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CYTOLOGY

1

PREPARATION AND IDENTIFICATION SLIDES OF MITOTIC DIVISIONS WITH ONION ROOT TIPS

1. Collect the root tips of onion.
2. Cut the specimen in saline solution and collect the Pieces into cornoy solution.
3. Leave for 2-12 hours for proper fixation.
4. Remove from the fixative and keep the Pieces in acetocarmine or aceto-orcein. Gently warm and then transfer over to the slide.
5. Place a cover slip over the piece and tap it slowly with a fine glass rod.
6. Cells dissociate by tapping and exhibit nuclei in different stages of cell division.
7. Identify different stages of mitosis basing on the characters of the chromosomes.
8. Label the slides for further observation and study.

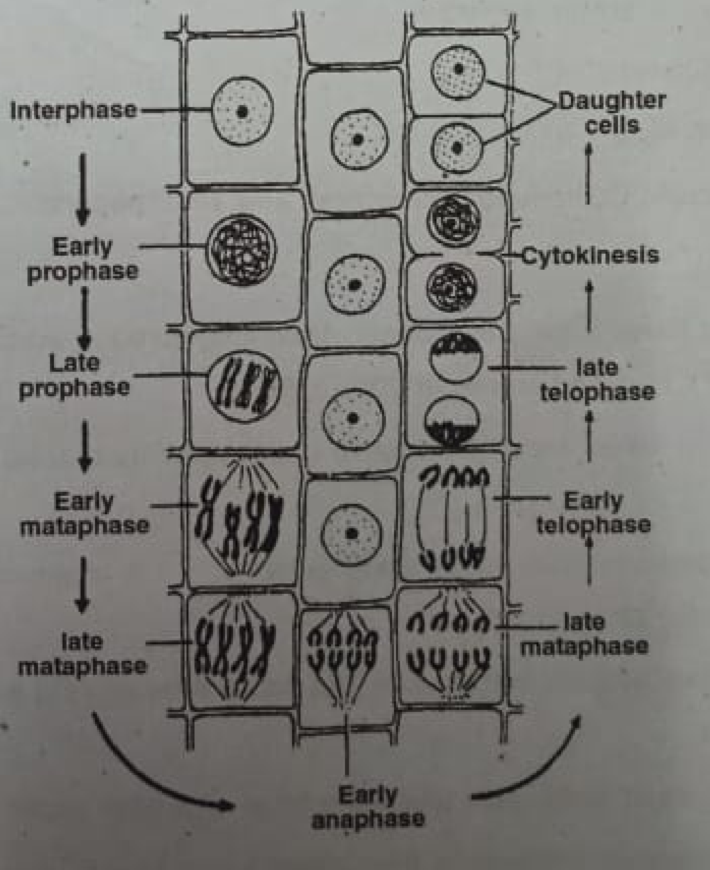


Fig: Different Mitotic stages in onion root tips

2

PREPARATION AND IDENTIFICATION OF DIFFERENT STAGES OF MEIOSIS IN GRASSHOPPER TESTES.

Introduction: Squash technique is the easiest method applied to study different stages of meiotic chromosomes in the testicular cells of grasshopper. Here slight pressure is applied over the cover slip placed over the pre-stained testicular lobe of grasshopper or locust.

Aim : Preparation of the slide with testicular chromosomes of grasshopper using squash technique for the study of various stages of meiosis.

Materials required:

1. Testicular lobes collected from grasshopper.
2. Clean and dry Slides and cover slips.
3. Acetyl alcohol.
4. Aceto-carmine or Aceto-orcein stain.
5. 2N - hydrochloric acid.
6. Pasteur pipettes.
7. Spirit lamp, Forceps, Compound Microscope, and filter papers.

Procedure:

1. Obtain testicular lobes from grasshopper previously fixed in acctic alcohol (ethnol: acetic acid at 3:1 v/v) and stored in 70% alcohol.
2. Wash the testis in water and then release the tubules by tearing with a dissection needle.
3. Transfer the tubules onto a watch glass containing 1% aceto-carmine or aceto-orcein and stain for 10 to 15 minutes.
4. Transfer one or two tubules onto a slide and add a drop of 45% acetic acid. Place a cover slip over it.
5. Remove the excess of acetic acid using the edge of a filter paper.
6. Place the slide between 2 sheets of filter paper.

7. Using the blunt end of a pencil, gently tap on the cover slip by applying pressure with the thumb.
8. The tubules flatten thereby spreading cells and chromosomes.
9. Seal the edges of the cover slip with nail polish and observe under a compound microscope.

OBSERVATIONS AND RESULTS:

Meiosis actually consists of two division processes: Viz., Meiosis I and Meiosis II. Meiosis I constitutes the reduction division where the chromosomal number is reduced by half. Meiosis II resembles mitosis. At the end of the two divisions four cells are formed, each with half the number of chromosomes as seen in the parent cell.

Provided in practical 3(i-b) can be seen in pages-7(IV)

3

IDENTIFICATION AND STUDY OF THE FOLLOWING SLIDES

(I) DIFFERENT STAGES OF MITOSIS AND MEIOSIS
(II) LAMP BRUSH AND POLYTENE CHROMOSOMES

2a.

3. (I) IDENTIFICATION OF STAGES FROM PREPARED SLIDES SHOWING MITOSIS AND MEIOSIS

(I) MITOSIS (EQUAL CELL DIVISION):

1. Mitosis occurs in somatic cells and is responsible for the growth of the body.
2. Daughter cells produced are equal in both quality and quantity.
3. Number of chromosomes in the daughter cells is similar to that of the mother cell.
4. D+ifferent stages of mitosis can be observed from the fresh squash prepared from the onion root tip.

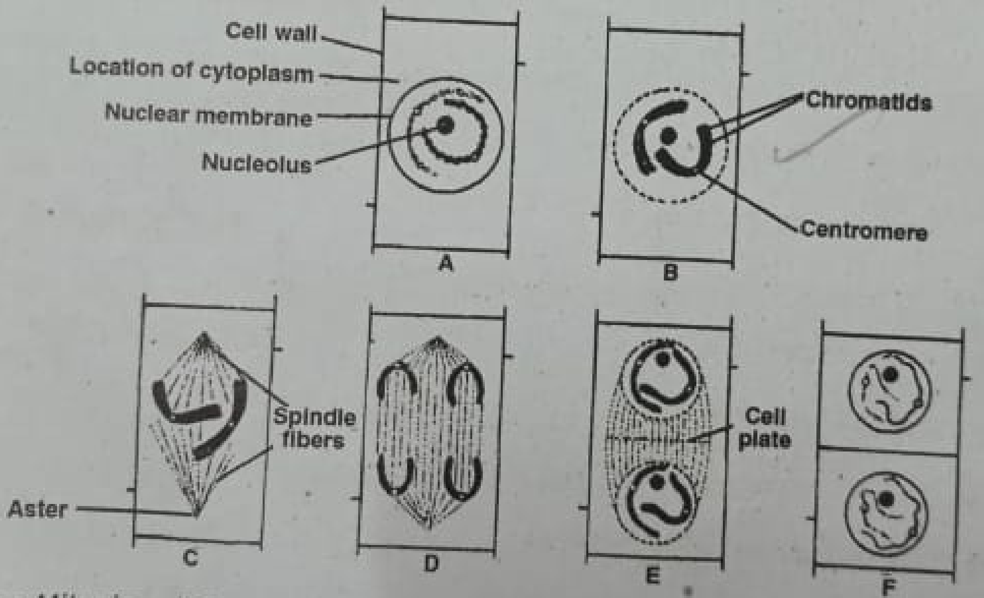


Fig : Mitosis - different stages : (A) Early Prophase (B) Late prohase (C) Metaphase (D) Anahase (E) Telophase (F) Interphase

1. INTERPHASE is identified by the presence of

1. Much grown cell becoming conspicuous from the rest of the cells.
2. Enlarged nucleus is intact with nuclear membrane, chromatin reticulum and nucleolus
3. Increase in nuclear and cytoplasmic quantities is generally observed.
4. Synthesis of proteins, replication of centrioles, DNA, chromosomes occur
5. Centrioles divide to initiate the process of cell division.

2.b

3. **PROPHASE** is identified by the presence of

1. Much enlarged nucleus with increased quantity of nucleoplasmic contents.
2. Chromatin net work breaking up into definite number of long coiled thread like chromosomes.
3. Chromosomes become short due to spiralization, condensation and dehydration.
4. Length wise split of the chromosome resulting in chromatids attached to the undivided centromere.
5. Nuclear membrane starts breaking up while nucleolus disintegrates and disappears.
6. Two centrioles separate and move towards poles initiating the formation of mitotic spindle.

3. **METAPHASE** is identified by the presence of

1. Total disappearance of nuclear membrane.
2. formation of asters and mitotic spindle.
3. arrangement of chromosomes along the periphery of equitorial plate.
4. formation of discontinuous fibres between aster and centromere of chromosomes.

4. **ANAPHASE** is identified by the presence of

1. Division of centromere resulting in the formation of daughter chromatids.
2. formation of inter-chromosomal fibres in between the two chromatids.
3. movement of the daughter chromatids towards asters.
4. daughter chromosomes appearing in the shape of V, U, J.

5. **TELOPHASE** is identified by the presence of

1. Daughter chromosomes reaching the asters.
2. Despiralization and hydration of chromosomes at the asters result in the formation of chromatin network. Chromosomal and inter chromosomal fibres along with mitotic spindle disappears.
3. Formation of nuclear membrane and nucleolus at the asters thus resulting in two daughter nuclei
4. Appearance of deep constriction at the centre of the cell initiating the cytokinesis.

3.(II) MEIOSIS (REDUCTION DIVISION) – I (HETEROTYPIC DIVISION)

1. This division occurs in germ mother cells resulting in the formation of gametes
2. Daughter cells may be equal as in spermatogenesis or unequal as in oogenesis.
3. A single diploid cell produces four haploid cells.
4. Helps in maintaining the diploid nature of the organisms through generations.
5. New characters are formed in the progeny due to crossing over in prophase.

1.c

(i) LEPTOTENE OF PROPHASE – I

1. Formation of indistinct chromosomes which are thin, thread like with chromomeres as beads.
2. Appearance of flower bouquet inside the nucleus by the congregation of chromosomes.
3. Formation of spindle fibres in between the centrioles which have separated and migrated to opposite poles to form meiotic spindle.

d

(ii) ZYGOTENE OF PROPHASE – I

1. Synapsis or Pairing of maternal and paternal chromosomes(homologous) to form diads/bivalents.
2. Two chromosomes lye close to each other as in zip.
3. Zipping starts either from the centre to the ends or in opposite direction.
4. A special synaptonemal complex is formed of proteins to facilitate genetic recombination.

(iii) PACHYTENE OF PROPHASE – I

1. Chromosomes split lengthwise to form chromatids and are united at the centromere thus forming the tetrads.
2. Further condensation and spiralization make chromosomes to attain definite shape
3. Crossingover occurs at certain points between non sister chromatids facilitating genetic recombination.
4. The points of crossover are the chaismata.
5. Breakage of chromatids at chaisma and exchange of genetic material between the chromosomes.

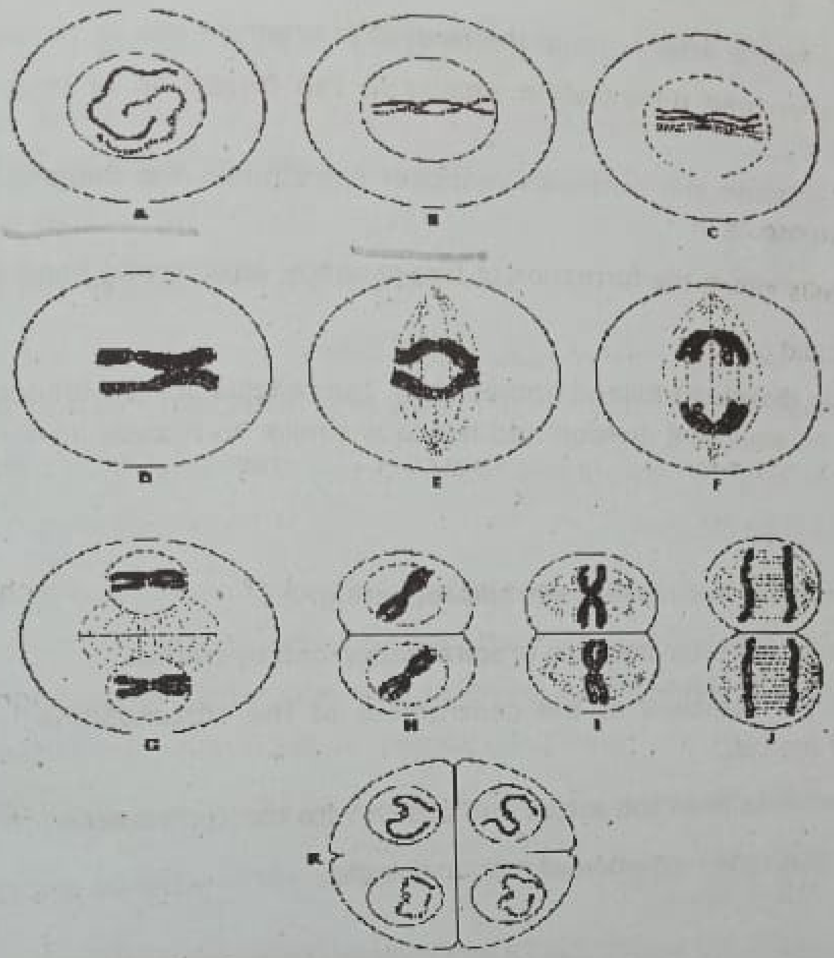


Fig : Meiosis-I (A) Prophase - I - Leptotene,(B) Prophase - I - Zygotene (C) prophase-I - Pachytene and diplotene (D) Prophase - I-Diakinesis (E) Metaphase-I, (F) Anphase-I, (G) Telophase - I (H) Prophase-II; (I) Metaphase-II; (J) Anphase-II; (k) Telophase - II

(iv) DIPLOTENE OF PROPHASE – I

1. Homologous chromosomes separate except at the points of chaismata
2. Tetrads appear in the form of 'O, G, 6' because of attachment at chaisma

(v) DIAKINESIS OF PROPHASE – I

1. Chromosomes moving apart due to terminalization of chaismata towards telomere.
2. Further spiralization and condensation of chromosomes result in short rod like structures.
3. Nucleolus and nuclear membrane break up and disappear.
4. Chromosomes float freely in cytoplasm.

(vi) METAPHASE –I

1. Chromosomes arrange on the equitorial plate forming the spindle with their centrioles on either side.

(viii) TELOPHASE – I

1. Diads reaching the aster regroup themselves to organize into a nucleus.
2. Despiralization and dehydration results in the formation of long thread like chromosomes.
3. Nuclear membrane and nucleolus reappear constituting the daughter nucleus at each aster.
4. Cytokinesis results in the formation of two daughter cells having haploid nucleus.

MEOISIS – II

(Homotypic division) follows immediately, the telophase – I with out any gap or interphase. This is an equitorial division and hence is similar to mitosis involving the four phases.

PROPHASE – II :

1. Nuclear membrane and nucleus start disappearing.
2. Chromosomes are visible because of spiralization and hydration.
3. Chromatids are attached at the centromere of the chromosome lying at the periphery of the cell.
4. Spindle fibres arise from the asters and attach with the centromere.
5. Centrioles initiate the formation of mitotic spindle.

METAPHASE – II :

1. Chromosomes move to the equitorial plate forming the perfect mitotic spindle.
2. Centromere divides resulting in the formation and separation of daughter chromosomes.
3. Inter chromosomal fibres appear between the two daughter chromosomes.

ANAPHASE – II :

1. Daughter chromosomes start moving to the aster because of contraction of spindle fibres.
2. Elongation of inter chromosomal facilitate the easy separation and movement of chromosomes.
3. Haploid chromosomes with exchanged genetic matter appear in various shapes.

TELOPHASE – II :

1. Daughter chromosomes reach the asters.
2. Nuclear membrane and nucleolus reappear and chromosomes elongate to form the network.
3. Cytokinesis divides the cell thus resulting in the formation of four daughter haploid cells.

Lamp Brush chromosomes

1. They were first described by Walter Flemming in 1882.
2. These are the largest special form of **chromosomes** found in the growing oocytes (immature eggs) of tailed and tailless amphibians, birds and insects, except mammals.
3. They can be seen with naked eye.
4. They are characterized by fine lateral loops, arising from the chromomeres, during first prophase (diplotene) of meiosis.
5. These lateral loops give them the brush-like appearance and hence are called lamp brush chromosomes.
6. Each chromosome has longitudinal axis formed by a single DNA molecule. Several hundred bead-like chromomeres are distributed on it in a linear fashion. From each chromomere arise two symmetrical lateral loops capable of expansion or contraction in response to various environmental conditions.
7. Lamp brush chromosomes can easily be separated in toto from oocyte nucleus.
8. They are involved in the synthesis of RNA, formation of yolk material in the eggs.

Fig : Lamp Brush Chromosomes

Polytene chromosomes

1. These are also giant chromosomes but relatively smaller than lamp brush chromosomes.
2. They were first observed in 1881 by E.G. Balbiani in salivary gland cells of Chironomus larvae. Balbiani rings of Chironomus contains 1000 to 2000 separate strands (corresponding to the degree of ploidy).
3. Later Gay (1956) observed strands 200 to 500 A in diameter in sectioned Drosophila salivary chromosomes. The individual fibres in band and inter band regions are similar in appearance, and are tightly packed.
4. Balbiani rings give the chromosome a fuzzy outlook. The Balbiani rings are rich in DNA and mRNA, and the formation and function of the Balbiani rings are similar to the puffs.
5. Puffs represent regions of active RNA synthesis (transcription). Puffing is due to the uncoiling of chromosome.
6. Nuclei of these cells are much larger than those of ordinary cells.
7. Chromosomes in these nuclei are so large that they are 50 to 200 times of a normal cell.
8. Carrying the genes formed of DNA and controlling the physiology of an organism, Shifting of heterochromatin in respect to euchromatin causing mutations in animals as well, Production of nucleolar material by heterochromatin and Synthesis of proteins indirectly are their functions

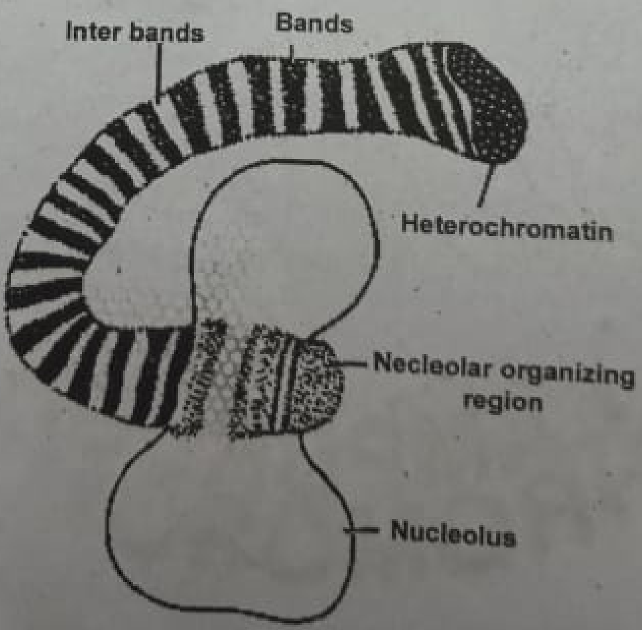


Fig : Structure of a polytene chromosome

GENETICS

1 PROBLEMS ON GENETICS - MENDELIAN INHERITANCE, LINKAGE AND CROSSING OVER, SEX LINKED INHERITANCE

1. PROBLEMS BASED ON MENDELIAN INHERITANCE

(A) DOMINANCE

Q. 1. Cross between red and white eyed drosophila, gives all red - a likely model is that eye color is controlled by one gene which has 2 alleles, red and white; also the red allele's phenotype is likely to be dominant. Therefore, choose appropriate symbols (one letter per gene, capital letter for allele with dominant phenotype).

Solution: All heterozygous dominant where gene for red eye is dominant over the gene for white eye. The parents are homozygous pure for the character. This explains simple dominance of a Monohybrid Cross. A pair of genes controls each of the contrasting character.

R - red allele. Dominant phenotype of red eyes.

r - white allele. Recessive phenotype of white eyes.

Cross 1 is then: RR × *rr* ——> all R*r* (all red eyes).

(B) PHENOTYPIC RATIO OF A MONOHYBRID CROSS

Q. 2. Cross made between a red-eyed F1 × red-eyed F1 gives F2: 36 red-eyed 13 white-eyed.

Solution: This cross indicates the self fertilization between the progeny of F1. The F2 results confirm the phenotypic ratio of Monohybrid cross i.e., 3:1.

Genotype of F1 organisms : Rr × Rr

Gametes of F1 organisms: R and r

Checker board of 2/2 for a monohybrid cross :

♀ \ ♂	R	r
R	RR	Rr
r	Rr	rr

Gamet	R	r
R	RR	Rr
r	Rr	rr

The results indicate 3/4 insects with red eyes and ¼ with white eyes. This is the phenotypic ratio of the monohybrid cross as proposed by Mendel.

(C) TEST CROSS

Q. 3. Cross between a long-eared mousexshort-eared mouse gives F1: 12 long-eared 10 short-eared.

Solution for cross 1: This is a model for TEST CROSS. Long earned mouse is crossed to a short earned one gives some long and some short. Ear length is controlled by one gene with 2 alleles, long and short. From this data, we cannot determine which allele has the dominant phenotype.

Presuming that the long allele expresses the dominant phenotype, the appropriate symbols are:

L - long allele. Dominant phenotype of long ears.

l - short allele. Recessive phenotype of short ears.

Basing on the data given for cross 1,

♀ ♂	l	l
L	Ll	Ll
l	ll	ll

Ll (heterozygous long) × ll (Homozygous short) ——> 1:1 long:short

This result is possible when the long eared mouse is a heterozygous one and the cross can be explained as a TESTCROSS. The heterozygous long eared mouse is crossed with a double recessive homozygous short-eared mouse. The organisms resulting out of this cross would be in the ratio of 1: 1

(D) INCOMPLETE DOMINANCE in *Flowers*

Q. 4. Cross 1 : Blue-flowered Plant ´ White flowered plant gives F1: all pale-blue-flowered.

Cross 2 : Pale-blue F1 × pale-blue F1 gives; F2: 27 blue, 49 pale : blue and 24 white.

Solution : This is an example of INCOMPLETE DOMINANCE. Hence the F1 organisms appear to be pale blue instead of blue. F2 ratio indicates the appearance of the three phenotypes in the ratio of 1:2:1 instead of 3:1. Hence the phenotypic and the genotypic ratio remains the same in incomplete dominance.

Blue X white gives all pale blue - a plausible model is that color is controlled by one gene with 2 alleles, blue and white. The color is determined by a semi-dominant mechanism, that is:

B - blue allele. Semi-dominant blue phenotype.

b - white allele. Semi-dominant white phenotype.

Therefore: BB - blue ; B*b* - pale blue ; *bb* - white

Cross 1 is then: BB × *bb* ——> all with B*b*, pale blue.

Cross 2 is then: Bb × Bb ——> 1:2:1; BB:B*b*:*bb* or: blue:pale;white

♀\♂	B	b
B	BB	Bb
b	Bb	bb

(E) LETHAL GENES in Mice

Q. 5. (a) cross 1: tail-less mouse × normal mouse gives F1: All tail less mice.

(b) cross 2: tail-less F1 (Tt) × tail-less F1(Tt) gives F2: 10 normal, 21 tail-less and 9 dead

Solution : This is a model for LETHAL ALLELES.

Tail-lessness and lethality are controlled by one gene with two alleles: T allele-dominant tail less lethalphenotype, *t* allele - recessive normal-tail pheno type.

Therefore:

TT - tail-less and dead

T*t* - tail-less and alive

tt - normal-tail and alive

Cross 1 is then: TT × *tt* ——> all Tt (tail-less)

Cross 2 is then: T*t* × T*t* ——> 1:2:1

1 – Tt – Tail less Dead

2 – Tt – Tail less Normal

1 – tt – Tailed Normal

♀\♂	T	t
T	TT	Tt
t	Tt	tt

R_1	T	T
ⓣ	Tt	Tt
ⓣ	Tt	Tt

(F) MENDEL'S EXPERIMENT-PREDICTION OF DOMINANT ALLELE

Q. 6. When true-breeding tall stem pea plants are crossed with true-breeding short stem pea plants, what is the phenotypic nature of the F1 and F2 generations and which is the dominant character.

Solution: This is the model for predicting the dominant allele. The F1 plants, produced by a cross of true-breeding (homozygous) parents differing in a single trait, are all heterozygous and display the dominant phenotype as shown in the Punnett square.

Cross of homozygous plants

Parent 1 TT X Parent 2 tt

	t	t
T	Tt	Tt
T	Tt	Tt

All F1 plants are heterozygous and they all have a tall stem.

Cross of heterozygous plants

Parent 1 X Parent 2
Tt Tt

	T	t
T	TT	Tt
t	Tt	tt

3/4 of the plants have tall stems

The F2 plants, produced by a cross of heterozygous parents, result in 3/4 of the offspring having the dominant phenotype, tall stems.

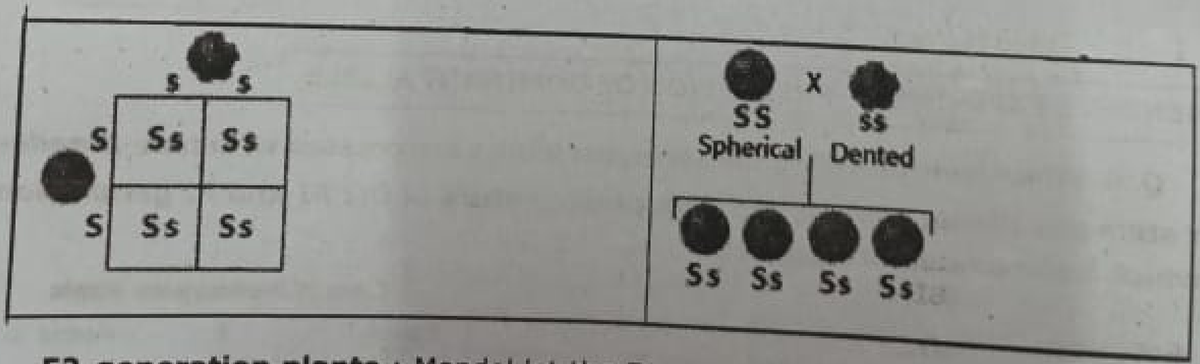
(G) MENDEL'S EXPERIMENT-LAW OF DOMINANCE

Q. 7. In Mendel's Experiment true-breeding pea plants with spherical seeds were crossed with true-breeding plants with dented seeds. (Spherical seeds are the dominant characteristic.) Mendel collected the seeds from this cross, grew F1-generation plants, let them self-pollinate to form a second generation, and analyzed the seeds of the resulting F2 generation. The results that he obtained, and that you would predict for this experiment are.

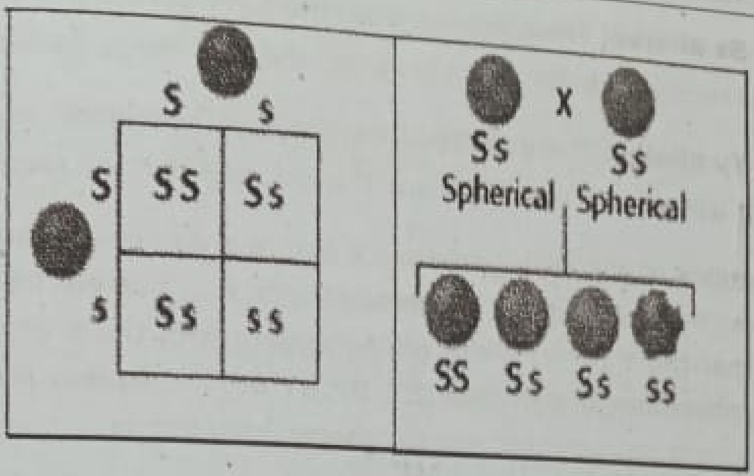
F1-generation plants:

Mendel crossed **SS** (spherical seeds) with ***ss*** (dented seeds.)

All of the gametes of the spherical seeded parent have the **S** (dominant) allele and all of the gametes of the dented seeded parent have the ***s*** (recessive) allele. All of the F1 generation plants will have the genotype of ***Ss*** (heterozygous), and all will be spherical seeded.



F2-generation plants : Mendel let the F1-generation plants self-pollinate to form a second generation and he analyzed the seeds of the resulting F2 generation. All F1-hybrid plants have the genotype ***Ss*** and all are spherical (dominant characteristic). The recessive alleles segregate during gamete formation. As a result, one out of four possible combinations of F2-generation plants will have the homozygous recessive genotype (***ss***).

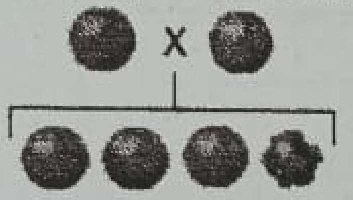


	S	s
S	SS	Ss
s	Ss	ss

(H) MENDEL'S EXPERIMENT

Q. 8. When do you expect a phenotypic ratio of 3:1 in the offspring of a mating of two organisms heterozygous for a single trait ?

Phenotypic ratio of 3:1



(3) are with dominant character and

(1) is with recessive character

Solution : (a) The alleles segregate during meiosis. (b) Mendel first proposed that alleles segregate from one another during the formation of gametes. Hence the gametes are always pure and receive allele for each character. (c) The alleles are identical. If the alleles were identical (SS x SS), all offspring would be the same. There would be a 4:0 segregation ratio. (d) The alleles are incompletely dominant. If the alleles are incompletely dominant, the heterozygotes would have an intermediate phenotype different from both the homozygous dominant (SS) and the homozygous recessive (ss). There would be three different phenotypes among the offspring, and the ratio would be 1 (SS) : 2 (Ss) : 1 (ss).

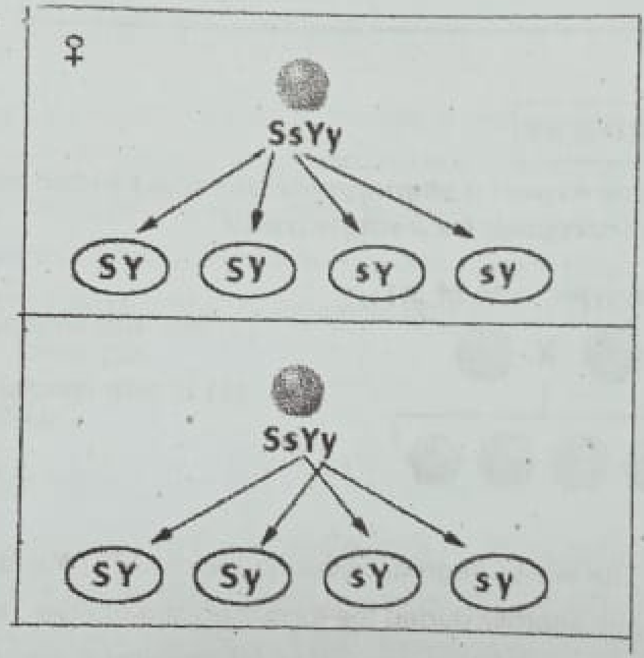
(I) PREDICTING COMBINATIONS OF ALLELES IN GAMETES OF PLANTS HETEROZYGOUS FOR TWO TRAITS.

Q. 9. A pea plant is heterozygous for both seed shape and seed color. S is the allele for the dominant, spherical shape characteristic; s is the allele for the recessive, dented shape characteristic. Y is the allele for the dominant, yellow color characteristic; y is the allele for the recessive, green color characteristic. What will be the distribution of these two alleles in this plant's gametes?

Assortment of Ss alleles: The gametes from the SsYy parent will receive one of the two alleles Ss. Half will receive the dominant S allele, and half the recessive's allele.

Assortment of Yy alleles: The gametes from the SsYy parent will also receive one of the two alleles Yy. Half will receive the dominant Y allele, and half the recessive y allele.

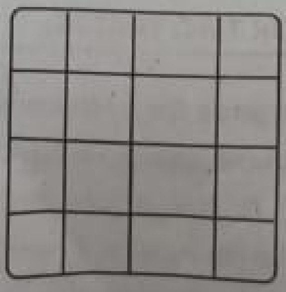
Possible combinations of alleles in gametes of plants are heterozygous for two traits: Since alleles of different traits segregate independently during gamete formation, there is the same probability that the Y (or y) allele will segregate with the S allele as with the s allele. Thus all four combinations of gametes (SY, Sy, sY, sy) are equally probable.



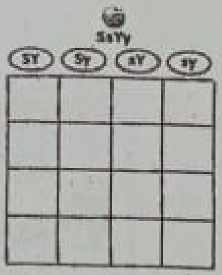
(J) PUNNET SQUARE - DIHYBRID CROSS

Q. 10. Construct a punnet square for the dihybrid cross, drawing Punnes square the P. 8. as to explain.

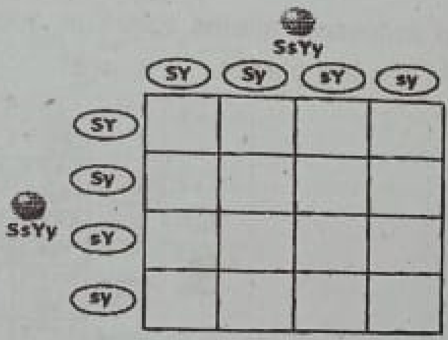
Since each parent produces 4 different combinations of alleles in the gametes, draw a 4/4 square punnett square.



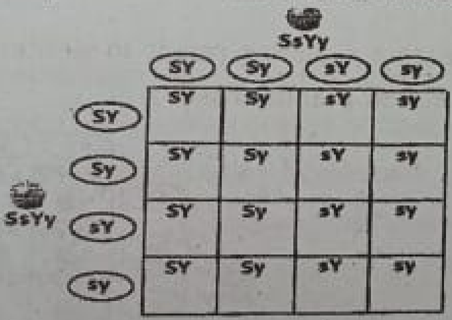
List the gametes for Parent 1 along the upper edge of the punnett square.



List the gametes for Parent 2 along the left edge of the punnett square.



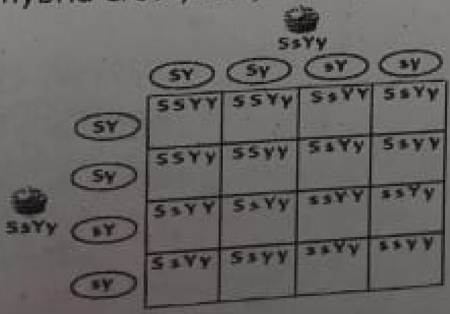
Fill out the squares with the alleles of Parent 1.



	SY	Sy	sY	sy
SY	SY	Sy	sY	sy
Sy	SY	Sy	sY	sy
sY	SY	Sy	sY	sy
sy	SY	Sy	sY	sy

Fill out the squares with the alleles from Parent 2.

The result is the prediction of all possible combinations of genotypes for the offspring of the dihybrid cross, *SsYy* × *SsYy*.



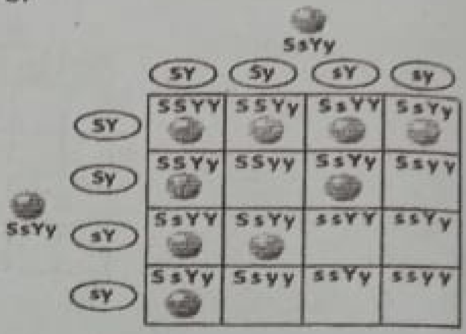
	SY	Sy	sY	sy
SY	SSYY	SSYy	SsYY	SsYy
Sy	SSYy	SSyy	SsYy	Ssyy
sY	SsYY	SsYy	ssYY	ssYy
sy	SsYy	Ssyy	ssYy	ssyy

NH_2

Predicting the phenotype of offspring Spherical, yellow phenotype. There are 9 genotypes for spherical, yellow seeded plants. They are:

SSYY (1/16), SSY*y* (2/16)

SsYY (2/16), SsY*y* (4/16)

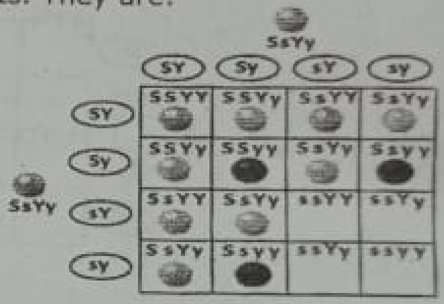


SsYy

SsYy	SY	Sy	sY	sy
SY	SSYY	SSYy	SsYY	SsYy
Sy	SSYy	SSyy	SsYy	Ssyy
sY	SsYY	SsYy	ssYY	ssYy
sy	SsYy	Ssyy	ssYy	ssyy

Spherical, green phenotype: Two recessive alleles result in green seeded plants. There are 2 genotypes for spherical, green seeded plants. They are:

Ss*yy* (1/16) Ss*yy* (2/16)

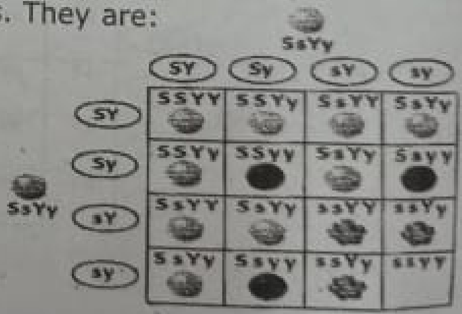


SsYy

SsYy	SY	Sy	sY	sy
SY	SSYY	SSYy	SsYY	SsYy
Sy	SSYy	SSyy	SsYy	Ssyy
sY	SsYY	SsYy	ssYY	ssYy
sy	SsYy	Ssyy	ssYy	ssyy

Dented, yellow phenotype : Two recessive s alleles result in dented seeded plants. There are 2 genotypes for dented, yellow seeded plants. They are:

*ss*YY (1/16) *ss*Y*y* (2/16)



SsYy

SsYy	SY	Sy	sY	sy
SY	SSYY	SSYy	SsYY	SsYy
Sy	SSYy	SSyy	SsYy	Ssyy
sY	SsYY	SsYy	ssYY	ssYy
sy	SsYy	Ssyy	ssYy	ssyy

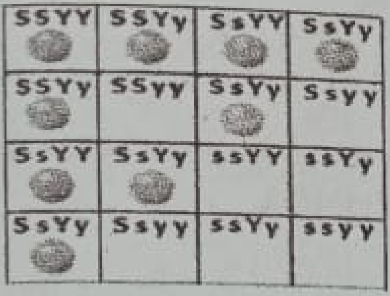
Dented, green phenotype: A ssyy plant would be recessive for both traits. There is only 1 genotypes for dented, green seeded plahts. It is: ssyy (1/16).

SsYy

SsYy	SY	Sy	sY	sy
SY	SSYY	SSYy	SsYY	SsYy
Sy	SSYy	SSyy	SsYy	Ssyy
sY	SsYY	SsYy	ssYY	ssYy
sy	SsYy	Ssyy	ssYy	ssyy

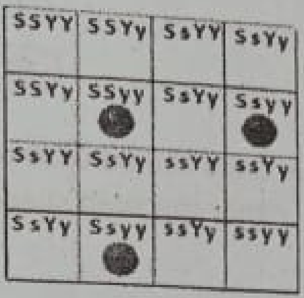
(K) DIHYBRID CROSS

Q. 11. Predict the phenotypic ratio of 9:3:3:1 for the offspring of a SsYy × SsYy dihybrid cross.



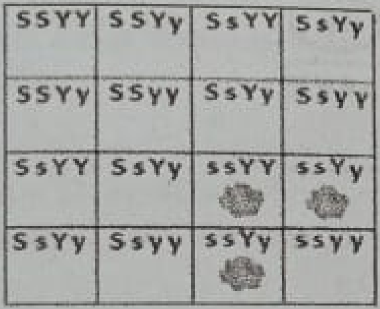
SSYY ●	SSYy ●	SsYY ●	SsYy ●
SSYy ●	SSyy	SsYy ●	Ssyy
SsYY ●	SsYy ●	ssYY	ssYy
SsYy ●	Ssyy	ssYy	ssyy

9 spherical, yellow



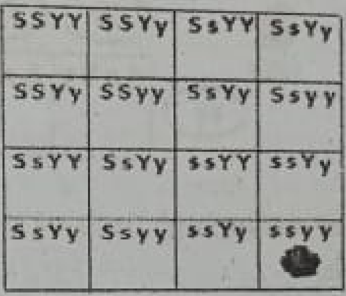
SSYY	SSYy	SsYY	SsYy
SSYy	SSyy ●	SsYy	Ssyy ●
SsYY	SsYy	ssYY	ssYy
SsYy	Ssyy ●	ssYy	ssyy

3 spherical, green



SSYY	SSYy	SsYY	SsYy
SSYy	SSyy	SsYy	Ssyy
SsYY	SsYy	ssYY ●	ssYy ●
SsYy	Ssyy	ssYy ●	ssyy

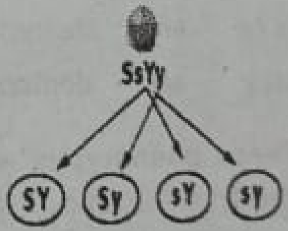
3 dented, yellow



SSYY	SSYy	SsYY	SsYy
SSYy	SSyy	SsYy	Ssyy
SsYY	SsYy	ssYY	ssYy
SsYy	Ssyy	ssYy	ssyy ●

1 dented, green

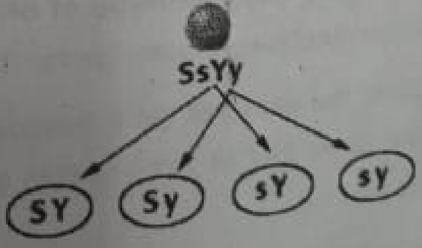
Q. 12. What is the genotypic nature of the gametes of a plant of genotype SsYy.



Assortment of alleles: Since the assortment of alleles occur, gametes will receive one of each pair (*Ss* and *Yy*) of alleles. All combinations of alleles will occur with equal probability (*SY*, *Sy*, sY, and *sy*) because alleles of different genes are assorted independently during gamete formation (meiosis.)

Each gamete has one gene for seed shape (S=spherical or *s*=dented) and one gene for color (Y=yellow or *y*=green) allele.

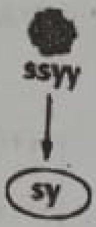
Q. 13. What are the genotypes of the offspring of a SsYy × ssyy test cross.



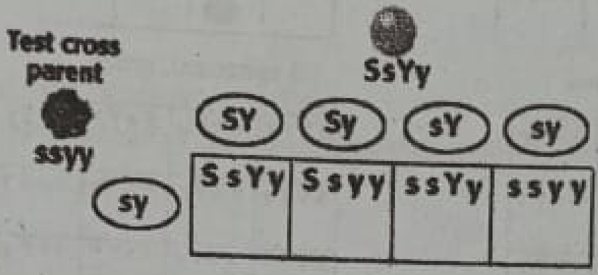
The expected phenotypic ratio of the progeny of a **SsYy × ssyy** test cross:

Alleles of the Ist parent: There are four combinations of alleles in the gametes from partent 1.

Alleles of parent 2: There is only one possible combination of alleles in the gametes from parent 2.



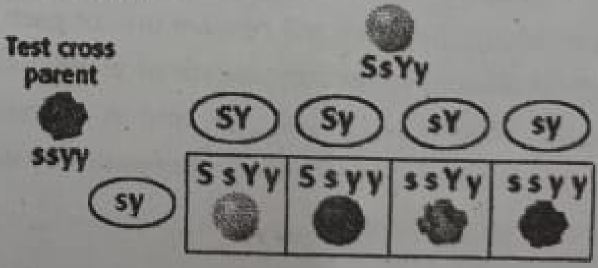
Test Cross: This cross can be used to determine if this spherical yellow seeded plant was heterozygous for either the seed shape or seed color trait.



Phenotype of offspring: Each of the genotypes of the offspring corresponds to a different phenotype.

SsYy	are	smooth,	yellow	seeded
Ssyy	are	smooth,	green	seeded
ssYy	are	dented,	yellow	seeded
ssyy	are	dented,	green	seeded

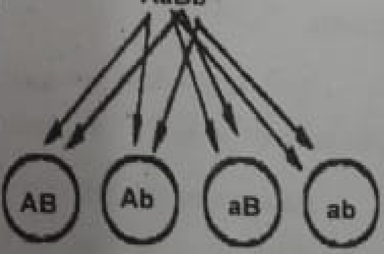
These phenotypes will appear in a predicted 1:1s:1:1 ratio.



Q. 14. In a dihybrid cross, AaBb ´ AaBb, what fraction of the offspring will be homozygous for both recessive traits?

Predicting the genotype of offspring: There are four possible combinations of gametes for the *AaBb* parent.

Half of the gametes get a dominant A and a dominant B allele; the other half of the gametes get a recessive a and a recessive b allele.



Both parents produce 25% each of AB, *Ab*, aB, and *ab*.

Possible gametes for each AaBb parent: Since each parent has four different combinations of alleles in the gametes, there are sixteen possible combinations for this cross. Therefore we use a 4 square by 4 square Punnett Square.

AaBb

		AB	Ab	aB	ab
	AB				
	Ab				
AaBb	aB				
	ab				

Alleles from both parents: Now we can predict the outcome of the genetic cross of *AaBb* × *AaBb*.

To determine the fraction of the offspring homozygous for both traits, first determine the genotype of an offspring homozygous for both traits. It would be *aabb*.

AaBb

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

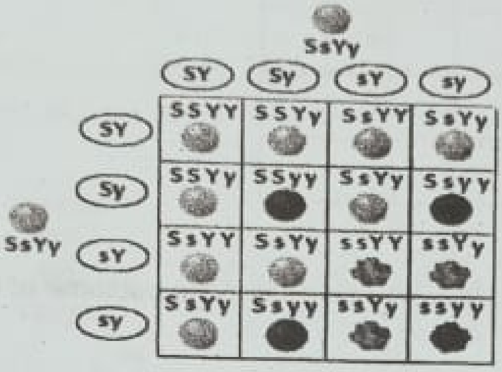
Offspring homozygous for both recessive traits: There is only one of 16 possible combinations with this genotype. The predicted fraction is therefore 1/16.

AaBb

		AB	Ab	aB	ab
	AB				
	Ab				
AaBb	aB				
	ab				aabb

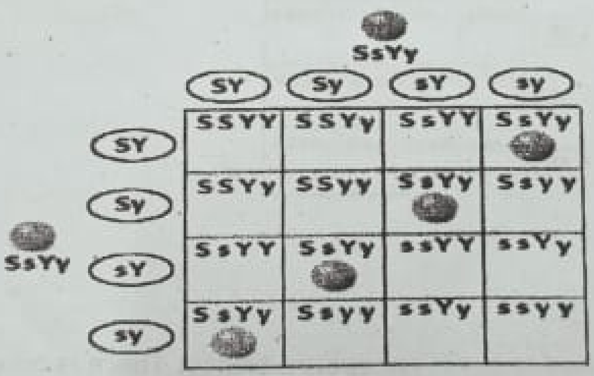
Q. 15. Following a SsYy × SsYy cross, what fraction of the offspring are predicted to have a genotype that is heterozygous for both characteristics?

Predicting the genotype of offspring: The solution for predicting the outcome of an *SsYy* × *SsYy* genetic cross was given in detail in problem 2 and problem 3. Review the answers to these problems if necessary. These are the possible combinations of gametes for this cross.



SsYy	SY	Sy	sY	sy
SY	SSYY	SSYy	SsYY	SsYy
Sy	SSYy	SSyy	SsYy	Ssyy
sY	SsYY	SsYy	ssYY	ssYy
sy	SsYy	Ssyy	ssYy	ssyy

There are 4 out of 16 possible combinations of gametes from an *SsYy* × *SsYy* cross with the genotype of SsYy. We would therefore predict that 4/16 (or 1/4) of the offspring of the cross would be heterozygous for both traits.



SsYy	SY	Sy	sY	sy
SY	SSYY	SSYy	SsYY	SsYy
Sy	SSYy	SSyy	SsYy	Ssyy
sY	SsYY	SsYy	ssYY	ssYy
sy	SsYy	Ssyy	ssYy	ssyy

2

STUDY OF LINKAGE THROUGH CROSSING OVER & GENE MAPPING

(A) Genetic Linkage. Linked **genes** sit close together on a chromosome, making them likely to be inherited together (left). **Genes** on separate chromosomes are never linked (center). But not all **genes** on a chromosome are linked. Genes that are farther away from each other are more likely to be separated during a process called homologous recombination (right).

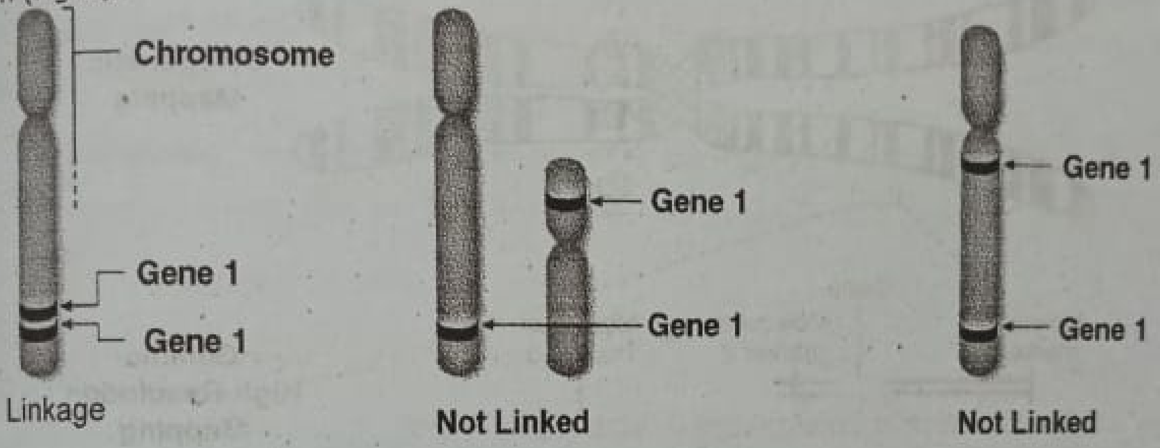


Fig : *Genetic and Physical Mapping*

(B) Homologous recombination: During the formation of gametes (eggs and sperm in pigeons), chromosomes go through a process called homologous recombination. First, the cell makes an identical copy of each chromosome. Identical copies are called sister chromatids, and they remain attached to one another.

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

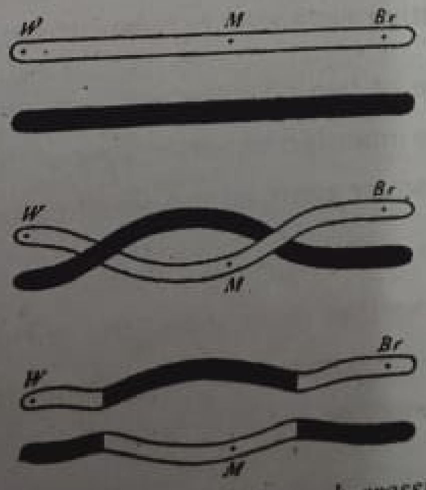


Fig. *Recombination through crossing over*

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

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

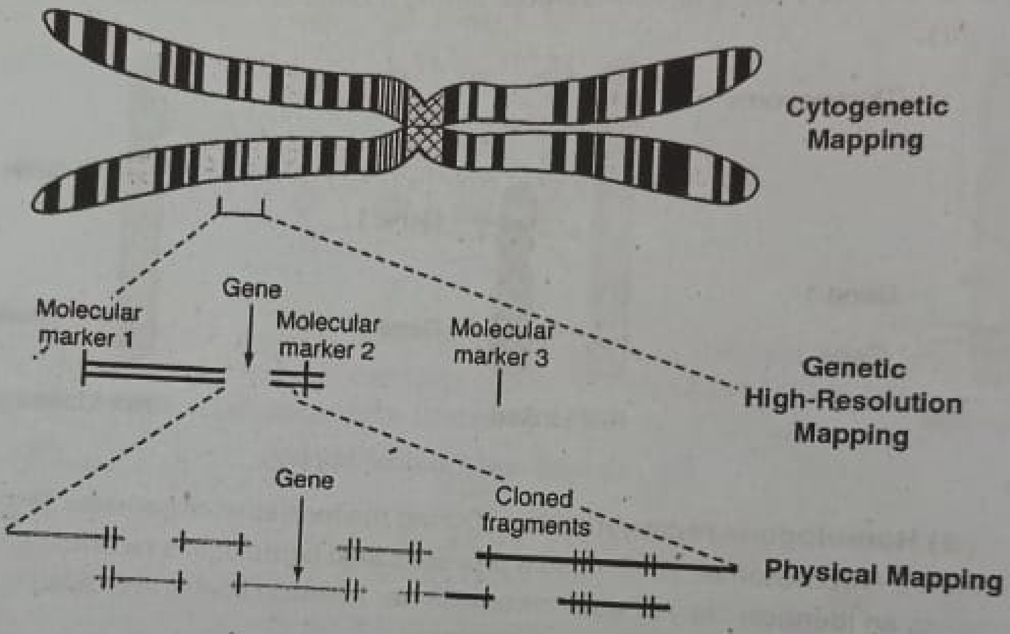


Fig : *Genetic and Physical Mapping*

(D) Genetic mapping using linkage: Linkage is used to find the location of a gene on a chromosome. By looking at how often different genes are inherited together, maps genetic can be created basing on the relative distances between them.

Since each gamete gets one of two possible versions of a chromosome, by random chance, two unlinked genes will be inherited together 50% of the time. Unlinked genes may be on different chromosomes, or so far apart on the same chromosome that they are often separated by recombination.

If two genes are inherited together more than 50% of the time, this is evidence that they are linked on the same chromosome. The closer together the genes are, the more frequently they will be inherited together.

Q. 16. Exceptions to the 9:3:3:1 ratio of offspring explaining the linkage.

A cross between tall, spherical-seeded plants and short, dented-seeded plants, produced many more than 1/16 short, dented-seeded plants in the F_2 generation. Explain the reason behind the result.

Solution: If it is a normal cross

F_1 offspring of unlinked alleles: In a dihybrid cross between plants with tall stems and spherical seeds (both dominant) and plants with short stems and dented seeds, all of the F_1-hybrid plants would have both dominant characteristics. The dihybrid cross is SSTT × sstt. All F_1-hybrids would be SsTt.

F_2 offspring for unlinked alleles : If the two traits are unlinked and the F1-plants are self-fertilized, in the F_2 generation plants we expect the 9:3:3:1 ratio of offspring:

9/16 tall plants with spherical seeds

3/16 tall plants with dented seeds

3/16 short plants with spherical seeds1/16 short plants with dented seeds.

The above result is due to the linkage of genes relating to the characters expressed.

F_1 offspring for linked alleles : An excess of parental genotypes in the F_2 generation (such as more than 1/16 short plants with dented seeds) can occur if the loci for the two traits are on the same chromosome, and linked to each other on the same DNA molecule.

If two traits are closely linked on the same chromosome, the alleles for the linked genes do not segregate during the formation of gametes. The two dominant alleles are linked on one chromosome, and the two recessive alleles are linked on the other, homologous chromosome.

F_2 offspring for linked alleles : To determine the genotype and phenotype for the offspring of a dihybrid cross when the traits are tightly we can use a Punnett Square to predict the outcome of this cross. The gametes of each parent will have the pairs of linked alleles, either ST or st. Since there are two combinations of alleles for each parent, we need a two square by two square Punnett Square. Fill out the Punnett Square linked

Note that 3/4 of the offspring are predicted to have both dominant traits, and 1/4 are predicted to have both recessive traits. With unlinked traits, only 1/16 are predicted to have both recessive traits.

SEX LINKED INHERITANCE

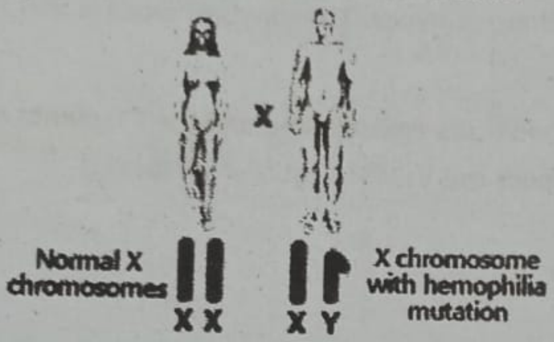
Q. 17. Hemophilia in humans.

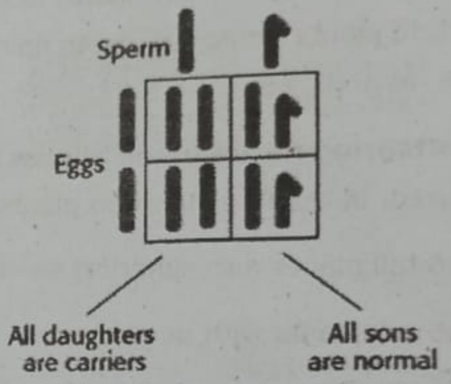
Hemophilia in humans is due to an X-chromosome mutation. What will be the result of a mating between a normal (non-carrier) female and a hemophilic male?

Solution: Genotypes and phenotypes of parents:

The eggs of the mother will all contain the normal **X** chromosome.

The sperm of the father will contain either the **X** chromosome with the mutation causing hemophilia or the **Y** chromosome.





Genotypes and phenotypes of offspring: All of the daughters inherit an **X** chromosome.

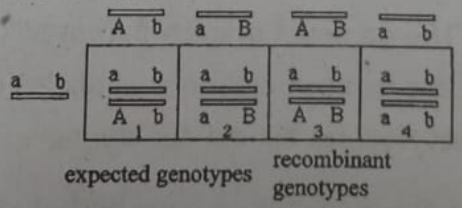
RECOMBINATION

Q. 18. Assume genes A and B are linked and are 50 map units apart. An individual heterozygous at both loci is crossed with an individual who is homozygous recessive at both loci. (a) What percentage of the offspring will show phenotypes resulting from crossovers and recombinations. (b) If you did not know genes A and B were linked, how would you interpret the results of this cross?

(a) 50 % because when the loci are at opposite ends of a chromosome they behave as if they were on different chromosomes.

Beginning with 100% $\frac{A\quad B}{a\quad b}$ recombination results in 50% $\frac{A\quad B}{a\quad b}$ and 50% $\frac{A\quad b}{a\quad B}$

The resulting cross gives results almost identical to a normal Mendelian dihybrid cross, which can be symbolized as follows:



expected genotypes recombinant genotypes

(b) It can be assumed that these genes are on separate chromosomes since they **appear** to segregate independently even though they are linked.

EMBRYOLOGY

1

STUDY OF T.S. OF TESTIS AND OVARY OF A MAMMAL

1. (a)T.S.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 seprate formed of intersticial cells.
3. Testosterone is the male hormone secreted by intersticial tissue.
4. Each chamber possesess a number of seminiferous tubules.
5. Germinal epthelium surrounds the seminiferouns tubule.
6. The cells divide by mitosis to produce spermatogonia.
7. Sperm mother cells then produced are 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 spermatigonia, spermatids and sperm cells.

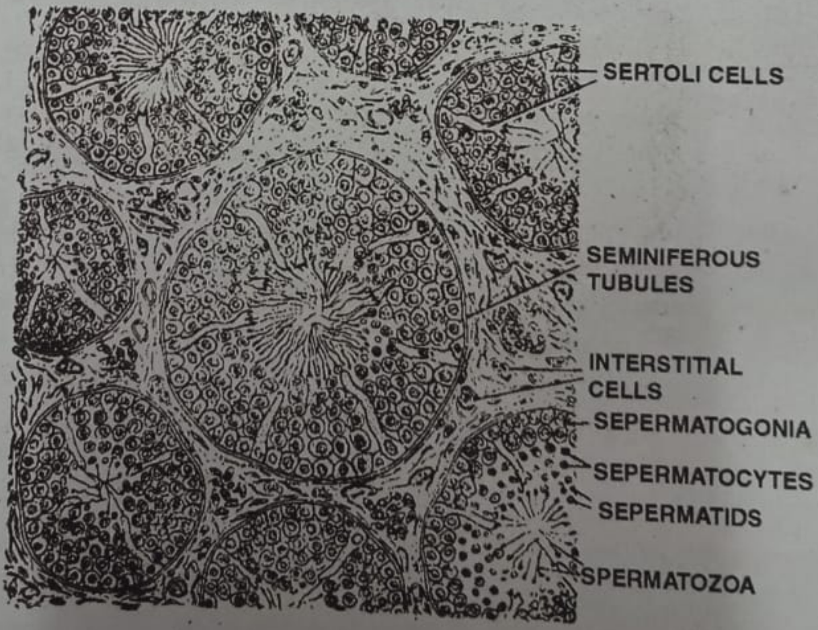


Fig : T. S. Testis

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1. (b)T.S.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 eprithellum.
5. In the stroma primary oogonia, secondary 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 Graffiam 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, secrating progesterone.

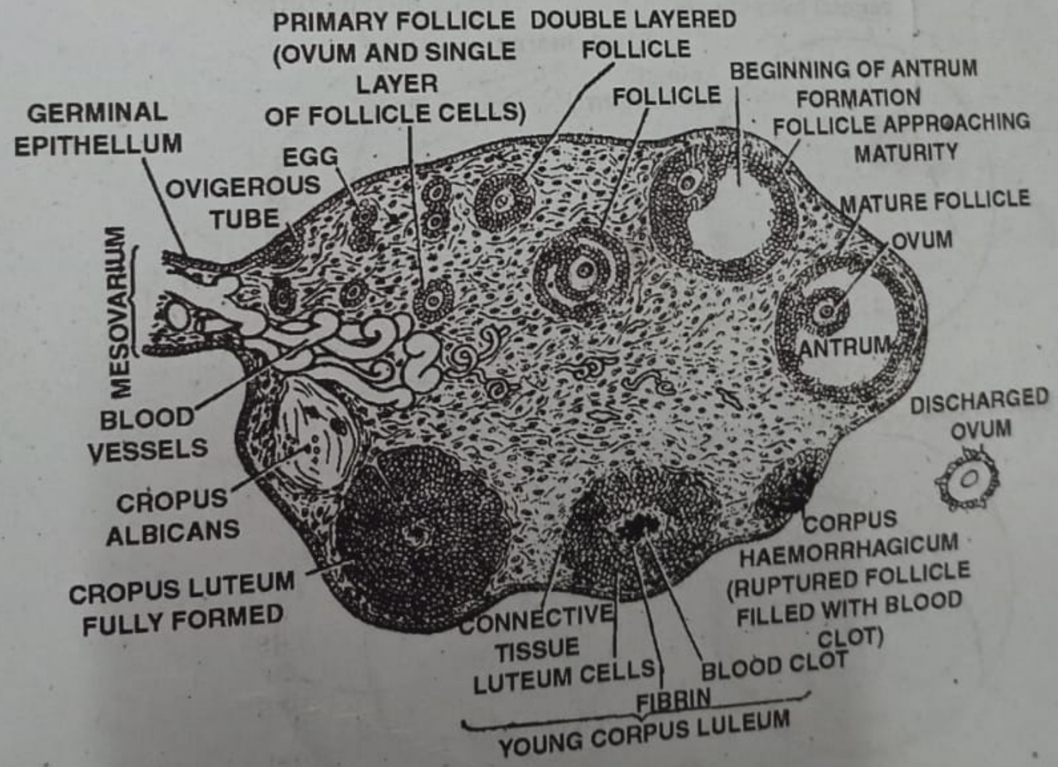

Fig : T. S. Ovary

2

STUDY OF DIFFERENT STAGES OF CLEAVAGES (2,4,8,16 CELL STAGES), MORULA, BLASTULA

2. (a) *Different Stages of Cleavage (2-Cell, 4 and 8-Cell)*

1. During development Zygotes undergo cleavage two to three hours after fertilization.
2. First cleavage is meridional, holoblastic, incomplete resulting in the formation of two blstomeres.
3. Second division is also meridional, holoblastic incomplete but results in the formation of four blastomeres.
4. Third division is equatorial, dividing the four blastomeres into four micromers towards animal hemispheres and four macromeres towards vegetal hemisphere.
5. Micromeres are small, pigmented while the macromeres are large and whitish with yolk.

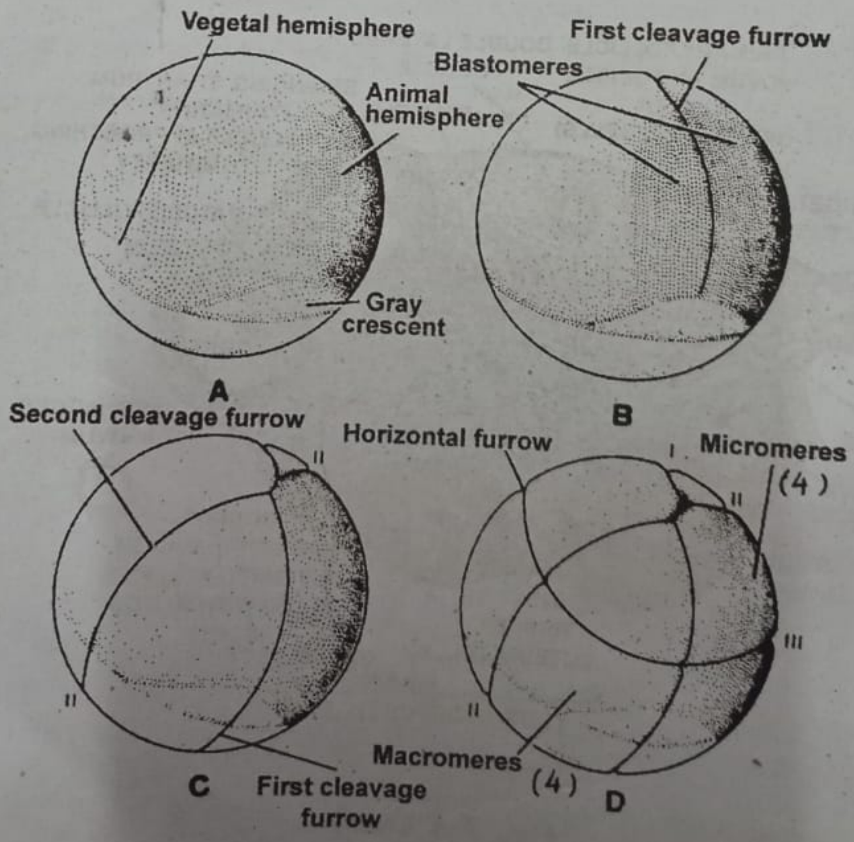

Fig : Stages of cleavage

(A) Zygote (B) 2 celled Stage (C) 4 Celled Stage (D) 8 Celled Stage

B.

2. (b)Morula

1. During development Zygotes undergo cleavage two to three hours after fertilization.
2. Morula is the first stage in the development of any vertebrate.
3. Morula is a solid cellular stage formed during the early stages of development. It appears round and ball like.
4. It may possess around eight or sixteen or thirty two blastomeres formed out of cleavage.
5. The small cells towards animal hemisphere are pigmented and are the micromeres.
6. Macromeres towards the vegetal hemisphere are larger, whitish and loaded with yolk.
7. Repeated divisions in the blastomeres result in the transformation of morula in to blastula.Presence of micromeres towards animal hemisphere, macromeres towards vegetal hemisphere, absence of any cavity inside, solid cellular nature are the features of morula.

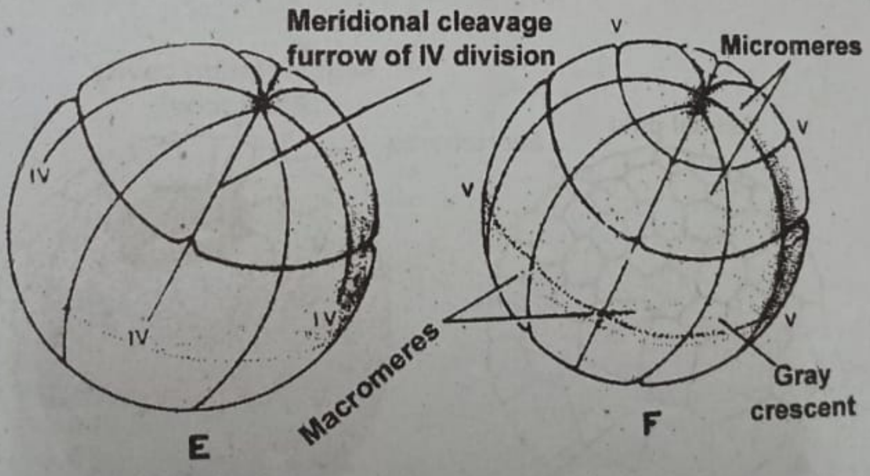

Fig : Morula

(E) 16 celled stage (F) 32 Celled Morula

2. (c) Blastula of Frog

1. Frog is a unisexual, sexually dimorphic, oviparous vertebrate with external fertilization and development.
2. During Development Zygotes undergo cleavage two to three hours after fertilization.
3. Morula is the first stage in the development of any vertebrate.
4. Blastula is the second stage in the development.
5. Micromeres towards animal hemisphere are very small, pigmented and more in number.
6. Macromeres are larger in size, lesser in number, whitish in colour and occupy the vegetal hemisphere.
7. Vertical section shows an eccentric cavity called blastocoel surrounded by many layers of micromeres.
8. Blastocoel is filled with fluid which is absorbed subsequently. Presence of eccentric blastocoel, micromeres arranged in the form of an arch, macromeres forming the base of the blastocoel are the characteristic features of the blastula of frog.

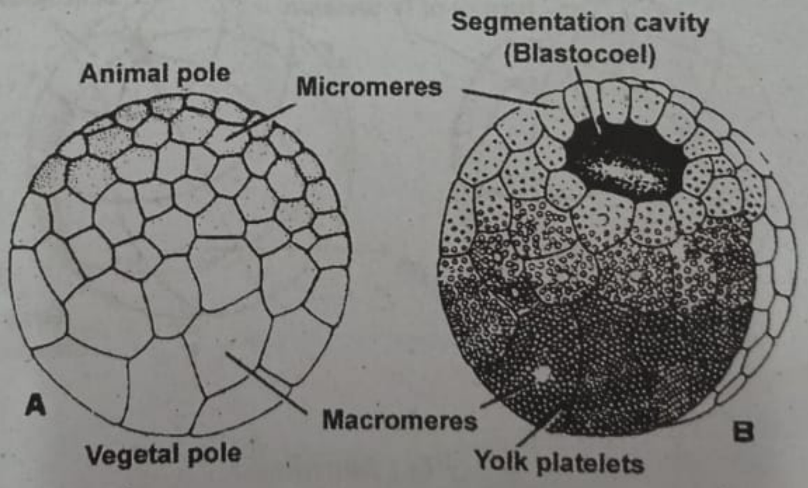

Fig : Blastula of frog

(A) Blastula (B) Vertical Section of Blastula showing blastocoel

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3

STUDY OF CHICK EMBRYOS OF 18 HOURS, 24 HOURS, 33 HOURS AND 48 HOURS OF INCUBATION

3. (a)18H.Chick Embryo

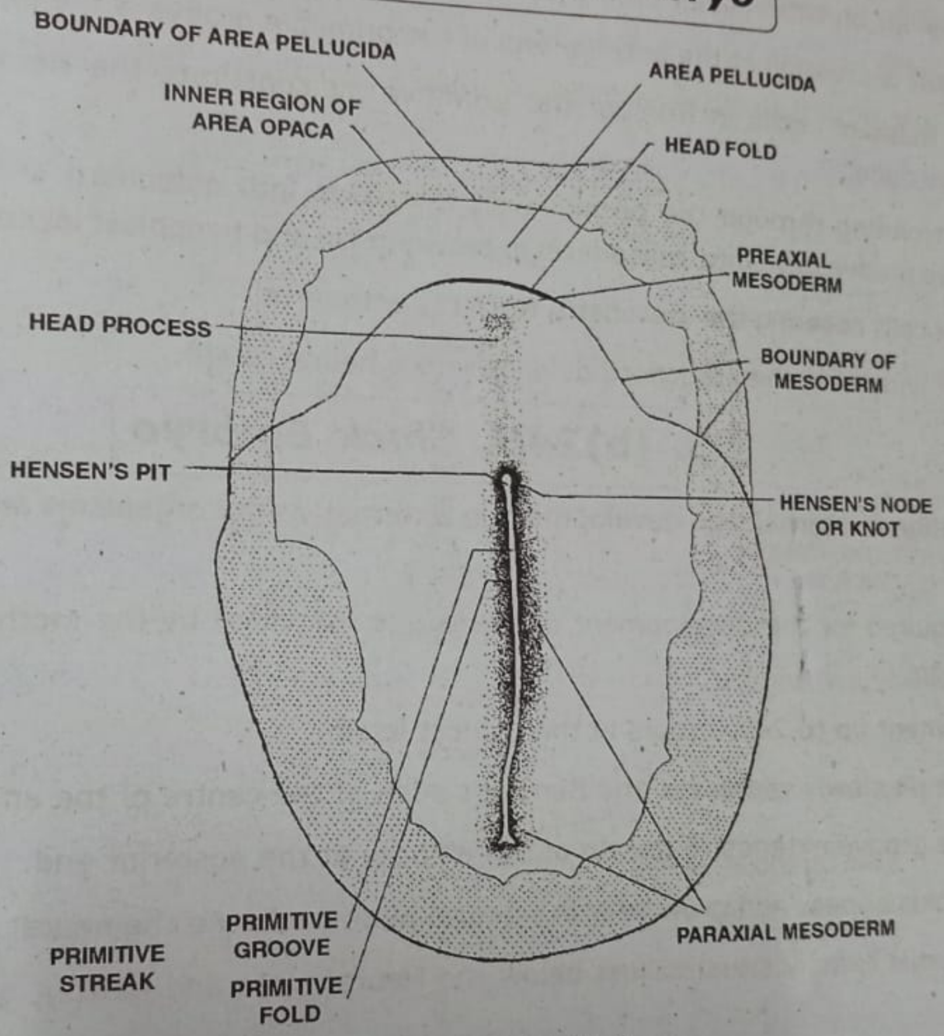

Fig : 18th Chick Embryo

1. Fertilization is internal and development is external in chick as the organisms are oviparous in nature.
2. Heat required for the development of the egg is provided by the mother bird during incubation.
3. Development up to 24h. occurs in the oviduct itself.
4. By 18h. entire surface of the embryo is composed of epiblast.
5. Embryo is seen as a blastoderm in the form of a disc over the yolk material.

6. The central part of the blastodisc has separated from the yolk to form into a central, transparent, multilaminar area called area pellucida.
7. Peripheral portion of the embryo is the area opaca, a non-transparent cellular layer living in close association with the underlying yolk material.
8. The central fold of epiblast having a deep groove is the primitive groove.
9. The two lips on either side of the groove are the primitive folds.
10. A shallow depression at the anterior end of the primitive groove is the primitive pit.
11. Dense mass of cells in front of the primitive pit constitute the Hensen's node or primitive node.
12 Cells involuting through the primitive pit transform into notochord and through the primitive groove form into mesoderm in between epi and hypoblast layers.
13. Epiblast cells covering the mesoderm form the ectoderm.
14. Epiblast lying over the notochord develop into a neural plate.

3. (b)*24H. Chick Embryo*

1. Fertilization is internal and development is external as the organisms are oviparous in nature.
2. Heat required for the development of the egg is provided by the mother bird during incubation.
3. Development up to 24h. occurs in the oviduct itself.
4. Primitive pit slowly regresses and hence is seen at the centre of the embryonic disc.
5. Primitive groove extends to the tip of the embryo at the posterior end.
6. Neural folds appear and grow over the neural plate enclosing the neural groove.
7. Notochord is exactly situated just below the Neural plate and in front of the primitive pit.
8. Mesoderm formed from the involuted cells of the epiblast is differentiated into somitic, intermediate and lateral mesoderms.
9. Formation of the mesodermal somites is initiated at 20h. of incubation from the somitic component of the mesoderm.
10. By 24h. four to five pairs of mesodermal somites are formed in the embryo.
11 Up grown neural folds fuse middorsally to form the neural tube opening out through anterior neuropore. The epiblast along with the anterior neural tube lifts up to form the head lobe.
12. Deep notch between the head lobe and the underlying yolk material is the subcephalic pocket.

13. Because of the separation of the neural tube from the epiblast, the upper most layer of the blastoderm is called the ectoderm.

14. Along with the neural plate, the endoderm also lifts up to contribute to the formation of the foregut. The opening between the fore gut and the yolk is the anterior intestinal portal.

15. Primary heart is formed as a horse shoe shaped tube from the fusion of the amniocardiac vesicles.

16. Groups of cells forming into blood islands can be seen in the opaca region.

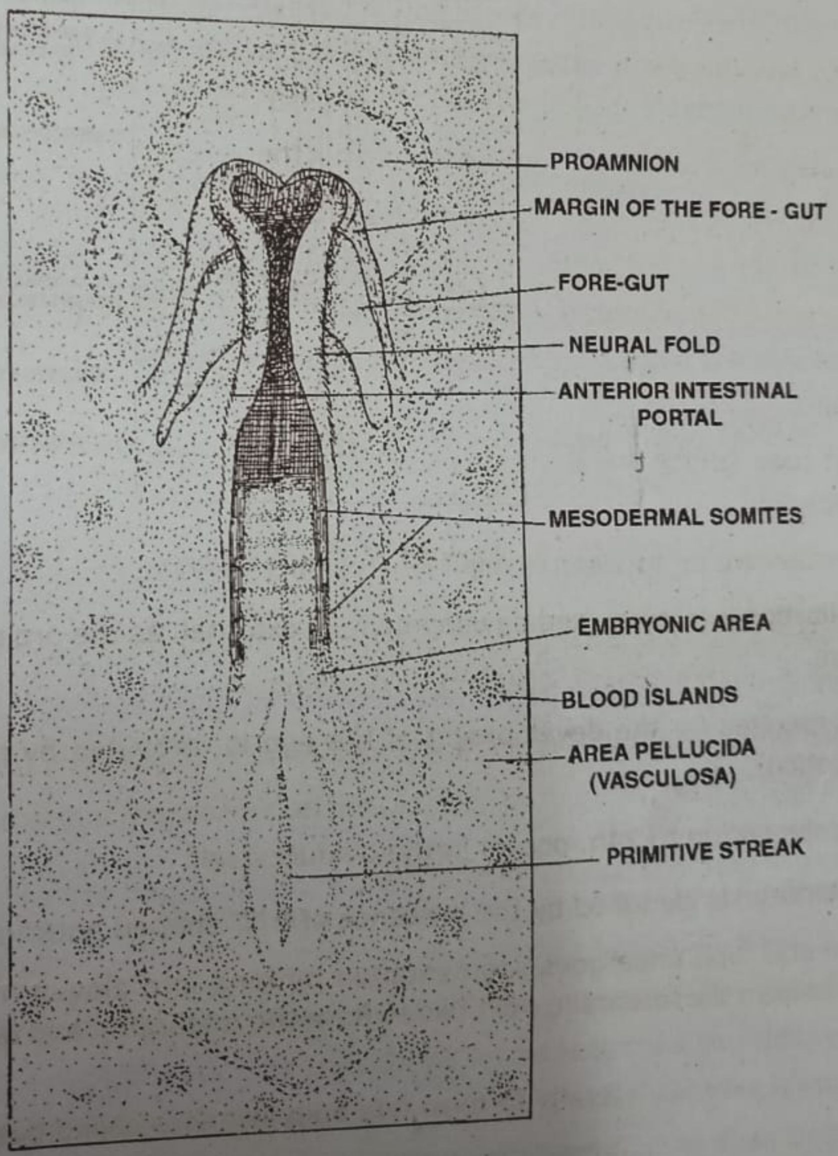

Fig : 24 H. Chick Embryo

3. (C)33H. Chick Embryo

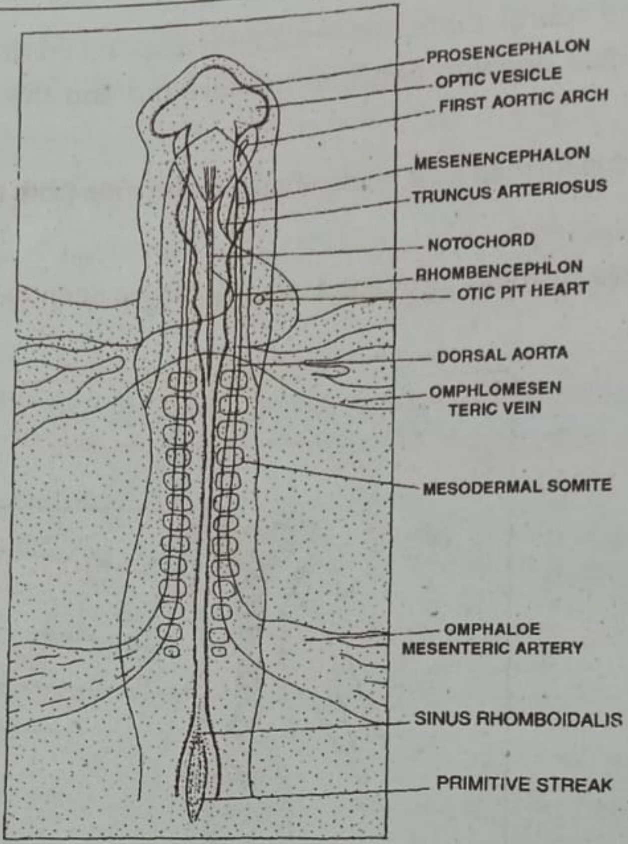

Fig : 33 H. Chick Embryo

1. Fertilization is internal and development is external as the organisms are oviparous in nature.
2. Heat required for the development of the egg is provided by the mother bird during incubation.
3. Development up to 24h. occurs in the oviduct itself.
4. 33h. embryo is identified by the presence of a 'T' shaped anterior brain region.
5. The neural tube undergoes segmentation forming into eleven neuromeres. First three unite to form the forebrain, next two to form the midbrain, and last six to form the hind brain.
6. Fore brain extends laterally to form two bulb like sacs called optic vesicles.
7. Hensen's node and pit regresses back to remain as a mass of cells at the posteior end.
8. Neural folds expand around the pit forming sinus rhomboidalis.
9. Anterior end of the primitive heart is forked into the ventral aortae. They extend along the foregut, takea loop and grows back as dorsal aortae to the posterior end and spreads over the yolk as omphalomesenteric arteries.

10. Posteriorly, the sinus venosus of the heart divides and expands over the yolk as omphalomesenteric veins.
11. 13 to 14 pairs of somites are formed from the mesoderm on either side of the body.
12. Auditory placode appears as a thickened cell plate at the 11 neuromere region.

3. (d)48H. Chick Embryo

1. Anterior end of the embryo undergoes torsion and hence has lost its symmetry while the posterior end shows bilateral symmetry.
2. Cranial and cervical flexures in the embryo makes prosencephalon to bend and lie at right angles to the mesencephalon. The nerve cord takes a deep curvature at the neck region.
3. Twenty eight pairs of somites are formed.
4. Endoderm extends into the head fold as foregut and into the tail fold as hindgut. Endoderm of the fore and hind guts comes near to the ectoderm to form oral and anal plates.
5. Horse shoe shaped heart also undergoes flexion resulting in the shift of posterior atrium over the ventricle.
6. Three pairs of aortic arches are formed between the dorsal and ventral aortae.
7. Omphalomesenteric veins forming from the blood capillaries of the opaca region located over the yolk sac open into the sinus venosus. The dorsal aortae unite behind the last pair of gill slits and passes back as a single aorta and finally divide to spread over the yolk sac as omphalo mesenteric veins.
8. Simultaneously anterior and posterior cardinals are formed and open into the sinus venosus.
9. Outer layer of the optic vesicles invaginate into the opticoel to form the optic cup. Ectoderm present over the optic cup transforms into a lens placode. This invaginates and fits into the optic cup as lens vesicle.
10. Pronephros is formed as a solid rod from the intermediate mesoderm.
11. Intermediate mesoderm of the last somite region form into tubular mesonephric ducts.
12. Head amniotic fold is formed from the somatopleure at the anterior end covering the head. It grows towards posterior end.

NH_2

OH NH_3

13. Yolk sac and Allantois are formed from the hindgut region at the posterior end of the embryo.

14. Three pairs of gill pouches are formed from the pharyngeal region. First gill pouch fuses with ectodermal invagination to form the first gill slit i.e. the spiracle.

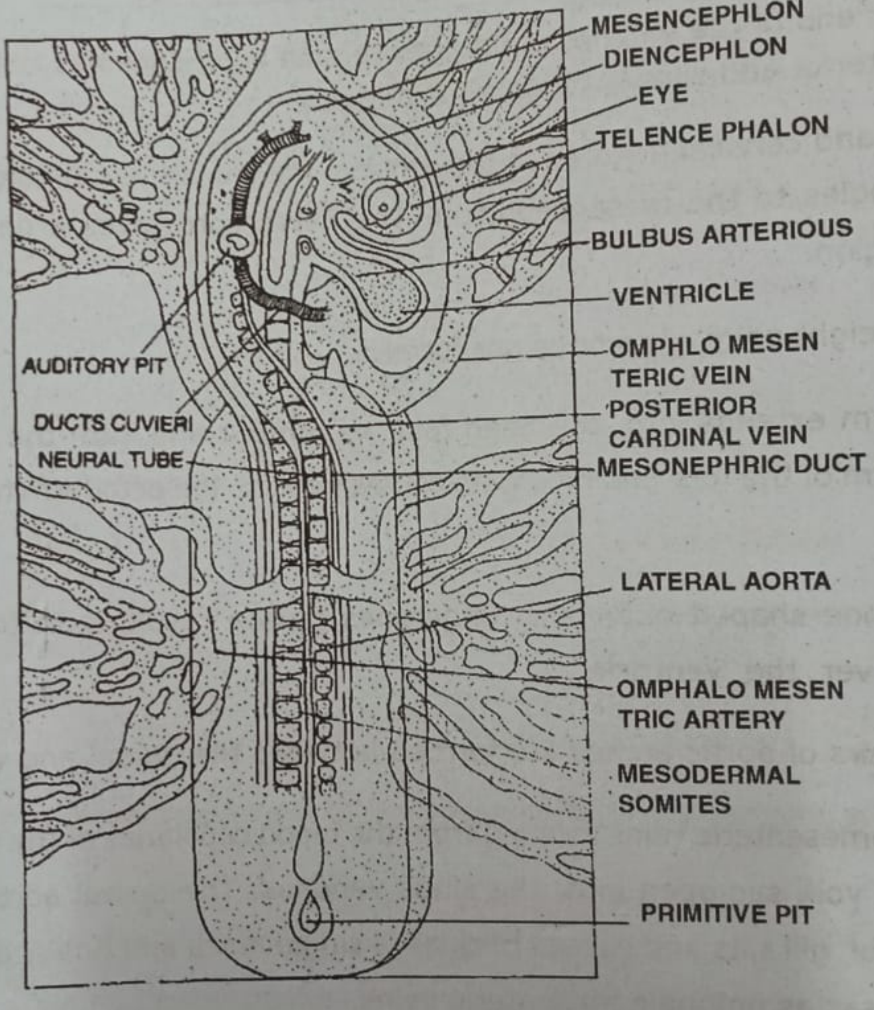

Fig : 48 H. Chick Embryo