General Biology Lab 4

College of Environmental sciences
Dep. Environmental Health.
First stage
Basma Bashar

Wednesday 18/12 /2024

Study of live and fixed cells

- **living cells**

Studying of living cells is done by preparing sections or slides or wet mount. A group of dyes are used in this type of preparation, which are known as **live stains.** These dyes do not affect the living cell directly and last for a long time, so there is sufficient time to study the living cell before it dies. Among these dyes are Neutral red, which is used to stain the cytoplasm, Methylene blue, which selectively stains the Golgi complex, and Janus green, which stains mitochondria.

- **Preparation of a slide of live animal cells (**squamous epithelial cells of the lining of the mouth).

Tools :Q-tips, water, glass slide, glass slide cover, compound microscope, Methylene blue dye.

Method:

1-Scratch the inner lining of your mouth and the inner surface of your cheek with Q-tips.

2-Place a portion of this preparation on a glass slide in the middle of a drop of water and stain it.

3-Cover what you have prepared with the slide cover, and examine it under the microscope.

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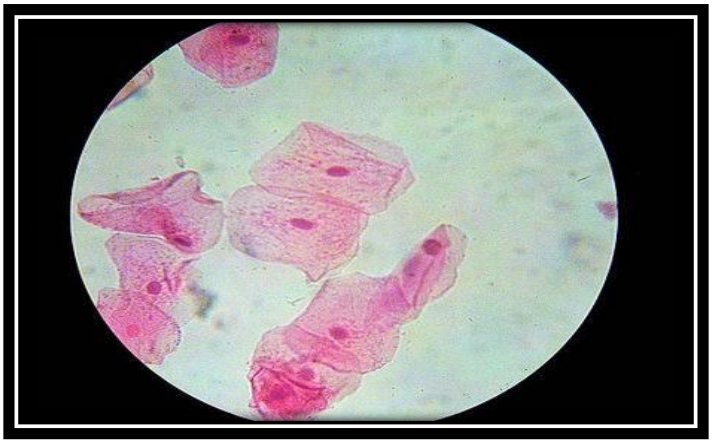

squamous epithelial cells

- **Fixed cell**

Fixed cell: is a cell that is killed by physical or chemical methods, keeping its structural components in a similar condition as much as possible for the state they were in the organism's body or close to that.

Steps to prepare permanent slides for samples:

1-Dissection : It means kills and taking the parts to be studied from animal or plant samples.

2- Fixation: It is killing cells while preserving the synthetic components in a condition as similar to natural as possible using special solutions

The Fixation process stops the process of fragmentation and decomposition resulting from the activity of bacteria and fungi, as well as stopping the process of self-decomposition of the tissue by enzymes. Fixation solutions include Ethyl alcohol and Formalin.

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3-Washing

The sample must be washed after fixation in order to remove any remaining trace of the fixative on the sample.

4-Dehydration

It is the removal of all water content from tissue because its presence hinders the paraffin from interfering with the cells well. It is used Alcoholic solutions of increasing concentration, ending with 100% absolute alcohol. In this process, the sample is not placed directly in the concentrated solution so that the cells do not shrink and become damaged . The time required the water to leave the sample varies in each step depending on the concentration of alcohol from 30 minutes to 3 hours.

Its common types are:

A- Ethyl alcohol

B- Methyl alcohol

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5- Clearing

It is the process of removing the dehydrating solution from the sample and replacing it with a solution (such as xylen or benzene) that mixes with the wax, making it clear and transparent, free of traces.

6-Embedding: In this step the sample is placed in molds containing pure paraffin wax and then cooled until the paraffin solidifies.



7- Trimming:

In the previous step, wax molds for the sample are prepared, and in order for the sample to be in a suitable position so it is trimmed in this step.

8- Sectioning or microtoming :

The sample is cut into very thin slices using a Microtome which comes in different types depending on the nature of the sample and the type of material embedded in it. There are sliding microtome, rotary microtome, overhead microtome, and sections The good ones are usually in the form of strips or a series of sections.

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It is preferable to place these strips on a black plate to make it easy to distinguish them and take the appropriate ones to place on the glass slide.

9- Staining :

After placing the sections on the glass slide, they are pasted with a transparent material such as egg white, and the wax is then removed with Xylol . The slide is passed through a series of alcohols decreasing concentrations, ending with water, and then stained with specific stains , such as Eosin which stain the cytoplasm and Hematoxylen stain the nucleus.

10- Mounting :

means placing the prepared section on a clean glass slide and then covering it with the slide cover. Most of the Mounting materials are either gum or resin.

11- Labeling:

This means placing a sticky note on the slide with its contents written on it, such as (the name of the sample studied and the name of the company supplying it).