EXPERIMENT

AIM

Prepare a temporary mount of onion root tips to study mitosis.

THEORY

Somatic growth of both plants and animals takes place by increase in the number of cells. These cells divide mitotically into two daughter cells wherein number of chromosomes remain unchanged in the daughter cells from that in the maternal cells. The doubling and halving of genetic material in the cycle of cell growth and its division is called cell cycle.

Cell cycle consists of four different stages, i.e. G_1, S, G_2 and M or D.

G_1 or Gap Phase-I Period of growth and increase in cell mass, chemical preparation for DNA synthesis. It is considered to be the longest phase, which takes about 10-11 hours to get completed.

***S* or Synthetic Phase** The cell synthesises genetic material DNA from raw materials, so DNA gets duplicated before cell enters into division phase. It lasts for 7-8 hours.

G_2 or Gap Phase-II Cell duplicates materials other than DNA to be passed to daughter cells like mitochondria, Golgi apparatus and cytoplasmic inclusions. It lasts for 4-5 hours. G_1, S and G_2 phases together constitute interphase.

M-Phase or D-Phase The cell divides during this phase and it lasts within 1-2 hours. Cell division phase has two main parts:

Cytokinesis: The division of cytoplasm into two daughter cells is known as cytokinesis.

Karyokinesis: The division of nucleus into two nuclei is known as karyokinesis.

Karyokinesis is of two types:

- Mitosis (Equational division)
- Meiosis (Reductional division).

Mitosis: Mitotic cell cycle in animals was first described by W. Fleming in 1882 and in plants, it was first described by Strasburger, again in 1882. Mitosis is a kind of cell division where two daughter cells are produced which are genetically identical to each other as well as to the parents cell. It is thus, the basis of continuation of unicellular as well as multicellular organisms. It occurs in somatic cells, helping in growth as well as repair of worn out tissues.

Meiosis: Meiosis takes place in the germ cells to produce gametes. As a result of meiosis four daughter cells are produced which have half the number of chromosomes as the parent cell and hence it is known as reductional division

In plants, such divisions rapidly take place in meristematic tissues of root and shoot apices, where stages

of mitosis can be easily observed. The onion root tip tissues show the dividing cells in different stages. Also, root tip tissue of onion is the meristematic region. The cells here keep dividing all the time which leads to the growth of roots in the deeper layers of soil.

REQUIREMENTS

Onion bulbs, wide mouthed glass bottle, corked tube, petridishes, scissors, forceps, needies, methy alcohol, acetic acid, N/10HCl, acetocarmine, distilled water, spirit lamp, compound microscope slides, blotting paper, coverslips, etc.

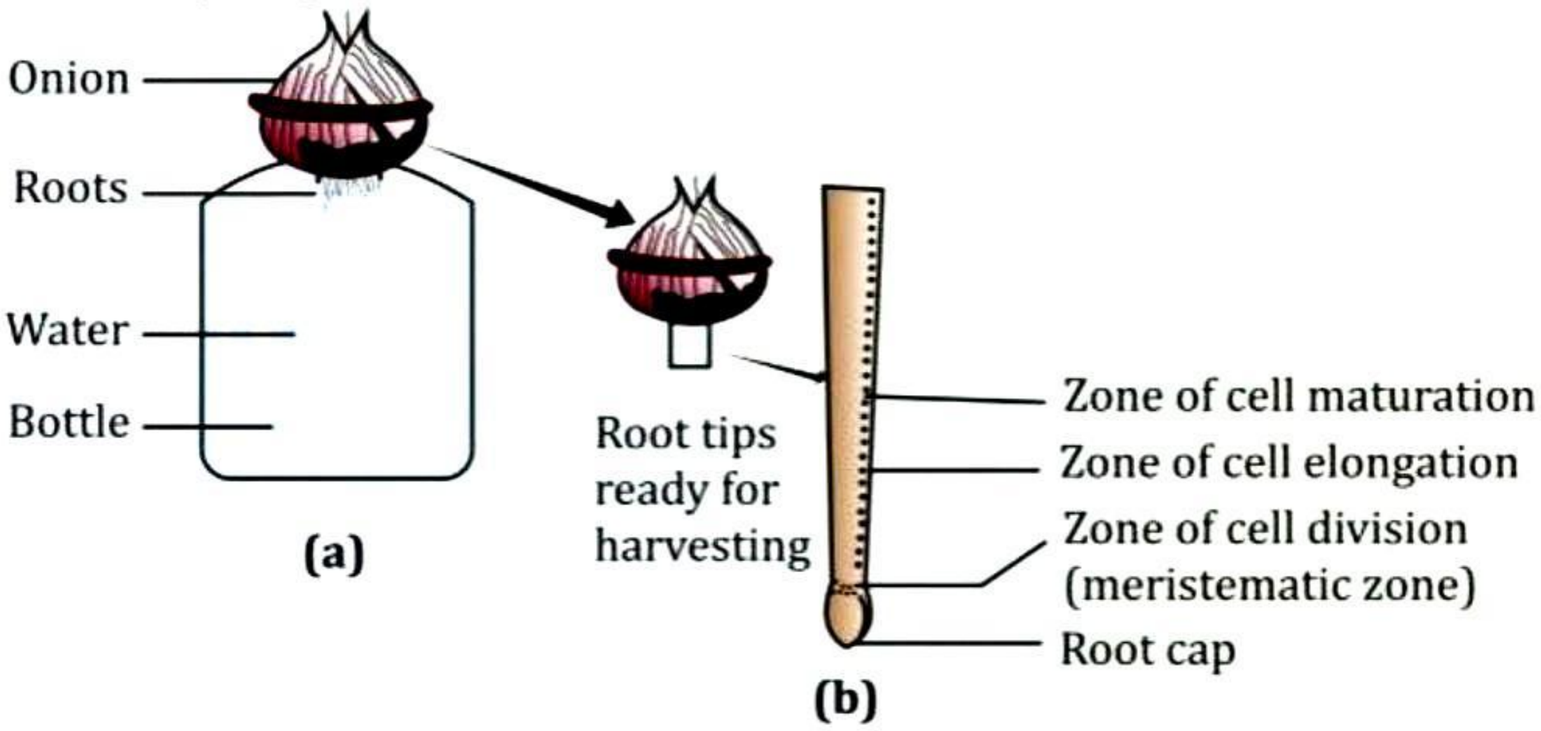
PROCEDURE

1 Growing Onion Root Tips

(i) Select medium sized onion bulbs and carefully remove the dry roots attached to them

(ii) Place thern in a bottle filled with water in such a way that only roots touch the water level

(iii) Check the water level in the bottle everyday and add few drops of water periodically in order to compensate any evaporation losses.



Method of growing onion roots and different zones of root apex

(iv) Keep them in this position for about 3-6 days. New roots may take 3-6 days to grow.

(v) Cut 2-3 cm long freshly grown roots, 2 hours after the sunrise and keep harvested roots in fixative (glacial acetic acid and ethanol in 1: 3 ratio) for 24 hours.

Note: As onion root tips have a cell cycle of 24 hours, i.e. they divide after 24 hours and this division usually takes place about 2 hours after sunrise. So, it is taken as available time.

(vi) Transfer roots from fixative to 70% alcohol.

(vii) These root tips are ideal material for the study of mitotic cell division.

Preparation and Study of Slide

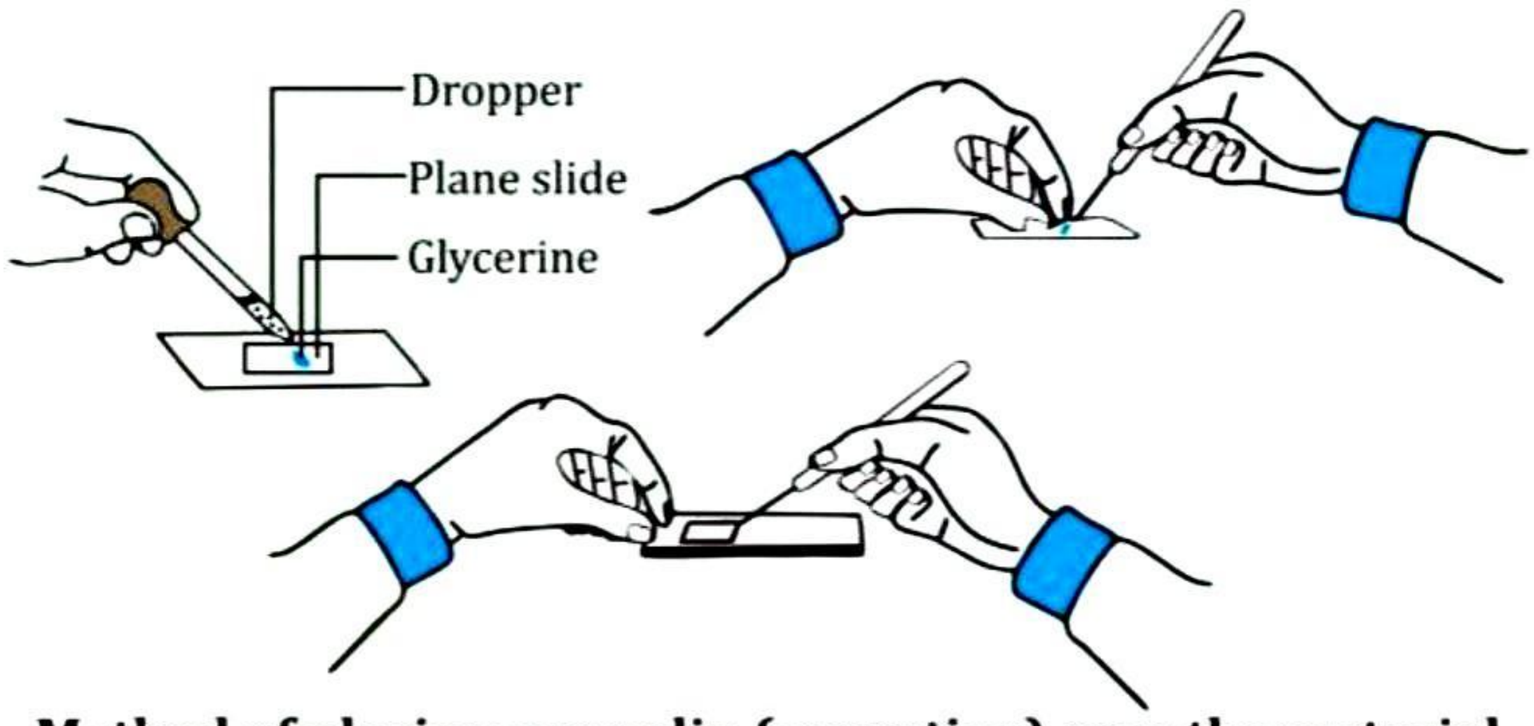
(i) Take one or two preserved roots and wash them thoroughly in water on a clean slide.

(ii) Place one drop of N/10HCl on the root tip followed by 2-3 drops of acetocarmine or acetoorcein stain on it. Leave it for 5-10 minutes.

(iii) Slightly warm the slide on spirit lamp. Care should be taken that the stain is not dried up.

(iv) Blot the excess stain using blotting paper.

(v) Now, cut the comparatively more stained 2-3mm tip portion of root and retain it on slide and discard the remaining portion.



Method of placing coverslip (mounting) over the meterial

(vi) After 10-20 seconds, put one or two drops of water and blot them carefully using blotting paper

(vii) Gently squash the root by tapping the coverslip with the blunt end of a needle so that meristematic tissue of the root tip below the coverslip is properly smashed and spread as a thin layer of cells.

(viii) Make sure that there are no air bubbles under the coverslip.

(ix) Now, gently warm the slide over a flame for few seconds and carefully seal the margins of the coverslip using molten paraffin wax or nail polish.

(x) This slide is now ready for the study of mitosis.

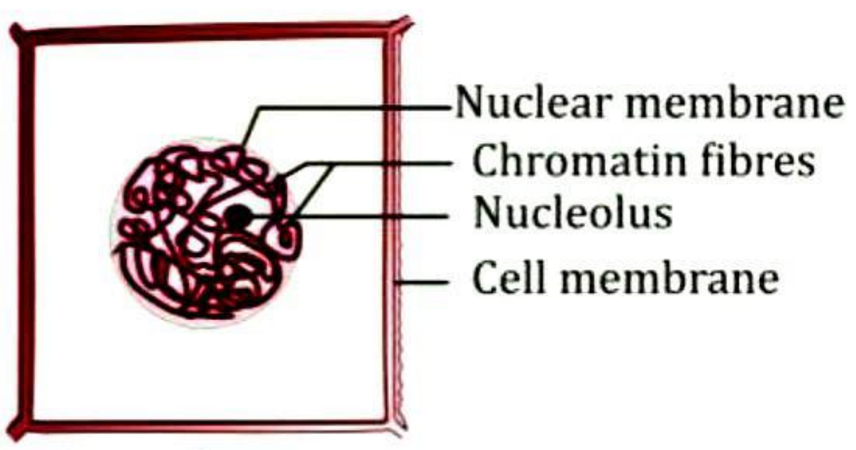
(xi) Observe the slide first under lower magnification and then under higher magnification to search the field microscopic for area having a few dividing cells and detailed features of mitosis. Examine different stages of mitosis under the compound microscope.

OBSERVATIONS

Under lower magnification of the microscope, rectangular cells with pink nucleus are seen scattered. Under higher magnification of the microscope different phases of mitosis become distinct. The stages of mitosis can be broadly categorised into two main parts, i.e. karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm and ultimately of the cell). Those cells which are not in the phases of cell division are considered to be in the interphase. You will observe that most cells in a microscopic field are in interphase.

Interphase

1. It is a non-dividing phase of the cell cycle occuring between two successive cell divisions.
2. The cells are mostly rectangular, oval or even circular in shape.
3. The nucleus is homogenous and looks granular in origin.
4. Nuclear envelope is distinct.
5. Chromatin fibres appear in the form of an interconnected mass within the nucleus.
6. Nucleolus is well observed inside the nucleus.



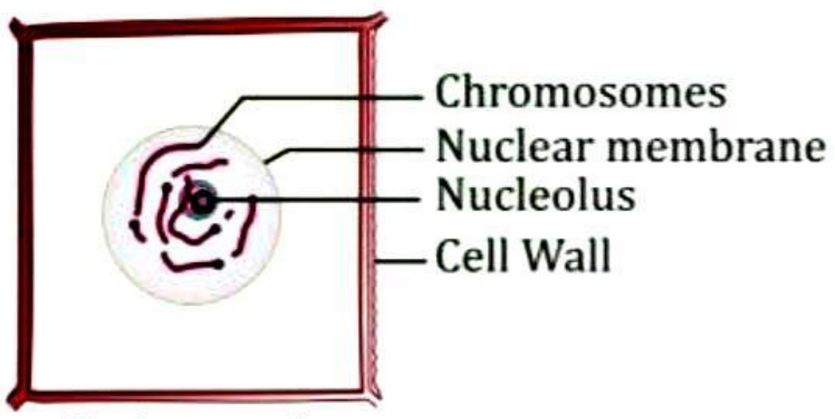
Interphase stage

Stages of Mitosis

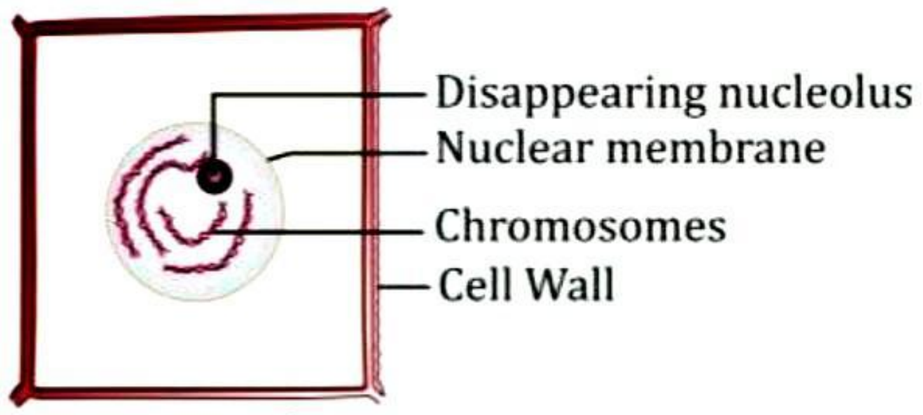
(i) Prophase

1. Nucleus is enlarged and occupies most of the cell volume. Intact nuclear outline is seen.

2. Chromatin network gets condensed and appears as long thread-like structures called chromosomes.
3. Nuclear membrane may start disappearing.
4. Nucleoli may or may not be visible.
5. If the cell is in early stage of prophase, then the chromatin fibres are very thin. However, in the cells at late prophase, comparatively thicker chromatin fibres would be visible.



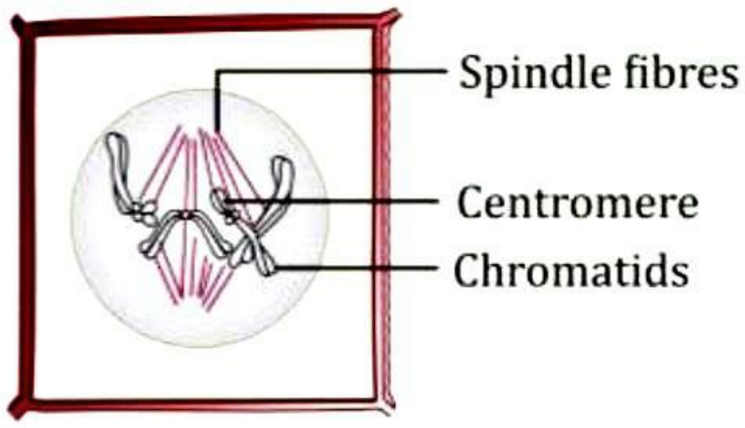
Early prophase
(a)



Late prophase
(b)

(ii) Metaphase

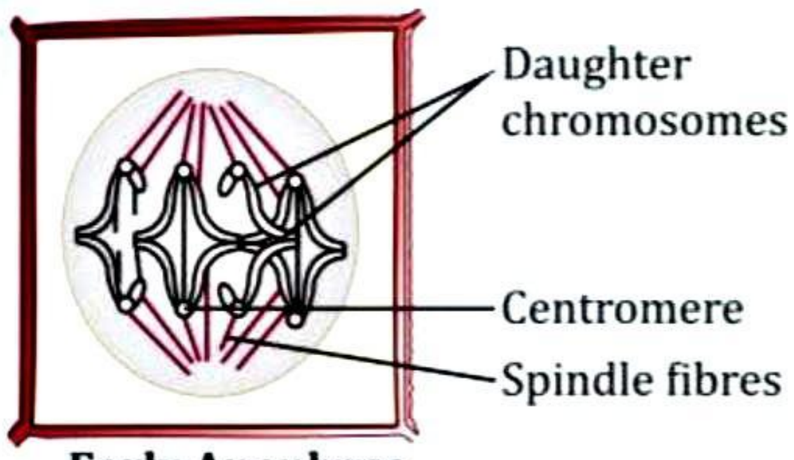
1. Chromosomes become shorter and thicker and hence become distinct and clearly visible under the compound microscope
2. Nuclear membrane completely disappears
3. Chromosomes orient themselves towards the equator with their centromeres arranged on an equatorial line forming metaphase plate. Out of two chromatids of each chromosome, one chromatid faces one pole and the other chrornatid faces the opposite pole
4. Series of spindle fibres attach the centromeres to the opposite poles.
5. Nucleolus is not observed during metaphase.



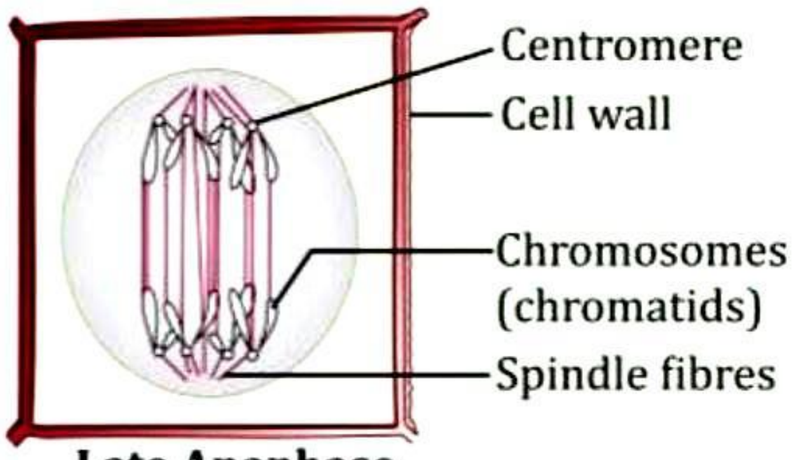
Metaphase stage

(iii) Anaphase

1. The two sister chromatids of each chromosome separate die to the spliting of ceritromeres and move towards the opposite poles.



Early Anaphase
(a)



Late Anaphase
(b)

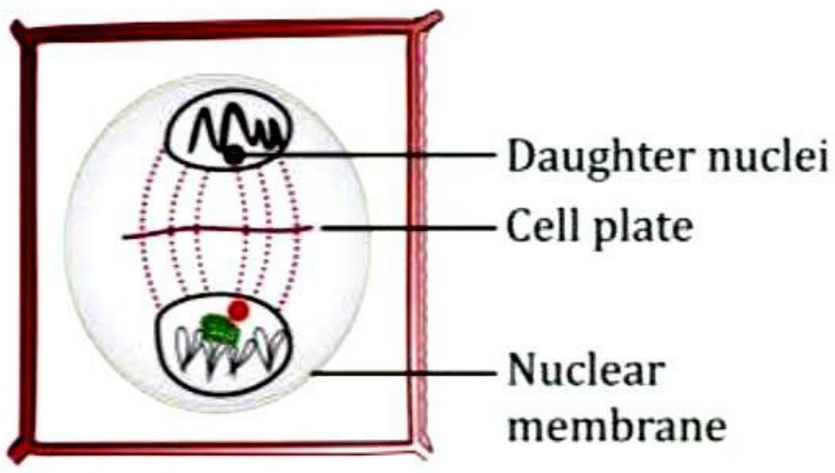
Anaphase stage

2. The daughter chromosomes (separated chromatids) appear in V, J, L and I shapes depending upon the position of the centromere in the chromosomes.

3. Anaphase is designated as early, mid and late stages depending upon the position of moving chromosomes toward the opposite poles.

(iv) Telophase

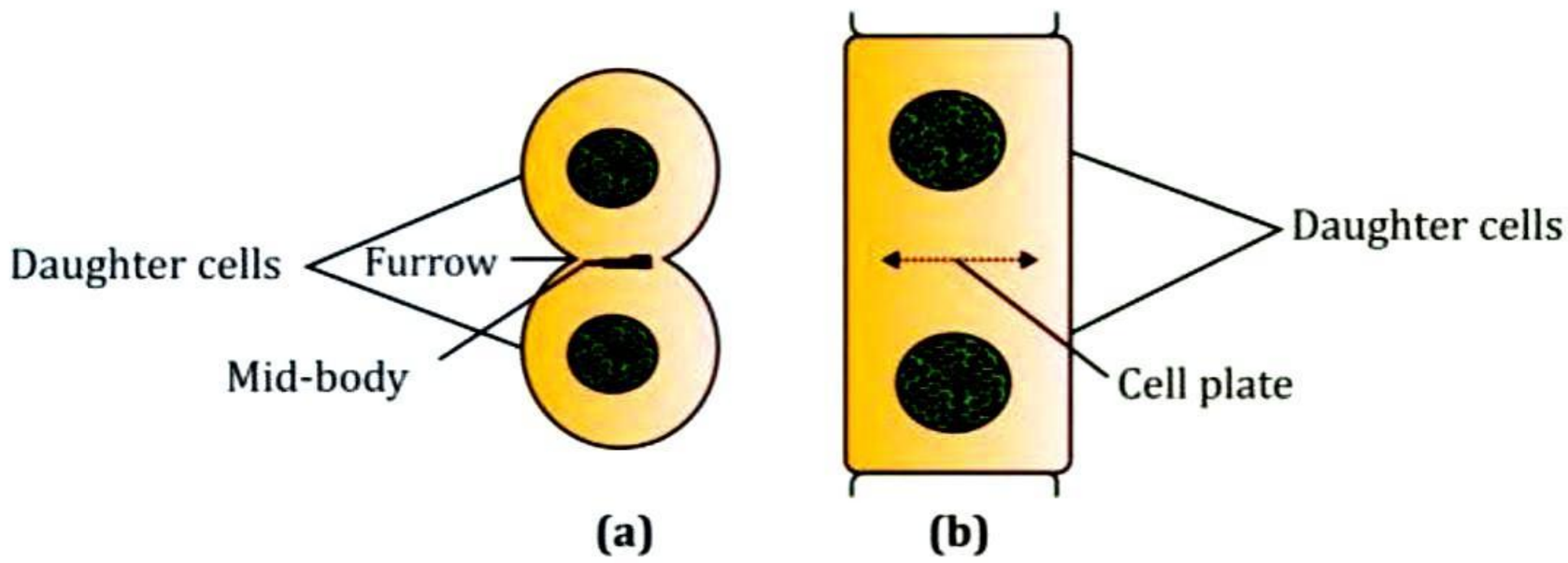
1. Chromosomes reach to the opposite poles, lose their individuality and look like a mass of chromatin again.
2. Nuclear membrane reappears to form the nuclei of two future daughter cells.
3. Nucleolus gets reconstituted.
4. Two daughter nuclei are thus formed and appear to be similar to the parent nucleus both quantitatively and qualitatively.



Telophase stage

Cytokinesis

In plant cells, cell plate is formed in the middle after telophase. The plate can be seen to extend outward to ultimately reach the margin of the cell and divide the cell into two equal halves. Such cell plates are characteristic of plant cells. In animal cell, furrow appears in the membrane at both sides and grows inward to divide cells into two.



Cytokinesis: (a) Furrowing in animal cells
(b) Cell plate formation in plant cells

RESULT

At the stages of mitotic cell division are clearly visible in the slide prepared from onion root tips.

PRECAUTIONS

1. Always clean the slide and coverslip thoroughly before and after use.
2. Care should be taken that there should be no air bubbles under the coverslip.
3. The base of the onion bulb should exactly be in contact with water, while growing the roots.

4. Always filter the acetocarmine stain before use.
5. Material should be mounted on the centre of slide.
6. Also the material should only be warmed gently, i.e. do not allow solution to boil on slide while warming.

VIVA VOCE

Q1. Why mitosis is also known as equational division?

Ans. The mosis is also known as equational division because the number of chromosomes in the daughter cell is equal to the chromosome number in parent cell.

Q2. Why do we use onion root tips or grasshopper larva for studying mitosis?

Ans. Onion root tips or grasshopper larva are often used for studying mitosis because these tissues have actively dividing cells and we can easily show up all the stages of mitotic cell divisions in them.

Q3. Why is early morning considered as an ideal time to harvest the onion root tips for study of mitosis?

Ans. This is because root tips have active cell divisions in morning. When we cut such material we get all stages of cell division in one material.

Q4. Suggest the name of few tissues which are suitable for the study of mitosis.

Ans. We can select the dividing cells of-

(i) Tail of tadpole larvae.
(ii) Bone marrow tissue of any vertebrate.
(iii) Epithelial cells of gills of fishes.
(iv) Shoot apices of plants.
(v) Root tip cells of any herbaceous plant.
(vi) Grasshopper larvae.

Q5. Why do we prefer monocotyledonous material for cytological studies?

Ans. Monocotyledonous plants are preferable because they have large sized chromosomes which are better visible in light microscope.

Q6. Which stain is used for studying chromosomes?

Ans. Acetocarmine is generally used for studying chromosomes as it stains chromosome deep red while cytoplasm remains unstained.

Q7. From where do spindle fibres originate?

Ans. Spindle fibres originate from centrioles in case of animal cells, but in plant cells, these originate from the cytoplasm. These are proteinaceous in nature.

Q8. In which stage of cell division chromosomes are seen best?

Ans. Chromosomes are best seen in metaphase stage, because at this stage chromosomes are thickest and shortest and exist in condensed form.

Q9. Suppose a cell has 44 chromosomes. How many chromosomes will it have after mitosis?

Ans. In mitosis, each of the daughter cells produced have the same number of chromosomes (44 chromosomes) as that of parent cell.