**The Cell Cycle: Preparation of a microscope slide from root tip cells and the analysis of the effect of colchicine on mitosis**

**Introduction**

All dividing cells go through a series of processes known as the cell cycle. In nearly all organisms, the structure of individual chromosomes can only be examined during mitosis. The standard technique for the examination of dividing cells outlined below will allow the visual identification of different stages of mitosis.

There are many variations on the basic method but all involve at least three important stages: - fixation, staining and preservation. The aim of fixation is to kill the material rapidly so that the internal structures are preserved in a lifelike form. Staining is necessary because without it the colourless chromosomes are difficult to distinguish from the equally colourless cytoplasm. The cell preparation can be made permanent by taking it through alcohol and placing it in a suitable mountant.

In this experiment, the sample, broad bean, *Vicia faba* (2n=12) will be pre-treated to facilitate the observation of the chromosome complement. The pre-treatment will inhibit spindle activity during division. You will examine all stages of mitosis in normal root tip cells and also root tip cells which have been pre-treated with colchicine. Colchicine is used to enhance chromosome condensation and also increase the number of cells in metaphase. Eventually all dividing cells treated with colchicine will enter and stop their cycle in metaphase. Colchicine treatment thus is an effective way of studying chromosomes and determining the population of dividing cells. In this species there is one large pair of sub-metacentric chromosomes and 5 pairs of smaller acrocentric chromosomes.

Root tips of Vicia faba (broad bean) have been treated for 4 hrs in 0.05% colchicine solution. Control root tips were left in water. The root tips will be cut from the seedlings and fixed. You will stain these root tips using Feulgen reagent. This technique stains chromatin and chromosomes pink-red.

You must prepare root-tip squashes of both the control and colchicine-treated root tips. The method is outlined on the next page. Make the following observations using the 40x objective. (This is NOT an oil immersion objective).

**Safety Notes:**

There is a detailed COSHH statement for this practical exercise. There are not excessively hazardous procedures but fume hoods should be used when needed (for acetic acid absolute ethanol (3:1) fixation and pouring acids) and students must wear gloves and goggles unless looking through the microscope. Ethanol is harmful if swallowed and highly flammable- handle with care. Feulgen reagent will stain living material- avoid contact with skin. Acetic orcein is potentially harmful as it a fairly strong acid.

Students will be handling thin cover slips that can easily be broken; care should be taken when handling.

**Method**

You should work in pairs. One member of the pair should prepare the control slides and one member should prepare the colchicine-treated root tip slides.

First cut off about six 2 cm root tips and fix in acetic acid absolute ethanol (3:1) for 10 minutes using a chemical hood.

1. ***Staining with Feulgen Technique***
2. Treat root tips with 1 M HCL at 60 degrees C (in water bath- record actual temperature) for 12 minutes. (note: pour acid in hood)
3. Rinse in tap water
4. Stan in Schiff’s reagent (basic fuchsin) for 15 minutes at room temperature.
5. Rinse in sulphited water
6. Rinse in tap water
7. To prepare temporary root-tip squash slide:
8. Cut off tips (approx. 1mm) of several roots with a sharp razor and place in the centre of a slide. Add 1 or 2 drops of 1% or 2% acetic-orcein
9. B) Place a coverslip over the root tip and tap several times to disperse the cells, using the blunt end of a pencil or biro.
10. Check under the microscope to see whether the cells are dispersed into a monolayer. If not, tap again.
11. Place several layers of filter paper onto the slide. Press down onto the coverslip on a flat surface with the thumb to squash the root tip. Press downwards. Do NOT twist.
12. (Note that this slide can be made semi-permanent by sealing the coverslip with nail varnish).

**Observation and analysis**

1. Determine the MITOTIC INDEX (MI) for each slide:

MI= number of cells in mitosis /number of cells counted.

Note: approximately 100 cells in a specific region in the meristem (region of cell division) should be counted to obtain a statistically significant result.

1. Identify cells undergoing mitosis and non-mitotic interphase cells. Try to identify all stages. Record the number of cells in each stage. Present your results in a table.
2. Comment on the differences between the colchicine and control preparations. Use statistical analysis. Explain HOW colchicine affects mitosis.
3. Draw and label nuclei in different stages of mitosis (Only draw what you see! )Your drawings should be large and convey the maximum information using the minimum amount of lines. Note the chromosome complement for one metaphase cell. Show the detailed morphology of as many chromosomes as possible. Can you count the number of chromosomes? Can you see chromatids and centromeres? Can you see nucleoli in the interphase nucleus? Annotate your drawings to explain what is happening in cytological, biochemical, and molecular biological terms.
4. Briefly explain the theory of the Feulgen Technique.