DEVELOPMENTAL BIOLOGY AND MOLECULAR BIOLOGY

M.Sc. Zoology

PRACTICAL – II

SEMESTER-I, PAPER-VI

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DEVELOPMENTAL BIOLOGY AND MOLECULAR BIOLOGY

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
M.Tech., Ph.D.,
Vice-Chancellor I/c
Acharya Nagarjuna University.

SEMESTER 1
PRACTICAL - II
106ZO24- DEVELOPMENTAL BIOLOGY AND MOLECULAR BIOLOGY

DEVELOPMENTAL BIOLOGY

1) Identification of shrimp larvae.
2) Frog developmental stages – egg, 4 and 8 celled stage, blastula, gastrula and tadpole larva.
3) Chick embryonic stages – 18hour, 24hour, 36hour, 48 hour and 72 hour embryo.
4) Embryos of rat, rabbit and pig.
5) Estimation of calcium and phosphorus in egg shell.
6) Estimation of carbohydrates and proteins in egg.

MOLECULAR CELL BIOLOGY

1) Identification of different stages of Mitosis and Meiosis.
2) Observation of Mitosis in Onion root-tip cells.
3) Observation of Meiosis in Cricket/ Grasshopper testis.
4) Preparation and Staining of Blood Smear.
5) Buccal Smear preparation for identification of Barr body.
6) Observation of permanent slides of Cytology.

(106ZO24)

Model Question Paper
M.Sc. (Zoology) Practical Examination (Semester - I)

DEVELOPMENTAL BIOLOGY AND MOLECULAR BIOLOGY

Max marks:70

1) Estimation the total amount of calcium in the given samples (20 M)
2) Preparation and staining of blood smear (15M)
3) Observation of mitosis in onion root tip (10M)
4) Identify the Spotters (5X3=15)
    a) Embryo of rat
    b) metaphase
    c) chick 18 hr embryo development
    d) Diakinesis
    e) 4 celled stage of Embryo
5) Record & Viva (10 M)

SEMESTER-I (106ZO24)
PRATICAL PAPER TITLE: DEVELOPMENTAL BIOLOGY AND MOLECULAR BIOLOGY

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DEVELOPMENTAL BIOLOGY

1) **IDENTIFICATION OF SHRIMP LARVAE**
2) **FROG – STRUCTURE OF OVUM:**
3) **FROG -V.S BLASTULA**
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5) **FROG – TADPOLE LARVA**
6) **CHICK- EMBRYO DEVELOPMENTAL STAGE OF 18 HOURS**
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8) **CHICK EMBRYO DEVELOPMENTAL STAGE OF 36 HOURS**
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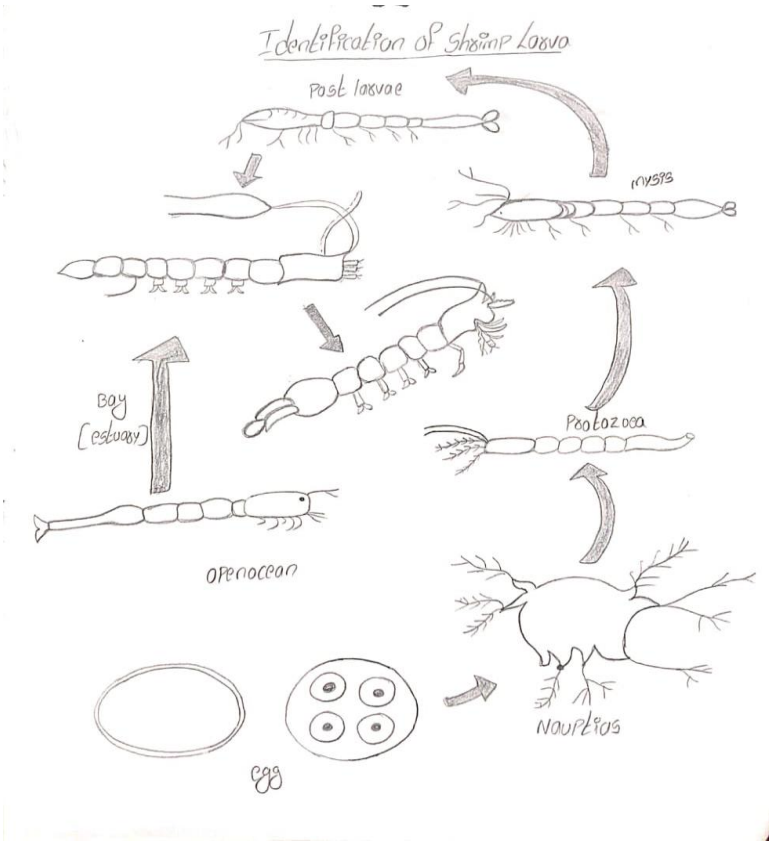
12) **EMBRYO OF PIG**
13) **ESTIMATION OF CALCIUM EGG SHELL**
14) **ESTIMATION OF CARBOHYDRATES**
15) **ESTIMATION OF PROTEINS**

MOLECULAR CELL BIOLOGY

1) **IDENTIFICATION OF DIFFERENT STAGES OF MITOSIS**
2) **IDENTIFICATION OF DIFFERENT STAGES OF MEIOSIS**
3) **OBSERVATION OF MITOSIS IN ONION ROOT-TIP CELLS**
4) **DEMONSTRATION OF MITOTIC CELL DIVISION IN GRASSHOPPER TESTES**
5) **PREPRATION AND STAINING OF BLOOD SMEAR**
6) **BUCCAL SMEAR PREPARATION FOR IDENTIFICATION OF BARR BODY**

1) IDENTIFICATION OF SHRIMP LARVAE



In their natural environment, adult shrimp make their way offshore to clean, stable oceanic water. Here they mature, mate, and the females spawn their eggs. within 14 hours the eggs hatch and the nauplii body strongly photogenic swim towards the surface.

These larvae will pass through three distinct stages i.e. Naupliar, protozoal, or zoeal and mysis before metamorphosis into post-larval shrimp.

Their diet ranges from the hereditary yolk sac, during the early naupliar stage to phytoplankton (Microscopic plant organisms) and then to zooplankton (Microscopic animals). Finally, at mysis stage and beyond. The shrimp is able to eat a wide variety of organisms. Including Artemia (brine shrimp).

During this period, the larvae drift with the currents. A small precent o them are swift into the boys and estuaries by the currents. Hear the post larvae remain, through their survival monthsuntil to mature and seek the offshore spawning grounds. It has been estimated that only one percent of those spawned in nature and reach the adult stage.

Larval stages and their identification

It is important that the aqua culturist be able to aeration not only the major stages of meta morphosis but also the sub-stages. This allows the requirement of food tanks etc. to be anticipated accurately and for the correct rate feeding to be made.

Nauplius stages

The Indo-pacific species (monodon indices, and japonicus) have 6 nauplius sub stages, where the new world species (vannamei and stylirostris) have only 5 sub stages. a general pattern of 3 sub stages for each of the zoeal and mystics stages is universally reported with only a few exceptions. The duration of each stage various with the species and raring temperature.

The nauplii hatch from the egg infolded over position, but quickly straighten out. After several minutes they began to swim, slowly at first, but within half an hour, they swim more brickly. It accomplished by movement of the three pairs of appendages in paddle like fusion. Which produces as zing-zing roll of body. They swim briefly and then rest. The nauplii are strongly phototrophic and swim in the direction of the light source. A rapid response by the nauplii to the light source indicates that they are in good health. When at rest the nauplii are suspended in perpendicular position within, the dorsal side of the body down ward and the appendages handed upward during the last naupliar sub- stage, the body becomes somewhat flattened.

Protozoal stage

In the zoeal stage swimming is accomplished with the first and second antennae, in the naupliar stage, but they are now aided by the well-developed first and second maxillipeds. The swimming stock is slower than that of nauplii the movement appears less jerky. The characteristic of the zoea is their continuous feeding. The culturist can judge how well zoea are feeding by their contractions of the digestive track and the presence of the long tail of faeces. Active feeding and a continued prompt response to a light source are indicating of healthy zoeae. Towards the end of the last zoeal substage. The body becomes slightly flexed.

Mysis stage

In the mysis stage, the antennae are reduced and swimming becomes q function of the pereiopods, with some assistance from the three pairs of maxillipeds in swimming the mystics body is flexed with the head lowered movement in a back ward direction. In this stage there is less tendency for the mysis to be attracted to light.

Post larvae

During the first 4-5 days of post larval life, the animals are planktonic. In subsequent stages, they can be seen to cling to the walls of the tank or well take up a completely demersal life by substage (7- day old post larvae) the larvae of burrowing species often are able to burrowing sand. Feeding by the post larvae is accomplished by the chelated pereopods, which are able to grasp and hold food pleopods are used in swimming.

Identification of protozoal stage

the size ranges given beloware based on measurement of P. Japonicus

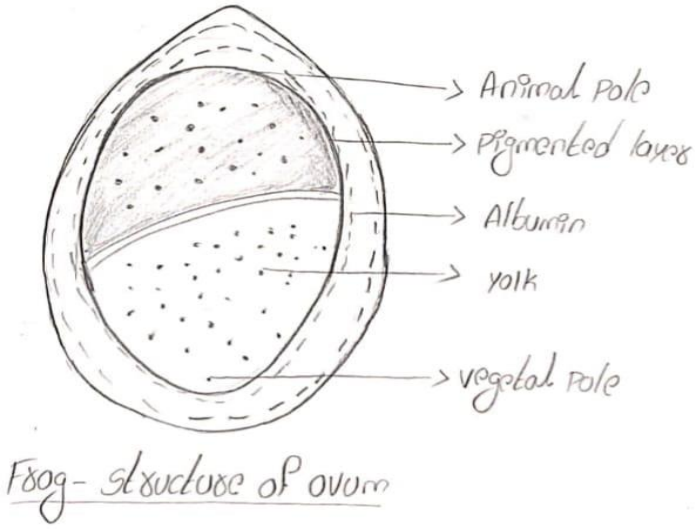
Z.1	1. Body length = 0.86 –1.32mm 2. Body flattened; carapace distinct 3. 1st&2nd maxillae and 1st& 2nd maxillipeds functional 4. Sessile eyes present 5. Furcal process present 6. Digestive track present
Z.2	1. Body length 1.33 – 2.13mm 2. Stalked eyes present 3. Rostrum developed 4.Supra orbital spines developed 5. Abdominal segments appear
Z.3	1. Body length 2.14 – 2.70mm 2. Abdominal segmentation distinct, dorsal and ventral 3. Rudiments of uropod's present

Identification of mysis sub-stages

The size ranges given below are based on the measurements of P. Japonicus

M_1	1. Body length 2.67-3.4mm 2. Body shrimp like on shape 3. Pereiopods well developed 4. Uropods well developed 5. Primordial pleopods buds developed 6. 1st & 2nd antennae reduced
M_2	1. Body length 2.99 -3.90mm 2. Unsegmented pleopod buds developed
M	1. Body length 3.70 – 4.57mm 2. Pleopods developed segmented.

2) FROG – STRUCTURE OF OVUM



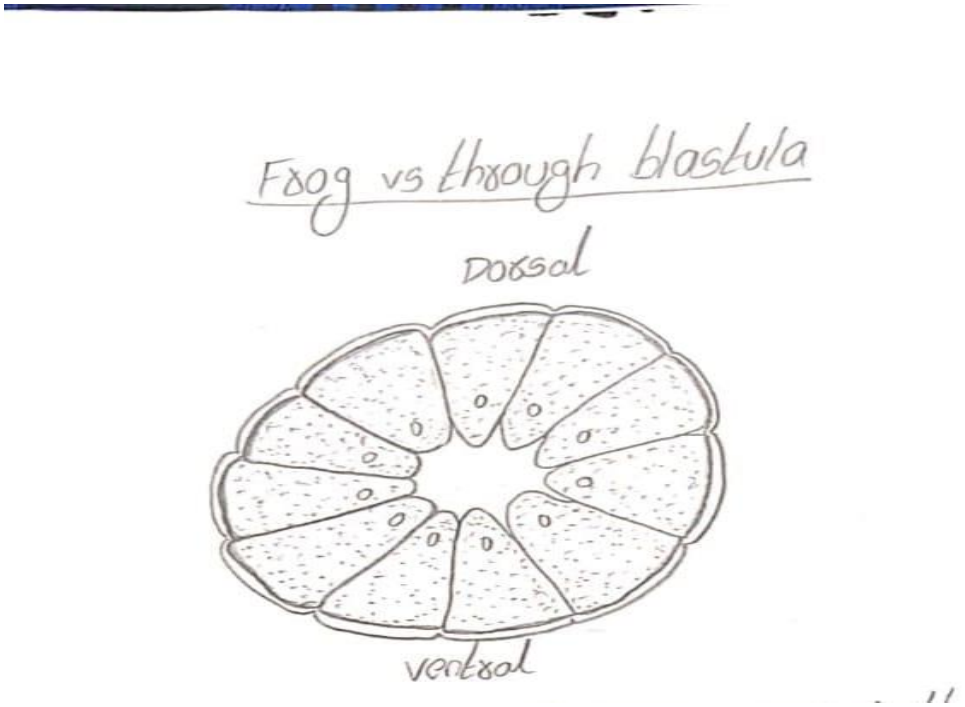
Identification points

- The ovum is rounded in shape and covered by albumen layer, chlorine and vitalline membrane.
- It measures about 1.6mm in diameter.
- One half of the zygote is pigmented black and remaining is almost white.
- Vitelline membrane swells up on the exposure to water the inner space contain parts which are minute in size and provide oxygen by manufacturing food material to embryo.
- The cytoplasm of the egg contains yolk and nucleus.
- The blackish brown pigment granules of melanin assemble forming a superficial pigmented layers of animal hemispheres.
- The centre of the pigmented area is the animal pole and the opposite end is vegetal pole.
- Soon after fertilization, the embryo rotates with in the vitelline membrane so that the animal hemisphere is the uppermost.

Result:

According to the above points the given slide is identified as frog - structure of ovum.

3) FROG -V.S BLASTULA



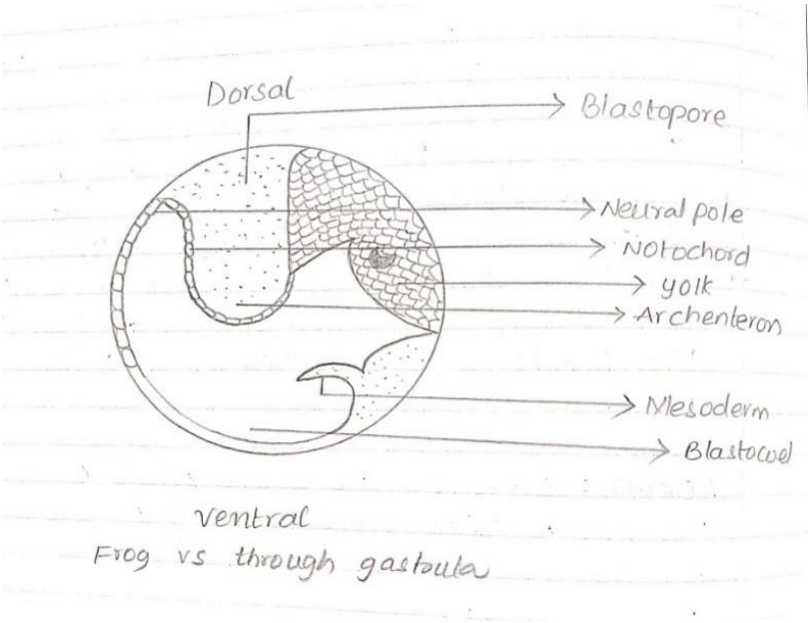
Identification points

- The frog cleaves and form blastula at eight celled stages.
- The blastula contains blastocoel cavity surrounded by unequal blastomeres.
- The smaller blastomeres are called as micromeres upper half and contains dark pigment.
- The layer blastomeres are called as macromers found in more than lower half and loaded with yolk.
- The lower side or vegetal hemisphere is composed of large yolk megamers, because of their large size the blastocoel is laying towards the animal pole.

Result:

According to the above points the given slide is identified as Frog- v.s through blastula.

4) FROG- V.S GASTRULA



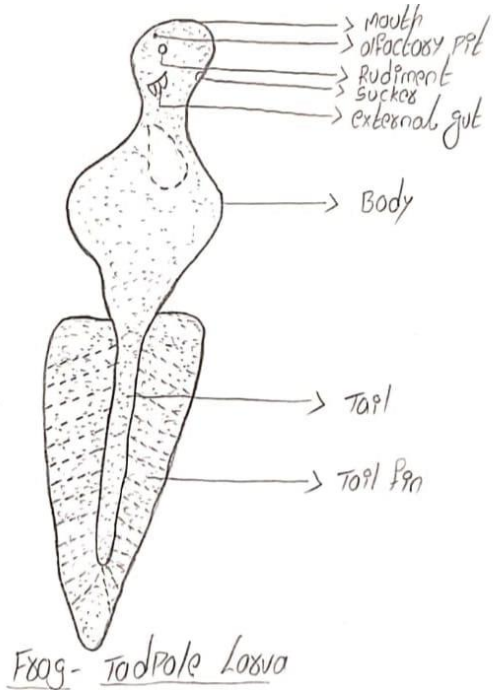
Identification points

- Gastrulation is a rearrangement of cells already present in the blastula. This completely recognises embryo during this process three germinal layers are formed.
- The future prospective organ forming cells are organised at their proper places in their gastrula stage.
- The gastrulation organises occurs by epiboly, blastopore involution and invigilation. during this process the mesodermal and notochord cells migrate inside forming roof of archenteron.
- The v.s shows that gastrula is composed of three layers the outer layer is ectoderm, inner endoderm, middle mesoderm is present separating them on either side of notochord.
- The other structure seen in section are dorsal lip of blastopore, yolk plug, ventral lip of blastopore, chordal cells and neural plate. The blastocoel is reduced to the development of archenteron.
- Gastrula has three germ layers namely ectoderm, endoderm, and mesoderm from which various organs are derived.
- The ectoderm gives rise to epidermis, cutaneous glands, nervous system, eye parts and lining of mouth cavity and cloaca.
- Endoderm forms the lining of alimentary canal, liver, pancreas, lungs and urinary bladder.
- Mesoderm gives rise to muscular connective tissue. Vascular system, genital organs, excretory organs, skeleton and notochord.

Result:

According to the above points the given slide is identified as frog- v.s through gastrula.

5) FROG – TADPOLE LARVA



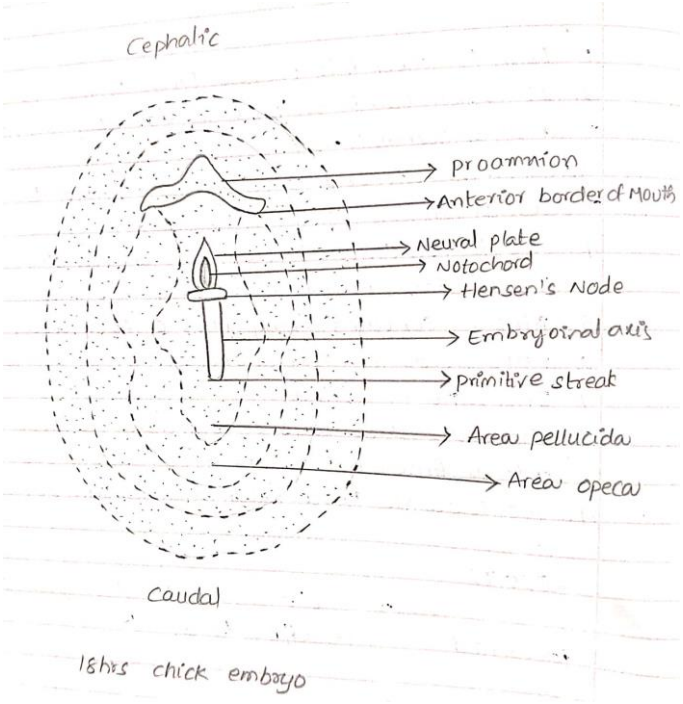
Identification points

- Egg hatches into tadpole larvae within 48 hours.
- It is whitish in colour with dark pigment granules and 5-6mm long.
- Larva is differentiated into body and tail with caudal fin.
- The larva contains rudiments of eyes, olfactory pit, gill clefts, stomodaeum, cloaca and myotomes.
- Mouth contains horny jaws or horny teeth
- Larva feeds on vegetation.
- Intestine is coiled.
- There are 3 pairs of external feathery gills which act as functional respiratory organs.
- The tadpole larvae metamorphoses into adult.

Result:

Based on the above points, the given slide is identified as frog –tadpole larva.

6) CHICK- EMBRYO DEVELOPMENTAL STAGE OF 18 HOURS



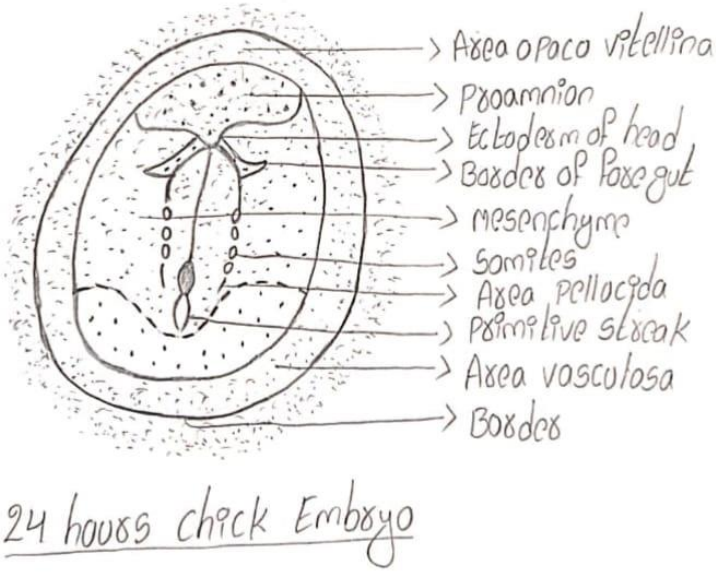
Identification points

- After 18 hours of incubation the notochord has become markedly elongated, forming a prominent structure.
- The notochord extends towards cephalic region in the midline from hensen'node.
- 18 hours incubated embryo is observe in the head process stage.
- At the tip of the notochord, neural process stage is seen.
- In front of notochord and neural plate, there is a space called as "pro-amnion"
- The embryonic areas i.e area pellucida and area becomes more prominent.
- The primitive streak gradually decreases in size.

Result:

According to the above points the given slide is identified as "Whole amount of 18 hours chick embryo".

7) CHICK EMBRYO DEVELOPMENTAL STAGE OF 24 HOURS



24 hours chick Embryo

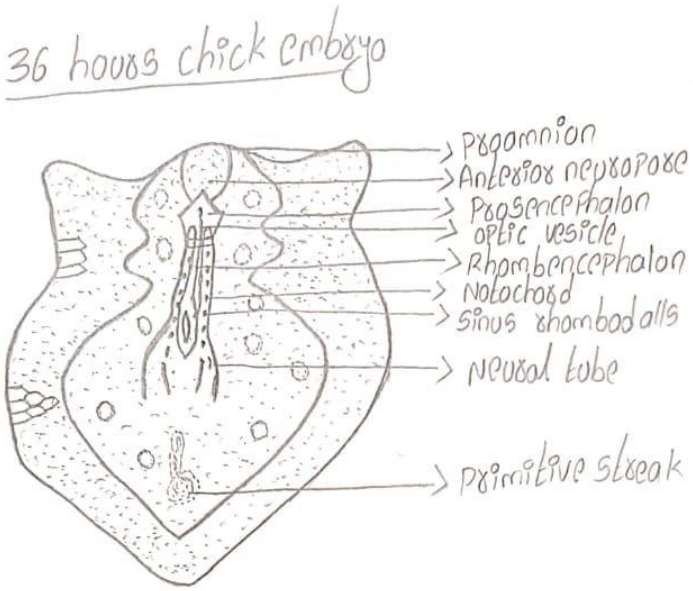
Identification points

- At 24 hours of incubation, the folding of the neural plate is much more clearly marked in stained and transparent preparation of entire embryo neural folds appear as a pair of dark bands.
- The neural folds at the cephalic and are more prominent than at caudal end.
- Foregut is also established at the anterior end. The gut caudal to fore gut is called as midgut and the opening from midgut into foregut namely anterior intestinal portal also appears.
- In the middle 4 pairs of somites are seen. The Hensen's node is pushed caudally and primitive streak is further reduced.
- Other structures seen are area pellucida, area opaquevitelline, ectoderm of head, mesoderm, blood is land area vesiculas, notochord, mesenchyme and pro-amnion.

Result:

According to the above points the given slide is identified as chick embryo development stages of 24 hours.

8) CHICK EMBRYO DEVELOPMENTAL STAGE OF 36 HOURS



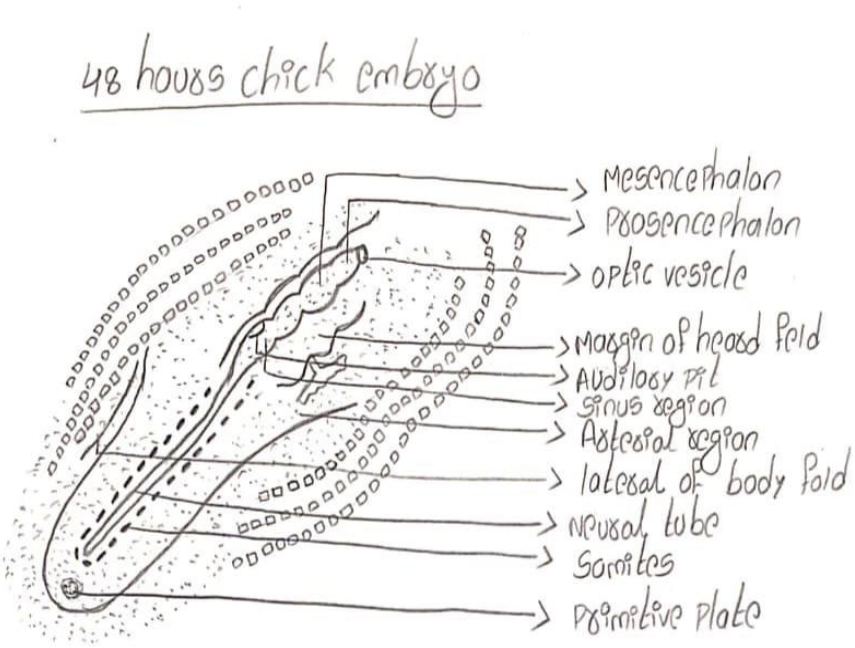
Identification points

- 36 hours incubated embryo shows some of the fundamental steps in the formation of central nervous system and circulatory system.
- Various neuromeric enlargements form brain region are observed.
- The brain differentiated into prosencephalon (fore-brain), mesencephalon (mid-brain), rhombencephalon (hind-brain).
- The optic vesicles are established as paired lateral outgrowths of the prosencephalon**.**
- Infundibulum is formed as a part of depression in the prosencephalon.
- 12 pairs of somites are formed.
- Anterior omphalomesenteric veins are developed.
- Primitive streak becomes shorter because of the lengthen of the neural tube.
- Mid-region of the heart is considerably dilated and bent to the right.
- Pro- amnion, neural tube, notochord, sinus rhomboidalis and sinus rhomboidalis ans terminals are also present.

Result:

Based on the above points, the given slide is identified as “chick embryo developmental stage of 33 hours”

9) CHICK EMBRYO DEVELOPMENTAL STAGE OF 48 HOURS



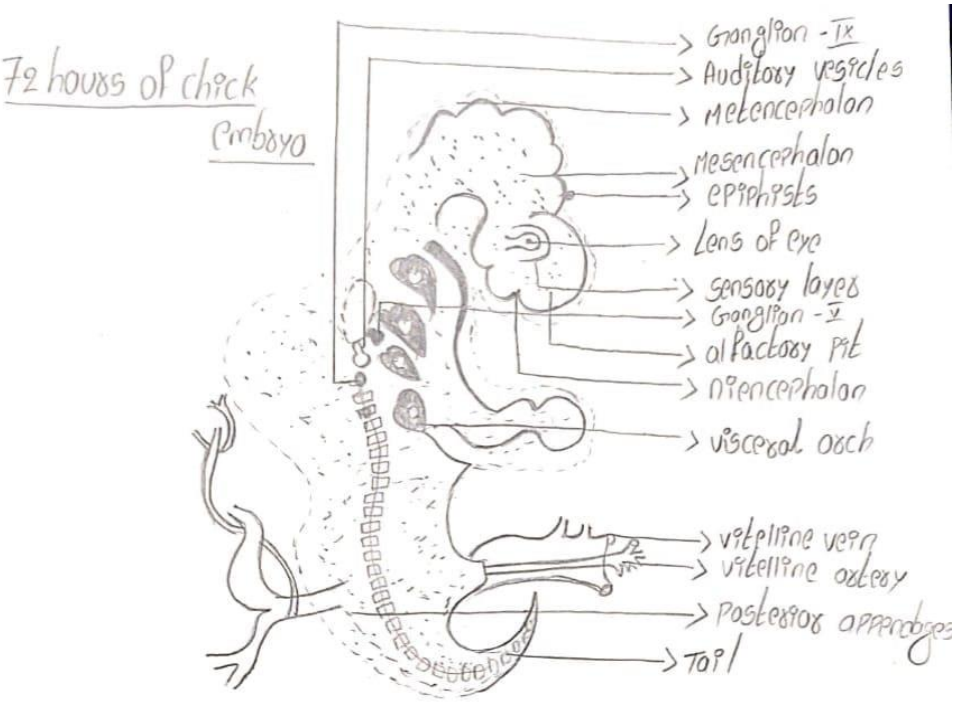
Identification points

- 48 hours of incubated embryo show cranial flexure and torsion.
- The cephalic region of embryo is twisted in such a way that left side comes to lie next to the yolk and right side away from the yolk.
- Brain is differentiated into prosencephalon, mesencephalon, meta cephalon and myelin cephalon
- The heart becomes chambered, it is differentiated into ventricular, arterial and sinus regions truncus arteriosus also developed.
- Auditory pit also makes its appearance.
- Vitelline vessels communicate with omphalomesenteric vein, omphalo mesenteric arteries and extra embryonic vascular plexusare well developed.
- The primitive streak diminishes to a small primitive plate. Head fold margin develops.
- 19 pairs of smoites ate formed.
- Neural plate becomes well developed.
- Lateral mesoderm is also observed. After 18 hours of incubation, the embryo develops rudiments of most of the organs.

Result:

According to the above points the given slide is identical as chick embryo of 48 hours.

10) CHICK EMBRYO DEVELOPMENTAL STAGE OF 72 HOURS



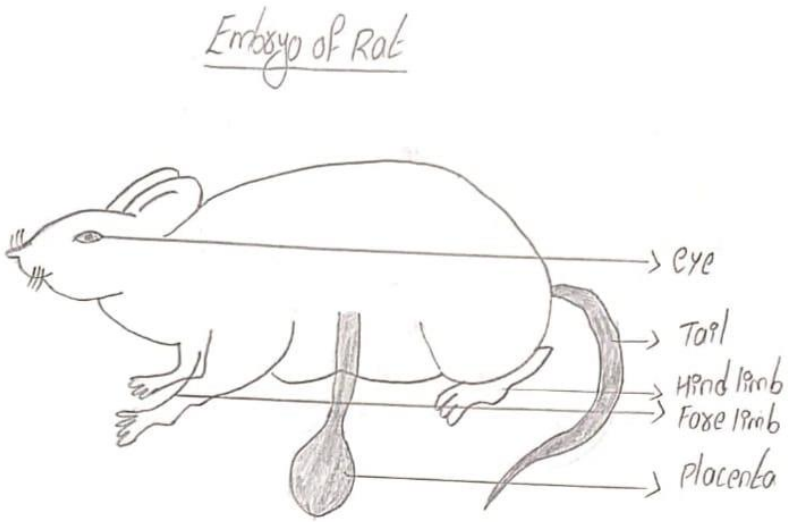
Identification points

- After 72 hours of incubation, body is affected throughout by torsion and entire body is turned through 90.
- The torsion is complete well posterior to the level of heart but the caudal portion of the embryo is not turned on its slide.
- Due to cranial and cervical and cervical flexures the long axis of the embryo shows nearly right-angled bends in the mid brain and in the neck region.
- Visceral arches developed.
- Mid body becomes concave.
- Mandibular arches form caudal boundary of oral depression and becomes more distinct.
- Nasal pits appear as shallow depressions.
- Appendages rudiments also make their appearance.
- Cephalization is going on metencephalon is also developed.
- In the eye, lens, sensory and pigmented layers are differentiated.
- The number of somite's increase to 36 pairs of vitelline arteries and vitelline veins also appear.

Result:

Based on the above points the given slide is identified as "chick embryo development stage of 72 hours".

11) EMBRYO OF RAT



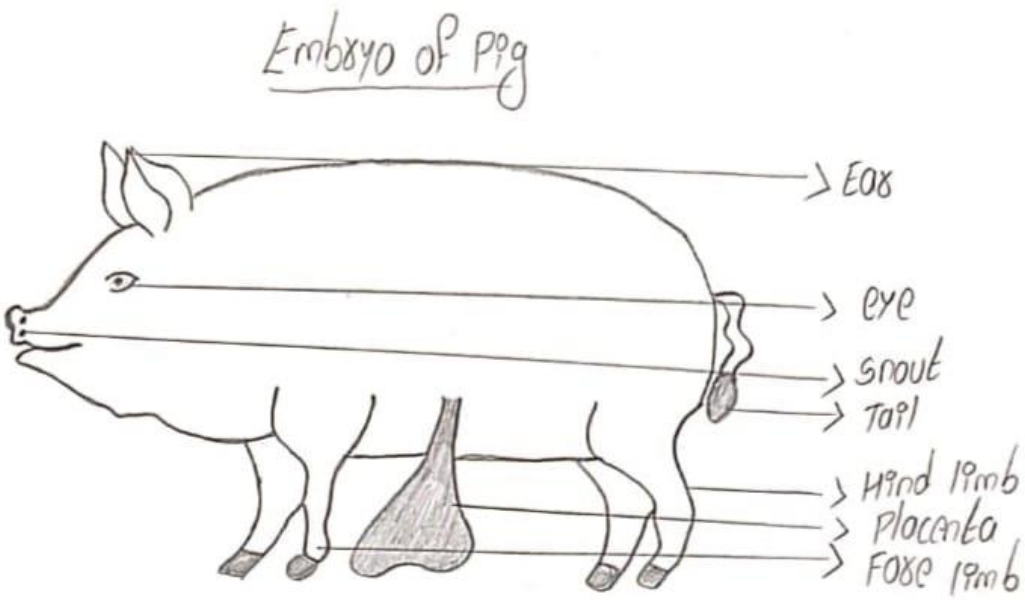
Identification points

- This embryo shows extrinsic implantation.
- Between the foetus and uterus connection is present.
- Head is developed and get its shape.
- Fore-limb and hind-limb are formed.
- Ears are not developed completely.
- Placenta is discoidal and haemochorial.
- Gestationperiod is 24 days.

Result:

According to above points the given spotter is identified as “embryo of rat”.

12) EMBRYO OF PIG



Identification points

- This embryo shows cotyledonary placenta.
- Between the foetus and uterus there is connection is present.
- Head gets its shape.
- Eyes are completely developed.
- Gestation period is 149 days.

Result:

According to the above points the given specimen is identified as “embryo of pig”.

13) ESTIMATION OF CALCIUM EGG SHELL

Aim

To estimate the amount of calcium in shell, incubated egg yolk, extra embryonic region of incubated egg of chick.

Apparatus

Test tubes, beaker, burette, pipette, conical flask, incubator, spectrophotometer.

Method

Clerk- colic method

Reagents

1. **0.01N potassium permanganate**,
   0.4gms of potassium permanganate was dissolved in 100ml of distilled water.
2. **4% of ammonium oxalate**
   4gm of ammonium oxalate was dissolved in 100ml of distilled water.
3. **Dilute ammonia**
   Dilute ammonia in 2ml of conc. ammonia was mixed with 8ml of distilled water.
4. Conc. Sulfuric acid.
5. Conc. Hydrochloric acid.

Principle

the calcium in the sample is precipitated directly as oxalate. The amount of oxalate is directly proportional to the amount of calcium in the sample.

S.NO	NAME OF THE SAMPLE	BURETTE READING		VOLIME OF KMN04
		INITIAL	FINAL	
1	Blank	0.0 0.5	0.5 1.0	0.5 0.5
2	Shell	0.0 0.0 0.0	26.5 25.5 25.5	26.5 25.5 25.5
3	Unincubated egg yolk	0.0 0.0 0.0	18.5 18.5	18.5 18.5
4	Incubated embryonic (a) Extra Embryonic (b) Embryonic	1305 0.0 7.8	27.0 7.8 15.6	13.5 7.3 03.5

Method of calculation:

Amount of calcium present in the sample

= (x-b) x 0.2 x 100\2

0.2 -constant

x -titrate volume of sample

b -titrate volume of blank

unit – mg of calcium / 1000ml of sample

Shell:

Amount of calcium present in the sample

= (x-b) x 0.2 x 100 / 2

x = (25.5 – 0.5) x 0.2 x 50

= 250mg calcium / 100ml ab sample

Unincubated egg yolk:

Amount of calcium present in the sample

= (x-b) x 0.2 x100 / 2

= (18.5- 0.5) x 0.2 x 50

= 18 x 0.2 x 50

= 180 gm ab calcium / 100ml ab sample

Procedure

Shell

10mg of shell was taken and was dissolved in maximum amount of conc. HCl. To this 2ml of distilled water and 1ml of 4% $(NH_4)_2C_2O_4$ was added. It was incubated in boiling water bath for one minute. After that it was titrated against 0.01N potassium permanganate until the pink colour persist for one minute and then disappears. It represents the end point. The process is repeated until the concurrent reading was obtained.

Incubated egg yolk

10% of homogenate was prepared in distilled water 3ml of the above solution was taken, to this add 2ml of distilled water and 3ml of (NH_4) C_3O_4 was added. This above mixture was taken and kept for about 30 minutes and centrifuged at 1500 pm (rotations per minute) for about 15 min. the residue is than washed with 3ml of dilute ammonia and again centrifuged at 1500rpm for 5min and the supernatant was discarded. The residue was collected to this add 2ml of conc. $H2SO_4$ than the test tube was placed in boiling water bath for one minute.

Preparation of blank

The blank was prepared by distilled water and 2ml of sulphuric acid. They are added and titrated against 0.01N potassium permanganate solution.

Extra embryonic

Amount of calcium present in the sample

(x-b) x 0.2 x 100 / 2

(13.5 – 0.5) x 0.2 x 50

13 x 0.2 x 50

130 mg ab calcium / 100 ab sample

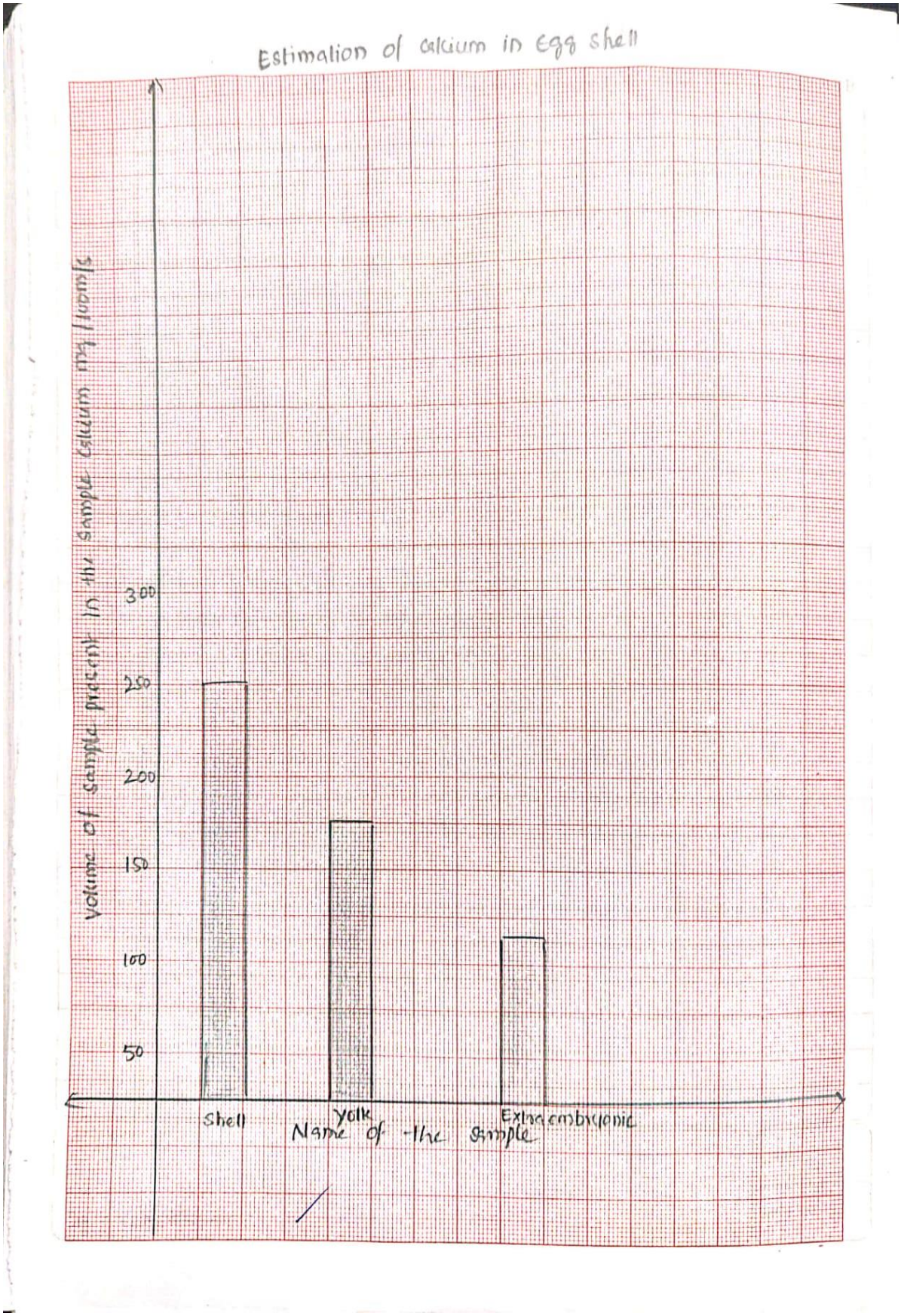
Discussion

- some minerals are also required for the normal proteins these minerals serve as structural and functional elements. Calcium, potassium phosphorus, sodium, magnesium, iron etc. are the major elements required.
- Calcium is one of the most important major elements which help in structural and physiological activities of the animal calcium is mainly present in bones and muscles along with phosphors. Calcium is also useful in growth and muscle construction, blood clotting excitability of nerves etc. enzymes take ATP as of actomyosin and actionsternase succinyl dehydrogenase also required calcium for their activities.
- Calcium is abundantly found in milk, cheese, egg, vegetables, butter and some fruits. The normal requirements of calcium in the body are 1gm per day in our diet.
- Eggs are good source of calcium carbonate in the shell. It is mostly present in the form of calcium carbonate in the shell. In the egg laying animals the shell is formed of $CaCO_3$ and calcium phosphate in small animals. In the present experiment we have estimated the amount of calcium in the shell of unincubated egg yolk. Embryonic membrane of egg yolk. It was formed that $CaCO_3$ it abundantly presents in the egg shell and calcium phosphate in small amounts.

Results

the total amount of calcium present: in the shell- 250; unincubated egg yolk-180; extra embryonic -130; embryonic-73.

S.NO	Name of the sample	Amount of calcium mg / 1000ml of sample
1	Shell	250
2	Unincubated egg yolk	180
3	i) Extra embryonic	130
	ii) Embryonic	73


Estimation of calcium in egg shell
Volume of standard KMnO4 required to titrate the sample calcium in the sample / ml
300
250
200
150
100
50
Shell
Yolk
Extra embryonic
Name of the sample

14) ESTIMATION OF CARBOHYDRATES

Aim

To estimate the proportions of carbohydrate in different fish tissue

Introduction

Carbohydrates are generally are the hydrates carbon. The Carbohydrates are widely distributed both in animals and in plant tissue in plants. They are produced by (a) photosynthesis (b)carbohydrates may be defined as polyhydroxy aldehyde, ketone and their derivatives into 4 types.

Monosaccharides

Monosaccharides are Simple Sugars. They are soluble in water. They are polyhydroxy aldehydes (or) Ketone which cannot further hydrolyse to simple sugars.
E.g.: Glucose, Fructose

Disaccharides

These are two molecules of same as different monosaccharides on hydrolysis
E.g.: Lactose, Sucrose

Oligosaccharides

Oligosaccharides are sugars which yield and 2-10 monosaccharides. molecules on hydrolysis

E.g.: Maltase

Polysaccharides

polysaccharides are non-sugar giving more than 10 monosaccharides on hydrolysis. Eg:
Homopolysaccharides – starch
Heteropolysaccharides Mucopolysaccharides.

Method Carrol et al (1951) method.

Reagents

<u>10% Trichloroacetic acid</u>
10 Trichloroacetic acid is dissolved in100m/of distilled water.
<u>Anathrone Reagent</u>
1g of thiourea and 500mganthrone powder are dissolved in 100ml of 72%. Sulphuric acid.

Procedure

- 10% homogenate of desired tissue were prepared in 10%. TCA (Trichloroacetic acid). The homogenates were centrifuged at 1000 rpm for 15 mins.
- 0.5m/ of supernatant4ml of anthrone reagent was added and boiled for minutes15 minutes.
- the contents were cooled by Keeping the test-tubes in cold water bath.

Preparation of the blank

In 50ml of distilled water 4ml of anthrone reagent was added.

Inference

The experiment has been conducted to estimate the amount of carbohydrates present in different tissue of fish, muscle, prawn muscle and carb muscle.

The Crab muscle had high contents of Carbohydrates of 127/100 followed by prawn muscle with 5.4mg / 100mg then in fish muscle, tissue18.144 mg/100- Crab muscle is metabolic site organisms hence its shows high contents of carbohydrate storage than in another, tissue of prawn and fish.

Result

The amount of carbohydrates in the Sample i.e Prawn - (13.464 mg/100mg), Crab (8.064mg/100mg) fish (1.51mg/100mg).

Method of calculation

Amount of carbohydrates(%) O.D of sample x amount of carbohydrates on standard x 100 / O.D of standard x wet weight of tissue

Prawn = 0.187 / 0.5 x 0.72 / 2 x 100 = 13.464mg/gm.wt
Crab = 0.112 / 0.5 x 0.72 /2 x 100 = 8.064mg/gm.wt
Fish = 0.021 /0.5 x 0.72 / 2 x 100 =1.51 mg/gm.wt

Observation table

S.NO	Name of the sample	Weight of the tissue	O.D values
1	Prawn	2gm	0.187
2	Crab	2gm	0.112
3	Fish	2gm	0.062

15) ESTIMATION OF PROTEINS

INTRODUCTION

proteins are the important receiver for the living organism's proteins are available through different food resources like beans, meat, egg, etc almost everything that occurs in the cell involved one or more proteins are among the most abundant in their functions.

Protein carryout cellular reactions. They are linear polymers of high molecular weight. All proteins contain carbon, nitrogen, hydrogen and the presence of nitrogen distinguished from them carbohydrates and the molecular weight of protein varies from about 12,000 Daltons to several million Daltons.

Amino acids are building blocks of proteins The structure of amino acids are as follows:

Aim

to estimate the amount of proteins in the given sample.

Method

Lowry-etal method (1951)

Requirements

Test-lubes
Centrifuge machine
Centrifuge tube
Homonizer
Spectrophotometer
Test-tube stand

Preparation of reagents tube

In a test-tube takes 4 gm of NaOH dissolving in 100ml of the distilled water
0.1N NaOH dissolved in 100ml of distilled water

Reagent A

Dissolve 2gms of Na_2CO_3 in 100ml of 0.1 NaOH

Reagent B

Dissolve 500mg of cupric Sulphate and 1gm of, Sodium potassium titrates in 100ml of distilled water.

Reagent C

Take 50ml of reagent A and add 1ml od reagent B.

Reagent D

Prepare 1:1 ratio of Follen - phenol reagent that is 1ml of Follen's reagent &1ml of distilled water.

51. of TCA

Take 5% of TCA and dissolved in 100m/of distilled water

Standard Bovine Serum Albumin Solution

Take 5mg of BSA and dissolve in 25ml of 1Ni.e. 0.2mg/ml of concentration.

Principle

- This method is above 10 times more Sensitive than 1st method.
- When the tissue is homogenized with 5% of TCA
- The tissue constituents are precipitated when the residue is dissolved by 1N NaOH
- The carbonyl groups of the protein's molecules react with copper and potassium of reagent-c there by forming copper potassium borate complex.
- The reagent D is called "Follin-phenol" is guite complex phosphomimic acid tungstate.
- The protein constitutes aromatic amino acid.
- Tryptophan copper potassium barite complex together with thyroxin & phenolic compounds present in proteins reduce to phosphomolybdate of Follin phenolic reagent to produce dark brown in colour.

Procedure

Take about 5mg of fresh tissue is homogenized with 5ml of 5% TCA &centrifuge the contents for about 10-15 mins. at the rate of 3000rpm. The supernatant is discarded. The precipitate is dissolved by adding 1ml of 1N NaOH from this pipette about 0.2ml of solution and 5ml of Reagent-c is added then 0.5ml of reagent D is added keep it outside for about 30min& the colour intensity is read at 520nm by using spectrophotometer (or) colorimeter.
The colour intensity is proportional to the amount present in the sample.

Preparation of standard

Take 0.2ml BSAstandard solution. Add 5ml of reagent-C & Keep it aside for about 10-15 min. then add 0.5ml of Follin-phenol reagent keep it aside for 15min. the colour intensity id observed.

Preparation of Blank

0.2ml of 1NaOH &, 5ml of reagent- A are added. To this 2.5ml of reagent-B is added and set table

E.g. :5ml of the starch solution contain 25mg of Protein

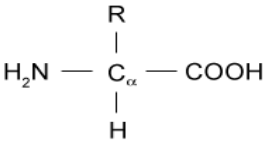
→0.2ml of starch solution contain 1mg protein
→0.4ml of starch solution contain 2mg protein
→0.6ml of starch solution contain 3mg protein
→0.8ml of starch solution contain 4mg protein
→ 1ml of starch solution contain 5mg protein

Calculation
Formula

Amount of proteins present in the sample
= Amount of protein present in sample of sample / OD of sample x weight of Tissue OD.

Result

Amount of proteins present in the sample is 75 mg/ml



S.NO	VOLUME OF THE SAMPLE SOLUTION	CONCENTRATION OF PROTEIN	OPTICAL DENSITY VALUE
1	0.2ml	1mg	0.1
2	0.4ml	2mg	0.2
3	0.6ml	3mg	0.3
4	0.8ml	4mg	0.4
5	1ml	5mg	0.4
6	1.2ml	6mg	0.6
7	1.4ml	7mg	0.7
8	1.6ml	8mg	0.8
9	1.8ml	9mg	0.9
10	2ml	10mg	1

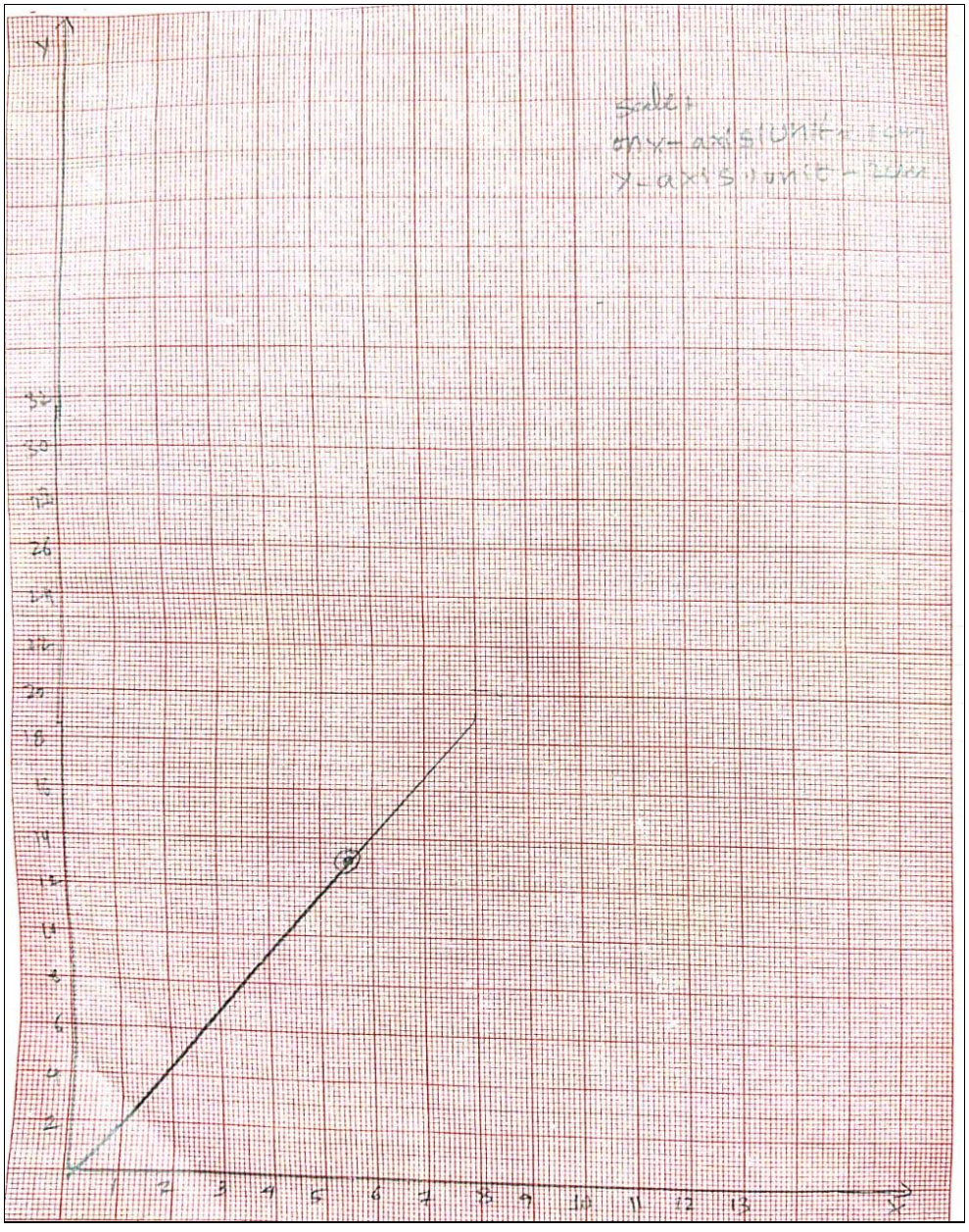
The calibration curve is plotted
Concentration on x -axis and optical value on y- axis

Calculation

The amount of proteins present in the sample is calculated as follows
= O.D of sample x Amount of present in sample x 100 / O.D of standard x volume of sample
Optical density of the sample = 1.33
Optical density of the standard = 0.7
Amount present in the sample = 0.2mg/ml
Volume of the sample = 30ml
1.33/0.7 x 0.2/30 x 1000
0.01266 x 1000
12.66mg/ml

Calculation

Optical density value of the given sample = 0.15
0.2ml of sample contains = 1.5
Hence ml of sample contains = 1x1.5/ 0.2
= 7.5mg
1ml of standard solution obtained from 100mg tissue
So 100mg of tissue contains 7.5mg of proteins
100mg (or)1gs of tissue contains = 7.5
Hemce the given sample contains 75mg /g

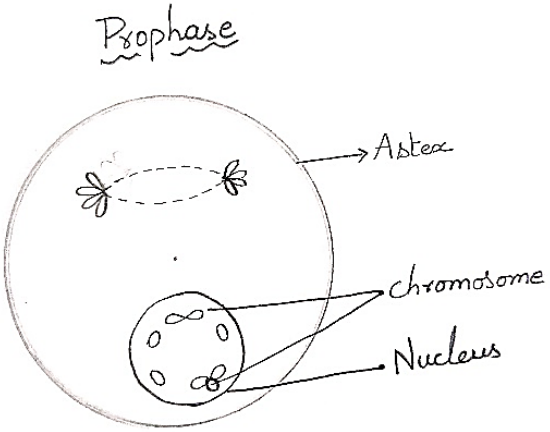


1) IDENTIFICATION OF DIFFERENT STAGES OF MITOSIS

MITOSIS: Mitosis is also called as somatic cell divisionor equational division. The process of cell division on where by chromosomes ate duplicated and distributed equally to the daughter cells is called mitosis. It helps to maintain the constant chromosome number in all cells of the body. Mitosis process complete in stages.

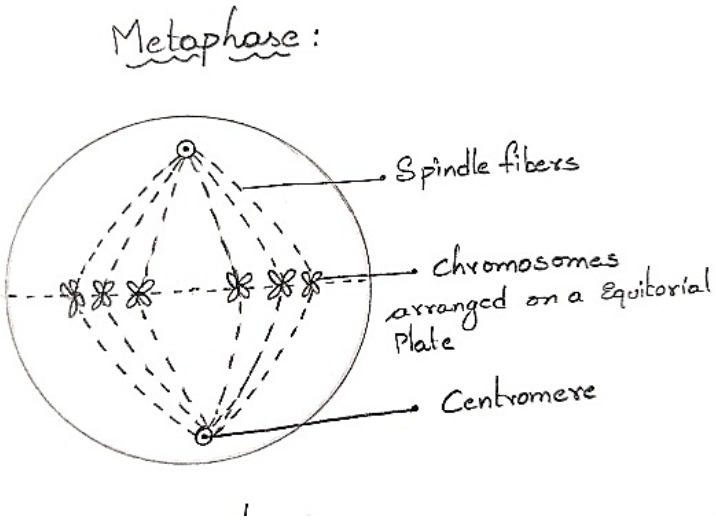
These are following stages: Prophase, Metaphase, Anaphase, Telophase, Cytokinesis.

STAGE 1: PROPHASE



- The nucleus becomes elongated
- DNA Synthesis is completed
- It comes after interphase
- Chromosome become filamentous thin and distinct
- Prophase may be early or late
- In late prophase each chromosome divides into Sister chromatids which are attached at centromere or Kinetochore
- Nuclear membrane and nucleus disappear
- The cell now enters into metaphase Identification
- Presence of Filamentous chromosome indicates the early prophase.
- Sister chromatids attached at centromere indicates the late phase.

STAGE 2: METAPHASE

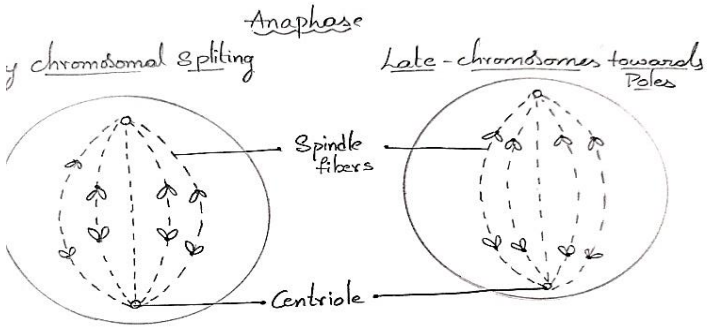


- The spindle tubules start attached with centromeres early metaphase. appearing and get of chromosomes in early metaphase.
- Chromosomes moles actively int became Arranged at the Equatorial plate or at the centre metaphase.
- Specially the in animals the centrosome helps to formation of spindle apparatus
- In plants centromeres are missing
- Centromere has two centrioles which separate and each occupies opposite site of the nucleus
- After metaphase the next phase is anaphase.

Identification

Centromers attach with spindle fibres in early metaphase chromosomes arranged at equatorial plate.

STAGE 3: ANAPHASE

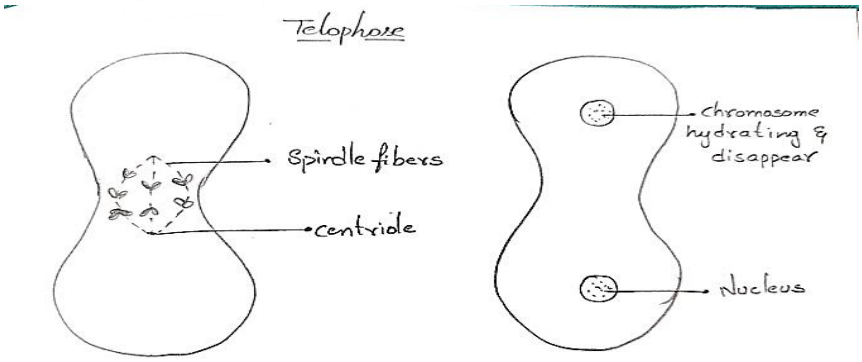


- Each chromosome Splits at the position of Centromere forming sister chromatids (or) daughter chromosome it shows early anaphase.
- The sister chromatids move towards poles with centromere facing periphery white arms towards each other showing late anaphase.
- Depending upon the position of centromeres. the chromosomes maybe I, J, L, V Shaped (metacentric, sub-metacentric, telocentric, acrocentric).

Identification

- chromatids Separate and starts moving toward poles in early anaphase.
- chromatids are sub-equatorial in late anaphase.

STAGE 4: TELOPHASE

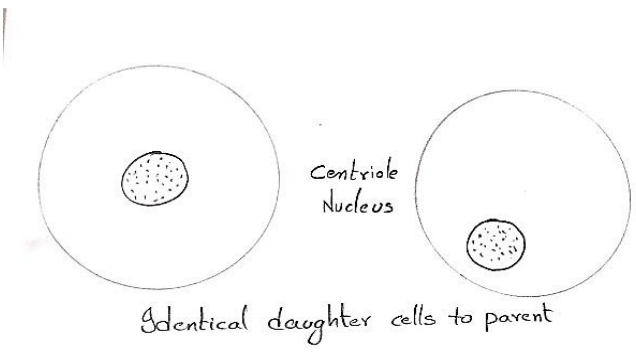


- It is the reverse of prophase
- The chromosomes aggregate at the poles.
- Spindle fibre's start disappearing.
- The new nuclear membrane starts to reappear around each set of chromosomes.
- The nucleolus reorganised.

Identification

- Chromosomes reach at poles in early telophase.
- Chromatids become surrounded by nuclear membrane.

STAGE 5: CYTOKINESIS



- After the appearance of nuclear membrane two daughter nuclei a
- Cytoplasm divides into two halves.
- The division of cytoplasm is called cytokinesis.

Identification

- Since there are two daughter cells indicates the cytokinesis stage.

2) IDENTIFICATION OF DIFFERENT STAGES OF MEIOSIS MEIOSIS

Meiosis is a reductional division which involves 2 successive divisions forming 4 daughter cells each having haploid no of chromosomes in first meiotic division, reduction in chromosomes occur without separation of chromatids. The stages involved are Prophase (with 5 sub stages).

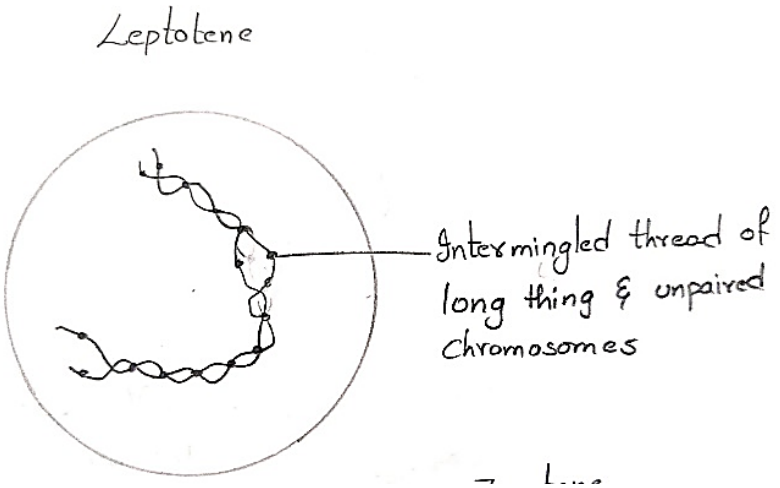
Prophase1
Metaphase1
Anaphase1
Telophase1

In second meiotic division separation of chromatids of chromosomes occur 4 haploid cells daughter cells with haploid number of chromosomes are formed during spermatogenesis in males and oogenesis in females. The stages of second mitotic division are.

Prophase II
Metaphase II
Anaphase II
Telophase II

Prophase 1

Leptotene:

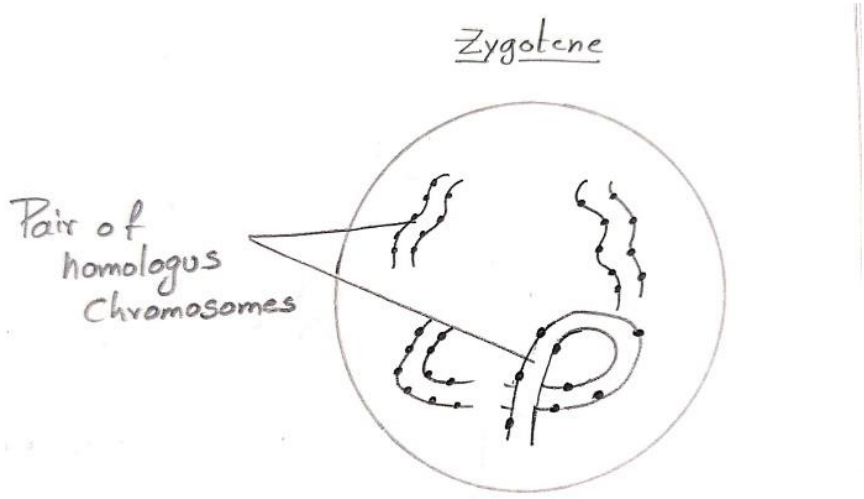


- This is the 1 stage of meiosis
- chromosomes appear as a long thread-like structures interwoven together.
- chromosomes given bead like appearance called chromosomes.
- Each of the chromosomes are drawn towards nuclear membrane near centriole.

Identification

Since chromosome thread like and give beaded appearance and it is identified as leptotene stage of prophase I.

Zygotene 1

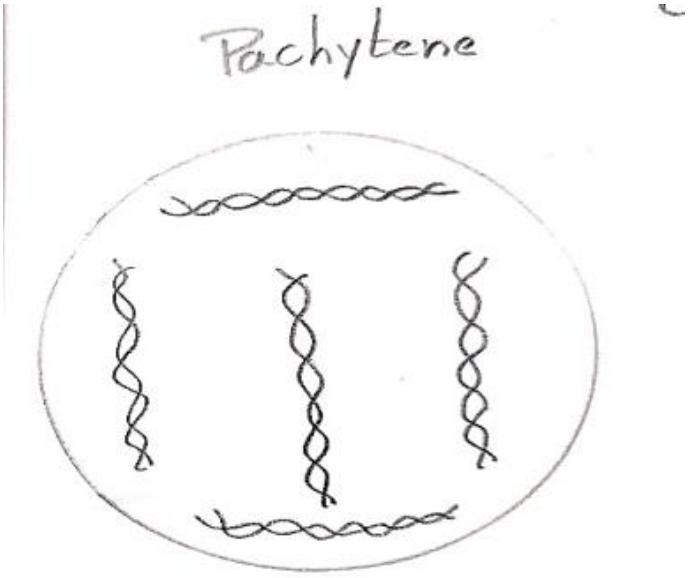


- This stage is characterized by pairing homologous Chromosomes.
- Homologous chromosomes pair by a process called synapsis pairing starts from many points on the chromosomes. The chromosomes are called as bivalent.
- Bivalent become shortened thickened by coiled and condensation.
- Pairing occurs in a zipper like fashion between two chromosomes at one region.

Identification

Since above chromosomes show pairing of homologous chromosomes hence it is identified as zygotene stage of prophase1.

Pachytene

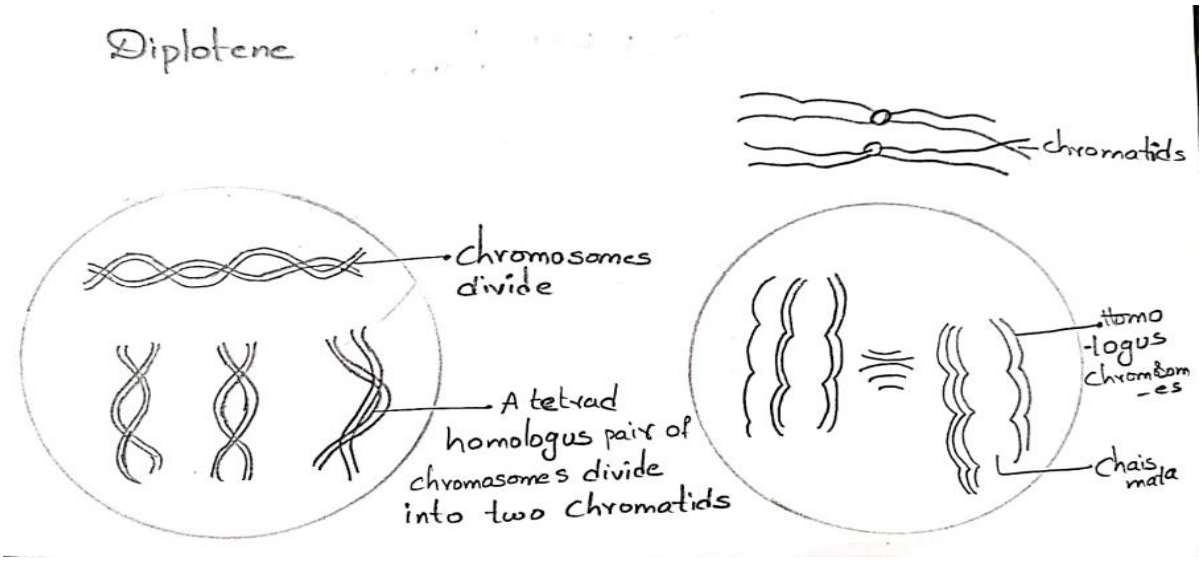


- Chromosomes became shortened and coiled.
- Chromosomes appear as thickened thread like Structure, haploid in number each thread has two homologous chromosomes closely applied as each other.
- Crossing over is accompanied by the chiasmata formation.

Identification

Since the above chromosomes showed, shortened, bivalent, and crossing over hence it is identified as Pachytene stage of Prophase1.

Diplotene

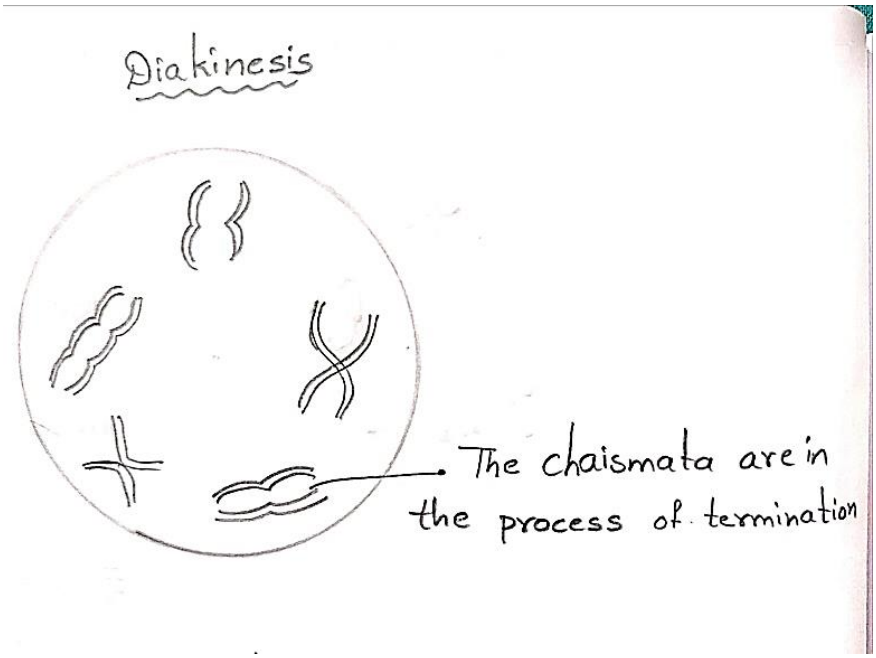


- This stage is characterized by thickening, shorten-Ing and condensation of chromosomes.
- Homologous chromosomes start separating one end separation from Starts from centromere and travel towards the end this is known as terminalisation.
- Double nature of chromosome become distinct and hence the name of diplotene.
- Homologous chromosomes remain in constant at certain points is called chiasmata.
- Chiasmata formation is characteristic feature of diplotene stage.

Identification

Since the above chromosomes shows condensation double Structure and chiasmata formation hence it is identified as diplotene Stage of chromosome.

Diakinesis

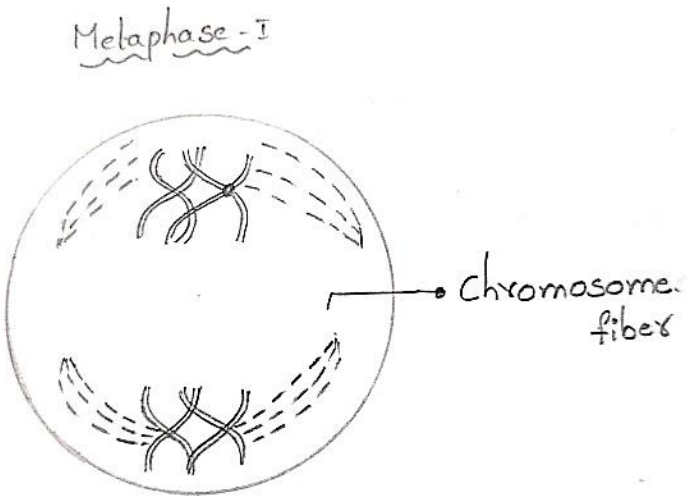


- Chromosomes become more compacted and condensed.
- chromosomes appear as rounded bodies and they can be easily counted.
- Bivalents have a tendency to move near the nuclear envelope.

Identification

- since the above chromosomes shows rounded appearance of hence it is identified as the Stage of diakinesis.

Metaphase1

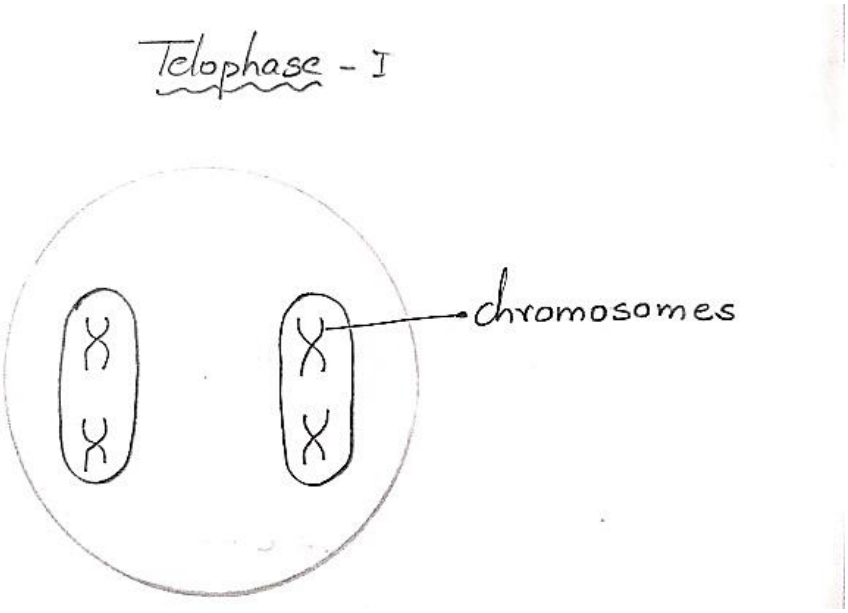


- Chromosomes of bivalent from Equatorial plate moves towards the poles
- Each poles have haploid number of chromosomes.

Identification

- As the chromosomes move towards the poles it is identified as the anaphase1.

Telophase1



- Nuclear membrane appears around group of Chromosomes of poles.
- After formation of nuclei chromosomes pass into a small interphase before the second mitotic division as a result of above dyad is formed.

Identification

Chromosomes at pole and formation of dyad hence it is identified as telophase1.

Meiosis II:

Prophase II:

- The chromatids appear as dyads.
- Nuclear membrane and nucleoli disappear

Metaphase II

- The chromosomes get oriented in the equatorial region of the spindle and their centromeres are attached to the chromosomal fibres. Each chromosome is easily seen.

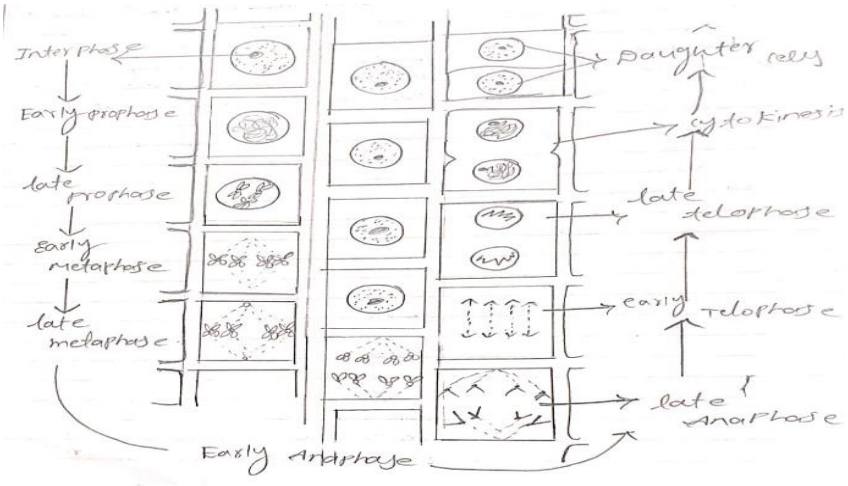
Anaphase II

- The spindle fibres contact at the homologous chromosomes separate and move towards the opposite poles.
- Each chromosome consists of two chromatids attached to 1 centromere.
- The two groups of the daughter chromosomes in each cell have started moving apart towards the opposite poles of the spindle.

Telophase II

- The 2 groups of daughter chromosomes in each haploid cell have reached the polies of each the spindle.
- The two haploid daughter cells formed as the second mitotic division.
- Four haploid cells formed as the single diploid cell.

3) OBSERVATION OF MITOSIS IN ONION ROOT-TIP CELLS



Aim

demonstrate the mitotic cell division in onion.

Materials required

Onion roots, slides, cover slips, forceps, blade, microscope, watch glass, spirit lamp, blotting paper.

Chemicals

1NHcl, distilled water, Acetocarmine stain.

Procedure

- Place 2-3 root tips on a watch glass.
- Add 2 drops pd 1NHCl and gently warm it
- Bloat the HCl with blotting paper and then add 2-3 drops of acetocarmine stain and warm it.
- Allow the root tip in stain for 5-10 min
- Take the stained root tips on a slide with 2-3 drops of glycerine.
- Place the cove slip over the slide with in a blotting paper and squash it.

Observation

Examine the slide under the microscope the cells and their chromosomes become spread out and distinct. Search out for different early and late mitotic stages such as interphase, prophase, metaphase, anaphase, telophase and cytokinesis.

4) DEMONSTRATIONOFMITOTICCELLDIVISIONIN GRASSHOPPER TESTES

Aim

To demonstrate the mitotic cell division in grasshopper testes by squash method.

Apparatus

Animal (grasshopper), scissor, blade, forceps, needle, Slide, coverslip, microscope.

Chemicals

chloroform. Nacl, carnoy's Fluid, acetocarmine saline Stain

Procedure

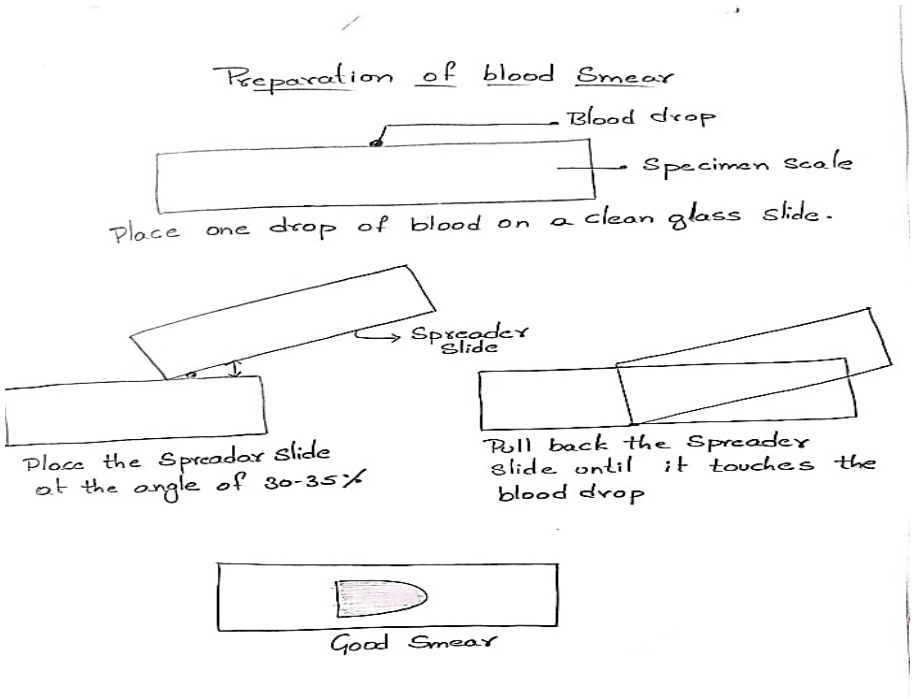
- Collect the grasshopper in dissecting dish having normal saline.
- Remove testes lobes in the acetocarmine.
- Take Stain the testes lobes in the acetocarmine

- Take testes lobes over a Slide and cover with a cover slip and tap gently. with needle or pencil and heat the slide over the flame of a Spirit lamp, now took a piece of blotting paper on Cover slip and apply a uniform pressure by your thumb.

Observation

Examine the slide under the micro- scope, the cells and then Chromosomes becomes Spread out and distinct such out for different Early and late meiotic stages such as prophase, metaphase, anaphase, & telophase.

5) PREPRATION AND STAINING OF BLOOD SMEAR



Aim

To identify the different stages of white blood cells in human blood.

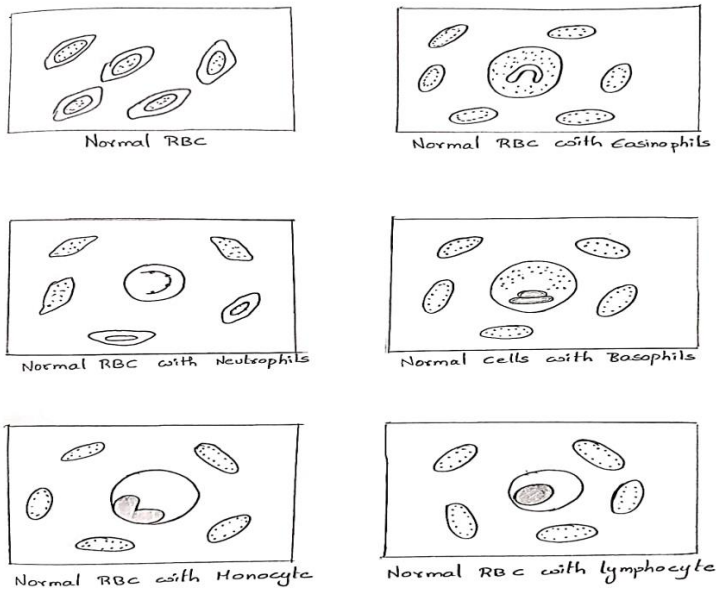
Requirements

Glass slides, sterilised needle, drop of blood, spirit, Giemsa stain or Leishman stain, distilled water, cotton, microscope.

Principle

WBC are the type of blood Cell that have a nucleus but no Pigment. They are important in defending the body against disease because they produce antibodies against any foreign particle (or) antigens WBC Can be divided into 2 types - granulocytes & agranulocytes granulocytes consist of neutrophils; eosinophils & basophil.

Neutrophils do not stain with either acidic or basic dyes they have many lobed nuclei and are called polymorphonuclear leucocytes. They constitute about 76.9% of the total leucocytes found eosinophils have lobed nucleus with cytoplasmic granules that stain with acidic dyes. they Constitute 1 to 4% of the total leucocytes count basophils has a lobed nucleus & the cytoplasm contains granules which Stain with basic dye. It comprises 0% to 4% of the leucocyte count granulocytes consists of lymphocytes and monocytes in which granules are not present with a nucleus monocyte comprises lymphocytes comprises of the total leucocyte count.



Procedure

- Obtain two clean microscope slides, spirit & sterilised needles.
- clean the finger with spirit and puncture
- place a small drop of blood at the end slide.
- use the second slide to make a thin blood as directed below.
- place the second slide at the angle of 30-35° and touch the slide with the blood drop.
- move the Spreader drop to action along Spread by capillary action along the edge of the slide.
- Immediately push the slide away from the blood drop making the slide making the thin smear.
- That should dry quickly as we move away from the drop.
- A perfect smear & good smear will have a feathered edge and separate RBC when you view under microscope.

Staining the blood smear

- Place thoroughly blood smear on horizontal staining rack
- Fix the Smear with methanol for 10 seconds.

- Stain the blood smear with Giemsa or Leishman's Stain for 10 seconds and rinse with distilled water for 1 minute air dry and examine under microscope using low power at first and then high power.
- Observe as many types of WBC observed basophils, eosinophils, neutrophils Blood cells as possible pay close attention to size frequency and nuclear features.

Result

RBC & Types of WRC observed Basophils, Eosinophils, Neutrophils are observed.

6) BUCCAL SMEAR PREPARATION FOR IDENTIFICATION OF BARR BODY

In human beings, the sex can be identified by observing the nucleus of their resting cells of their female a darkly stained chromatin body is observed on the slide in the interphase nucleus. The chromatin Barr body after the name Murry Barr in 1990.

Aim:

To identify the presence of Barr body in human buccal cavity.

Principle

Buccal epithelial cells especially the Barr body structure which are considered to play a major role in sex determination.

Requirements

Slide, microscope, methyl blue, cover slips, tooth picks, needle, buccal epithelial cells, carnoys fixative.

Procedure

- Wash your mouth with sterile water to prepare mucous gently rub the inside of the cheek cells
- Take the cells with the help of flat rounded piece of wood (or) toothpick and transfer the scrapping over a clean glass slide.
- Then made a thin film of cells on the slide with needle and keep them for air-drying
- Air dried smear was kept in carnoys fixative on for 30-35 minutes
- Then the methylene blue stain or Giemsa stain was poured and allowed to stain for 20-25 minutes.
- After staining the slide was washed with distilled water to remove the excess stain.
- Finally, the slide was kept for air-drying and then observed under the microscope.

Observation

The female epithelial cells dark stained heterochromatin Barr body is observed usually periphery of the nucleus clearly high-power microscope.

Barr body

In mammal's males are the heterogametes (x y) homogameties (xx) as the female has two copies but x chromosome is achieved by the random inactivation of one of the two X-chromosome. The heterochromatinised X-chromosome appears as a darkly Stained body attached to the nucleus membrane are inside of the nucleus this Phenomenon was discovered by Murray Barr or now called as Barr body.
+-In human body this can be early demonstrated in female cells in the buccal cavity mucosa no Barr bodies is seen in the Somatic Cells of Turners female (45x0), are is seen in Klinefelter male (47xxy), two in (47+xxy) females three in (48+ xxx).

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