SATAVAHANA UNIVERSITY

ZOOLOGY IV SEMESTER

MANUAL FOR PRACTICAL EXAMS

1. Preparation and identification of stages of slides of mitotic division with onion root tips

Aim: To study and demonstrate mitosis by preparing a mount of onion root tip cells.

Why is onion root tip used to demonstrate mitosis in this experiment?

It is because of the meristematic cells that are situated in the tip of the roots that render the most desirable and suitable raw material to study different stages of mitosis. Onion is a monocot plant. Monocotyledonous plants possess large chromosomes that are clearly visible. Hence their root tips are used. The period of time taken for mitosis varies as it is dependant on cell type and type of species.

Materials Required: Compound microscope, Acetocarmine stain,Water,Burner,N/10 Hydrochloric acid, Filter paper, Coverslip, Aceto alcohol (Glacial acetic acid and Ethanol in the ratio 1:3),Glass Slide,Onion root peel,Forceps,Blade,Watch glass,Dropper,Needle,Vial

Procedure Of The Experiment

- Place an onion on a tile
- With the help of a sharp blade, carefully snip the dry roots of the onion
- Place the bulbs in a beaker containing water so as to grow the root tips
- It may take around 4 to 6 days for the new roots to grow and appear
- Trim around 3 cm of the newly grown roots and place them in a watch glass
- With the help of forceps, shift it to a vial holding freshly prepared aceto-alcohol i.e., a mixture of glacial acetic acid and ethanol in the ratio 1:3.
- Allow the root tips to remain the vial for one complete day
- With the help of forceps, pick one root and set in on a new glass slide
- With the help of a dropper, allow one drop of N/10 HCl to come in contact with the tip of the root. Additionally, add around 2 to 3 drops of the acetocarmine stain.
- Heat is lightly on the burner in such a way that the stain does not dry up.
- Excessive stain can be carefully treated using filter paper
- The more stained part of the root tip can be trimmed with the help of a blade.
- Discard the lesser stained part while retaining the more stained section.
- Add a droplet of water to it
- With the help of a needle, a coverslip can be mounted on it
- Gently tap the coverslip with an unsharpened end of a needle in order for the meristematic tissue of the root tip present under the coverslip to be squashed properly and to be straightened out as a fine cell layer
- The onion root tip cells' slide is now prepared and ready to be examined for different stages of mitosis
- Observe and study mitosis by placing the slide under the compound microscope. Focus as desired to obtain a distinct and clear image.

Mitosis

In mitosis, the nucleus of the Eukaryotic cells divides into two, subsequently resulting in the splitting of the parent cells into two daughter cells. Hence every cell division involves two chief stages:

- Cytokinesis – Cytoplasm division
- Karyokinesis – Nucleus division

Stages Of Mitosis

The various stages of mitosis are:

1. Prophase

- The process of mitosis is initiated at this stage wherein coiling and thickening of the chromosomes occurs
- Shrinking and hence the disappearance of the nucleolus and nuclear membrane takes place
- The stage reaches its final state when a cluster of fibres organize to form the spindle fibres.

2. Metaphase

- Chromosomes turn thick in this phase. The two chromatids from each of the chromosomes appear distinct
- Each of the chromosomes is fastened to the spindle fibres located at its centromere
- Chromosomes align at the centerline of the cell

3. Anaphase

- Each of the chromatid pair detaches from the centromere and approaches the other end of the cell through the spindle fibre
- At this stage, compressing of the cell membrane at the centre takes place

4. Telophase

- Chromatids have reached the other end of the cell
- The disappearance of the spindles
- Chromatin fibres are formed as a result of uncoiling of daughter chromosomes
- The appearance of two daughter nuclei at the opposing ends due to the reformation of the nucleolus and nuclear membrane
- At this phase, splitting of the cell or cytokinesis may also occur.

Observations and Conclusion

- The slide containing the stained root tip cells is placed on the stage of the compound microscope, changes taking place is noted and sketched.

The different phases of mitosis such as prophase, metaphase, anaphase and telophase can be observed.

2. Preparation and Identification of meosis stages in gross hopper testis

Principle: Meiosis is a type of cell division in which the number of chromosomes is halved (from diploid to haploid) in the daughter cells, i.e., the gametes. The division is completed in two phases, meiosis I and meiosis II. Meiosis I is a reductional division in which the chromosomes of homologous pairs separate from each other. Meiosis II is equational division resulting in the formation of four daughter cells. Stages of meiosis can be observed in a cytological preparation of the cells of testis tubules or in the pollen mother cells of the anthers of flower buds.

Requirements:

Permanent slides and compound microscope

Procedure:

The testes of the grasshopper are removed and fixed in Carnoy's fluid. After 2–14 hours, the testes are transferred to 10% alcohol and stored.

Squash Preparation

1. 1 or 2 lobes of the testes are removed.
2. The testes are placed on a glass slide.
3. Apply 1 to 2 drops of acetocarmine stain.
4. With a sharp blade, the teste lobes are cut into minute pieces and kept for 10 minutes.
5. The slide is then gently covered with a coverslip, taking care so that air bubbles are not formed.
6. Warm the slide gently and place it between 2 folds of filter paper.
7. Press the material with the tip of the finger and remove the excess stain, which comes out on the sides of the coverslip.
8. The slide is observed under the microscope.

Place the slide on the stage of the microscope and search for the dividing cells using lower magnification. When dividing cells are located observe them under higher magnification.

Interphase

This phase is usually present in animal cells. The cells in this stage are physiologically active. No DNA replication takes place.

Prophase-I

a. Leptotene. The chromosomes are long, standard, and uncoiled. They are densely formed on 1 side of the cell. Only 1 sex chromosome occurs in the males, which normally replicates later and hence appears as a dark skin body.

b. Zygotene. Homologous chromosomes pair by a process called synapsis. Pairing starts from many points on the chromosome. The chromosomes are called bivalents. Bivalents become shortened and thickened by coiling and condensation. Synapsis of a chromosome is cemented by a complex called synaptonemal complex, which facilitates crossing over.

c. Pachytene. Crossing over takes place between nonsister chromatids. Crossing over is accompanied by the chiasmata formation.

d. Diplotene. Condensation of chromatid material is greater. Each chromosome can be distinguished separately.

e. Diakinesis. Homologous chromosomes begin to coil and become shorter and thicker. Chromosomes are fully contracted and deeply stained. The ‘X’ chromosome is rod-shaped, univalent, and easily distinguishable from the rest of the chromosomes.

Metaphase-II

The chromosomes get oriented in the equatorial region of the spindle and their centromeres are attached to the chromosomal fibers. Each chromosome is easily seen. Maximum concentration occurs at this stage.

Anaphase-II

The spindle fibers contract and the homologous chromosomes separate and move toward the opposite poles. Each chromosome consists of 2 chromatids attached to 1 centromere.

Telophase-I

The separation of homologous chromosomes is completed. They reach the opposite poles. Two distinct daughter nuclei are formed. The daughter nuclei formed contain only half the number of chromosomes present in the parent cell. Cytokinesis may occur after the completion of telophase.

Prophase-II

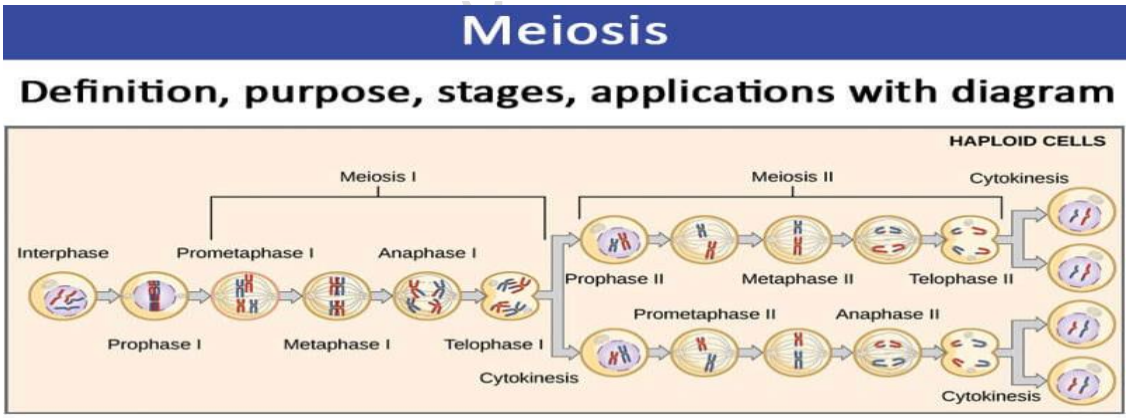
The chromosomes with 2 chromatids become short and thick. This is the stage of the second meiotic division. The nuclear membrane and the nucleolus are absent. The spindle is formed and the chromosomes are arranged on the equator.

Anaphase-II

The spindle is formed. The centromeres of the daughter chromosomes are attached to the spindle fibers. The 2 groups of the daughter chromosomes in each cell have started moving apart toward the opposite poles of the spindle.

Telophase-II

The 2 groups of daughter chromosomes in each haploid cell have reached the 2 poles of the spindle. The 2 haploid daughter cells formed as a result of first meiotic division divide again by the second meiotic division. Four haploid cells are formed from a single diploid cell.



II. Genetics problems: (As per your convenience you can add more problems)

PROBLEMS ON MENDELIAN INHERITANCE:

Mendelian Inheritance

Gregor Johann Mendel (1822-1884) is called the 'father of genetics' as he was the first man to conduct decisive experiments in heredity and to formulate the basic laws of inheritance. The study of Mendel's principles of heredity is known as mendelism. Unfortunately his remarkable piece of work remained unattended for several years, until 1900. It was in the beginning of 20th century, Mendel's work was red is covered by three different persons Hugo Devries of Holland, Von TSChermak of Australia and Karl Correns of Germany.

Mendel selected sweet pea i.e. *Pisum sativum* as plant material for his experiments.

Monohybrid Cross:- It is a cross between two parents differing in a pair of contrasting characters for a single trait. In this phenotypic ratio 3:1 and genotypic ratio 1:2:1.

Dihybrid Cross:- It is across between two parents with two pairs of contrasting characters in this, phenotypic ratio is 9:3:3:1 and genotypic ratio is 1:2:2:4:1:2:1:2:1.

Pheno type:- The external appearance of an individual resulting from the interaction between genotype and environment.

Geno type:-The genetic constitution of an organism with respect to the alleles under organism with respect to the alleles under consideration.

Back Cross:- When F_1 individuals are crossed with either of the it is called Back Cross. Resulting Progeny's ratio will be 1:1.

Test Cross:- If the F_1 hybrid is crossed with recessive parent it is called Test Cross. Progeny will be in the ratio of 1:1.

Mendel's Laws of inheritance

1 Law of Dominance :- In a monohybrid cross, plants having a pair of contrasting characters for a trait are crossed with each other. Among the two different characters one is dominant and other one being a recessive character. In F_1 generation, i.e. first filial generation, the dominant character was expressed. This is called law of dominance.

2 Law of Segregation :- In the second filial generation i.e. in F_2, characters become separated or segregated. Thus the factors responsible for hereditary characters are independent units, which although enter the crosses together, but segregate out again as distinct characters.

3 Law of Independent assortment :- Two or more than two allelomorphic pairs of characters of an organism behave independently of each other in inheritance. Thus their assortment is completely independent.

Problems on Monohybrid Cross

Q1. What will be the appearance of (a) F_1 and (b) F_2 progenies when a pure (homozygous) tall pea plant is crossed with a pure (homozygous) dwarf pea plant?

[Tallness (T) gene is dominant over dwarf ness (t)

Solution:- Pure (homozygous) tall pea plant = TT

Pure (homozygous) dwarf pea plant = tt

a)

Parents	:	♂	X	♀
Phenotype	:	Pure tall	X	Pure dwarf
Genotype	:	T T	X	t t
Gametes	:	T		t
F_1 Generation	:	T t		Heterozyous tall

Thus, the off springs of F_1 generation will be heterozygous tall.

b) Here the F_1 hybrids i.e. heterozygous tall (Tt) are self pollinated which may result into following possibilities.

Parents : Heterozygous tall X Heterozygous tall

Genotype : Tt X Tt

Gametes : 

Off spring of F_2 generation : 1 homozygous, tall; 2 heterozygous and tall

♀ \ ♂	T	t
T	TT	Tt
t	Tt	tt

off springs of F_2 generation: 1 homozygous tall, 2 heterozygous tall and 1 homozygous dwarf

Results:- a) The result of F_1 would be the production of heterozygous tall (Tt)

b) The result of F_2 would be the production of tall and dwarf in a ratio of 3:1 (Phenotypic ratio) and its genotypic ratio falls under 1:2:1 with pure homozygous tall (TT) heterozygous tall (Tt) and pure homozygous dwarf (tt).

2Q. In rabbit, the coloured coat (C) is dominant to albino coat (c). What type of offspring would you expect if you cross a pure line coloured rabbit, with an albino rabbit? Show both genotypes in the first and second generations.

Parents : ♂ X ♀

Phenotype : Pure tall coloured X albino

Genotype : C C X c c

Gametes : C c

F_1 : C c heterozygous coloured rabbit

Selfing i.e : F_1 C c X F_1 C c

Gametes : C c C c

♀ \ ♂	C	c
C	C C	C c
c	C c	cc

Result :- The resulting genotype of F_1 would be Cc (heterozygous) and F_2 would be CC, Cc and cc (coloured pure), heterozygous pure coloured and pure albino.

3 In man, brown eyes are dominant to blue two brown eyed people have a blue eyed child. What will be the genotypes of the parents?

Parents : ♂ X ♀

Phenotype : Brown eyed X Blue eyed

Genotype : BB X bb

Gametes : B b

F_1 : Bb heterozygous brown eyed

Parents genotype : B b X B b

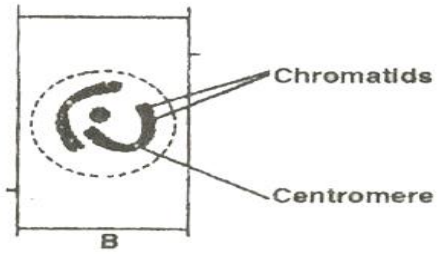
Gametes : B b B b

♂ / ♀	B	b
B	BB	Bb
b	Bb	bb

Result:-A blue eyed child would be resulted from two brown eyed parents with genotype of heterozygous brown eyes i.e Bb. Both the parents are with the same genotype Bb. Then one of the children with blue eyes can be expected or resulted.

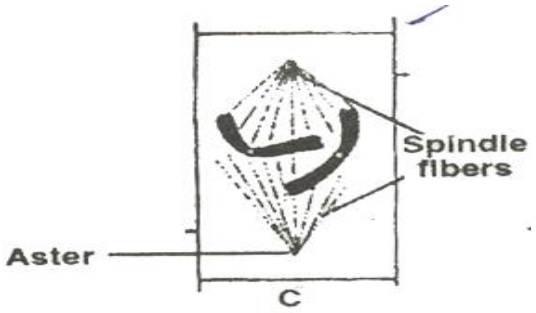
III.A. Mitosis stages:

1. **Mitosis-Prophase**



1) The nucleus becomes enlarged. DNA synthesis is completed. Prophase comes after interphase.
2) Chromosomes become filamentous, thin and distinct.
3) Prophase may be early prophase or late prophase.
4) In late prophase, each chromosome divides into sister chromatids attached at centromere or kinetochore.
5) Nuclear membrane and nucleolus disappear and cell enters into metaphase.

2. Mitosis-**Metaphase**



1) The spindle tubules start appearing and get attached with centromeres of chromosomes in early metaphase.

2) Chromosomes more actively and become arranged at the equatorial plate or centre in metaphase.

3) Specially in animals, the centrosome helps in the formation of spindle apparatus.

4) Centrosome has two centrioles which separate and each occupies opposite sides of the nucleus.

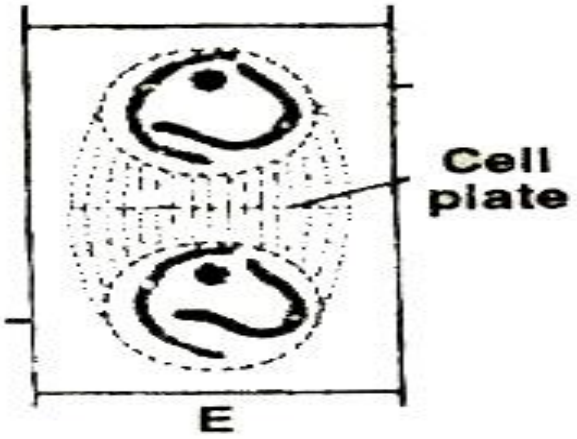
3. Mitosis-Anaphase

1) Each chromosome splits at the position of centromere, forming sister chromatids or daughter chromosomes showing early anaphase.

2) The sister chromatids move towards poles with centromeres facing periphery while arms towards each other showing late anaphase.

3) Depending upon the position of centromeres, the chromosomes may be V-shaped, J-shaped, I-shaped i.e., metacentric, sub-metacentric and acrocentric, respectively.

4. Mitosis-Telophase



1) The chromosomes reach towards poles in early telophase.

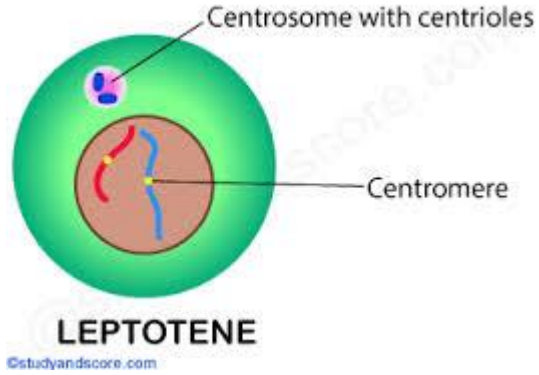
2) Nuclear membrane and nucleolus reappear.

3) Chromatids reach at poles-Early telophase.

4) Chromatids become surrounded by nuclear membranes-Late telophase.

III.B. MEOSIS STAGES:

1. **Meosis- leptotene:**



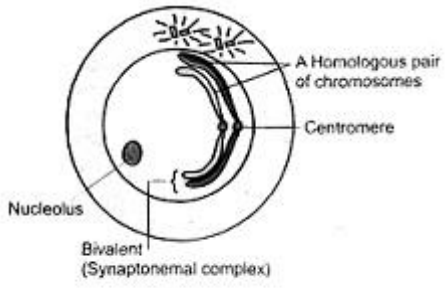
1. Chromosomes are visible as long and slender threads, they are much finer than those in mitotic prophase. Although, each chromosome has replicated and consists of two sister chromatids, these chromatids are not visible so that the chromosomes generally appear as single and individual structure.

2. Along the length of chromosomes, bead like structures called chromomeres may be seen. The arrangement of chromosomes in the nucleus is not always random. In some plants, the chromosomes are clumped to one side of the nucleus leaving the remaining part of the nucleus clear; this is called synizesis.

3. In animals, the chromosomes appear polarized where the ends of chromosomes are drawn together towards that part of the nuclear membrane close to the centriole and they seem to be attached with the envelope. Such polarization called bouquet stage may persist until pachytene.

4. It is now well known that each chromosome is attached at both of its ends to the nuclear envelope via a specialized structure called an attachment plaque.

2. Meosis Zygotene:



(b) Zygotene

1. Zygotene stage is considered to begin when intimate pairing (synapsis) between the homologous chromosomes is initiated. Pairing often starts when the homologous ends of the chromosomes are brought together on the nuclear envelope, and continues inwards in a zipper like fashion.
2. In other cases, synapsis may begin at any or several contact points, called zygomeres, and proceeds from these points in both the directions. Synapsis is highly precise and specific and occurs between all homologous sections leading to **"gene-to-gene pairing".**
3. If homologous segments are present on non-homologous chromosomes, as in case of interchanges, they pair together forming a multivalent. In case of inversion, gene-to-gene pairing occurs through loop formation.
4. If there are more than two homologous chromosomes (auto-polyploids, polysomics), they all may pair to form a multivalent, but at any given point, pairing is, as a rule, two-by-two. It is not known exactly what causes the homologous parts of the chromosomes to become precisely aligned during zygotene.
5. It has been suggested that the specificity for pairing is mediated by the axes of chromosomes which are involved in the formation of a specialized structure called synaptinemal complex (or synaptonemal complex).
6. It has been shown that total DNA is not replicated during the S phase of pre-meiotic interphase. The completion of synthesis of the remaining part (about 0.3%) of DNA occurs during zygotene; it is believed to play role in chromosome pairing.

3. **Meosis -Pachetene:**

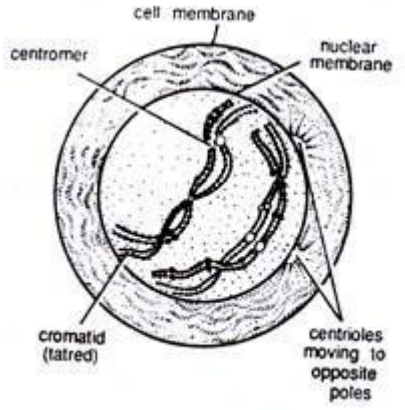


Fig. 32. PACHYTENE

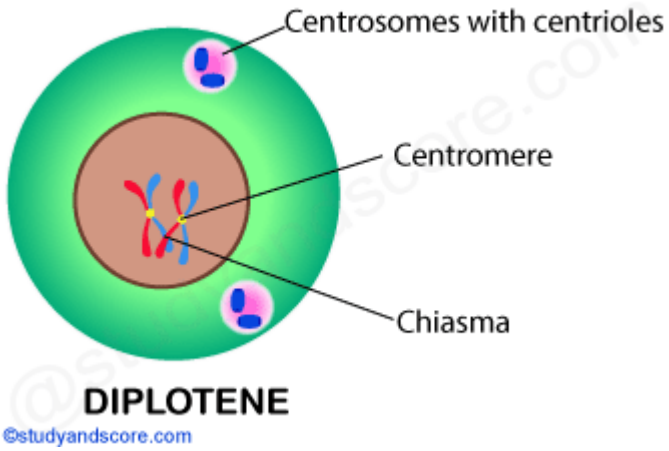
1. Pachytene stage begins just when the synapsis has completed. The paired chromosome structures are called bivalents. If there are more than two homologues in paired condition, they are called **"multivalents",** e.g., trivalents, quadrivalents, pentavalents etc.
2. The unpaired chromosomes are called univalents. Each bivalent has four strands (2 chromatids of each of the two chromosomes), therefore, it is also called "tetrad". However, it is not possible to see the four strands of the bivalents.

3. Chromosomes are visible along the length of bivalents; their pattern can be used to identify specific bivalents or their segments. Synaptinemal complex can be observed between synapsed chromosomes with the help of electron microscope.

4. At pachytene stage, recombination nodules appear at intervals on the synaptinemal complex, and they are thought to mediate crossing over. Exchange of chromatids is invisible at this stage but they subsequently result in chiasmata.

5. Nucleoli are observable during pachytene; in many species they are united to form one large nucleolus. A very small amount of DNA replication (ca. 0.3%) occurs during pachytene; this is believed to be a form of repair replication related to the process of crossing over.

4. **Meosis -Diplotene:**



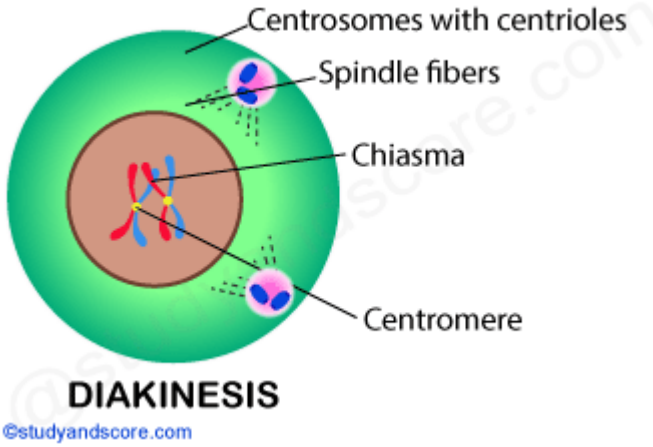
1. After pachytene, the paired homologues begin to move apart; this stage is called diplotene. Synaptinemal complex dissolves but the two homologous chromosomes in each bivalent remain joined by one or more chiasmata which represent the sites where crossing over has taken place .

2. The number of chiasmata per bivalent depends on the species and on the length of chromosomes. In Viciafaba upto 12 chiasmata have been observed in the long chromosomes. At this stage, the chromatids in each chromosome become visible.

3. Due to the forces that repel the homologous chromosomes, the chiasmata slowly shift towards the telomeres and decline in number; this process is called chiasmaterminalization. Therefore, at later stages of diplotene, the actual position and number of crossovers cannot be determined. The terminalization process may be complete, partial of absent; in the last case, chiasmata are called localized chiasmata.

4. Chiasmata terminalisation stats in this phase.

5. **Meosis -Diakenesis:**

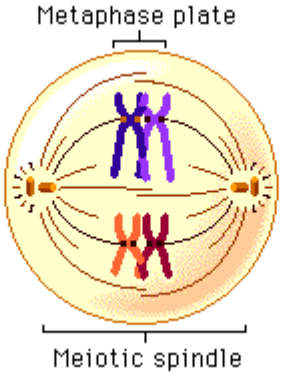


1. Contraction of chromosomes continues and it reaches the maximum at the end of this stage. Chiasmaterminalization is complete and all the chiasmata are normally located at chromosome ends .

2. Bivalents may take various forms, such as, open ring, closed ring, rod, and cross bivalents.

3. At the end of diakinesis, nucleolus begins to disappear. Bivalents move close to the nuclear membrane and become evenly distributed. Therefore, diakinesis is an ideal stage for counting the chromosomes.

6. **Meosis -Metaphase-I:**



1. Chromosomes are coiled to the maximum extent, and they appear smooth in outline. Nucleolus is absent and the nuclear membrane has disappeared, its components becoming a part of the endoplasmic reticulum. Chiasmaterminalization has already been completed.

2. Spindle is formed and the centromeres of the two chromosomes is each bivalent are attached with the chromosomal fibres of opposite poles. Bivalents move towards equatorial plate mainly due to the contraction and relaxation of the chromosomal fibres.

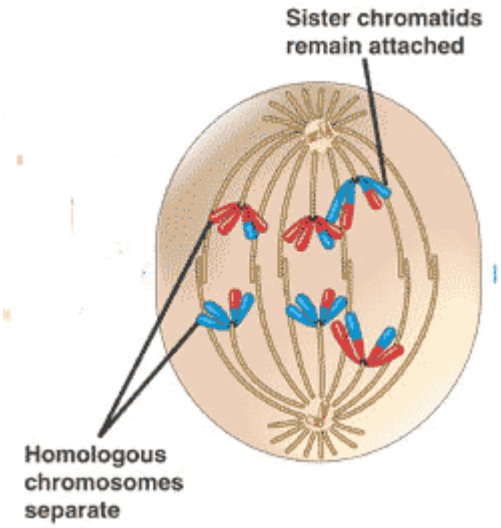
3. The two chromosomes of each bivalent coronet in such a way that their centromeres point towards the opposite poles and lie on either side of the equatorial plate, while the chiasmata lie on the plate itself . Thus the two homologous centromeres in each bivalent are located at equal distance from the equatorial plate and their respective poles.

4. Although the centromere of each chromosome is divided into two parts, it functions as a single centromere. The metaphase I (MI) differs from the metaphase of mitosis in many respects. In mitosis, single chromosomes are arranged on equatorial plate and the sister chromatids are held together by functionally undivided centromeres to which the spindle fibres are attached on both the sides.

5. But in meiosis, spindle fibres are attached on only one side of the centromere of each chromosome of the bivalent. Kinetochore fibres of sister chromatids point in the same direction in contrast to mitosis where they point towards opposite poles.

6. The shape of the bivalents at metaphase I may vary according to the number and presence or absence of chiasma and the centromere position in the chromosome: it may be a closed ring, open ring or rod shaped.

7. **Meosis -Anaphase-I:**

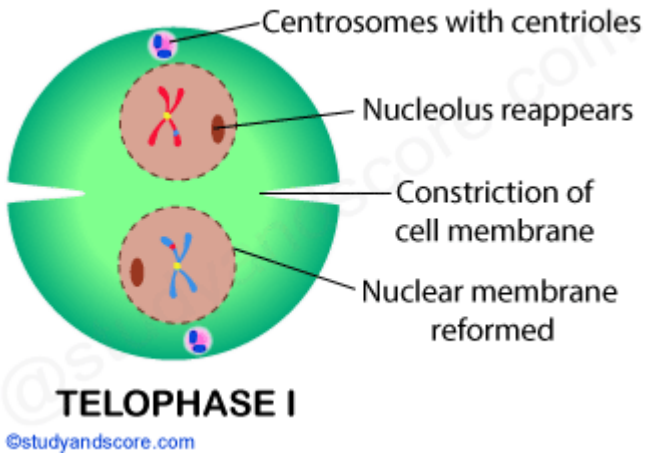


1. The anaphase I (AI) begins when the chromosome ends of bivalents loose their connections and the two homologues forming a bivalent move towards opposite poles, i.e., disjunction of homologous chromosomes occurs. The chromosome separation is **"reductional"** when crossing over has not occurred.

2. But if crossing over has occurred, the separation is reductional only for the region between the centromere and the first chiasma; it is "equational" for the region distal to the first chiasma. If double crossing over involving the same two chromatids (2-strand double crossing over) occurs, the region between the two chiasmata shows equational division, while the rest of the regions show reductional division at meiosis I .

3. The number of chromosomes reaching each pole is half of the somatic number (2n) and is called the haploid or gametic chromosome number (n). The somatic number is expressed as 2n irrespective of the ploidy level (2x, 4x, etc.), while the gametic number is denoted by "n".

8. . **Meosis -Telophase-I:**



1. When the chromosomes reach the poles, telophase I is considered to begin. Each pole receives half the number of somatic chromosomes. This stage is quite variable in different species. During this stage, the chromosomes may be partly uncoiled and the nuclear membrane may be formed.

2. Later, cytokinesis may produce two cells which remain attached together; they are called dyad, e.g., in barley, maize, Tradescantia, grasshopper etc. In other cases, telophase I is absent and the two groups of chromosomes at anaphase I directly pass on to prophase II, e.g., in Trillium. In Paeonia, chromosome coiling is retained and cytokinesis is postponed until after the second meiotic division.

9. Lampbrush chromosomes:

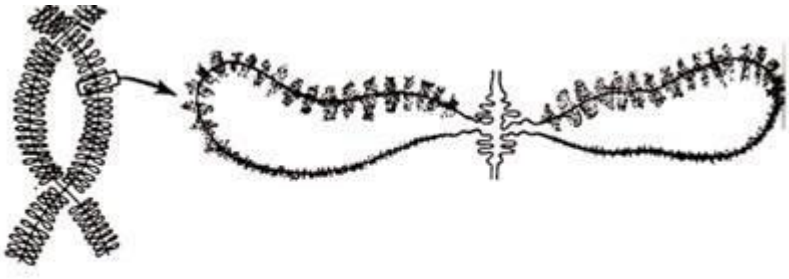


Fig. 9.13 Lampbrush Chromosome with a pair of loops highly magnified.

1. Lampbrush chromosomes are special type of giant chromosomes found in the nuclei of oocytes of many vertebrates, such as fishes, amphibians, reptiles and birds during the prolonged diplotene stage of first meiosis. They are also found in the nucleus of Drosophila spermatocyte. Lampbrush chromosomes were first observed by Flemming in 1882 and given the name by Ruckert in 1892.

2.These chromosomes may sometime become even larger than the polynemic or polytenic chromosomes of salivery glands of dipterans. The largest chromosomes may sometime be as long as 1 mm in urodele amphibians. These chromosomes consist of main axis and many fine lateral projections or loops which give them the appearance of a test tube brush or lampbrush.

3. Actually, the main axis consists of four chromatids or two bivalent chromosomes and the chromonemeta of these chromatids give out fine loops at the lateral sides. Only the kinetochore bears no lateral loops. Ris (1957) studied the loops with electron microscope and suggested that the loops were integral parts of chromonemata which are extended in the form of major coils.
4. Generally one to nine loops may arise from a single chromomeral area. The size of loop varies from an average of 9.5μ in frog to nearly 200 μ in newt. These loops probably consist of one DNA double helix from which fibrils project which are covered with loop matrix consisting of RNA and proteins.
5. Loop formation is interpreted by Gall (1958) as a reversible physiological change which is probably non-genetic. The number of pairs of loops increases in meiosis till it reaches a maximum in diplotene. After diplotene stage, the number of loop pairs gradually decreases and the loops disappear at Metaphase 1.
6. Physiological studies indicate that the loops of lamp-brush chromosomes and balbiani rings of polytene chromosomes are the sites of active genes.

10.Polytene chromosomes:

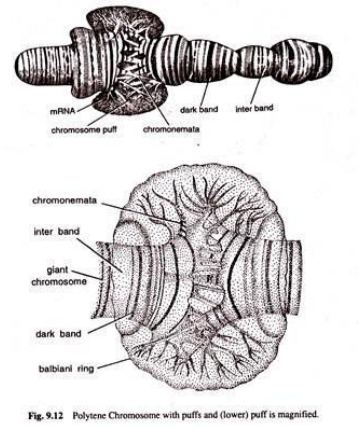


Fig. 9.12 Polytene Chromosome with puffs and (lower) puff is magnified.

1. The giant chromosomes consist of a bundle of chromonemal fibrils which arise by a series of about 10 consecutive duplications of the initial chromonemata that increase the DNA content about 1,000 times the DNA content of somatic cells. Because of the multi-stranded condition, these chromosomes are called polytene chromosomes.

2. The polytene chromosomes bear along their entire length a series of dark bands alternated by light bands or interbands. The dark bands are narrow or broad disc shaped structures. They are euchromatic in nature and contain large amount of DNA, small amount of RNA and certain basic proteins. They are feulgen positive and absorb ultraviolet (UV) light of 2600 A.

3. The light bands or interbands are fibrillar, feulgen negative, heterochromatic regions containing small amount of DNA. large amount of RNA and acidic proteins and they absorb little amount of UV light.

4. The number, distribution and localization of discs or bands are notably similar in homologous polytene chromosomes of Drosophila. The centromeres of all these chromosomes fuse to form chromocentre in Drosophila. During certain developmental stages, the single bands or adjacent bands of polytene chromosomes produce local reversible swellings which are called ‘chromosomes puffs’ or bulbs.

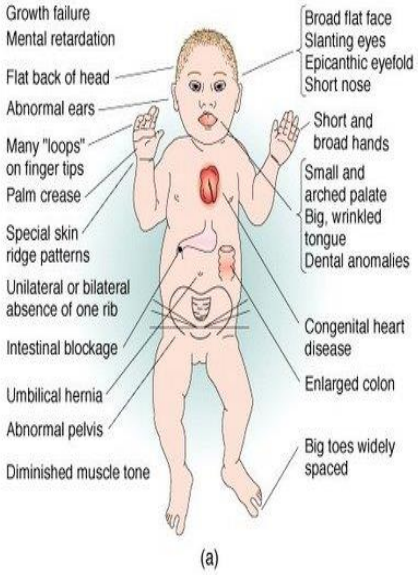
5. The chromonemata of polytenic chromosomes give out many series of loops laterally. These loops or rings are known as the balbiani rings and they are rich in DNA and RNA

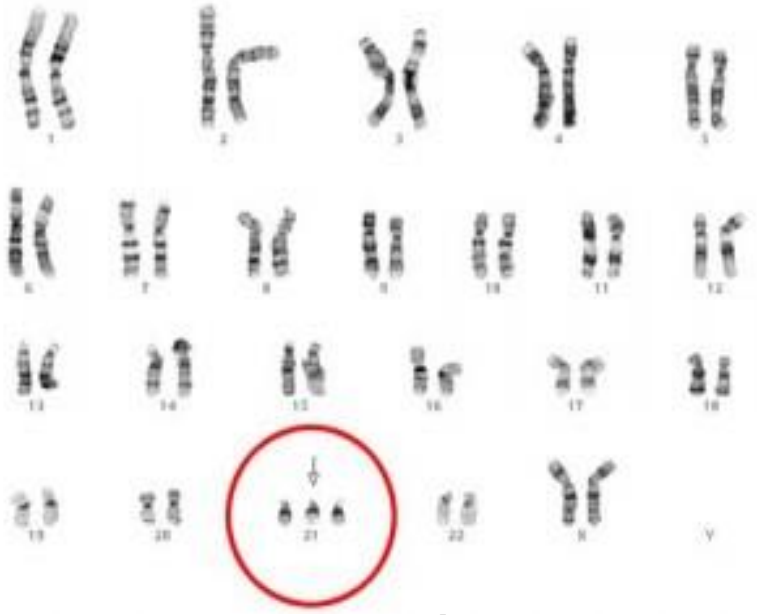
III.C.GENETICS

1. **Klinefelter Syndrome :-** (44 + *XXY*)

1. It was first described by **H.F KLINEFELTER** in 1942.
2. The most common karyotype of this syndrome is 47, *XXY.*
3. The symptoms of the syndrome occurs more than one x chromosome is present along with a y chromosome.
4. All patients of this chromosome have one or more x chromatin bodies in their cells.
5. The persons with Klinefelter syndrome tend to be tall, sexual maturity is absent, tests remain very small but penis and scrotum are normal, voice is high pitched enlargement of breast, mental retardation is occur. Scanty body hair.
6. The victims possess an additional x chromosome with xy. So the chromosome make up is 44 + *XXY.*

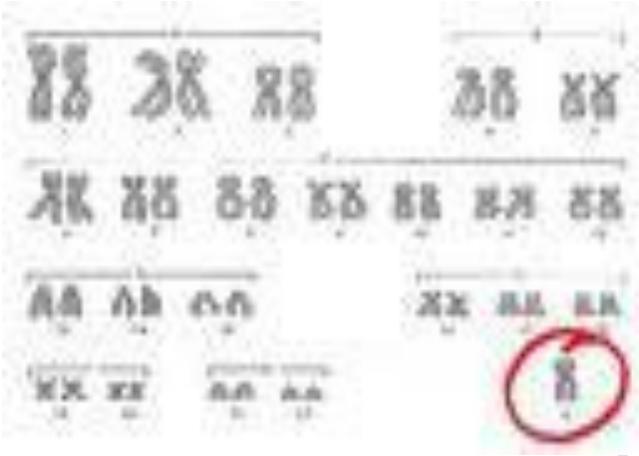
2 .Down Syndrome :- (21 – Trisomy)





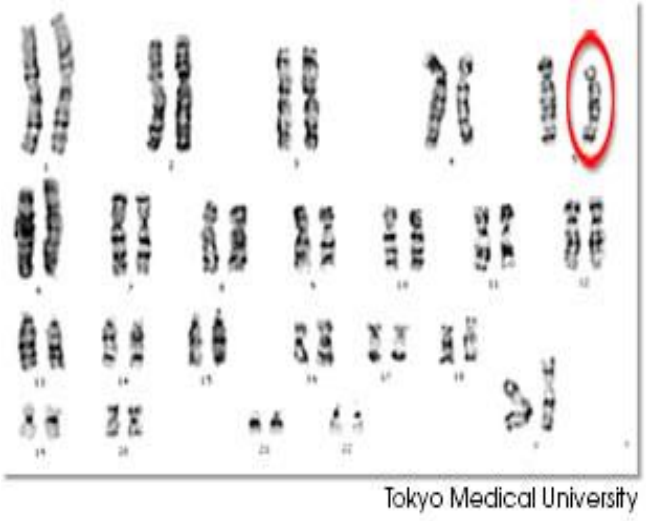
1. The Down syndrome is formerly known as Mangolism.
2. This disease was first described by **Longdon Down** in 1866.
3. This disease was the First autosomal aneuploidy. A small autosome added to the 21st pair cause the Down syndrome.
4. In this disease 47 chromosomes are present instead of 46 chromosomes. Trisomy of 21 is due to the primary non dis junction which can occur at the either of the two meiotic divisions.
5. After time of meosis, the homologous chromosomes of 21st pair fail to separate and are transmitted together into the egg. Such egg contains 23 + 1 chromosomes (Trisomy). When such egg is fertilized with a normal sperm of 23 chromosomes, the zygote has 46 + 1 = 47 chromosomes.
6. Patients of Down syndrome are short in stature about 4 feet tall.
7. They have an epicanthal fold, broad and short skulls, wide nostrils, large tongues with distinctive furrowing, stubby hands, with simain crease on the palm and a single crease on the 5th digit Mental retardation.
8. It is first chromosomal disease described in humans.
9. In this disease the average life span is about 16.2 years.
10. Down syndrome occurs once in about 750 live births amen European people.
11. Males with Down syndrome are sexually in competent and do not reproduce.
12. Effective females are capable of reproduction and giving birth to children. 50% of them may become again the patients.

3 . Turner's Syndrome :- (**45, X O**) :-



1. This is a monosomic x condition (45, X O) associated with an abnormal phenotype.
2. It was described by **H.H.Turner** in 1938.
3. It occurs in about 1 in 2500 live female births.
4. These people have sex chromatin negative as they carry only one X chromosome.
5. It is caused by monosomy (aneuploidy) where one chromosome is lost from one pair (2n-1).
6. This abnormality is due to 45 chromosomes instead of 46. The missing chromosome is one x chromosome. Hence the chromosome makeup 45, X O.
7. It is caused by non-disjunction of XX chromosomes when an abnormal egg without any X chromosome is fertilized by a sperm with X-chromosome, the resulting baby contains X O chromosomes.
8. They are sterile females.
9. They have female phenotypes. But there is no menstruation.
10. Breasts are poorly developed.
11. The neck is webbed, Broad chest, low set ears.
12. Intelligence is below average.
13. They are dwarf, mental retardation also occurs

4. Cri-du-chat Syndrome :-

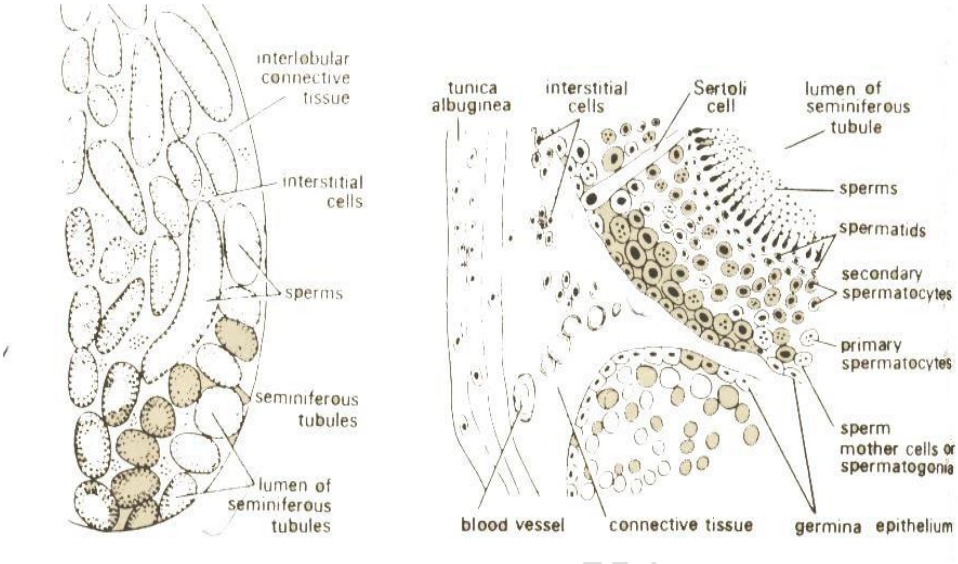


1. The chromosome of this disease is (46 XX 5P).
2. This is also known as cat cry syndrome.
3. It is discovered by **LEJEUNE**.
4. The infants with this syndrome are weak and cry like mewing of a cat.
5. Children are small head, broad face, saddle nose, widely placed eyes with epicanthic folds and physical and mental retardation.
6. The Cri-du-chat patients die in infancy or early childhood.
7. The short arm of chromosome 5 becomes translocated to chromosome 15.
8. Children inheriting the 5P chromosome expressed the Cri-du-chat syndrome.
9. The chromosome deficiency is in the short arm of chromosome 5 and is designated ^{2} $5P^{-}$. Karyo type (46 XX $5P^{-}$)

III.D. EMBRYOLOGY

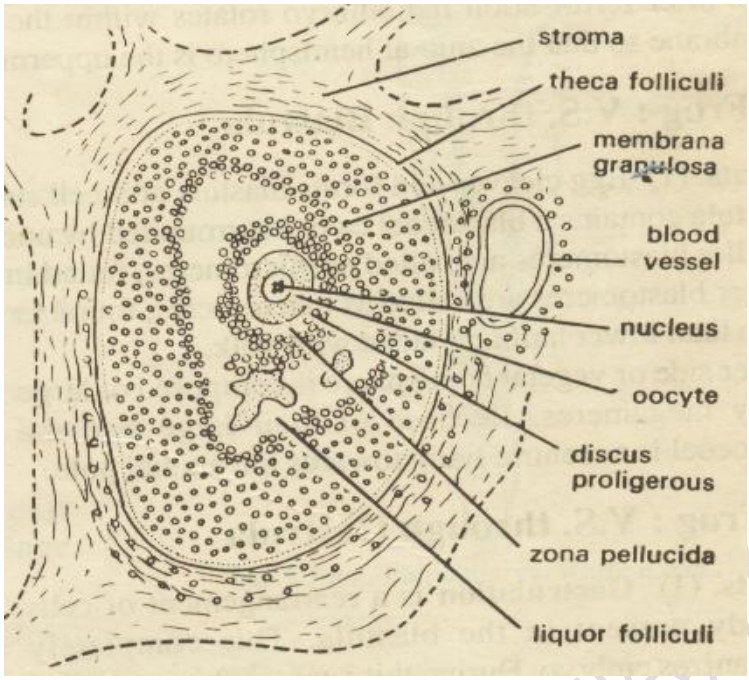
1. T.S Of Testis

T.S. of Mammalian testis



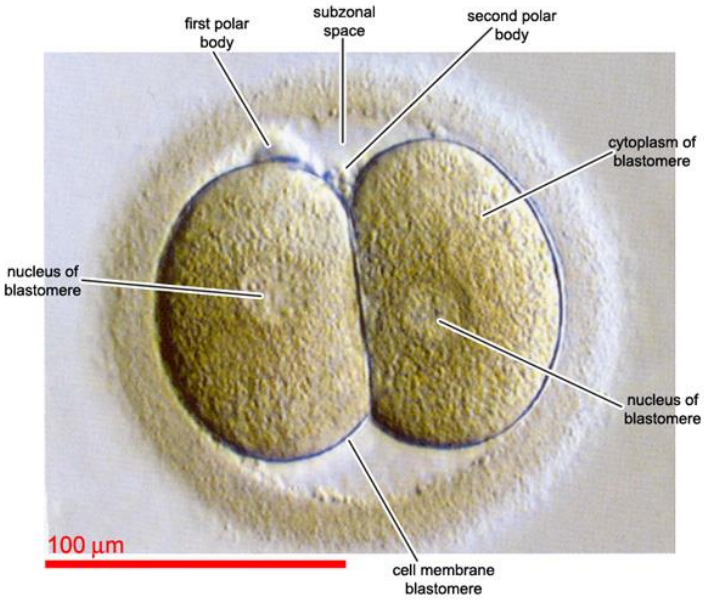
1. Capsular membrane surrounding the testis is the tunica albugemina.
2. Entire cavity of the testis is divided into a number of chambers by the septae formed of interstitial cells.
3. Testosterone is the male hormone secreted by interstitial tissue.
4. Each chamber possesses a number of somniferous tubule.
5. Germinal epithelium surrounds the somniferous tubule.
6. The cells divide by mitosis to produce spermatogonia.
7. Sperm mother cells then produced and released into the lumen of the tubule.
8. Large cells located in the septae are the sertoli cells of nutritive function.
9. The cavity of the tubule is loaded with primary spermatogonia, secondary spermatogonia, spematids and sperm cells.

2. T.S. of Mammalian ovary



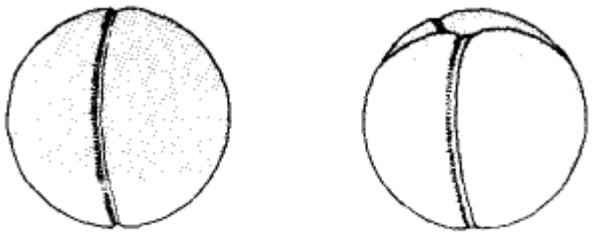
1. Ovary is surrounded by a connective tissue membrane called tunica albugenia.
2. The cavity of the ovary is filled with stroma composed of connective tissue and spindle cells.
3. Group of cells entangled in the stroma tissue constitute follicles.
4. Each follicle is surrounded by nutritive epithelium.
5. In the stroma primary oogonia, seconday oogonia, mature ova and blood capillaries can be observed.
6. Follicular cells capable of developing into ova are formed from germinal epithelium.
7. In mammals, the mature follicle is called Graffian follicle. It is surrounded peripherally by cellular mass called cumulus oophorus.
8. Attached to cumulus oophorus is the ovum surrounded by a cavity called antrum.
9. Carpus leuteum is the yellow mass of glandular tissue formed at the place of release of ovum, secreting progesterone.

3. 2cell stage



1. The first cleavage event occurs approximately 30 hours after fertilisation and results in a 2-cell embryo.
2. In frog development the first cleavage occurs shortly after the zygote nucleus forms. A furrow appears that runs longitudinally through the poles of the egg , passing through the point at which the sperm entered and bisecting the grey crescent. This divides into two halves forming the 2-cell stage.

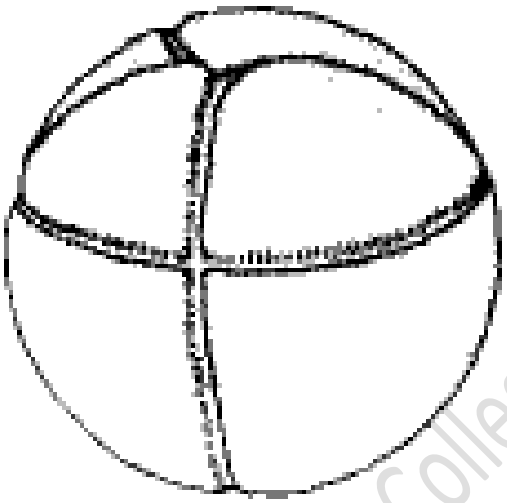
4. 4 cell stage



2-cell stage 4-cell stage

1. The 4-cell embryo is the result of a second cleavage event, and occurs at approximately 40 hours after fertilization. The individual cells are called blastomeres. At this stage, the process of embryonic genome activation is initiated in human embryos, and lasts until the 8-cell stage. The resulting morula stage marks the first morphological indication of a break in radial symmetry.
2. In frog development the second cleavage forms the 4-cell stage. The cleavage furrow again runs through the poles but at right angles to the first furrow.

5. 8 cell stage

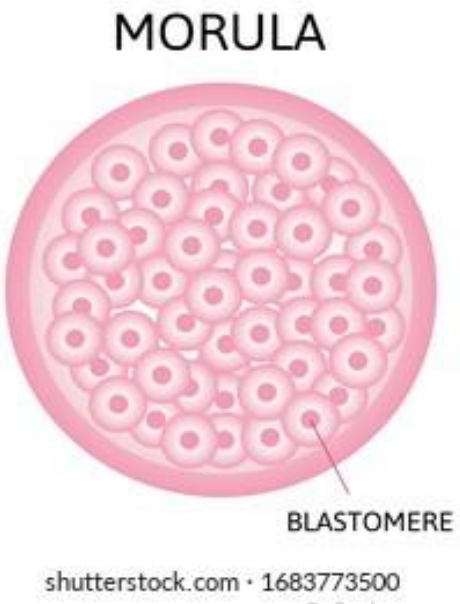


1. Cleavage is holoblastic and rotational. Humans having Holoblastic cleavage with equal division.

2. At the eight-cell stage, having undergone three cleavages the embryo goes through some changes. At this stage the cells begin to tightly adhere in a process known as compaction. Recently, it has been proposed that in placental mammals the cells become more likely to contribute to one of the first two cell types to arise, the inner cell mass or trophectoderm, depending on their position within the compacted embryo. A single cell can be removed from a pre-compaction eight- cell embryo and used for genetic testing and the embryo will recover.

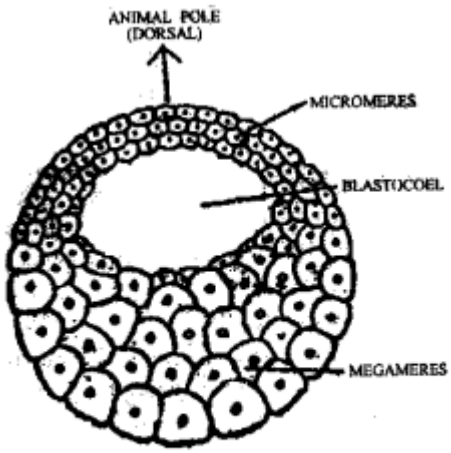
3. Most of the blastomeres in this stage become polarized and develop tight junctions with the other blastomeres. This process leads to the development of two different populations of cells: Polar cells on the outside and apolar cells on the inside. The outer cells, called the trophoblast cells, pump sodium in from the outside, which automatically brings water in with it to the basal (inner) surface to form a blastocoel cavity in a process called cavitation. The trophoblast cells will eventually give rise to the embryonic contribution to the placenta called the chorion. The inner cells are pushed to one side of the cavity (because the embryo isn't getting any bigger) to form the inner cell mass (ICM) and will give rise to the embryo and some extraembryonic membranes. At this stage, the embryo is called a blastocyst.

6. Morula



4. **Morula**, solid mass of blastomeres resulting from a number of cleavages of a zygote, or fertilized egg. Its name derives from its resemblance to a mulberry (Latin: *morum*).
5. A morula is usually produced in those species the eggs of which contain little yolk and, consequently, undergo complete cleavage.
6. Those blastomeres on the surface of the morula give rise to extra-embryonic parts of the embryo. The cells of the interior, the inner cell mass, develop into the embryo proper.
7. In humans, the morula is composed of 60 or more cells. As the number of cells in a morula increases, the zygote develops in a blastocyst, a hollow bubblelike structure, which eventually becomes implanted in the uterine lining.

7. Blastula of frog



(Blastula of Telolecithal frog Eggs)

1. The morula stage gives rise to a stage called the blastula which is a hollow ball like structure.
3. Blastulation At the end of cleavage the solid ball of cells give rise to blastula which consists of a number blastomeres. The characteristic features of the blastula stage are the presence of a well defined cavity called the blastocoel. This is the beginning of the primary body cavity. The process of the formation of blastula is called blastulation.
4. The blastula of frog is called amphiblastian as the cavity is confined to only the animal pole. The vegetal pole however is composed of a solid mass of non pigmented yolky cells.
5. In the thirty two cell stage, the blastula consists of a single layer of cells and is called the early blastula. The pigmented cells (micromeres) are found in the anterior half while the yolky megameres are present in the posterior half. As has been already pointed out, the blastocoel lies entirely in the anterior half.
6. The blastula of frog is hollow and has a very well developed blastocoel. It is said to be a coeloblastula. As segmentation proceeds, the number of cells in the blastula increase; so also the blastocoel. The floor of the blastocoel is flat while its top portion is arched. The roof is made up of three to four layers of pigmented micromeres while the floor is formed by yolky megameres. Between the micromeres and the megameres and along the equator is found a group of cells which are intermediate in size (between megameres and micromeres). These cells constitute the germ ring. The germ ring is formed in the region of the grey crescent.

a. Gastrula of frog

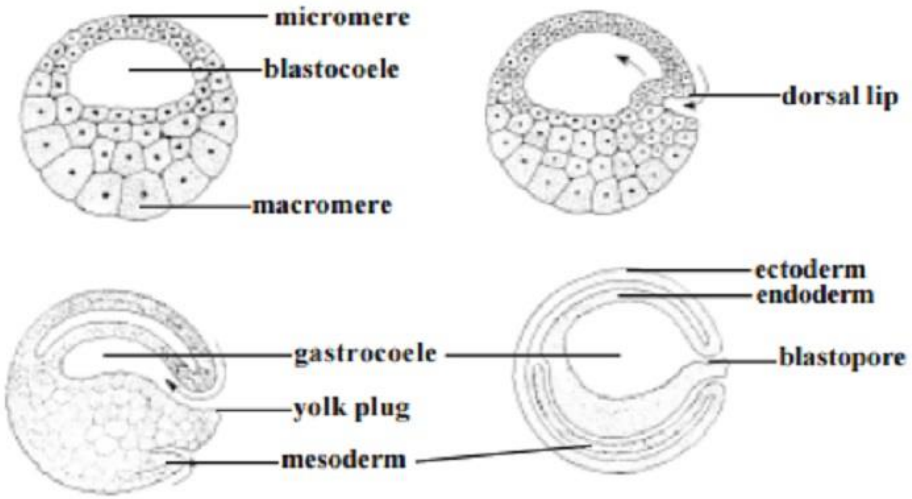
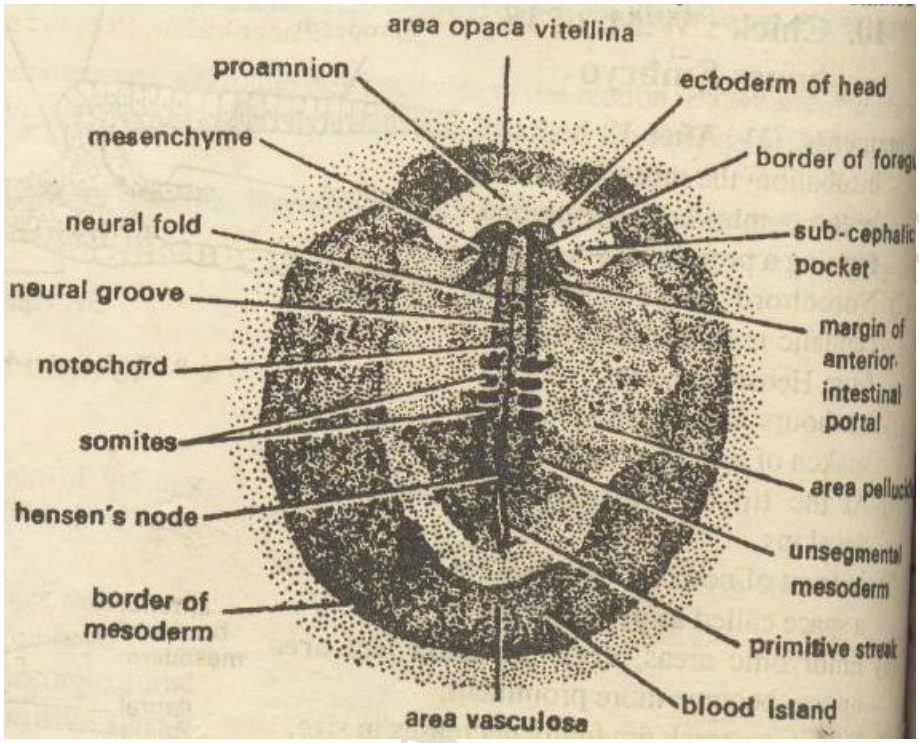


Fig. Gastrulation of Frog

1. The process of gastrulation converts the blastula into a spherical, bilaterally symmetrical, triploblastic gastrula.
2. It involves dynamic movement and rearrangement of blastomeres. Such movements of blastomeres along specific paths during gastrulation are called as morphogenetic movements. Three types of morphogenetic movements can be found- invagination, involution and epiboly.
3. Invagination: Invagination is an active infolding of blastomeres. During invagination, few blastomeres near grey crescent are pushed inward to form a slit or groove. The opening of this groove is called as blastopore and the cavity is called as gastrocoel or archenteron. The blastopore gradually assumes a crescentic shape. Finally it becomes circular. The region dorsal to the blastoporal opening is called the 'dorsal lip'. The lower edge may be called the 'ventral lip'. Due to enlargement of archenteron, blastocoel is gradually reduced.
4. Involution: Involution is the process of rolling in movement of blastomeres. During this process the micromeres multiply and migrate to the dorsal lip of blastopore and roll inside or turn into the archenteron and arrange themselves on the roof of the archenteron.
5. Epiboly: Epiboly means growth of one layer of cells over another. During epiboly, micromeres of animal pole divide rapidly and move over the macromeres of vegetal pole. This layer forms ectoderm. As a result of these morphogenetic movements, three primary germ layers are formed.

b. 24 Hours Chick Embryo

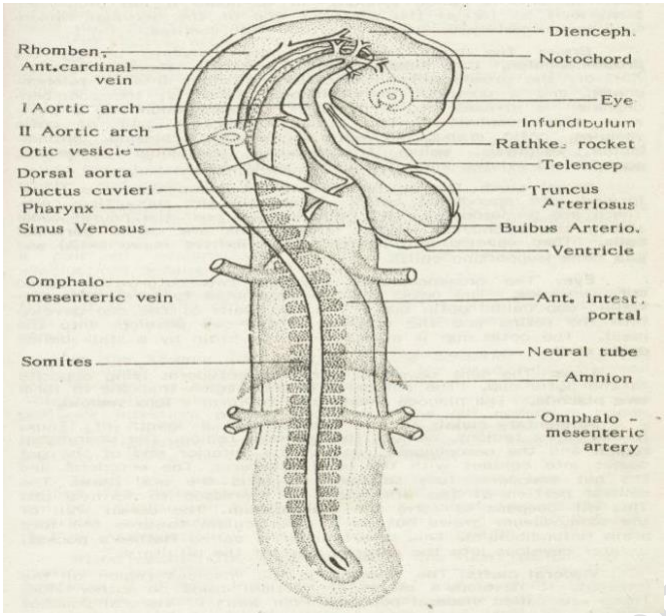


A twenty – four hour Old Chick Embryo is obtained after 24 hour (One day) of incubation. It has the following Salient features:

1) It is characterised by the presence of four pairs of somites.
2) The Shape of the embryo is Oval. It lies flat on the yolk with the ventral surface facing the yolk.
3) The blastoderm has two regions, namely a Central area pellucida and a peripheral area Opaca. The area pellucida is transparent and the area opaca is dull.
4) The area pellucida has two regions, namely embryonic area and extra-embryonic area.
   a) The embryonic area develops into the body of the embryo.
   b) The extra-embryonic area develops into the foetal membranes.
5) The area opaca has two regions, namely Inner vasculosa and outer vitellina
   a) Inner vasculosa contains blood islands.
   b) Outer area vitellina has no blood islands. It lies in close contact with yolk.
6) The ectoderm on either side of the medium dorsal line becomes thickened and elevated to form longitudinal folds called neural folds. They enclose a groove called neural groove.
7) The gut is formed of fore-gut only.
8) The mesoderm is proliferated by the primitive streak.
9) Primitive streak remains as a mid-dorsal thickening at the posterior end of the

blastoderm. It is enclosed by sinus rhomboidalis. It is in its process of degeneration.

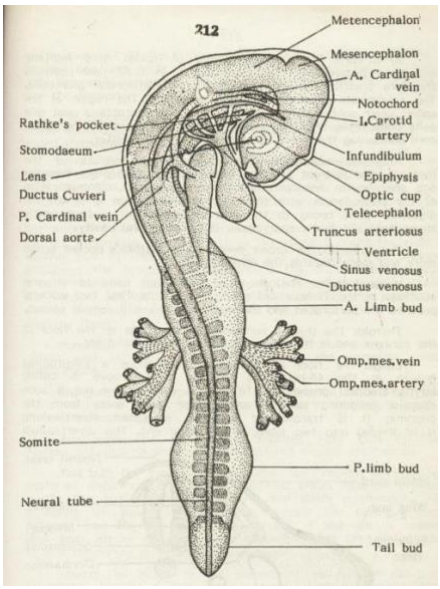
c. The 48 Hour Old Chick Embryo



The forty eight hour old chick embryo is obtained after 48 hours (Two days) of incubation It has the following features:

1) It is shaped.
2) It has 24 pairs of somites
3) The brain has three region, namely the prosencephalon, the mesencephalon and the rhombencephalon.
4) The epithelial cells of spinal cord are transformed into ependymal cells.
5) The fore gut attains a length of 1 ½ mm. It has three regions, namely pre-oral region, the pharyngeal region and the Oesophageal region.
6) The region of the fore gut lying above and behind the laryngo-tracheal groove develops into the Oesophagus.
7) Mid gut open widely on the yolk.
8) The heart is '**S**' shaped. The valves are not yet formed. Four region can be Visualised in the heart. They are sinus venosus, atrium, ventricle, and bulbus cordis.
9) Amnion and Chorion begin to develop in the 48-hour old embryo in the form of amniotic head fold and amniotic lateral fold. The embryo is covered over by the above two folds as far back as the Omphalomesenteric arteries.

d. 72 Hour Old Chick Embryo



A Seventy-two hour Old Chick Embryo in obtained after 72-hours (3 days) of incubation. It has the following salient features:

1) It lookes like the mirror image of Question mark (?)

2) The embryo has 35 pairs of somites.

3) The alimentary canal consists of the fore-gut, mid-gut and the hind-gut.

4) The infundibulum grows deeper. The Rathke's pocket grows more and more towards the infundibulum.

5) The lung buds are well developed.

6) The liver develops as two evaginations from the floor of the gut.

7) The tail region develops a posterior outgrowth in front of the anal plate. This out growth is the tail bud.

8) The optic cup increases in size. The lens vesicle is detached from the ectoderm.

9) The limb-buds are clearly visible. They appear as broad swellings on either side of the embryo.

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