



HISTOPATHOLOGY - FIRST COURSE

LAB 10 :THEORY OF HISTOLOGICAL STAINING

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Third Stage

المرحلة الثالثة

محاضرة رقم 10

Lecture No. 1

الجانب العملي

Practical

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The aims of a lecture:

- The aims of a lecture on the theory of histological staining are to explain the fundamental principles and practical applications of using dyes to visualize and differentiate cell and tissue components under a microscope.

Theory of histological staining

The theory of staining is very complex subject and is not fully understood.

Therefore many theories have been put forth to explain the facts. Histological staining is based on some chemical and physical properties. Numerous special staining techniques rely on the fundamental chemical processes of oxidation and reduction. In special staining techniques, oxidizing or reducing agents act on tissue sections, so that subsequently applied dyes or reagents can bind to or react with particular tissue elements, resulting in visualization.

:There are two theories

A-Physical theories

1-Simple solubility e.g. Fat stains are effective because the stain is more soluble in fat than in 70% alcohol

Absorption: This is a property by which a large body-2 attracts to itself minute particles from a surrounding medium

B) Chemical theories

the acid dyes stain basic elements (Cytoplasm) and basic dyes stain acidophilic material (nucleus)

STAINING

The sections, as they are prepared, are colourless and different components cannot be appreciated. Staining them by different coloured dyes, having affinities of specific components of tissues, makes identification and study of their morphology possible.

Basophilic : Substances stained with basic dyes

Acidophilic: Substances stained by acid dyes

Vital staining : Staining of structures in living cells, either in the body (in vivo) or in a laboratory preparation (in vitro). e.g. Janus green is taken up by living cells and stains the mitochondria.

:Types of staining

1-Metachromatic staining

differentiate particular tissue components by staining them a different color to that of original dye are certain basic dyes belonging to aniline group that will Some of the common metachromatic dyes are:

Methylene blue, Methyl violent Thionin, Crystal violent,
Toluidine blue

2- Progressive staining

Stain applied to the tissue in strict sequence and for specific times

3- Regressive staining

Tissue is first overstained and then the excess stain is removed from all structures

4-Histochemical staining

Staining which is used to indicate the chemical composition of the tissue or cellular elements

:Staining Methods

Direct staining Application of simple dye to stain the tissue-1

.in varying shades of colours

Indirect staining It means use of mordant of facilitate a-2

particular staining method or the use of accentuator to improve

.either the selectivity or the intensity of stain

Mordants : Substance that causes certain staining reactions to take place by forming a link between the tissue and the stain. e.g. Ammonium and Potassium alum .for hematoxylin

Accentuators : are substances that causes an increase in the selectively or in the staining power of dye. e.g .KOH .in Mehtylene blue

:Dyes are classified

A) Natural dyes

1. Haemotoxylin : This is the most popular dye used as a nuclear stain.

It is derived from the long tree mainly found in Mexico. It develops staining properly after oxidation. It is a weak dye and to make it give sharp stain a mordant is needed

2. **Carmine** : It is a scarlet dye made from the ground bodies of cochineal beetles.

B) Synthetic dyes

Most of these are in Aniline base and derived from coal

1-Basic dyes :are cationic dyes and stain nuclei, basophilic granules or bacteria

2-Acidic dyes are anionic dyes and stain mainly cytoplasm, eosinophilic granules **Decolourization Partial** or **complete removal of** stain from tissue sections. When the colour is removed selectively (usually with microscopic control)

Differentiation In regressive staining differentiation is the

—removal of washing out of the excess stain until the colour is retained only in the tissue components to be studies

Impregnation is the deposition of salts of heavy metals on
. or around cells, tissue constituents

It has followed characteristics

— Structures demonstrated are opaque and black-1

The colouring matter is particulate-2

The deposit is on or around but not in the element-3

Questions

- 1 Why is staining necessary in histology
- 2- What is the basic principle behind how dyes bind to tissues?
- 3-Staining Techniques and Methods

Any questions?

*Thank
You*