(iv) When the heart first develops in the embryos of Amphibians, reptiles, Birds and Mammals, it is 2-chambered as in the embryos and Adult of fishes. In later stages of Embryonic development in Amphibians, reptiles, Birds and Mammals the heart become, 3-chambered. This condition is retained in Adults of Amphibians and most reptiles. In Birds and Mammals the heart becomes 4-chambered in the last embryonic stages to continue as such in the Adults. .

(v) Modern Scientists have discovered "Biochemical recapitulation" also for example, fishes mainly excrete Ammonia. Adult Amphibians Excrete urea, but their tadpoles excrete Ammonia like the fishes Birds excrete uric acid, but their embryos excrete first Ammonia and then urea during earlier stages

9. Evidences from Taxonomy -

Plants and Animals show a great diversity of form. They also show some similarities amongst themselves. It is on account of these differences and resemblances that the taxonomists have been able to arrange the living things in to smaller and larger groups. Thus the taxonomists have recognized that there are about 250,000 different kinds of plants. These different kinds are called species. The members of each species are recognized by their close resemblance to one another, their differences being of a minor type. Amongst species also there are differences and resemblance of varying degree some species resemble one another more closely than they resemble others and form a closely related group similarly; there are other groups of closely resembling species. The members of each group resemble each other more closely than they resemble the members of other groups. Each such group of species is called a genus. Genera also show different degrees of resemblances amongst themselves. Those that resemble one another more closely than others are placed in a large group called a family.

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Families are grouped in to larger units called orders, orders into classes and classes in to Phyla. Evolution has a ready explanation for this system of grouping or classifying plants and animals in groups indicates relationship.

Missing Links

The transitional fossil forms which show characteristics of two different groups of living animals are called missing links. Missing links are know between different existing groups from fishes to amphibians, from amphibians to reptiles and from reptiles to birds and mammals.

(A) Ichthyostega : It is a primitive fossil amphibian included in **Stegocephalia** (= Labyrinthodontia). Its fossils process piscine as well as amphibian characteristics and is regarded as a connecting link between the two.

(B) Seymouria : It is a missing link between amphibians and reptiles. Its amphibian characters are short limbs and labyrinthine teeth. The reptilian characters include two sacral vertebrae, an interclavicle and cleidoic eggs.

Archaeopteryx : Archaeopteryx is a missing link between reptiles and birds.

Connecting links

1. **Viruses :** These are described as connecting link between nonliving and living.
2. **Euglena :** Euglena is a protozoan. connecting link between plants and animals

3. **Proterospongia :** It is a colonial protozoan. It is regarded as connecting link between unicellular and multicellular animals.
4. **Peripatus :** Peripatus is a connecting link between Annelida and Arthropoda.
5. **Neopilina :** Neopilina belongs to class Monoplacophora of phylum Mollusca.
6. **Balanoglossus :** Balanoglossus belongs to subphylum Hemichordata. It is an intermediate between nonchordates and chordates.
7. **Chimaera :** It is a connecting link between cartilaginous fishes and bony-fishes.
8. **Lungfishes :** Lungfishes Neoceratodus, Lepidosiren and Protopterus are connecting link between fishes and amphibians.
9. **Prototheria :** The egg laying mammals, Echidna and Ornithorhynchus, the only living prototherians of class Mammalia, are connecting link between reptiles and mammals.

HISTORY OF EARTH FORMATION

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▶About 375 million years ago, around Carboniferous Period, all the present day continents formed a single, giant land mass called **Pangae.** This singular or landmass supercontinent remained till the beginning of Mesozoic Era.

▶About 180 million years ago, i.e., near the end of Triassic Period, **this land mass got split into two landmasses**

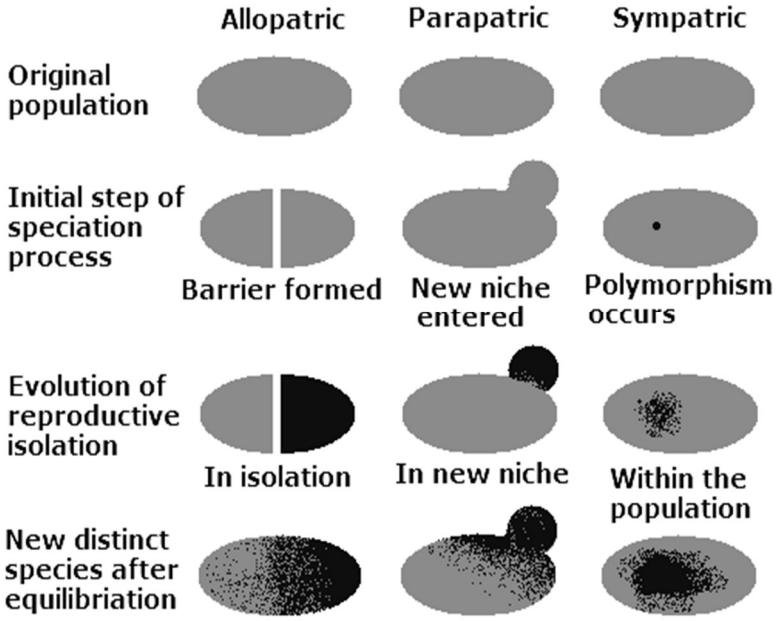
1. **Laurasia :** The northern half consisting of North America, Europe and Asia.
2. **Gondwana : The Southern land mass formed of South America, Africa Australia, Antarctica and India.**

▶**About 135 million years ago**, i.e. near the end of Jurassic Period, a rift appeared separating South America from Africa and South America drifted westward. **India also broke loose, moved northward and rammed into Asia**

▶About 65 million years ago, i.e., near the end of Mesozoic Era, North America separated from Europe and Madagascar isolated from eastern Africa. Westward drift of North and South America resulted in the widening of North and South Atlantic oceans.

▶About 40 million years ago. This collision caused uplifting of the Tibetan Plateau and Himalayan Mountains. Because of rotation of land masses North Atlantic and Indian oceans increased in size.

SPECIATION



ALLOPATRIC SPECIATION

Allopatric speciation occurs when the new species evolves in geographic isolation from the parent species. The species range becomes subdivided by a barrier such as a new mountain range or the change in the course of a river. Gene flow between the two subpopulations becomes impossible, allowing evolution to proceed independently in each. Natural selection may favor different genotypes on either side of the barrier and random genetic drift and mutations could contribute to divergence. **Over time, divergence may proceed to the point that, were the two populations to meet again, they would not be able to interbreed and speciation would be complete.**

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This form of speciation may take place most readily in small populations at the extreme edge of a species range (the isolated population is smaller than the parent population). The peripheral population could become isolated, for example, during contraction of the main species range in response to changing climate. The isolated population would be subject to the founder effect and could be genetically different from the parent population. The combined effect of a small atypical population and extreme environmental conditions can cause rapid and extensive genetic reorganization through random genetic drift and strong natural selection, or, in other words, a genetic revolution.

Distinct peripheral isolates have been observed on islands. For example, the Papuan kingfisher of the *Tanysiptera* genus has three almost indistinguishable forms on mainland New Guinea and strikingly different island species derived from isolates. Speciation in the cichlid fishes *(Haplochromis)*in Lake Victoria is thought to have occurred through the isolation of small founder populations caused by changes in the drainage pattern of the rivers feeding the lake. Small changes in, for example, male coloration and courtship behavior have resulted in the formation of as many as 170 reproductively isolated species.

PARAPATRIC SPECIATION

This form of speciation occurs where the speciating populations are contiguous and hence only partially geographically isolated. They are able to meet across a common boundary during the speciation process. Where a species occupies a large geographical range it may become adapted to different environmental (e.g. climatic) conditions in different parts of that range. Intermediates,or hybrids, will be found but the large distances involved prevent the two types from merging completely.

For example, the herring gull *Larus argentatus* is a ring species whose distribution covers a large geographical area. Westwards from Britain towards North America its appearance changes gradually, but it is still recognizable as the herring gull. Further west in Siberia it begins to look more like the lesser black-backed gull *Larus fuscuss.* From Siberia to Russia and into northern Europe it becomes progressively more like the lesser black-backed gull. The ends of the ring meet in Europe and the two geographical extremes appear to be two good biological species.

SYMPATRIC SPECIATION

Sympatric speciation describes a situation where there is no geographical separation between the speciating populations. All individuals are, in theory, able to meet each other during the speciation process. This model usually requires a change in host preference, food preference or habitat preference in order to prevent the new species, being swamped by gene flow.

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In theory it can occur where there is a polymorphism in the population conferring adaptation to two different habitats or niches. Reproductive isolation could then arise if the two morphs had a preference for 'their' habitat. There is some evidence for this in natural populations. For example, caterpillars of the ennine moth**, feed on apple and hawthorn trees**. Females prefer to lay their eggs on the species on which they were raised.;

Caterpillars also prefer to feed on the plant on which their mothers were raised and adult moths prefer to mate with individuals from the same plant. **The apple and hawthorn types are not completely isolated, but may represent an intermediate point in ongoing sympatric speciation**.

A change in host-plant preference can happen very rapidly. The apple maggot, *Rhagoletis pomonella,* was first found on apples in 1864 having moved from its previous host, hawthorn (coincidentally, both hosts used by the ermine moth). Like the ennine moth, mating, feeding and egg-laying takes place on the host plant so the two forms no longer meet in the wild. Although only 100 generations old, they have also evolved extensive enzyme differences. Again, reproductive isolation is not complete, and may never become so as the apple and hawthorn forms are able to mate. Some evolutionary biologists would wish to see them classed as separate species.

▶**Example of sympatric speciation occurs in plants through polyploidy**. **Polyploidy is the spontaneous duplication of the entire genome resulting in an individual with a multiple of the original chromosome number**. Polyploidy is common in plants, where it often results in larger, more vigorous forms. It is usually fatal in animals, although some amphibians are polyploids. The polyploid plant is no longer sexually compatible with the parent population but is able to establish a distinct population which may occupy a different habitat. The sand dune grass, *Spartina townsendii,* is a polyploid derived from the

original *S. angliea.*It is more vigorous than the parent and has colonized large areas of sand dune in Britain.

Incipient speciation Darwin considered varieties to be incipient species. There is a continuous range between slightly different races and fully diverged species. Divergence occurs when two populations undergo different genetic change with time. This may be due to directional selection for different phenotypes in the two populations. However, even if the selection on the phenotype (e.g. bill size, optimum temperature, etc.) is in exactly the same direction in both populations, which is unlikely, a different combination of alleles may finally be selected in each population. For example, suppose two genes *A* and B both code for similar enzymes, and there is selection to produce less enzyme. Now *AABB* has too much enzyme, and either *A* or *B* must be lost. *AAbb* may become fixed in one population while *aaBB* becomes fixed in another, *(aabb* is lethally deficient because it has no enzyme at all.) There are also differences which arise slowly by chance (drift or mutation). When the two populations are sufficiently different in appearance, behavior, ecology or genetics, they may be considered to be separate species

MODES OF SELECTION

THERE ARE THREE MODES OF SELECTION

Stabilizing selection

Stabilizing selection removes individuals deviating too far from the norm, and maintains the population near the optimum. In a stable environmental niche most individuals are well adapted and new types or deviants ate likely to be less fit. For example, house sparrows *(Passer domesticus)* killed in a severe snowstorm in New York were found to have wings markedly longer or shorter than the mean. Human babies with birth weights deviating furthest from 3.6 kg are more likely to die or suffer damage at or soon after birth because they are either too big to pass through the birth canal, or too small to survive, This selection is now very much reduced in countries where medical support is available

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- **Some facts about Stabilizing selection**:-

A. Operate in constant or unchanging environment.

B. Introduce homogeneity in population.

C. favor average or normal individuals.

D. checks accumulation of mutations that may lower the fitness of species in unchanging environment.

E. Tends to arrest variance and evolutionary change.

F. it operate rarely because environment is rarely constant

Directional selection

Some facts about Directional selection

1. Operate when environment is changing in one direction.
1. Favors those mutation which increase fitness in the changing environment.

Mutation – Natural and induced mutations. chromosome number and forms, structural rearrangements; Polyploidy.

STRUCTURAL ORGANIZATION OF CHROMOSOMES

Morphological features of normal metaphase chromosomes

- The **primary constriction,** which indicates the position of the centromere, stains densely and joins all four **arms.**
- **Secondary constrictions** are pale staining and usually represent **nucleolar organizer regions (NORs),** the positions of tandemly repeated rRNA genes.
- Interstitial secondary constrictions are found on human chromosomes 1, 9 and 16, whilst distal constrictions appear on chromosomes 13, 14, 15, 21 and 22.
- Telomeric chromosome segments found distal to NORs are termed satellite regions because they may appear detached from the main body of the chromosome.
- The **satellite regions** of different chromosomes are often found grouped together (satellite **association),** because the nucleolar organizers contribute to a common **nucleolus.**
- Other secondary constrictions can be induced by growing cells under abnormal conditions and are termed *fragile sites* **Chromosome polymorphisms** (heteromorphisms) are heritable morphological features of chromosomes which vary within a population but have no phenotype. **Examples include areas of variable heterochromatin, *inversions* and rare fragile sites. These can be used as cytogenetic markers**

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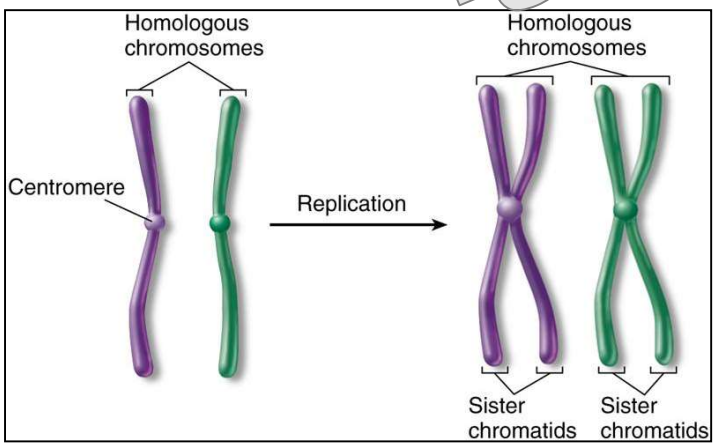
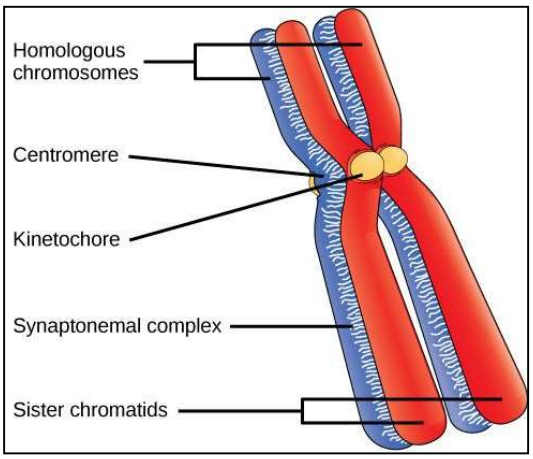
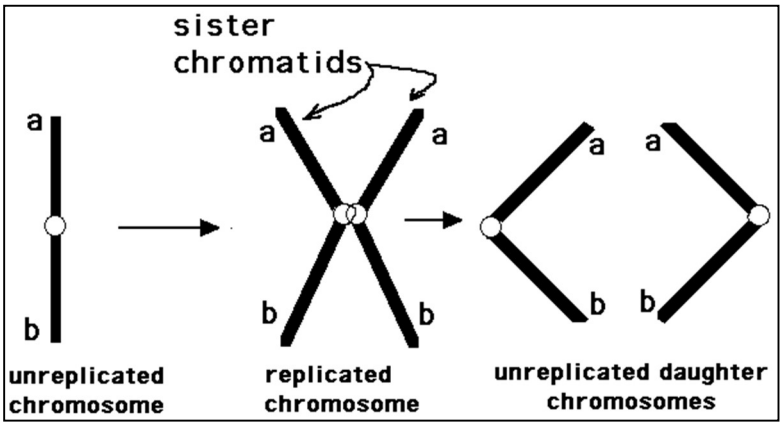






►**Chromosome parameters.**

All known eukaryotes possess more than two chromosome per haploid set, so it is necessary to define a system of descriptive nomenclature which allows individual chromosomes to be distinguished from others in the same nucleus.

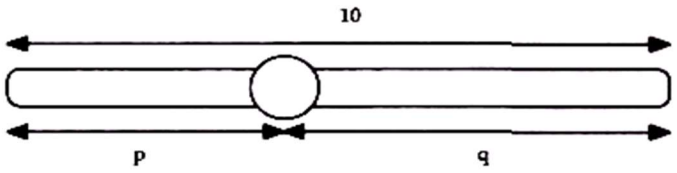
►**The nomenclature which is used is summarized below and specifies the type and position of the centromere and the relative lengths of the chromosome arms.**

Most chromosomes can be defined in terms of three parameters**, the d-value, the r-value** and the **centromeric index (i),** which delimit regions of the chromosome in which the centromere can be placed irrespective of chromosome size.

►**The d-value** is the difference in length between the long and short arms of the chromosome, i.e. *d - q -p,* where *q* and p are the lengths of the long and short arms, respectively, and the total length of the chromosome has been divided into 10 arbitrary units.

►**The r-value** is the ratio of the lengths of the two chromosome arms, i.e. ***r = q/p***.

►**The centromeric index** is the distance from the centromere to the tip of the short arm, expressed as a percentage of the total chromosome length, i.e. *i* - 100p/(p + *q).*



Measurements used to define the morphological parameters *d*, *r* and *i*.

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►The lowest number of chromosomes in nematode: ***Parascaris univalens***, where the haploid n = 1;

►The highest number of chromosomes in Adder's Tongue Fern ***Ophioglossum reticulatum***with an average of 1262 chromosomes.

►The highest number of chromosomes in animals in ***Acipenser brevirostrum*** at a mere 372 chromosomes

Selection Of Chromosome Banding Techniques

C-banding	A Giemsa-based technique which includes incubation with barium hydroxide. Identifies regions of heterochromatin and is therefore useful for determining the position f centromeres.
Cd- banding	A technique for identifying kinetochores
D-banding	A technique for identifying regions of DNase I sensitivity, generally corresponding to regions of open chromatin which are potentially transcriptionally active
G-banding	The most widely applied chromosome banding technique, the reproducible pattern of bands being the basis of the international standard human and mouse cytogenetic maps. Chromosomes are partially digested with trypsin and incubated with Giemsa's staing (a mixture of methylene blue, eosin and other dyes dissolved

	in methanol) **Chromosomes are G-banded to facilitate the identification of structural abnormalities. Slides are dehydrated, treated with the enzyme trypsin, and then stained**
G12-banding	G-banding carried out at high pH, which stains mouse and human chromosomes differently, allowing them to be discriminated, e.g. in somatic cell hybrids
N-banding	A technique for specifically staining nucleolar organizer regions, e.g. silver nitrate staining
Q-banding	A banding technique using the fluorescent dye quinacrine. This produces a similar pattern to G-banding, but also causes areas of heterochromatin to fluoresce brightly and is useful, e.g. for identifying the Y-chromosome
R-banding	A Giemsa-based technique including a heat-denaturation step which results in a reversed banding pattern from that obtained with conventional G-banding. This technique is useful in, e.g. identifying terminal deletions.
Replication banding :-	This technique involves the incorporation of bromodeoxyuridine into replicating DNA. A brief pulse during the S phase allows replication timing to be investigated and if prolonged, generates a banding pattern similar to Giemsa's stain, suggesting G-bands correlate to replication time zones Incorporation over two rounds of replication allows sister chromatids to be discriminated on the basis that one has bromidexyuridine incorporated in both strands and the other in only one strand. The chromatids then stain differently in the presence of Giemsa's stain and the fluorescent dye Hoechst 33258. This technique, known as harlequin staining, is useful for the detection of sister chromatid exchanges
T-banding	A variation of R-banding in which only telomeric DNA is stained. T-bands are particularly gene-rich
Leishman's Stain:	Principle Conventional staining techniques are used to uniformly stain chromosomes and leave the centromeres constricted, thus enabling the measurement of chromosome length, centromeric position, and arm ratio.

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KARYOTYPES AND THEIR CHANGES

1. The term karyotype describes the number, size and centromere position/arm length ratio of the chromosomes in a cell.
2. Autosomes are the chromosomes found in all individuals, irrespective of sex.
3. The number of chromosomes in a single set, as found in a gamete, is called the haploid number and is denoted by *n, so* diploids (which are zygotes formed by fusion of two haploid gametes) have 2n chromosomes.
4. Put another way, diploids have *n* pairs of chromosomes. In some species the two sexes have different numbers of sex chromosomes, so males and females can have different diploid numbers. The sex chromosomes may be noted separately (e.g. in a species where « = 8, females have two X

chromosomes, males have one X, and there is no Y chromosome, then in males 2n = 16 XO, and females are 16XX)

5. **The karyotype is usually a constant feature within a species, but variant types of chromosome carrying homologous information may exist in a species. These are known as chromosomal polymorphisms (many shapes).**
6. ►**Karyotypes may be very stable through evolution.** Most dragonflies have *n* = 13; snakes and birds both have about 18 large chromosomes and several **microchromosomes**.

MECHANISMS OF CHROMOSOMAL REARRANGEMENT

Rearrangement of chromosomes requires at least two breaks (four ends) in DNA rearrangement (chromosomes), followed by efficient rejoining and /or healing.

1. ⇨These break / rejoin may occur **by recombination**between DNA sequences repeated and dispersed throughout the genome.
2. ⇨Transposable elements dispersed throughout the genome may promote such rearrangements **directly by their transposition mechanism.**
3. ⇨Incorrect repair of ends of double-strand breaks caused **by ionizing radiation (or other mutagens**) is another source of rearrangement which can be demonstrated in cultured cells.
4. ⇨All linear chromosomes must have a telomere, special repeated sequences with associated proteins which protect the end of the chromosome from degradation. **Chromosome breaks which do not rejoin to a normal chromosome end or acquire a telomere of their own remain unstable and reactive and promote further cycles of chromosome breakage and possible rearrangement**. Terminal rearrangements involve the end of a chromosome, interstitial rearrangements are internal. The rearrangement may only affect one chromosome, or may involve transfer or exchange between two nonhomlogous (genetically different) chromosomes.

1. DELETIONS: -

Terminal deletions involve a single break, then the fragment with a centromere obtaining a new telomere. **The acentric fragment (no centromere) will be lost at cell division.**

1. ⇨**Interstitial deletions involve the loss of a region within the chromosome, followed by rejoining of the ends of the flanking pieces. This may occur by recombination between repeated sequences in similar orientation in the same chromosome to delete the intervening region as a closed circle**
2. ⇨⇨Alternatively, misaligned recombination between repeats on homologous chromosomes can create a duplication-deletion pair. Deletions are also produced by meiotic recombination in chromosome regions heterozygous for other rearrangements (e.g. translocations or inversions, see below).
3. ⇨⇨⇨Heterozygous zygotes carrying a deletion will be partially haploid, unless the region is duplicated elsewhere. In the fruitfly *Drosophila melanogaster* there are two loci which are dominant lethals if present in a single copy (haplolethals), 11 are deleterious and 41 give small flies (haplo-minutes) if haploid. Single copies of genes may often be inadequate for full fitness, and combined loss of about 3% of the genome is lethal, probably because of the combined effects of loss of one copy of many genes.
4. **Deletions are probably less harmful in polyploid species. Heterozygous deletions, especially if large, may inhibit meiotic pairing/synapsis, and so cause nondisjunction and lower fertility.**

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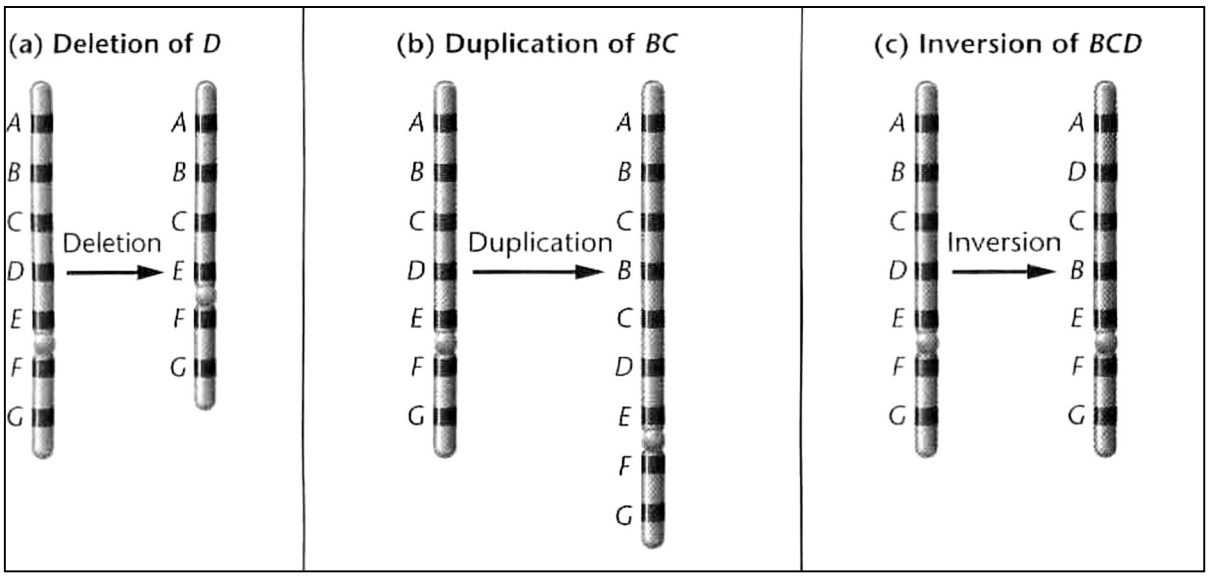

2.**DUPLICATIONS**

Since cellular life arose, and possibly before**, duplications have been the main source of new genetic material**, **followed by divergence and separate evolution of the two copies**. Sequencing entire eukaryote genomes consistently reveals large numbers of duplicate chromosome segments, both within and between chromosomes.

- **Occasionally a small translocation will survive in its new location as a duplication.** When selection acts for a large increase in copy number (amplification) of a particular gene duplications usually appear, often as microscopically visible heterogeneous staining regions containing many tandem repeats.
- **Examples are selection by insecticide on aphids to amplify the gene for an esterase which breaks down the insecticide, thus becoming insecticide resistant, and by chemotherapy cancer cells to amplify genes for a protein which pumps toxic drugs out of the cell.**
- **Duplications of the centromere do not survive because the two centromeres may orientate to opposite poles at division, breaking the chromosome. Duplications arc generally less harmful than deletions.**

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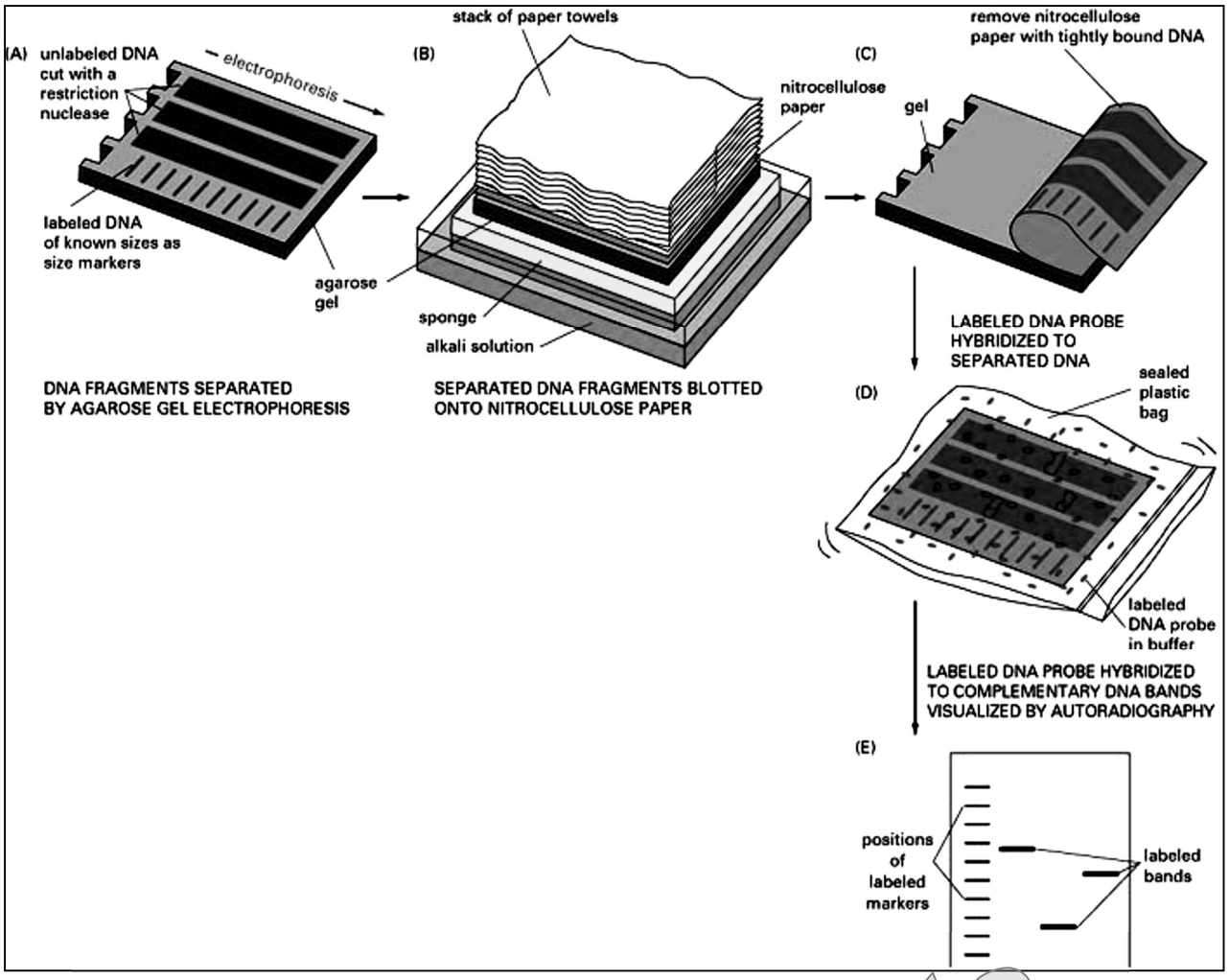
3. TRANSLOCATIONS

1. A translocation is the transfer of material between two nonhomologous chromo-somes. This usually involves a reciprocal exchange of the ends of chromosome arms .
2. Heterozygotes will only produce genetically balanced gametes when both original chromosomes or both rearranged chromosomes pass into the same gamete. Recombination complicates this segregation, but there is one arrangement, alternate disjunction, which ensures balanced gametes. This requires metacentric chromosomes, and no chiasmata (i.e. no recombination) between the translocation breakpoint and the centromere.
3. This is favored if the translocation breakpoints were near the centromere, and chiasmata are near the telomeres . (These conditions may arise by centric fusion as easily as by translocation.)

SOUTHERN HYBRIDIZATION



1. A sheet of nitrocellulose (or, alternatively, nylon) membrane is placed on top (or below, depending on the direction of the transfer) of the gel. Pressure is applied evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel), to ensure good and even contact between gel and membrane. Buffer transfer by capillary action from a region of high water potential to a region of low water potential (usually filter paper and paper tissues) is then used to move the DNA from the gel on to the membrane; ion exchange interactions bind the DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane.
2. The membrane is then baked, i.e., exposed to high temperature (60 to 100 °C) (in the case of nitrocellulose) or exposed to ultraviolet radiation (nylon) to permanently and covalently crosslink the DNA to the membrane.
3. The membrane is then exposed to a hybridization probe—a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye. In some cases, the hybridization probe may be made from RNA, rather than DNA.
4. After hybridization, excess probe is washed from the membrane, and the pattern of hybridization is visualized on X-ray film by autoradiography in the case of a radioactive or fluorescent probe, or by development of color on the membrane if a chromogenic detection method is used.

The Southern blotting technique is extremely sensitive. It can be used to map the restriction sites around a single copy gene sequence in any genome (even of man). It is used for DNA fingerprinting, preparation of RFLP maps, detection and identification of the transferred genes in transgenic individuals etc.

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Recently some new membrane materials, e.g., nylon membranes, have been developed which have the following advantageous features: **(1)** They are physically robust in comparison to nitrocellulose filter membranes, **(2)** both DNA and RNA become cross-linked to them by a brief exposure to UV light, which **(3)** saves the time needed for baking in vacua in the case of nitrocellulose membranes, and **(4)** the same membrane blot, e.g., a membrane onto which DNA/RNA has been transferred from a gel and cross-linked by UV exposure, can be used for search with more than one probe after removing the earlier probe by high temperature washing or some other denaturing procedure; in other words, the nylon membranes are reusable.

TOPIC 4 -NORTHERN HYBRIDIZATION

In this technique, RNAs are separated by gel electrophoresis, the RNA bands are transferred onto a suitable membrane, *e.g.,* diazobenzyloxymethyl (DBM) paper or nylon membranes, and immobilized; the bands are hybridized with radioactive single-stranded DNA probes, and the bands showing hybridization are detected by autoradiography.

Clearly, northern blotting hybridization is simply an extension of the Southern blotting technique The two techniques, however, show the following differences:

(1) in Southern hybridization DNA is separated by gel electrophoresis while in northern blotting RNAs are separated;

(2) as a result, in Southern hybridization DNA has to be denatured before blotting, while this step is not needed in northern hybridization; **(3)** nitrocellulose membrane is generally not used for northern, while it is often used for Southern hybridization; and finally **(4)** hybridization with the probe produces DNA: DNA hybrid molecules in Southern but RNA : DNA molecules in northern hybridization.

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Initially, specially prepared paper (diazobenzyloxymethyl, DBM, paper prepared by diazotization of aminobenzyloxymethyl paper) was used for northern blotting since RNA did not bind to nitrocellulose membrane. RNA becomes covalently bound. DBM paper due to which these blot-transfers are reusable, DBM is also equally effective in binding to denatured DNA, and is more efficient than nitrocellulose in binding to small DNA fragments.

Recently developed nylon membranes have superceded the use of DBM paper as they are robust, reusable and bind (by cross-linking) to RNA on a brief exposure to UV light. Northern hybridization is useful in the identification and separation of the RNA that is complementary to a specific DNA probe; this is a sensitive test for the detection of transcription of a DNA sequence that is used as probe.

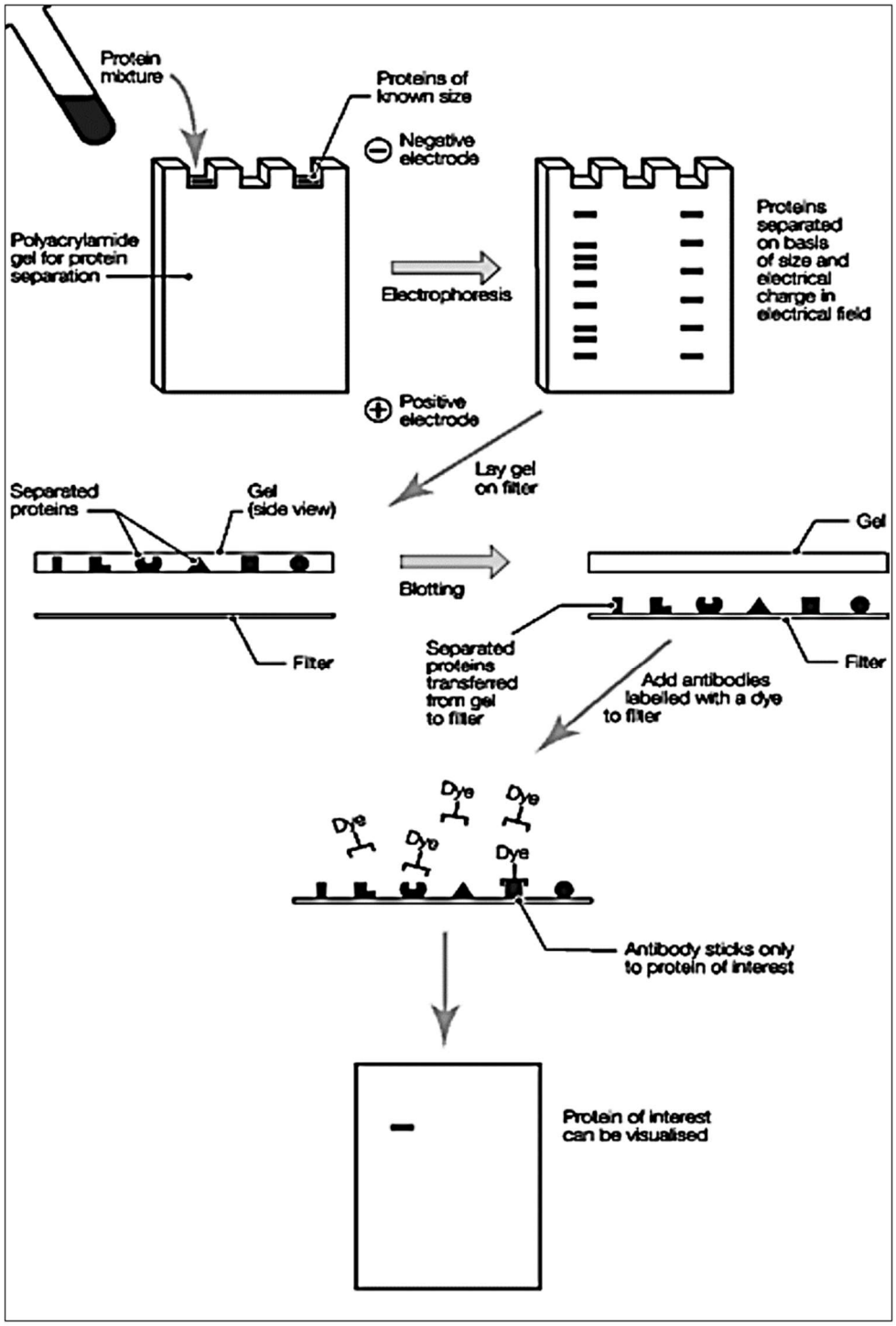
TOPIC 5 -WESTERN BLOTTING

In western blotting, proteins are electrophoresed in polyacryiamide gel, transferred onto a nitrocellulose or nylon membrane (to which they bind strongly), and the protein bands are detected by their specific interaction with antibodies, lectins or some other compounds.

The various steps of this technique are briefly described below.

1. Protein bands separated by polyacryiamide gel electrophoresis. The protein bands are transferred onto a nitrocellulose or nylon membrane; initially this was achieved by a capillary movement of buffer similar to Southern blotting (capillary blotting) but nowadays it is usually done by electrophoresis (electrophoretic blotting).

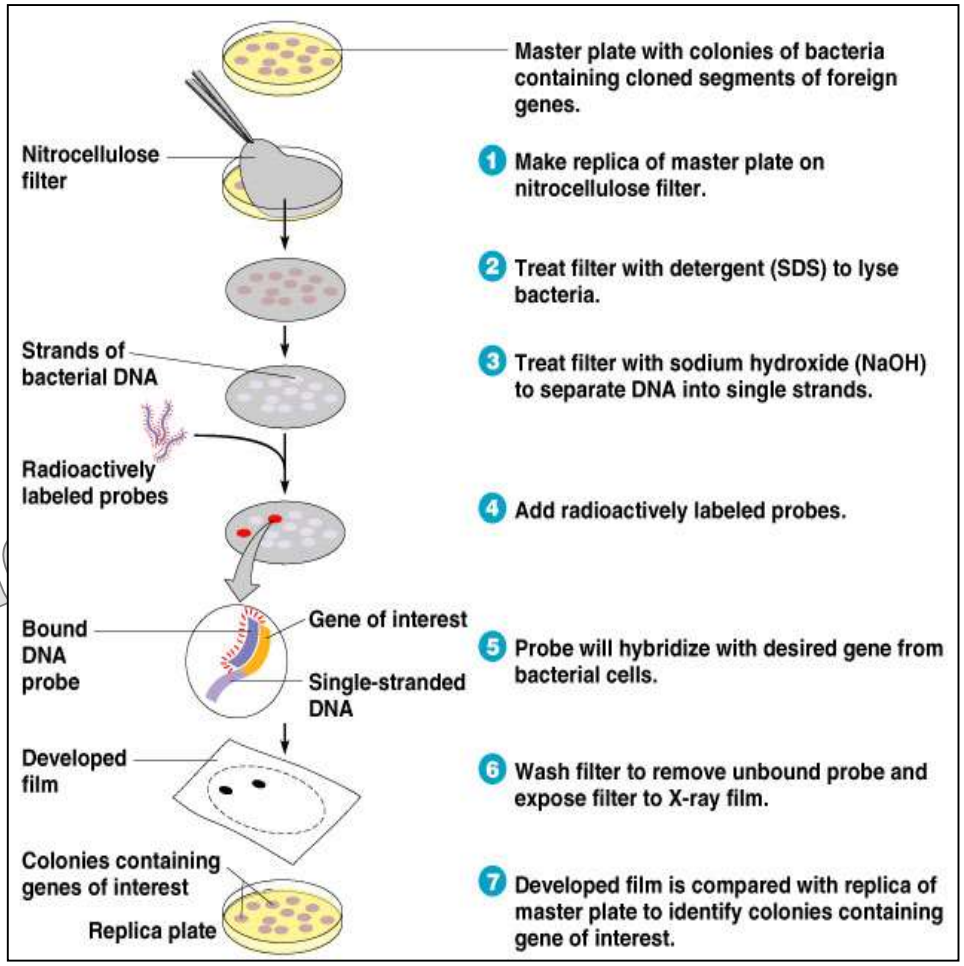
2. Electrophoresis has been applied for the blotting step in Southern and northern hybridizations as well; in such cases buffer of low ionic strength (to avoid overheating during electrophoresis) and nylon membranes (since nucleic acids bind to nitrocellulose membrane only under conditions of high ionic strength) are used. The electrophoretic blotting, both of proteins and nucleic acids is much faster and more efficient than capillary blotting.



The specific protein bands are identified in a variety of ways. (i) Antibodies are the most commonly used as probes for detecting specific antigens. (ii) Lectins are used as probes for the identification of glycoproteins. These probes may themselves be radioactive or a radioactive molecule may be tagged to them. Often the identification process is based on a 'sandwich' reaction. In such an approach a species-specific second antibody or protein A of *Staphylococcus aureus* (protein A binds to certain subclasses of IgG antibodies) or streptavidin (it binds to biotinylated antibodies) is used to bind to the antibodies bound to the protein bands. These second molecules may be labelled with radioactive, enzyme or fluorescent tags; a single preparation of these labelled molecules can be employed as a general detector for various probes.

TOPIC 6 -COLONY HYBRIDIZATION

This technique is used to identify those bacterial colonies in a plate, which contain a specific DNA sequence. These bacterial colonies are obtained from bacterial into which this sequence was introduced through genetic engineering, and the given sequence is represented by the probe used in the hybridization experiment. The procedure for colony hybridization is briefly described below.



1. The bacterial cells subjected to transformation are plated onto a suitable agar plate; this is the master plate.
2. The colonies of master plate are replica plated onto a nitrocellulose filter membrane placed on agar medium. For replica plating, a block of wood or cork, of suitable diameter for the master plate, is covered with velvet cloth. This block is sterilized and then lowered into the master plate till the velvet touches all the colonies; the block is withdrawn and gently lowered onto the nitrocellulose filter so that the bacterial cells sticking on to the velvet are transferred onto the filter. The master plate is retained intact for later use. A reference point is marked both on the master plate and the on replica plate to facilitate later comparisons.
3. After the colonies appear, the filter is removed from the agar plate and treated with alkali to lyse the bacterial cells. This also denatures the DNA released from these cells.
4. The filter is treated with proteinase K to digest and remove the proteins; the denatured DNA remains bound to the filter.
5. The filter is now baked at 80° C to fix the DNA; this yields the DNA-print of bacterial colonies in the same relative positions as those of the colonies themselves in the master plate.
6. The filter is now hybridized with the radioactive probe; the probe represents the sequence of DNA segment used for transformation. The unhybridized probe is removed by repeated washing,
7. The colonies whose DNA hybridizes with the probe are detected by autoradiography; only these colonies show up in the autoradiograph.

The positions of colonies showing up in the autoradiograph are compared with the masterplate to identify these colonies; these colonies contain the DNA segment in question. The colonies are then picked up for further studies. A variation of this procedure can be applied to phage plaques as well.

TOPIC 7 -DOT BLOT TECHNIQUE

This technique is used to detect the presence of a given sequence of DNA/RNA in the nonfractionated (not subjected to gel electrophoresis) DNA. Sample DNA's from several tissues/individuals can be tested in a single test-run. Dot blots are useful in detecting presence of the sequence being transferred in a number of suspected transgenic individuals, and presence of the specific mRNA in several such individuals or in different tissues of a single individual. The generalized procedure of dot blot is briefly described below.

1. The sample DNAs (or RNAs) from different individuals or tissues are transferred onto a nitrocellulose filter in form of dots; several samples are dot-blotted onto a single filter (Fig, III-l). A sample DNA or RNA is the total DNA or RNA extracted from an individual or a tissue.
2. The DNA is first denatured and then the filter is backed at 80°C to fix the DNA firmly onto the filter. The filter is now pretreated appropriately to prevent nonspecific binding of the probe to the filter (see, Southern Hybridization).
3. The filter is then treated with the appropriate radioactive single-stranded DNA probe under conditions favouring hybridization. The filter is now washed repeatedly to remove the free probe.
4. Dots having the appropriate DNA or RNA sequence will hybridize with the radioactive probes. These dots are detected by autoradiography; the intensity of dot in the autoradiograph corresponds fairly well with the extent to which DNA or RNA is represented in the sample.

The dots that show up in the autoradiograph denote the individuals or tissues in which the DNA or RNA sequence corresponding to the probe is represented. In case of transgenic individuals, Southern hybridization may then be used for the dot blot positive individuals to derive more precise information on whether and where the sequence in question is integrated into the genome.

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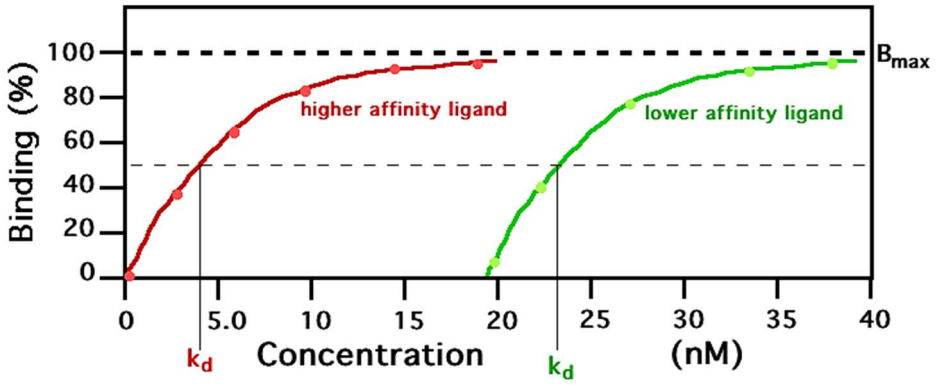
TOPIC 8 -Nitrocellulose filter-binding assay

This assay is no longer used widely, but it is rapid and simple, and can give a lot of information

It measures affinities between two molecules (often protein and DNA) using a filter. The filter is constructed of nitrocellulose paper, which is negatively charged. Since most proteins have a net positive charge, nitrocellulose paper is ideal for immobilizing proteins. DNA is negatively charged due to the phosphate backbone and will not "stick" to the nitrocellulose on its own, however, any DNA that has been bound by protein will stick. The exact amount of DNA "stuck" to the nitrocellulose is quantified by measuring the amount of radioactivity on the filter using a scintillation counter



Protein and DNA are mixed in a series of microfuge tubes in which the amount of DNA is kept constant, but the amount of protein is incrementally increased. These samples are allowed to equilibrate. After equilibration, an equal volume from each tube is squirted onto small, round, nitrocellulose filters which are arranged on a vacuum plate (a flat surface that has a vacuum applied from below to suck fluid downward). All of the protein will stick, but only the protein that has bound DNA will register in the scintillation counter.

Step	Comments
Incubate labeled DNA with protein	Allow enough time to allow the system to reach equilibrium
Filter the mixture through a filter disk made of nitrocellulose	Proteins bind to nitrocellulose, but DNA does not. Any DNA that is retained on the filter is there because it is interacting with the protein.
Dry the filters and count	

Neural Basis of Learning, Memory, Cognition, Sleep and Arousal.

Components (types) of Behaviour :-

1. Nature/innate

2. Nurture/learned

1. Nature/innate

A fixed action pattern (FAP) is a sequence of unlearned behavioral acts that is essentially unchangeable and, once initiated, is usually carried to completion.

- A FAP is triggered by an external sensory stimulus called a **sign stimulus.**
  - In the red-spined stickleback, the male attacks other males that invade his nesting territory.
  - The stimulus for the attack is the red underside of the intruder.
  - A male stickleback will attack any model that has some red visible on it.
- A proximate explanation for this aggressive behavior is that the red belly of the intruding male acts as a sign stimulus that releases aggression in a male stickleback.
- An ultimate explanation is that by chasing away other male sticklebacks, a male decreases the chance that eggs laid in his nesting territory will be fertilized by another male.

2.Nurture/learned

- Learning can be defined as an adaptive change in individual behaviour as a result of experience.
- The degree of permanence of newly acquired learned behaviour patterns depends on memory storing information gained from the experience.
- Learning alters the range of behaviours shown by an individual, and allows it to adapt to and control its environment.

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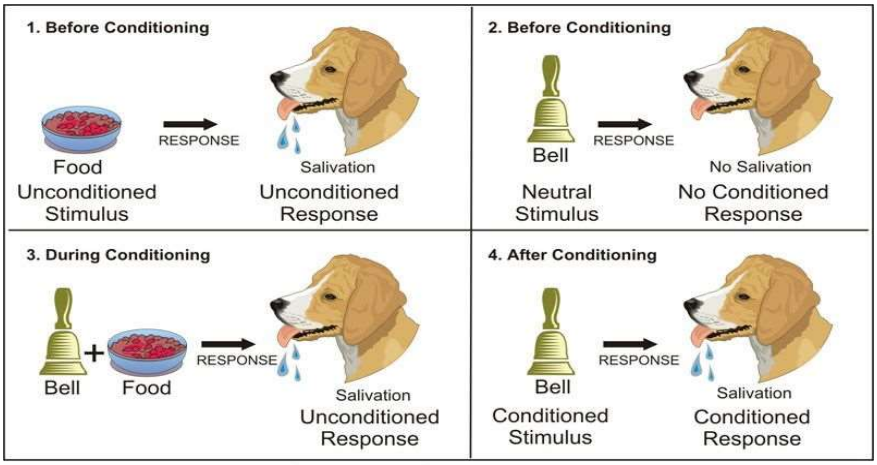
Types of learned behaviours

i. Habituation

- Ideally, an animal should recognize important cues or signals in its environment and act in response to them in order to adapt to constantly changing environment.
- **Continuous repetition of a stimulus not associated with reward or punishment (reinforcement) puts off any response to the stimulus.* E.g. birds learn to ignore scare crow. Particularly important is the reaction to new signals.
- The importance of these signals have to be assessed, and the animal has to work out how to react to them. This is where learning is important - a suitable response can be learnt, rather than a trial and error effort after each presentation of the same stimulus.
- Take for example a rat that has just experienced an aversive stimulus (e.g. a very bright light). Immediately after this event, the rat may be extra sensitive to other cues (such as noises or lights) that it would not normally respond to. This is **sensitization**, a period of increased responsiveness following a reward or punishment (or 'reinforcement').
- However, if the second stimulus is repeated without the reinforce, the response of the animal should become reduced, and the stimulus may even be completely ignored eventually. This is habituation. The animal learns not to respond to irrelevant stimuli.

- This decline in response is specific to a given stimulus. If a new stimulus is presented the animal will react (i.e. it has not ceased responding simply because it is tired). Animals will not habituate to relevant stimuli e.g. those associated with predators, food or mates.



Classical Conditioning

***Habituation is important* in the development of a behaviour in young animals and helping to understand neutral elements in the environment, such as movements due to wind, cloud, shadows, wave action etc. It is based in the nervous system and is not a form of sensory adaptation since the behaviour is permanent and no response is ever shown to the stimulus after the period of habituation.**

ii. Associative learning

Associative learning simply means that an animal learns to associate an event with a result. There are two types of associative learning: classical (Pavlovian) conditioning, and instrumental (operant or trial-and-error) conditioning.

Many animals can learn to associate one stimulus with another.

- **Associative learning** is the ability of animals to learn to associate one stimulus with another.
- For **example**, a mouse may have an unpleasant experience with a colorful, poisonous caterpillar and learn to avoid all caterpillars with that coloration.

1. Classical (Pavlovian) conditioning (conditioned reflex):is a type of associative learning.

- Researchers trained Drosophila melanogaster to avoid air carrying a particular scent by coupling exposure to the odor with an electrical shock.
- Drosophila has a surprising capacity for learning.
- **Pavlovian conditioning involves the association of events over which the animal has no control.** It is able to gain prediction of events in its environment. Pavlov's **dog** associated bell with the arrival of food and begins to salivate at the sound of a bell. At first, the dog didn't react to the stimulus alone, but after a few repetitions the stimulus was able to cause salivation, before the arrival of the food being given to the dog.

The animal learns to associate a conditioned stimulus (e.g. a bell), with the onset of an unconditioned stimulus (e.g. the arrival of food). An unconditioned response (e.g. salivation) would only occur in response to an unconditioned stimulus prior to learning.

- Birds avoid eating black and orange cinnabar moth larvae because of bad taste and avoid all similarly coloured larvae even though they may be nutritious.

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- Associative learning may play an important role in helping animals to avoid predators.
- Zebra fish, an Asian minnow, and pike, an American predatory fish, do not occur together in the wild.
- Researchers exposed zebra fish in an experimental group to an influx of 20 mL of water containing an alarm substance and then, 5 minutes later, to 20 mL of water with pike odor.
- Zebra fish had no innate negative reaction to pike odor, but learned to associate pike odor with the alarm substance.
- The zebra fish were conditioned to associate pike odor with the alarm substance.

2. Operant conditioning is also called trial-and-error learning.

- An animal learns to associate one of its own behaviors with a reward or a punishment.
- Trial-and-error activities give rise to responses which are reinforced either by rewarding (positive) or punishment (negative). The association of outcome of a response in terms of reward or punishment increases or decreases respectively future responses. Instrumental conditioning therefore involves association of events with control.
- Possibly the most famous set of experiments examining this sort of learning were done by followers of the school of B.F. Skinner. The classic tool is the Skinner box. Here, the animal is placed in the box, which has some sort of reward for conducting a behaviour. For example, if the animal presses a lever, it receives a food pellet. By trial and error, the animal learns that pressing the lever delivers the reward. In this way, the animal gains prediction and control over events in its environment. The animal learns to associate its own behaviour with a particular outcome. If the outcome is rewarding e.g. access to food, the animal learns to repeat the behaviour that resulted in food access previously. It therefore learns a conditioned response to obtain food (unconditioned response).
- An example is the mouse eating the poisonous caterpillar and learning to avoid such caterpillars in the future.

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3. Latent or exploratory learning

Animals explore new surroundings and learn information which may be useful at a later stage (hence latent) and mean the difference between life and death. E.g. In mice, knowledge of the immediate environment of its burrow may help it escape from a predator. At the time of acquiring this knowledge, it has no apparent value, hence *not all behavioural activities are apparently directed to satisfying a need or obtaining a reward.*

4. Insight learning

This is the highest form of learning which does not result from immediate trial-and-error learning but may be based on information previously learned by other behavioural activities. Insight learning is based on advanced perceptual abilities such as thought and reasoning.

Kohlar's work on chimpanzees suggested insight learning: when presented with wooded boxes and bananas too high to reach, the chimps stacked up the boxes beneath the bananas and climbed up to get them. This response appeared to follow a period of 'apparent thought'. Previous experience of playing with boxed (latent learning) may have increased the likelihood of the response.

5. Imprinting

- **Imprinting** is a type of behavior that includes learning and innate components and is generally irreversible.
- Imprinting is strongly controlled by genes. Young chicks must learn from parents' activities such as singing, nest building or following their mother.

- **There are three types of imprinting:**
- **Filial imprinting** involves activities that are learned by the young ones from their parents in early stages of life. Hunting by cheetahs, leopards and tigers is learned from parents in younger age.
- **Sexual imprinting** is the recognition of the opposite sex in the adult stage. Courtship display, such as singing, feather display and dancing must be learned during the growing stage by watching the adults.
- Peter Marler of the University of California by experiment demonstrated that chicks reared in isolation failed to develop courtship songs which they tried in adult stage. The intricate nest building by weaver birds must be learned by the individuals in the younger stage by watching the older individuals.
- **Social imprinting** happens during the brief but sensitive early period of life but it has great stability and influences the behaviour of the animals towards the others for the rest of life. Adult behaviour is the result of learning during the early stages of life
- **Social** Imprinting has a **sensitive period,** a limited phase in an animal's behavior that is the only time that certain behaviors can be learned.

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- An example of imprinting is young geese following their mother.
  - **In species that provide parental care, parent-offspring bonding is a critical time in the life cycle.**
  - During the period of bonding, the young imprint on their parent and learn the basic behavior of the species, while the parent learns to recognize its offspring.
  - Among gulls, the sensitive period for parental bonding on young lasts one or two days.
  - If bonding does not occur, the parent will not initiate care of the infant, leading to certain death of the offspring and decreasing the parent's reproductive success.
- **How do young gulls know on whom—or what—to imprint?**
  - The tendency to respond is innate in birds.
  - The world provides the *imprinting stimulus,* and young gulls respond to and identify with the first object they encounter that has certain key characteristics.
- **In greylag geese, the key stimulus is movement of the object away from the young.**
- A proximate explanation for young geese following and imprinting on their mother is that during an early, critical developmental stage, the young geese observe their mother moving away from them and calling.
- An ultimate explanation is that, on average, geese that follow and imprint on their mother receive more care and learn necessary skills, and thus have a greater chance of surviving, than those that do not follow.
- Early study of imprinting and **fixed action patterns** helped make the distinction between proximate and ultimate causes of behavior.
  - They also helped to establish a strong tradition of experimental approaches in behavioral ecology.

ERYTHROCYTES – Red Blood Corpuscle (RBC)

GENERAL STRUCTURE:

1.RBC is a circular, biconcave, non-nucleated disc. Advantages of biconcave disc:

(i) Allows considerable alteration in cell volume.

(ii) Allows easy folding of RBC on itself when it passes through capillaries.

(iii) Haemoglobin remains distributed in the centre of the RBC which facilitates optimal and quick exchange of gases (i.e. oxygen and carbon dioxide).

RBC depends entirely on glucose metabolism for its energy supply. Glucose is transported easily across the cell membrane by *facilitated diffusion*.

2. Structure: RBC contains haemoglobin, which takes pink colour with Leishman's stain. Below the cell membrane is a contractile layer of lipoprotein – '***spectrin***', which is arranged in a fibrillar manner. It maintains the shape and flexibility of RBC membrane and also contains specific blood group substance, the *antigen*.

3. Composition

(i) 62.5% water

(ii) 35% Haemoglobulin (29.5± 2.5 pg/RBC)

(iii) 2.5%:

(a) Sugar – glucose

(b) Lipids – cephalin, cholesterol and lecithin

(c) Protein – Glutathiones, albumin like insoluble protein, acts as a reducing agent, thus prevents damage of haemoglobin.

(d) Enzymes of glycolytic system; carbonic anhydrase and catalase.

(e) Vitamin derivatives

4. Diameter: 6.6–8.8 μm (average 7.3 μm)

Count

At birth: 6–7 million/mm^3.

Adults:

Male: 5–6 million/mm3. (average 5.5 million/mm^3.)

Females: 4.5–5.5 million/mm3. (average 4.8 million/mm3.)

Life span: 120 days

Site of destruction: Tissue macrophage system.

PRINCIPLE:

(i) When RBCs are placed in isotonic solution i.e., solution with same tonicity as that of plasma (e.g., 0.9% NaCl; 5% glucose; 10% mannitol and 20% urea solution), they remain suspended in it i.e., neither swell nor shink because the osmotic pressure of the isotonic solution is equal to the osmotic pressure within the RBCs.

(ii) RBCs, when placed in hypotonic solution (e.g., <0.9% NaCl), swell by absorbing water from outside and finally burst.

(iii) When RBCs are placed in hypertonic solution (e.g., >0.9% NaCl), fluid goes out of the cells and they shrink.

Red Blood Corpuscles -(Erythrocytes RBC)

RBC circular, Biconcave & non nucleated in mammals (RBC is circular only in outline)

► Exception -Camel, Llama & Embryo (RBC nucleated) nucleated RBC is oval shaped.

► RBC of frog-Biconvex nucleated.

► Formula of Haemoglobin $C_{3032}\ H_{4816}\ O_{872}\ N_{780}\ S_8\ F_4$,

► Molecular weight = 66000 and Only ferrous (Fe^{2+}) ion is functional in haemoglobin.

► Ratio of RBC in plasma is known as haematocrit value. it is in male 40% & in females 36%.

► Size of RBC in Man - 7.5 μ m, Size of RBC in Rabbit - 7.0 μm.

► RBC is present only in vertebrates. Amphibians has largest RBC while mammals has smallest

► **Largest RBC -Amphiuma *(Salamander)* (75 to 80μ)**

► **Smallest RBC - Musk deer *(Moschus moschiferus)***

► **Largest RBC in mammals - Elephant (9.4 μ)**

► **Size of Frog RBC - 35-40μ.**

Note: RBC is nucleated in - Bone marrow, But due to deficiency of vit B_{12}& folic acid nucleic acid does not formed & divisions are not take place & RBC remain large in size.

Change in size of RBC - Anisocytosis.

Change in shape of RBC - Poikilocytosis. Ex. In Uraemia Shrunken RBC is known as creanated RBC.

Due to deficiency of Fe RBC remain small in size. Ex. In Pregnancy.

Due to deficiency of folic acid RBC increases in size.

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VARIATIONS IN SIZE, SHAPE AND STRUCTURE OF RBC

1. **Anisocytosis** – Variation in the size of RBCs.
2. **Pikilocytosis** – Variation in the shape of RBCs.
3. **Spherocytosis** – Spherical RBCs; more fragile.
4. **Anaemia** – Reduction in number of RBCs less than 4 million/mm^3. or their content of haemoglobin less than 12 gm% or both.
5. **Polycythemis** – RBC count increases more than 6 million/mm^3

Causes:

A. Physiological:
(i) at birth
(ii) at high altitude due to chronic hypoxia

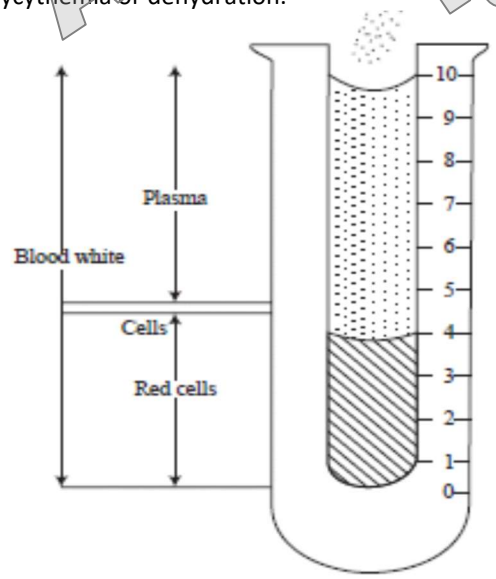
B. Pathological:
(i) congenital heart diseases which produces hypoxia
(ii) dehydration
(iii) shock
(iv) tumour of bone marrow, called **Polycythemia Vera (Erythraemia)**.

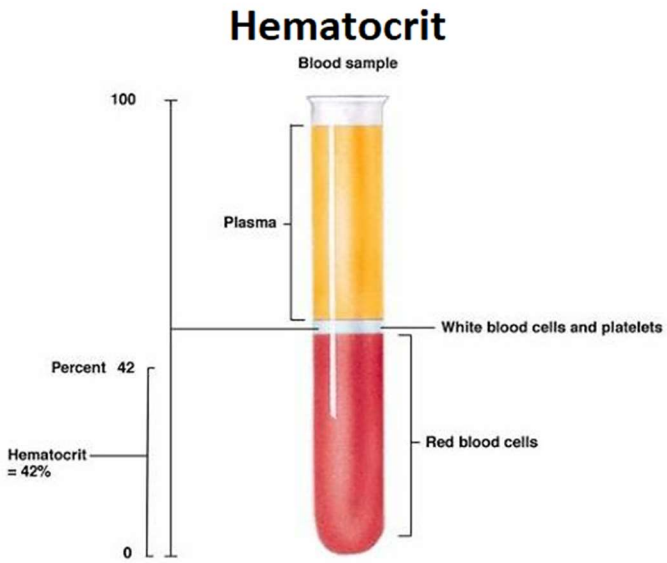
6. At Birth:
(i) RBCs are larger in size and RBC count is 6–7 million/ mm^3
(ii) PCV (packed cell volume) is 54%, because RBC count is more.

7. In **lead poisoning**, the following changes are seen in the RBCs:
(i) ***Punctate basophilia*** i.e., dots of basophilic material in the sytoplasm.
(ii) ***Cabots ring*** i.e., ring shape or figure of '8' material at the periphery of the cell.
(iii) ***Howell jolly bodies*** i.e., small nuclear fragments in the cytoplasm.

RBC INDICES (BLOOD STANDARDS)

1.Haematocrit (Hct) or packed cell volume (PCV). It measures the percentage by volume of packed RBCs in a whole blood sample after centrifugation. It is determined by putting uncoagulated whole blood in a Wintrobe tube which is calibrated (Fig. 11.1). The tube is centrifuged for 30 minutes at a speed producing 1500 g and the percentage is calculated for the volume of red cells. The Hct value is usually three times the haemoglobin value and is given as percent. A low Hct indicates such conditions as anaemia or overhydration; a high Hct denotes such conditions as polycythemia or dehydration.





2. **Mean Corpuscular Volume** (MCV) i.e., volume of a single RBC in cubic microns (μm^3). It can be computed as:

$$MCV = \frac{\text{PCV per 100 ml blood}}{\text{RBC count in million/cu mm}} \times 10\mu m^3$$

PCV per 100 ml means Hct % $= \frac{45}{5} \times 10\mu m^3$

= 90 μm^3 (range: 78-94 μm^3)

RBCs with normal volume, called **Normocytes**.
RBCs with less than normal volume, called **Microcytes**.
RBCs with more than normal volume, called **Macrocytes**.

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3. Mean Corpuscular Haemoglobin Concentration (MCHC) i.e., amount of haemoglobin expressd as percentage of the volume of a RBC or it is the haemoglobin concentration in a single RBC.

$$MCHC = \frac{Hb\ in\ gm\%}{PCV\ per\ 100ml\ blood} \times 100 = \frac{145}{45} \times 100$$

= 33% (or 33 gm/100 ml of cells) range ($35 \pm 3\%$)

If MCHC is within normal range, then RBC is **Normochromic**.

If MCHC is less than normal range, the RBC is **Hypochromic**.

MCHC is never more than 38%, because cell cannot hold haemoglobin beyond its saturation point i.e., metabolic limit of the cells haemoglobin forming mechanism,therefore, anaemia can 'never be hyperchromic'.

4. Colour Index (CI): It denotes the ratio of haemoglobin to RBC. It is an insignificant index, because normal range of RBC is very wide.

$$CI = \frac{Hb\ \%}{RBC\ \%}$$

$$= \frac{100}{100}$$

= 1 (range: 0.85-1.15)

14.8 gm% Haemoglobin = 100%

5 million/ mm^3 RBCs count = 100%

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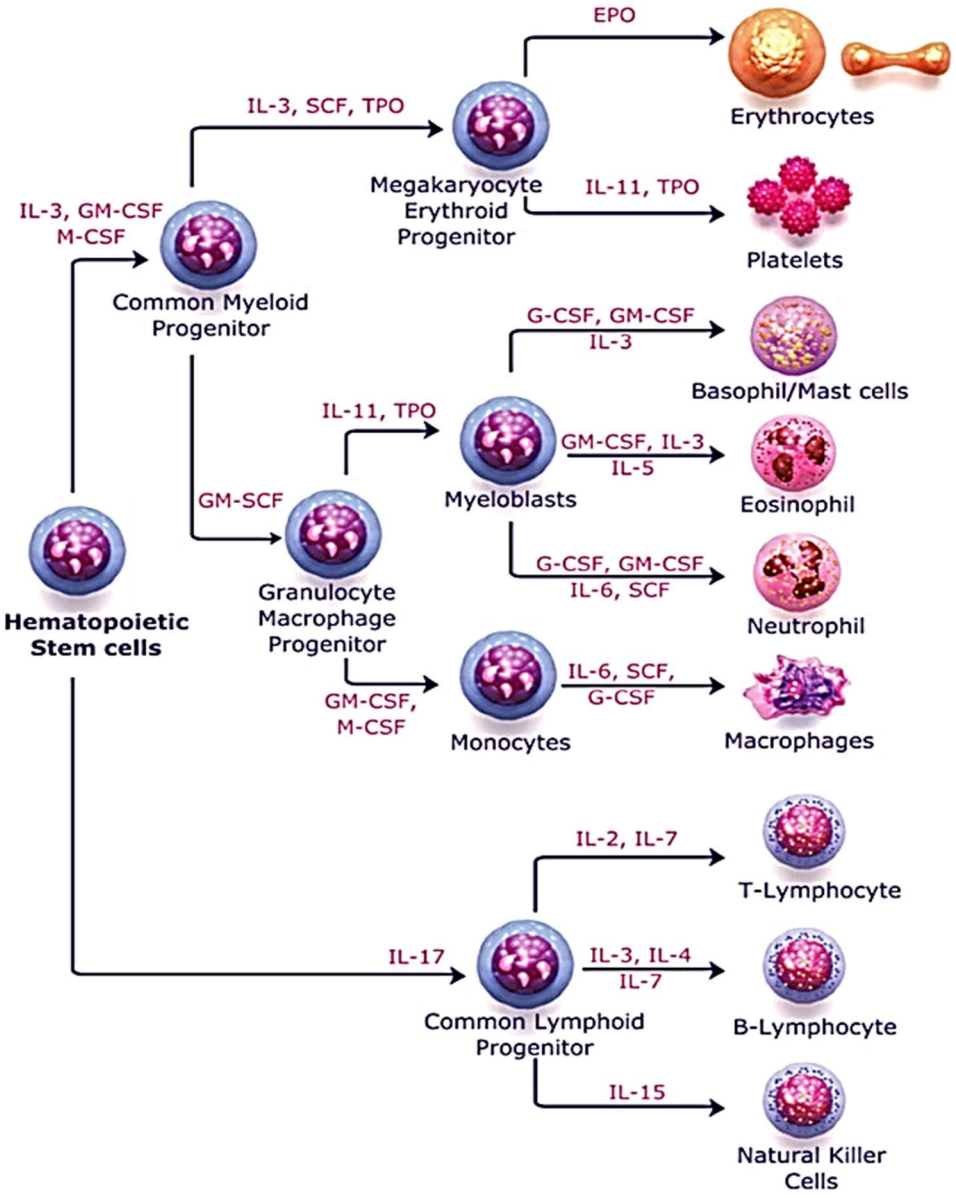
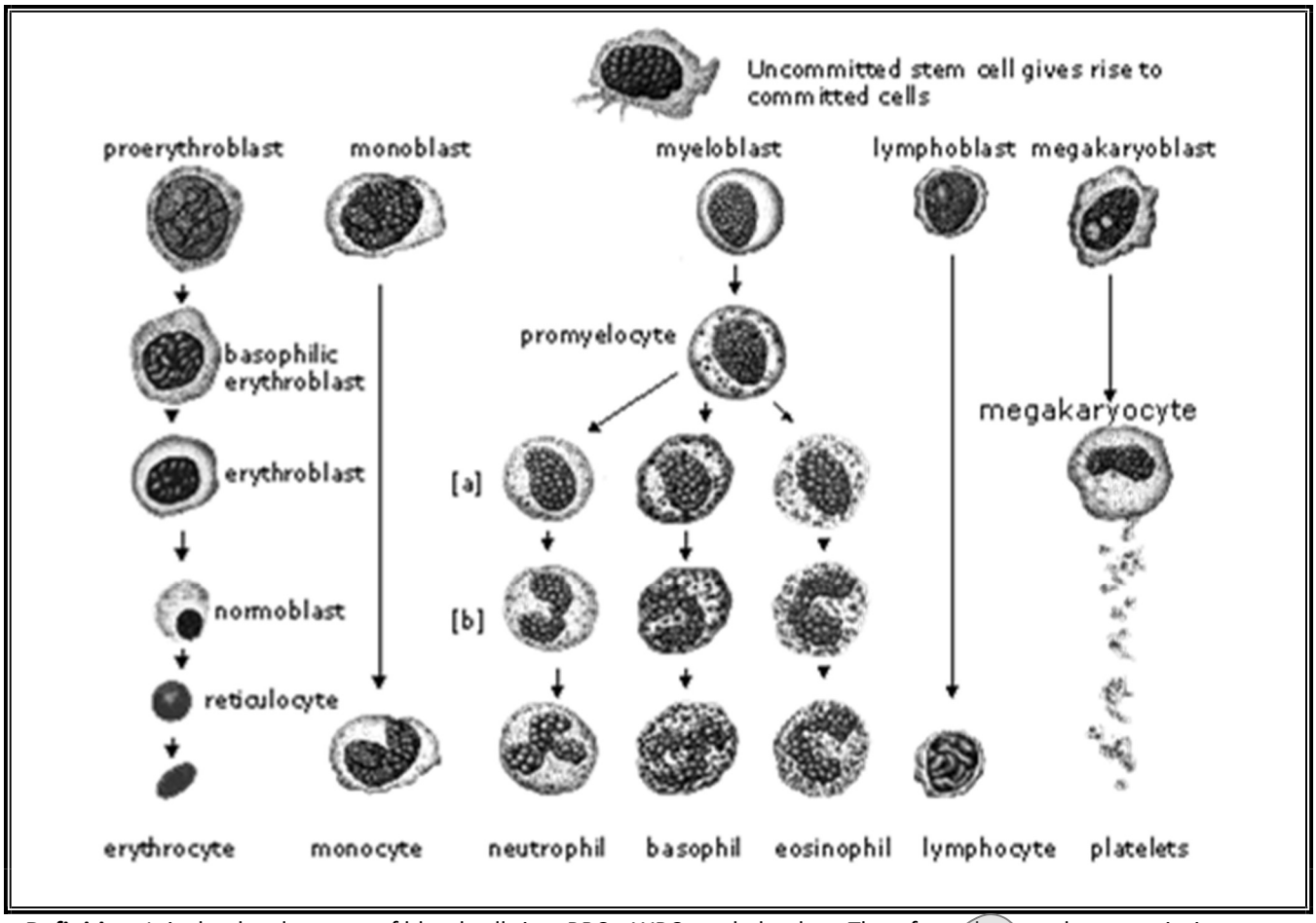
HAEMOPOIESIS





Definition: It is the development of blood cells i.e., RBCs, WBCs and platelets. Therefore, the term haemopoiesis includes:

(1)Erythropoiesis i.e. development of RBCs

(2) Leucopoiesis i.e. development of WBCs; and

(3) Megakaryocytopoiesis i.e. development of platelets.

ERYTHROPOIESIS

(A) During intrauterine life - 3 stages.

1. ***Mesoblastic stage:***In early embryo upto 3 months of foetal life, RBCs are formed from mesoderm of yolk sac or *area vasculosa.* Since erythropoiesis occurs within the blood vessel, therefore, this stage is also called ***intravascular***erythropoiesis.
   Endothelial cells proliferate and differentiate to form masses of nucleated haemoglobin bearing cells; these cells get detached from capillaries and finally lose their nuclei to give rise to non-nucleated discs.
2. ***Hepatic stage:***After 3 months of foetal life, liver and spleen are the site of blood formation. Nucleated RBCs develop from the mesenchyme between the blood vessels and the tissue cells.
3. ***Myeloid stage:***From the middle of foetal life, erythropoiesis occurs in the bone marrow. *Hepatic* and *myeloid stages* are ***extravascular***erythropoiesis.

(B) In children, erythropoiesis occurs in:
(1) All bones with red marrow (mainly), (2) Liver, and (3) Spleen.

(C) In adults, i.e. after 18-20 years of age, from red bone marrow which includes:
(1) ends of long bones like humerus and femur, because shaft is converted to yellow marrow.
(2) skull (3) Vertebrae (4) ribs (5) sternum and (6) pelvis
If marrow gets destroyed, then liver and spleen again become important sites of blood formation.

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Terminology		Nucleus	Cytoplasm	
			Staining	Haemoglobin
Haemocytologist (Stem cell)	↑	Very big – occupies almost whole of the cell with open chromatin, containing 4-5 nucleoli; deep basophilic	Rim all around the nucleus; deep basophilic	Absent
Pro-erythroblast	↑	Occupies 3/4 of cell volume; 2-3 nucleoli; chromatin open	Slightly more in amount; deep basophilic	Absent
Early normoblast	↑ 7 DAYS	Size decreases; *no nucleoli*; chromatin condenses	Further increase in amount; less basophilic	Absent
Intermediate Normoblast	↓	Nucleus size further decreases; chromatin further condenses	Marked cytoplasm, polychromatophilic staining	**Starts *appearing***
Late Normoblast (i) Early	↓	Nucleus very small with chromatin dot ***cart wheel appearance***	Increases markedly	Further increases in amount.
(ii) Late	↑	Nucleus degenerates, becomes uniformly deeply stained ***pyknotic***	Further increases; more acidic, less basophilic	Further increases in amount.
Reticulocyte		No nucleus; remnants of RNA present	Acidiophilic	Further increases in amount.
Erythrocyte	2 DAYS ↓	Nil	Acidiophilic	Further increases in amount.

STAGES OF ERYTHROPOIESIS

Stages of erythropoiesis are summarized in Table.

During the development of erythrocytes as the cell attains maturity, it shows the following characteristic features:

1. Reduction in the cell size.
2. Cytoplasm increases in amount and nucleus decreases in size.
3. Staining reaction of cytoplasm changes from deep basophilic to polychromatophilic (acidophilic plus basophilic) and finally to acidophilic; due to gradual reduction in RNA amount.
4. Initially, nucleus is very big in size with open chromatin and containing many nucleoli; with maturity of RBC, chromatin material condenses and degenerates finally leading to disappearance of nucleus and nucleoli.

ANAEMIAS

Definition: Anaemia is a clinical condition characterized by reduction in the number of RBCs less than 4 million/ mm^3. or their content of haemoglobin less than 12 gm% or both.

Classification:

1. *Dyshaemopoiesis* or ***abnormal haemopoiesis*** resulting in ***aplastic anaemia.***

Causes:

(i) X-rays irradiations
(ii) y-rays irradiation
(iii) Hypersensitivity of bone marrow to
  (a) cytotoxic drugs, sulpha drugs etc.
  (b) chemicals.

2. Hemolytic anaemias– anaemias due to excessive destruction of RBCs.

(i) Intrinsic (intra-corpuscular) defects hereditary in nature:
  (a) Congenital (or familial or hereditary) spherocytosis.
  (b) Haemoglobinopathies – sickle cell anaemia and thalassaemia.
  (c) Erythroblastosis foetalis.
  (d) Glucose-6-phosphate dehydrogenase (G6PD) deficiency.

(ii) Extrinsic (extra-corpuscular) defects acquired in nature:
  (a) Antigen-antibody reaction.
  (b) Infection *e.g.* malaria.

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STRUCTURE OF PLASMA MEMBRANE

⇨ Cell membrane is a dynamic fluid structure and most of their molecules are able to move about in the plane of the membrane.

⇨ Plasma Membrane is asymmetrical structure; the lipid and protein composition of the outside and inside differ from one another in ways that reflect the different functions performed at the two surfaces of membrane.

Characteristics of lipid bilayers:-

1. Lipids and Proteins are free to move about (diffuse) in the plane of the membrane and to **rotate** about an axis perpendicular to the membrane. The membrane is like a two-dimensional solution of proteins in a phospholipid solvent (Phospholipid Bilayer) ===> **Fluid**.

2. Proteins and phospholipids cannot flip from one side of the membrane to the other however.

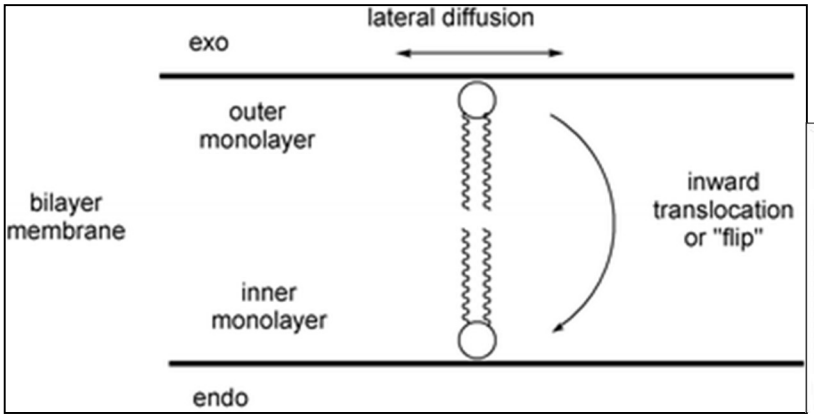
3. Fluidity of lipids depends on hydrocarbon tails of fatty acids: fatty acids with cis double bonds don't condense easily into solid state ==> bilayer is more fluid.

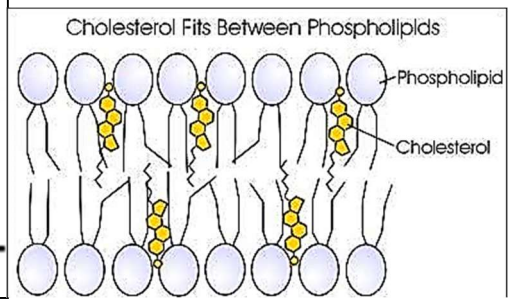
4. Steroid lipids (Cholesterol) make bilayers less fluid but also inhibits transformation to solid state at low temperature.

5. Membrane proteins are classified as either Integral or Peripheral

6. They tend to aggregate when surrounded on all sides by water, so that their hydrophobic tails are buried in the interior and their **hydrophilic heads** are exposed to water.

7. They have self sealing properties when they are torn from in between





membrane	% protein	% lipid	% carbohydrate
myelin	18	79	3
human erythrocyte plasma membrane	49	43	8
mitochondrial inner membrane	79	24	0
amoeba plasma membrane	54	42	4

Fluid Mosaic Model of the Cell Membrane:-

The most widely accepted model of the cell membrane is the "Fluid Mosaic Model". By this concept the cell membrane consists of a continuous, fluid, double layer of phospholipids. Proteins either are embedded in the bilayer or associated with either the cytoplasmic or extracellular face. Carbohydrates are linked to the proteins (glycoproteins) or lipids (glycolipids) only on the extracellular side. The phospholipid profiles of the cytoplasmic and extracellular layers differ. Cholesterol, in varying amounts depending on the cell type, lies within the membrane serving to stabilize it.

⇨Five important features of the fluid mosaic model of membrane structure proposed by **S.L Singer and G.L Nicholson**

1. fluid (can move)
2. mosaic (made up of a lot of differnt parts integrated together)
3. bilayer (helps regulate what goes in and out)
4. proteins (serve as markers on the surface for identification purposes and to allow certain molecules in and out)

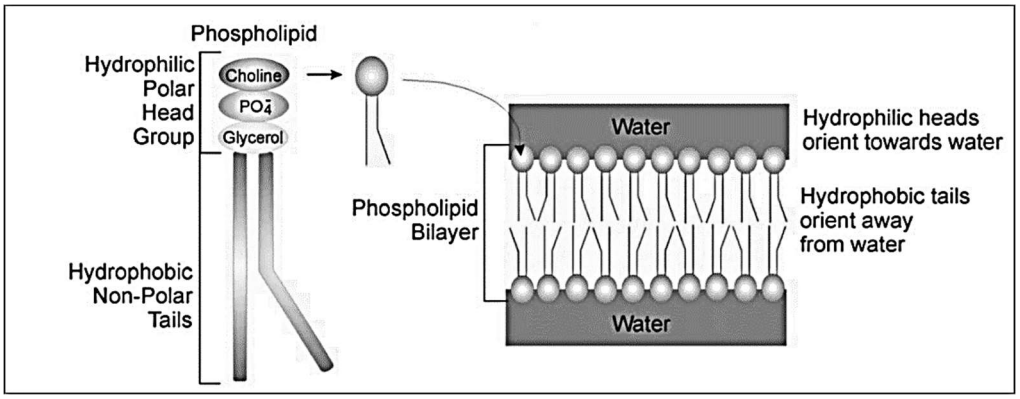
5. transport (passive and active, allows molecules across membrane)

Structure of Phospholipids

The Amphipathic Nature of Phospholipids

The basic structure of biomembranes is defined by their continuous components-the phospholipids. These molecules have an important attribute that allows them to form membranes. One part of them associates with water while the other part shuns it. As we see this underlies the basic organization of the biological membrane.

- Hydrophilic head--"likes" water--polar end
- Hydrophobic tail--"hates water"--non-polar chain of fatty acids
- Tail--length & number double bonds differs in different phospholipids



⇨Lipids constitute about 50% of the mass of most animal cell membrane and All of them in cell membrane are amphipathic.

⇨The most abundant lipids are phospholipids, which have a polar head group and two hydrophobic fatty acid tails differing in length 14-17 carbon. One tail usually has one or more double bonds**(unsaturated)**while other tail does not **(Saturated).**

⇨Difference in the length and saturation of the fatty acid tail are important because they influence the ability of phospholipids molecule to pack against one another and for this reason they affect the fluidity of the membrane

Four major phospholipid predominate in the P.M. of many mammalian cells.

1. **Phosphotidyl choline (PC),**
2. **Sphingomylin(SM),**
3. **Phosphotidyl serine(PS)**
4. **Phosphotidylethano lamine(PE)**

Other Phospholipid such as ionositol phospholipid are present in smaller quantities (but are functionally very important in cell signaling).-

****PC & SM are found in outer monolayer and PS & PE found in inner monolayer**.

***ionositol phospholipids are concentrated in cytoplasmic half of Plasma membrane of Eukaryotic cells.**

Fatty acid tail are of two types

1. Saturated fatty acids

♦ All C-C bonds are single bonds and Straight chain allows maximum interaction of fatty acid tails so Make membrane less fliuid(Solid at room temperature)

2. Unsaturated fatty acids

♦ Some C=C bond (double bonds) that Bent chain keeping tails apart and Make membrane more fliuid

♦ Polyunsaturated fats have multiple double bonds and bends and they are Liquid at room temperature

♦they are "Good Fats" which do not clog arteries (vegetable fats)

3. Membrane sterol

♦ Reduces membrane fluidity by reducing phospholipid movement so Hinders solidification at low (room) temperatures and Stabilizes the Membrane

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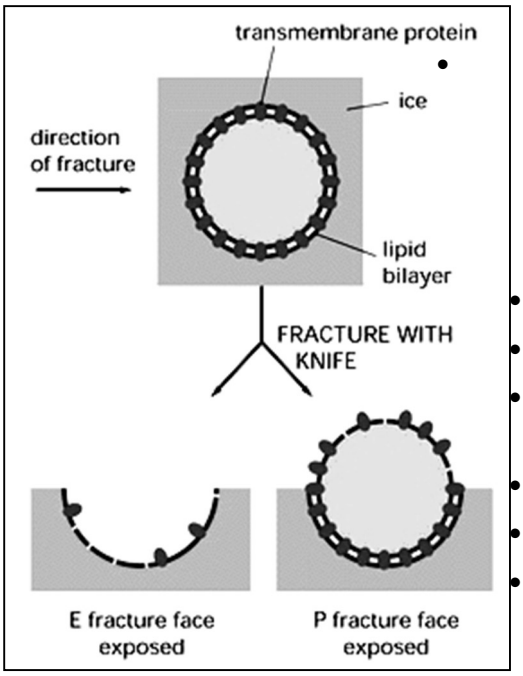
• Flat shape: Interdigitates between phospholipids

• **Cholesterols is Present in animal cell membranes**

⇨ **Absent in bacteria; and in most plants—(where Cell walls provide stability**)



Freeze-Fracture Technique

This technique is used to visualize the distribution of proteins in membranes. In this technique a membrane specimen is rapidly frozen to near liquid nitrogen temperatures (–196°C) to immobilize the sample. The specimen is then fractured with a cold microtome knife, which often splits the bilayer into monolayer and exposes the interior of the lipid bilayer and its embedded proteins.

- **The freeze fracture method involves the following steps:**
- Cryofixation of the specimen by rapid freezing.
- Fracturing of the specimen by cleaving it with a microtome knife or breaking it.
- Coating of freeze-fracture plane by heavy metals under high vacuum.
- Removal of the specimen from the replica.
- Observation of the replicas with a TEM.

Freeze etching is a slight modification of freeze fracturing through the process of controlled sublimation of water vapor (transition from the solid state to the gas state, bypassing the fluid state) to expose membrane surfaces or macromolecules just below the fracture plane.

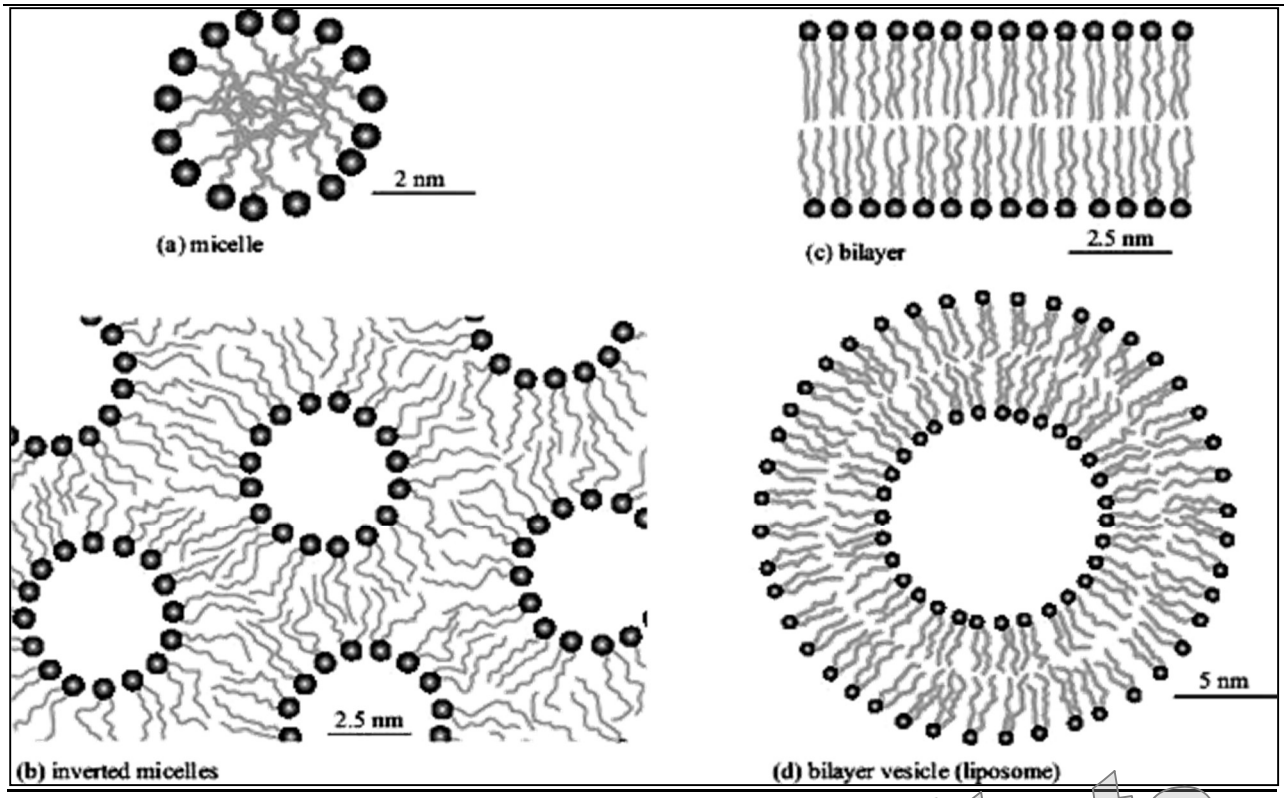
⇨ **X-ray diffraction** and **freeze fracture electron microscopy technique**, are needed to reveal the details of Plasma membrane organization.

Primary sterol found in fungi is ergosterol, while in some plants they are sitosterol, brassicasterol and avanesterol

Alternative phopholipids Conformation

1. Liposomes Size vary from 25 nm to 1 µm depending on how they are produced

Liposome-like structures underlie such things as LDL-particles and are being used in medicine among other areas.



- Liposomes are bilayered lipid vesicles
- Form by sonicating lipids in aqueous solution
- Vehicles for drug, nucleic acid, antibody delivery
- Used in cosmetics

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2. Micelles:

Micelle is formed when a variety of molecules including soaps and detergents are added to water. The molecule may be a fatty acid, a salt of a fatty acid (soap), phospholipids, or other similar molecules.

The molecule must have a strongly polar "head" and a non-polar hydrocarbon chain "tail". When this type of molecule is added to water, the non-polar tails of the molecules clump into the center of a ball like structure, called a micelle, because they are hydrophobic. The polar head of the molecule presents itself for interaction with the water molecules on the outside of the micelle.

What is Critical Micelle Concentration?

The proportion of molecules present at the surface or as micelles in the bulk of the liquid depends on the concentration of the amphiphile. **At low concentrations surfactants will favor arrangement on the surface**. As the surface becomes crowded with surfactant more molecules will arrange into micelles. At some concentration the surface becomes completely loaded with surfactant and any further additions must arrange as micelles. This concentration is called the **Critical Micelle Concentration (CMC)**

Membrane Fluidity

♦ Lipids have rapid lateral movement

♦ Lipids flip-flop extremely slowly

♦ Phospholipid molecules are synthesized in only one monolayer of a membrane. In ER special class of ER-membrane bound enzymes called **phospholipids translocators(Flippase)** catalyze the rapid flip-flop of specific phospholipids from the monolayer where they are made to the opposite monolayer

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Role of flippase and floppases

Although the barrier to rapid spontaneous flip-flop contributes to the maintenance of lipid asymmetry, other mechanisms must be responsible for the regeneration of lipid asymmetry. Perhaps the most significant contributors to

the maintenance and dissipation of transbilayer lipid asymmetry are proteins that catalyze the movement of lipids across the membrane. These activities are classified according to substrate specificity, requirements for energy, and direction of transport. Two classes of transport activities have been described that are responsible for the *ATP-dependent transport* of lipids. The most well-characterized activity, catalyzed **by flippase,** transports glycerophospholipid from the outer monolayer to the cytoplasmic surface of the plasma membrane. A second *ATP-dependent* activity, catalyzed by **floppases,** transports lipids in the opposite direction.

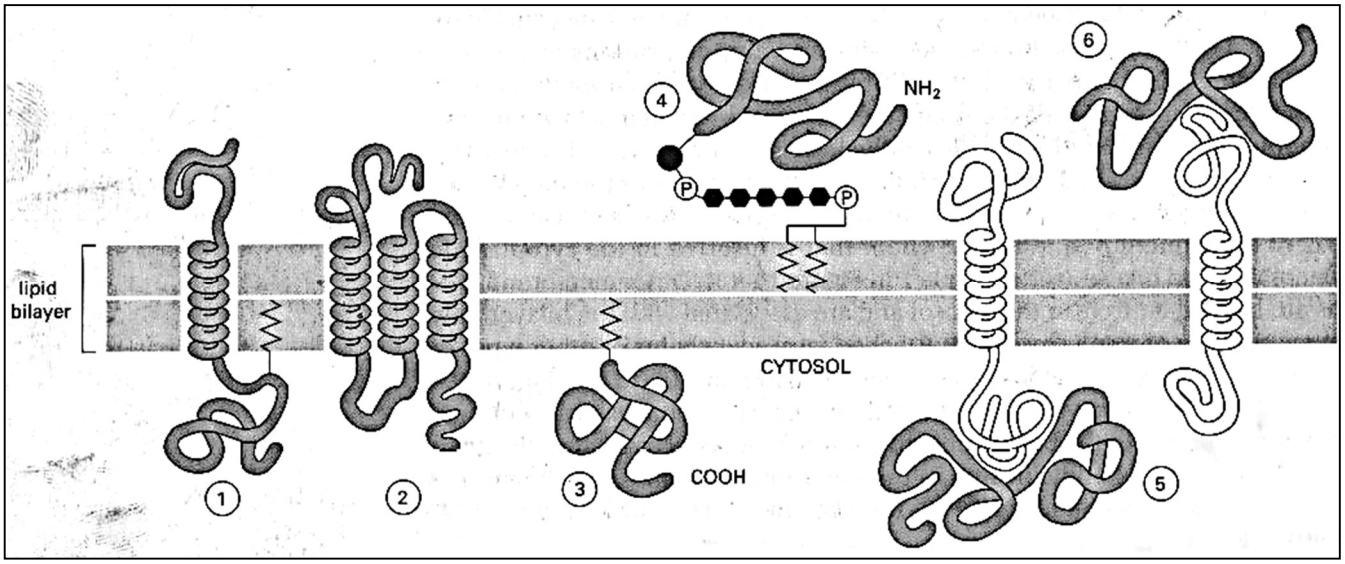
- ♦ Fluidity depends on lipid composition. **MORE UNSATURATION MORE FLUIDITY**
- ♦ For measuring the motion of individual lipid molecule, spin labeling is used. In this process groups containing unpaired electrons are tagged to lipid molecules whose spin create a paramagnetic signals. This can be detected by **electron spin resonance (ESR) spectroscopy.**

Membrane Proteins:

Because lipid molecules are small in comparison to protein molecules, there are always many more lipid molecules than protein molecules in membranes – about 50 lipid molecules for each protein molecules in a membrane that is 50% protein by mass. Different membrane proteins are associated with membrane in different ways.

Membrane proteins are classified as either Integral or Peripheral

- **Integral Membrane Proteins** are an intrinsic component of the membrane and extend from one side of the lipid bilayer to the other; they cannot be removed without disrupting the membrane. These proteins contain sequences of amino acids **with hydrophobic side chains (R-groups) which adopt an α-helical conformation as they pass through the lipid bilayer.** In the α-helix polar peptide groups are H-bonded to one another isolating them from the hydrophobic lipids; the hydrophobic side chains (R-groups) cover the surface of the α-helix where it contacts the lipids. It takes a sequence of approximatley 20 hydrophobic amino acids to traverse the hydrophobic portion of the lipid bilayer.
- **Peripheral Membrane Proteins:** bind through noncovalent interactions to the surface of the membrane, either to the lipid headgroups or to other membrane proteins; they can be removed by treatments (e.g. change in pH or ionic strength) which do not disrupt the membrane.



Type 1 & 2 are trans membrane proteins which are amphipathic like their lipid neighbors. Their hydrophobic region passes through the membrane and interacts with the hydrophobic tails of the lipid molecules in the interior of the bilayer. Their hydrophilic regions are exposed to water on one or the other side of the membrane.

Type 3 are located entirely in the cytosol and are associated with the bilayer only means of one or more covalently attached fatty acid chains by forming covalent attachments with three classes of lipids: **isoprenoid groups such as farnesyl and geranylgeranyl resiudes**; **fatty acyl groups such as myristoyl and palmitoyl residues;** and **glycoinositol phospholipids (GPIs).**

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⇨An isoprenoid group that contains 15 (farnesyl) or 20 (geranylgeranyl) carbon atoms attaches to a cysteine residue at the C-terminus via a *thioether* linkage.

⇨A myristic acid (14-carbon, saturated fatty acid) molecule attaches to a protein through *amide* linkage to the alpha-amino group of an N-terminal *Gly* residue. A palmitic acid (16-carbon, saturated fatty acid) molecule attaches to a *Cys* residue close to the N- or C-terminus via a *thioester* linkage.

⇨A glycosyl phosphatidyl inositol molecule (GPI) attaches to the C-terminal amino acid via an amide linkage.

GPI-linked proteins occur on the exterior surface of the plasma membrane

Type 4 entirely exposed at the external cell surface being attached to the bilayer only by a covalent linkage (via a specific oligosaccharide) to phosphotidyl ionositol in the outer lipid monolayer of the P.M. those are made as single pass trans membrane protein ins the ER, while still the ER, the trans-membrane segment of protein is cleaned off and Glycosyl phosphotidyl ionositol (GPI) is added, leaving the protein bound to the noncytoplasmic surface of the membrane solely by this anchor.

Type 5 & 6 bound to one or other face of membrane by monocovalent interaction with other membrane protein. they are called peripheral membrane protein also. They can be released from the membrane.

► **By exposure to solution of very high or low ionic strength or extreme pH, which interfere with protein-protein interaction but leave lipid bilayer intact.**

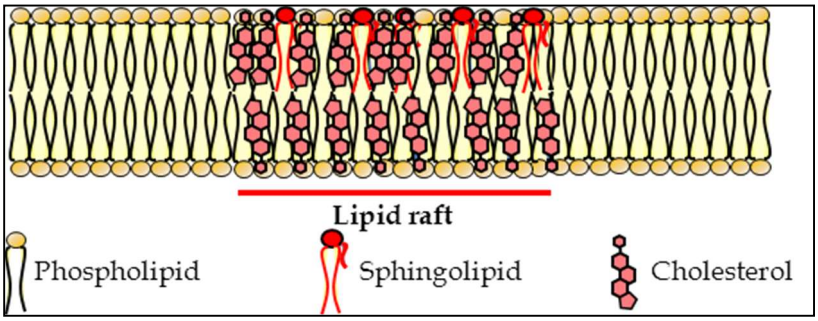
►The transmembrane domain in Trans Membrane proteins contact with hydrophobic environment of the lipid bilayer and are composed largely of amino acid residue with nonpolar side chains. Because the peptide bonds themselves are polar and water is absent, all peptide bonds in the bilayer are driven to form hydrogen bonds with one another. The hydrogen bonding between peptide bonds is maximized because of α-helix form of transmembrane domain (in most of the Transmembrane protein this is usual structure).

LIPID RAFT

- The plasma membrane of cells is made of a combination of glycosphingolipids and protein receptors organized in **glycolipoprotein microdomains termed lipid rafts.**
- These specialized membrane micro domains compartmentalize cellular processes by serving as **organizing centers for the assembly of signaling molecules, influencing membrane fluidity and membrane protein trafficking, and regulating neurotransmission and receptor trafficking.**
- **Lipid rafts are more ordered and tightly packed than the surrounding bilayer, but float freely in the membrane bilayer.**

Properties of lipid rafts

lipid rafts generally contain twice the amount of cholesterol found in the surrounding bilayer. Also, lipid rafts are enriched in sphingolipids such as sphingomyelin, which is typically elevated by 50% compared to the plasma membrane. To offset the elevated sphingolipid levels, phosphatidylcholine levels are decreased which results in similar choline-containing lipid levels between the rafts and the surrounding plasma membrane.



⇨Cholesterol interacts preferentially, although not exclusively, with sphingolipids due to their structure and the saturation of the hydrocarbon chains. Although not all of the phospholipids within the raft are fully saturated, the

- Symmetry in biology refers to the symmetry observed in organisms, including plants, animals, fungi, and bacteria. External symmetry can be easily seen by just looking at an organism.
- Internal features can also show symmetry, for example the tubes in the human body (responsible for transporting gases, nutrients, and waste products) which are cylindrical and have several planes of symmetry.
- While sponges and placozoans represent two groups of animals which don't show any symmetry (i.e. are asymmetrical), the body plans of most multicellular organisms exhibit, and are defined by, some form of symmetry. There are only a few types of symmetry which are possible in body plans. These are radial (cylindrical), bilateral, biradial and spherical symmetry.
- While the classification of viruses as an 'organism' remains controversial, viruses also contain icosahedral symmetry.

Symmetry as one of the criteria for study of evolution in animals:

- Symmetry is often selected for in the evolution of animals.
- Traditionally it has been suggested that bilateral animals evolved from a radial ancestor.
- Cnidarians, a phylum containing animals with radial symmetry, are the most closely related group to the bilaterians. Cnidarians are one of two groups of early animals considered to have defined structure, the second being the ctenophores.
- Ctenophores show biradial symmetry leading to the suggestion that they represent an intermediate step in the evolution of bilateral symmetry from radial symmetry.

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In such animals the part that moves forward (and usually contains the mouth) is termed anterior.

Radial Symmetry

▪ Rotational symmetry, also known as radial symmetry in biology, is the property a shape has when it looks the same after some rotation by a partial turn. An object's degree of rotational symmetry is the number of distinct orientations in which it looks exactly the same for each rotation.

▪ Organisms with radial symmetry show a repeating pattern around a central axis such that they can be separated into several identical pieces when cut through the central point, much like pieces of a pie. Typically, this involves repeating a body part 4, 5, 6 or 8 times around the axis – referred to as tetramerism, pentamerism, hexamerism and octomerism, respectively. Such organisms exhibits no left or right sides but do have a top and a bottom surface, or a front and a back.

▪ **George Cuvier classified animals with radial symmetry in the taxon Radiata (Zoophytes,which is now generally accepted to be an assemblage of different animal phyla that do not share a single common ancestor (a polyphyletic group).**

▪ Most radially symmetric animals are symmetrical about an axis extending from the centre of the oral surface, which contains the mouth, to the centre of the opposite (aboral) end.

Animals in the phyla Cnidaria and Echinodermata generally show radial symmetry, although many sea anemones and some corals within the Cnidaria have bilateral symmetry defined by a single structure, the siphonoglyph.

▪ Radial symmetry is especially suitable for sessile animals such as the sea anemone, floating animals such as jellyfish, and slow moving organisms such as starfish. Whereas, bilateral symmetry favours locomotion by generating a streamlined body.

Icosahedral and Spherical Symmetry

Icosahedral symmetry:

▪ **Icosahedral symmetry** occurs in an organism which contains 60 subunits generated by 20 faces, each an equilateral triangle, and 12 corners. Within the icosahedron there is 2-fold, 3-fold and 5-fold symmetry. Many viruses, including canine parvovirus, show this form of symmetry due to the presence of an icosahedral viral shell. Such symmetry has evolved because it allows the viral particle to be built up of repetitive subunits consisting of a limited number of structural proteins (encoded by viral genes), thereby saving space in the viral genome.

▪ **Spherical symmetry** is characterised by the ability to draw an endless, or great but finite, number of symmetry axes through the body. This means that spherical symmetry occurs in an organism if it is able to be cut into two identical halves through any cut that runs through the organism's centre. True spherical symmetry is not found in animal body plans. **Organisms which show approximate spherical symmetry include the freshwater green alga Volvox.**

▪ Bacteria are often referred to as having a 'spherical' shape. Bacteria are categorized based on their shapes into three classes: cocci (spherical-shaped), bacillus (rod-shaped) and spirochetes (spiral-shaped) cells. In reality, this is a severe over-simplification as bacterial cells can be curved, bent, flattened, oblong spheroids and many more shapes. Due to the huge number of bacteria considered to be cocci (coccus if a single cell), it is unlikely that all of these show true spherical symmetry. It is important to distinguish between the generalized use of the word 'spherical' to describe organisms at ease, and the true meaning of spherical symmetry. The same situation is seen in the description of viruses – 'spherical' viruses do not necessarily show spherical symmetry, usually they are icosahedral.

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Bilateral Symmetry

▪ Organisms with bilateral symmetry contain a single plane of symmetry, the sagittal plane, which divides the organism into two roughly mirror image left and right halves – approximate reflectional symmetry.

▪ Animals with bilateral symmetry are classified into a large group called the bilateria which contains 99% of all animals (comprising over 32 phyla and 1 million described species). All bilaterians have some asymmetrical features, for example the human heart and liver are positioned asymmetrically despite the body having external bilateral symmetry.

▪ The bilateral symmetry of bilaterians is a complex trait which develops due to the expression of many genes. The bilateria have two axes of polarity. The first is an anterior-posterior (AP) axis which can be visualised as an imaginary axis running from the head or mouth to the tail or other end of an organism. The second is the dorsal-ventral (DV) axis which runs perpendicular to the AP axis. During development the AP axis is always specified before the DV axis.

▪ Bilateral symmetry is associated with the term cephalization—meaning the specialization of the anterior end of the body to form the head where the nervous tissues, sense organs and feeding organs are concentrated. The opposite end is posterior; the back or upper surface is termed dorsal, and the under surface (usually toward the ground) is termed ventral (1.venter, belly). Structures on or toward the central longitudinal axis and those toward the sides are termed as medial and lateral respectively.

Biradial Symmetry & Segmentation

▪ **Biradial symmetry** is found in organisms which show morphological features (internal or external) of both bilateral and radial symmetry. Unlike radially symmetrical organisms which can be divided equally along many planes, biradial organisms can only be cut equally along two planes. This could represent an intermediate stage in the evolution of bilateral symmetry from a radially symmetric ancestor.

▪ The animal group with the most obvious biradial symmetry is the ctenophores. In ctenophores the two planes of symmetry are

- ▪ (1) the plane of the tentacles and
- ▪ (2) the plane of the pharynx.

▪ In addition to this group, evidence for biradial symmetry has even been found in the 'perfectly radial' freshwater polyp Hydra (a cnidarian). Biradial symmetry, especially when considering both internal and external features, is more common than originally accounted for.

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Type of Symmetry	Shown by	Chances of getting antimeres		Special feature
		Axis	Plane	
1.Spherical	Radiolarians Heliozoans	Any	Any	Homaxial apolar. Rare . Floating animals
2.Radial	Hydrozoans Scyphozoans	Only one axis Antero-posterior Longitudinal	Any	Monaxial heteropolar
3. Biradial	Anthozoans Ctenophors	Only one axis	Two planes Passing through mouth	Elongated, Oval mouth
4.Bilateral	Triploblastic animals	Only one Antero-posterior	One. Median Sagittal plane	Cephalization is present Advanced animals Show quick movements

Segmentation:

▪ In some animals, the body is externally and internally divided into segments with a serial repetition of at least some organs and the phenomenon is called as metamerism.

▪ In the annelids, arthropods, and chordates there is a linear repetition of body parts known as segmentation (metamerism); each repeated unit is a somite (metamere). In earth-worms the successive somites are essentially alike, but they are unlike in different body regions of a crayfish or insect.

▪ Metamerism is conspicuous both externally and internally in annelids, is mostly external with arthropods, and mainly internal in man and other chordates (vertebrae, body muscles, some blood vessels, and nerves).

Appendages, Skeleton, Sex and Embryonic Development

Appendages:

▪ Protruding parts that serve in locomotion, feeding, and other ways are termed appendages; examples are the tentacle of sea anemones, minute setae of earthworms, antennae and legs of arthropods, and the fins, legs, and wings of vertebrates.

Skeleton:

▪ Most land dwellers and many aquatic animals have a skeleton for support or protection; it may be internal (frog, man, etc.) or external (coral, crab, insect) and may be of either inorganic or material.

Sex:

▪ An animal containing both female and male sex organs in one individual is termed monoecious (also hermaphroditic); members of most higher phyla are dicoecious, each individual being either male or female.

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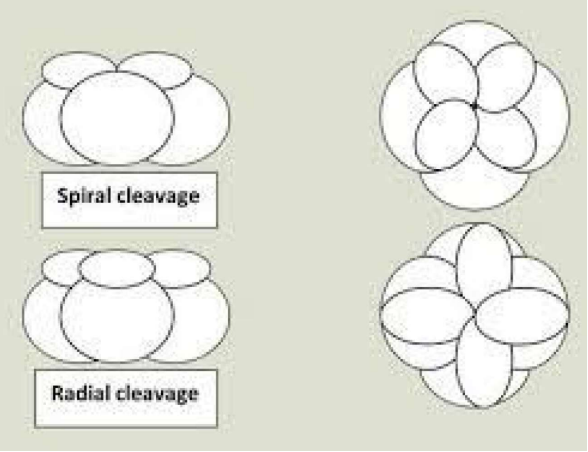
Embryonic development:

▪ Cleavage of the egg is complete or holoblastic in many invertebrates, amphioxus, amphibians, and mammals. In eggs with much yolk, cleavage is incomplete or meroblastic, confined to a limited part as in squids, insects, many fishes, reptiles, and birds.

Types of cleavage

1 Spiral Cleavage

2 Radial



1. Spiral Cleavage

- Spiral cleavage is seen in annelids, Arthropoda and Mollusca.
- It occurs in such a manner that the resulting daughter cells are not located exactly on top of one another; instead, they are located at a slight angle. Spiral cleavage is a characteristic of Protostomes(you are just about to study about this in following paragraphs) , and results in determinant cells (Cell that have a determined embryological fate early on during the development of the embryothis also is coming soon after this).
- In other words, determinant cells are programmed to become a specific type of cell, early on during the process.

2. Radial Cleavage

- Radial cleavage is found in echinoderms and vertebrates.
- It occurs in such a manner that the resulting daughter cells are located exactly on top of one another.
- Radial cleavage is a characteristic of Deutrostomes, and results in indeterminant cells (Cells that can individually give rise to a complete embryo, and they don't have a determined embryological fate early on during the development of the embryo).
- In other words, you can take a single cell from a developing embryo, and given the right condition, that single cell can give rise to a whole embryo (If you've taken embryology classes you've definitely heard of experiments like this done with frog embryo).