features.

The diseases caused by disorders of the immune system fall into two broad categories:

- immunodeficiency, in which parts of the immune system fail to provide an adequate response (examples include chronic granulomatous disease and primary immune diseases);
- autoimmunity, in which the immune system attacks its own host's body (examples include systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's disease and myasthenia gravis).

The body's capability to react to antigens depends on a person's age, antigen type, a state of physiological immunodeficiency, because both their innate and adaptive material factors and the area where the antigen is presented.[20] Neonates are said to be in

immunological responses are greatly suppressed. Once born, a child's immune system responds favorably to protein antigens while not as well to glycoproteins and polysaccharides.

Ecoimmunology, or ecological immunology, explores the relationship between the immune system of an organism and its social, biotic and abiotic environment.

Immunotherapy or **biological therapy** is the treatment of disease by activating or suppressing the immune system. Immunotherapies designed to elicit or amplify an immune response are classified as **activation immunotherapies,** while immunotherapies that reduce or suppress are classified as **suppression immunotherapies.**

In recent years, immunotherapy has become of great interest to researchers, clinicians and pharmaceutical companies, particularly in its promise to treat various forms of cancer.[1][2][3]

Immunomodulatory regimens often have fewer side effects than existing drugs, including less potential for creating resistance when treating microbial disease.[4]

Cell-based immunotherapies are effective for some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK Cell), cytotoxic T lymphocytes (CTL), etc., work together to defend the body against cancer by targeting abnormal antigens expressed on the surface of tumours cells.

TSWRAFPDCW, Bhongir SUBJECT: Zoology

GENETICS, PAPER-IV

FACULTY: K. Srilatha

Topic: Sex Linked Inheritance: Sex-Linkage in Drosophila and Man

The chromosomes present in the diploid cells of the majority of the sexually reproducing animals are of two types: autosomes bearing genes for somatic characters and sex chromosomes bearing genes for sex.

Sex chromosomes also carry some genes for non-sexual characters such as colour blindness and haemophilia.

Such genes which are always associated with sex chromosomes are called sex- linked genes. In man and Drosophila the sex chromosomes (X and Y) are unequal in size and shape, X being larger and rod shaped whereas Y is small and slightly curved. In birds and butterflies the sex chromosomes (Z and W) are also unequal in shape and size, Z being larger than W.

In Mendelian pattern of inheritance, the genes for contrasting characters were located on autosomes but not on the sex chromosomes. Secondly, the result of reciprocal cross is same as normal cross which is not the case with sex linked inheritance. There are three types of sex-linked genes depending upon their association with particular chromosome.

They are as follows:

(i)The genes which are located on X-chromosomes are called X-linked genes or sex linked genes.

(ii)The genes which are located on Y chromosomes

In order to understand the inheritance of character present in sex chromosomes, let us understand transmission of X-chromosome from male individual in Drosophila or in man. The X-chromosome from male individual will always pass to the daughter, while X-chromosomes from female individual will be distributed equally among the daughter and sons (Fig. 5.17).

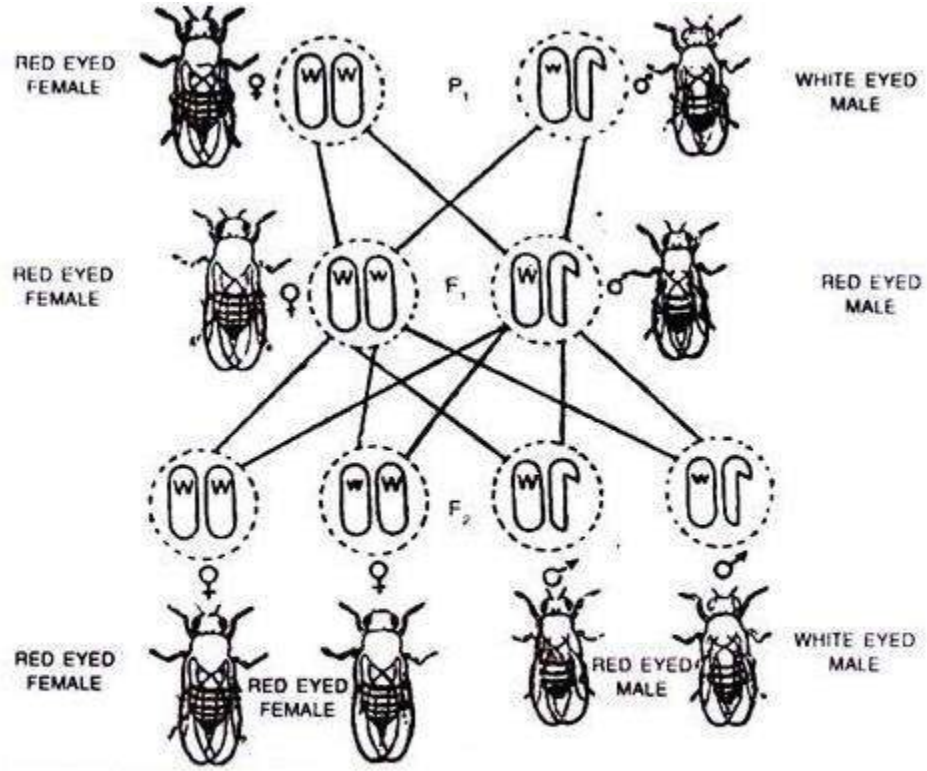


Fig. 5.17 Sex-Linked Inheritance in Drosophila. Cross between Red-Eyed Female and White-Eyed Male

A character from the father goes to the daughter (F_1) and then from daughter to grandson in the next generation (F_2). Such type of inheritance is also called as criss-cross inheritance. In this type of inheritance result of the reciprocal crosses are not identical as in case with Mendelian crosses.

Sex-Linkage in Drosophila:

T.H. Morgan (1910) for the first time discovered sex-linkage in Drosophila melanogaster. Morgan when experimenting noted the sudden appearance of one

white-eyed male (mutant form) in the culture of normal red-eyed Drosophila. This white-eyed male was crossed with red eyed female. The F_1 flies (both male and

female) were all red-eyed indicating that white eye colour is recessive to the normal red eye colour.

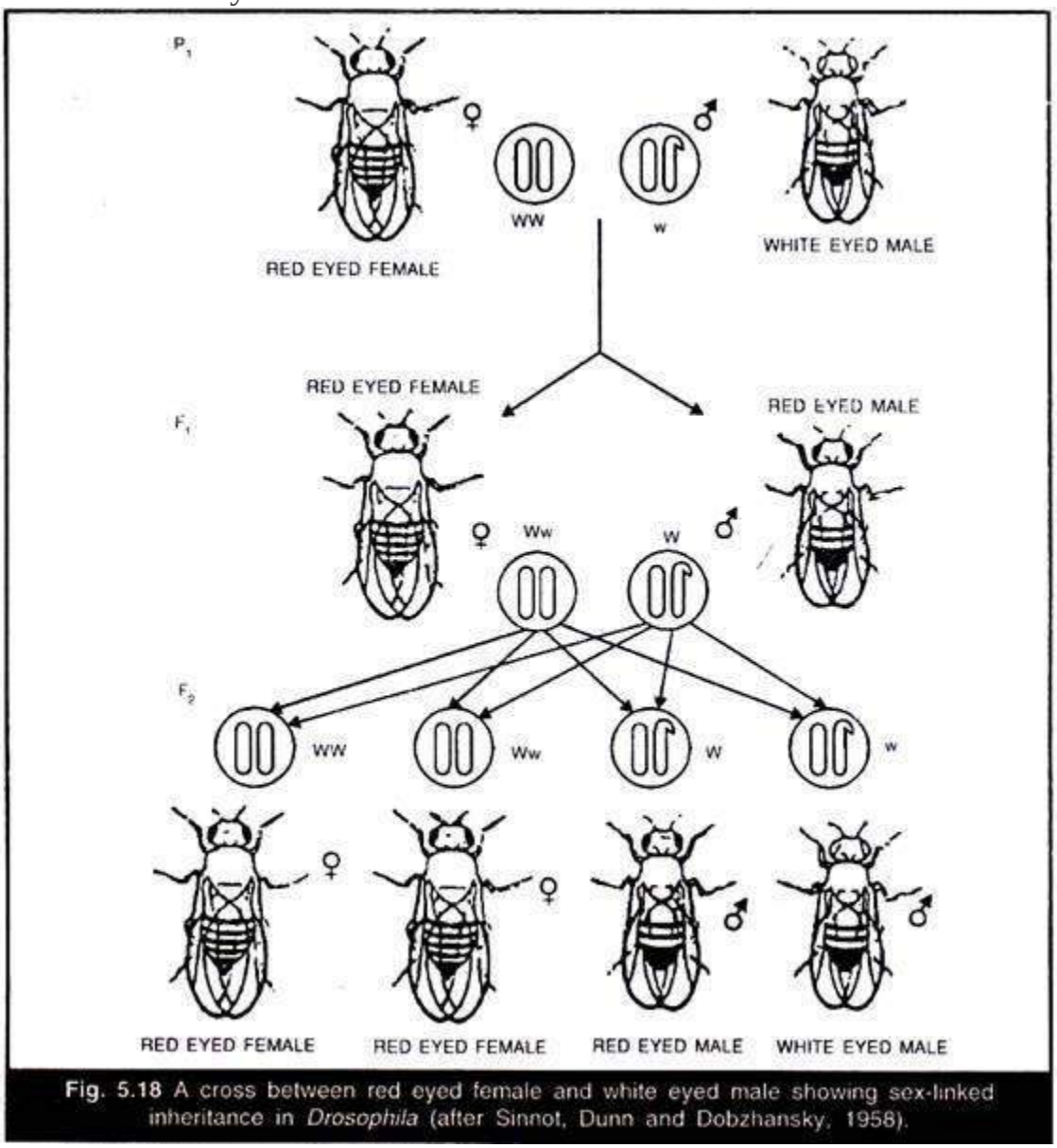


Fig. 5.18 A cross between red eyed female and white eyed male showing sex-linked inheritance in *Drosophila* (after Sinnot, Dunn and Dobzhansky, 1958).

When these F_1 flies were inter-crossed freely, the red-and white-eyed flies appeared in the ratio 3: 1 in the F_2 generation. White- eyed flies were male. Among the red eyed flies two-third were female and one-third were male. The females were all red eyed whereas 50% males were white eyed and the remaining 50% males were red eyed (Fig. 5.21).

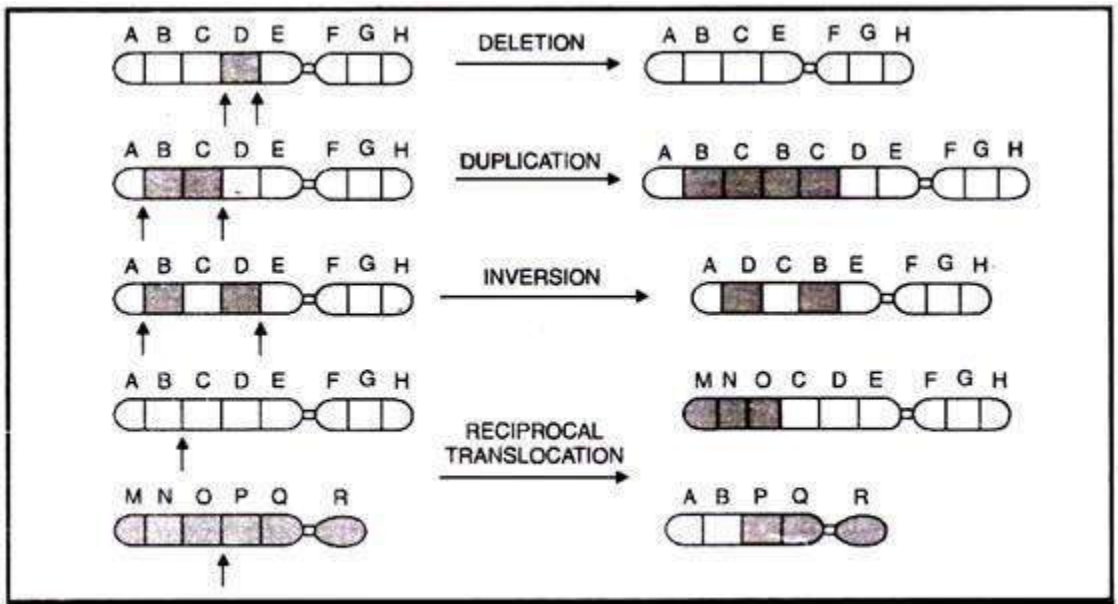


Fig. 5.21 Forms of chromosomal aberrations showing deletion, duplication, inversion and reciprocal translocation.

If a reciprocal cross is performed between white eyed female and red eyed male individual, all female individuals in F_t generation are red eyed and all male individuals, are white eyed. When these two types of individuals from F_1 generation are inter crossed, female population in F_2 generation will consist of 50% red eyed and 50% white eyed individuals. Similarly the male population in this generation consists of 50% red eyed and 50% white eyed individual (Fig. 5.19).

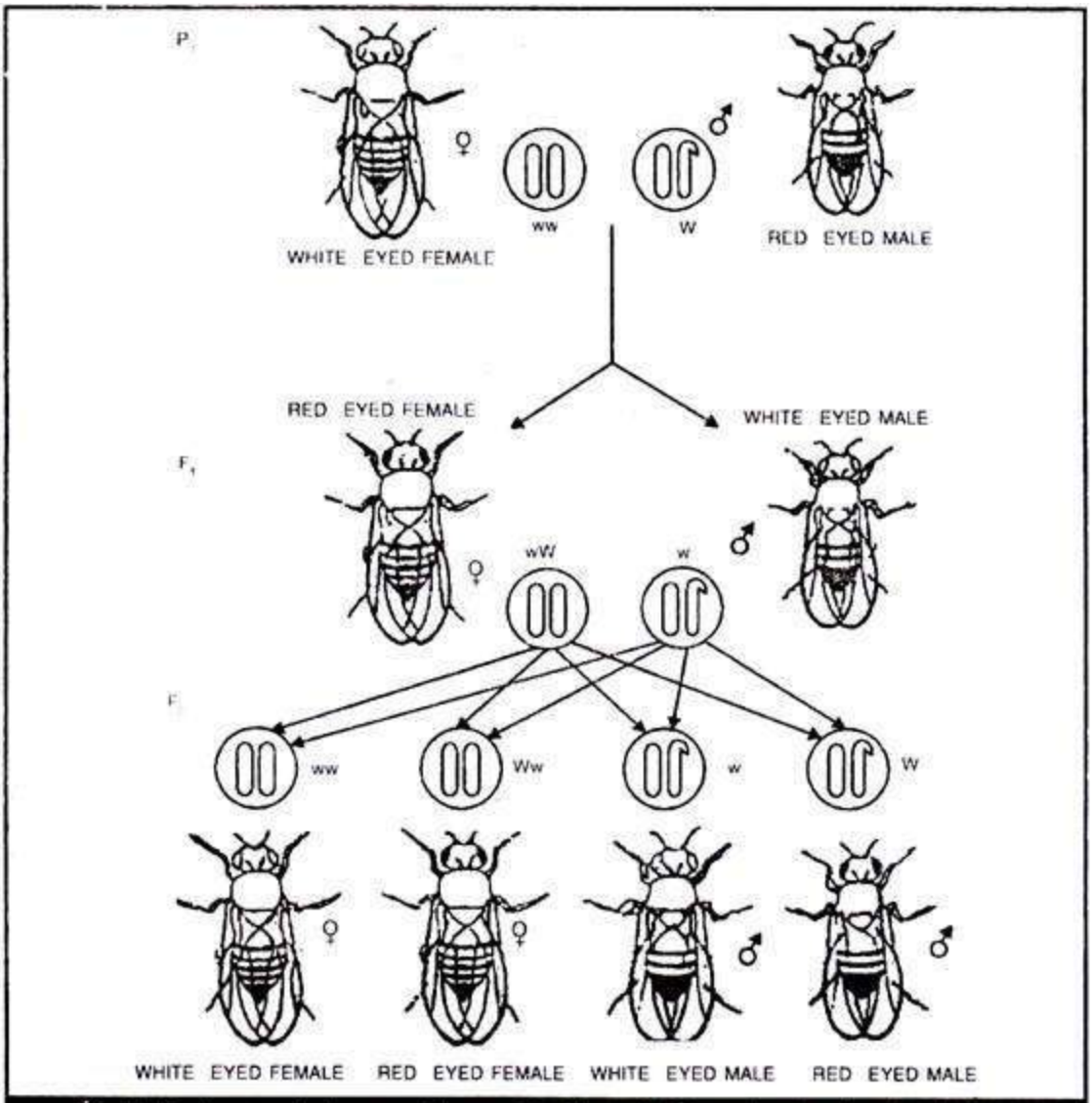


Fig. 5.19 A cross between white-eyed female and red-eyed male showing X-linked inheritance in *Drosophila* (after Sinnott, Dunn and Dobzhansky, 1958).

The inheritance of white-eye colour in Drosophila can be explained on the basis of the following assumptions:

(v)Gene for white eye colour in male Drosophila is located in X-chromosome and Y chromosome is empty, carrying no normal allele for eye colour.

(w)In white eyed female Drosophila there are two X chromosomes, each one bearing a gene for white eye colour (w). It transmits one gene for white eye colour

(x)to each offspring.

(iii) As we can see in the above reciprocal crosses, the gene for recessive white eye colour (w) passes by father on to daughter (F_1 generation). The daughter in turn passes this gene to her sons (F_2 generation). The character thus seems to alter or cross from one sex to the other in its passage from generation to generation. In other words, character is transferred from mother to son and never from father to the son.

Characteristics of Sex Linked Inheritance:

(a)It is a criss-cross inheritance as the father passes its sex-linked character to his daughter who in turn passes it to the grandson.

(b)Daughter does not express the recessive trait but act as carrier in the heterozygous condition.

(c)Female homozygous for recessive trait expresses the trait.

(d)Any recessive gene borne by the X chromosome of male is immediately expressed as Y chromosome has no allele to counteract.

Sex Linkage in Man:

In man about fifty six sex-linked genes have been reported, the most common examples are:

1.Red green colour blindness.

2.Haemophilia.

1. Red Green Colour Blindness:

Colour blindness is an example of sex linked character. Those who suffer from red green colour blindness cannot distinguish between red and green colour. The gene for this defect is located on X chromosome. It was first studied by Horner (1876). Colour blindness is recessive to normal vision.

(i) Normal Woman and Colour Blind Man:

When a normal woman is married to a colour blind man, their children (daughters and sons) have normal colour vision. But when their daughters were married to

normal man, 50% of their sons are colour blind and the remaining 50% are normal, while the daughters were all normal.

(ii) Colour Blind Woman and Normal Man:

If a colour blind woman marries a normal man, their daughters are normal but all their sons are colour-blind. When these F_1 daughters are married to colour blind men, colour blind sons and daughters are born in equal number.

2. Haemophilia (Bleeder's Disease):

Haemophila is another popular example of sex linked inheritance in human beings. It is caused by a mutant gene (h) present in X chromosome and recessive to normal gene and is, therefore, suppressed in heterozygous condition. Individuals suffering from this disease lack a factor responsible for clotting of blood. So in the absence of blood clotting substance, a minor cut or injury may cause prolonged bleeding leading to death. This disease in man is generally restricted to male members.

If a haemophilic man marries a normal woman, the daughter are all carriers (phenotypically normal but carries haemophilic gene in one on her X chromosome) but sons are normal. Such a carrier daughter, when marries a normal man transmits the haemophilic gene to half of her son (Fig. 5.20). A haemophilic woman is produced only if a carrier woman is married to a haemophilic man.

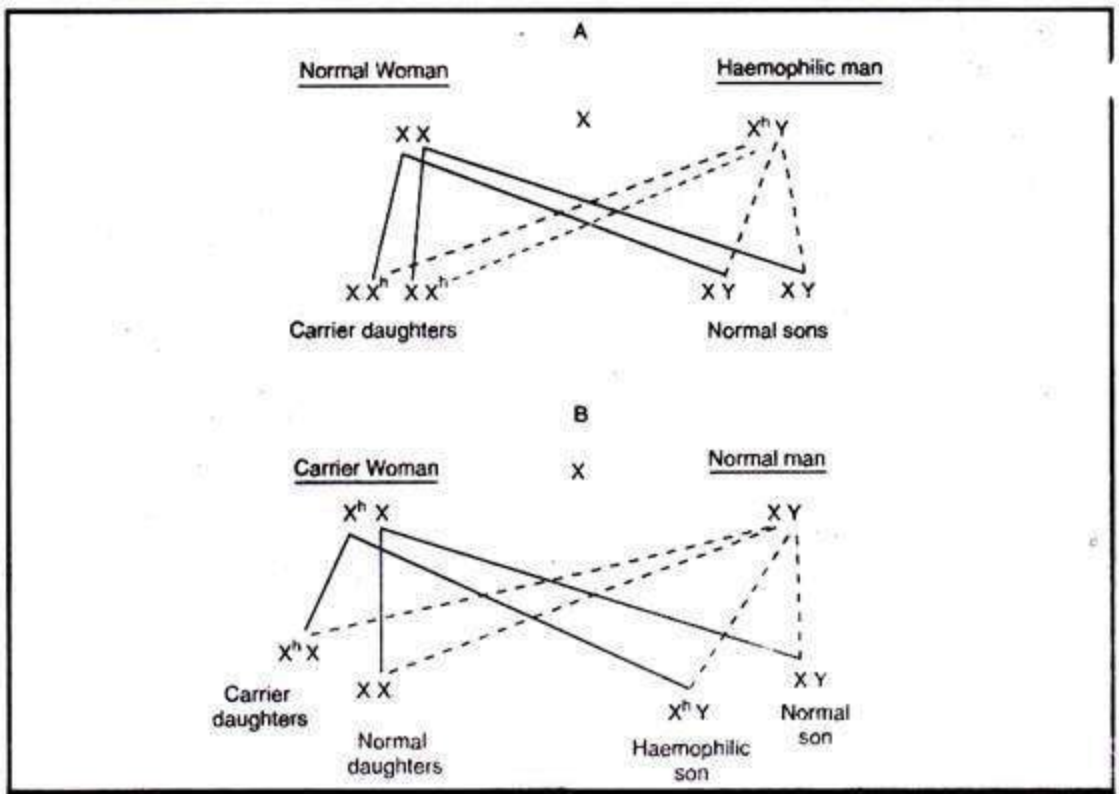


Fig. 5.20 Sex-linked inheritance of haemophilia. A : A marriage between normal woman and haemophilic man. B : A marriage between a carrier woman and a normal man.

Haemophilia is also called 'Royal disease' as it is found in certain royal families of Europe. Apparently the gene for haemophilia (h) arose as a mutation in a reproductive cell which produced Queen Victoria of England.

Generally Haemophilia is of two types:

(i)Haemophilia A:

It is the most common type and the patient lacks anti-haemophilic factor (AHF).

(ii)Haemophilia B:

It occurs in about 20% of the patients. It is due to lack of plasma thromboplastin component (PTC).