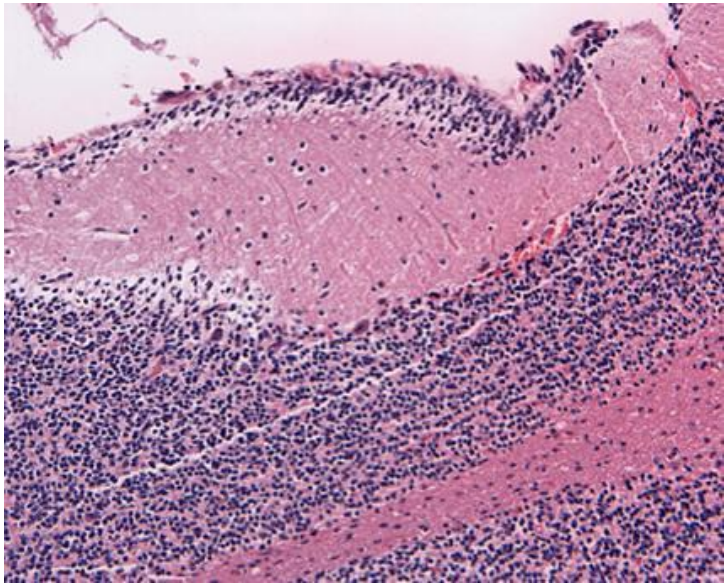


Tissue Staining

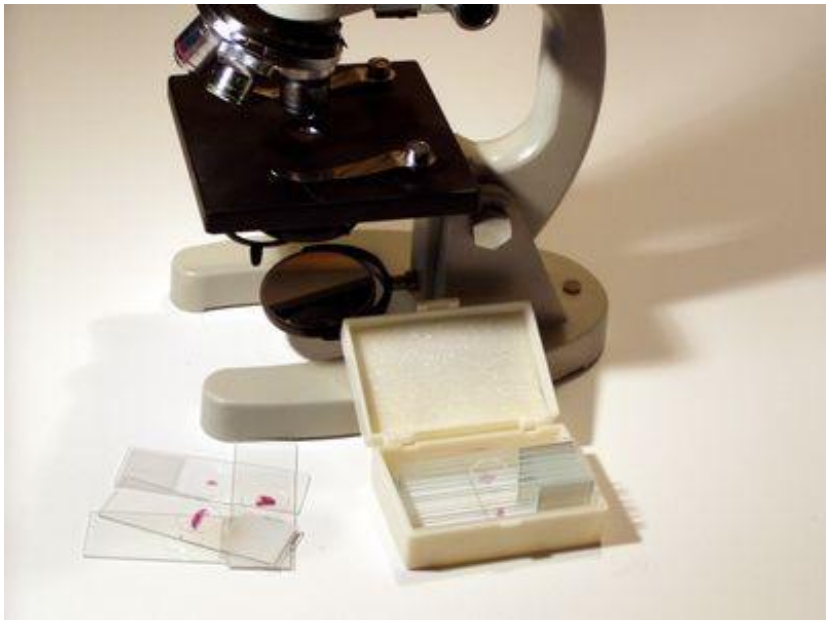
322 Histological Techniques

Learning Objectives Outline:

- Learning what is tissue staining?
- Knowing about the history of dyes and tissue staining
- Understand the method of staining of paraffin section
- Knowing about Hematoxylin and Eosin (H & E) Staining
- Other staining

What is tissue staining?

Staining is treating (tissue for the microscope) with a reagent or dye that makes certain cellular elements visible without affecting others



Write down 3 things we use dyes on it from our daily life?

History of Dyes

- Grew 1682 – stained plant tissue with cochineal

- Leeuwenkoek 1714 – stained muscle fibers with saffron

History of Dyes con.

- William Perkin 1856 – synthesized dye aniline violet

A.D. 1856 . . . . . Nº 1984.

Dyeing Fabrics.

LETTERS PATENT to William Henry Perkin, of King David Fort, in the Parish of Saint George in the East, in the County of Middlesex, Chemist, for the Invention of "PRODUCING A NEW COLORING MATTER FOR DYEING WITH A LILAC OR PURPLE COLOR STUFFS OF SILK, COTTON, WOOL, OR OTHER MATERIALS."

Sealed the 20th February 1857, and dated the 26th August 1856.

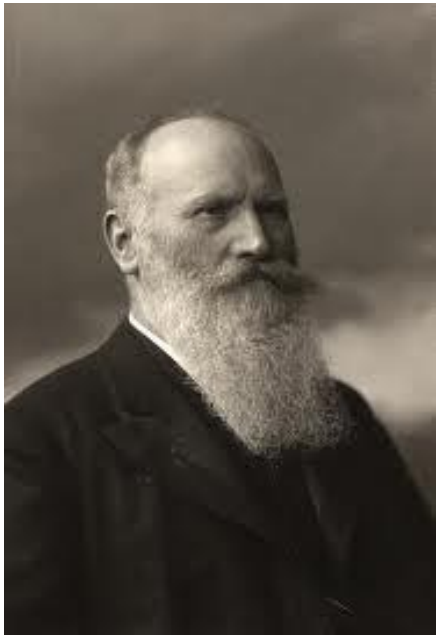
PROVISIONAL SPECIFICATION left by the said William Henry Perkin at the Office of the Commissioners of Patents, with his Petition, on the 26th August 1856.

I, WILLIAM HENRY PERKIN, do hereby declare the nature of the said Invention for "PRODUCING A NEW COLORING MATTER FOR DYEING WITH A LILAC OR PURPLE COLOR STUFFS OF SILK, COTTON, WOOL, OR OTHER MATERIALS," to be as follows:—

Equivalent proportions of sulphate of aniline and bichromate of potassa are to be dissolved in separate portions of hot water, and, when dissolved, they are to be mixed and stirred, which causes a black precipitate to form. After this mixture has stood for a few hours it is to be thrown on a filter, and the precipitate to be well washed with water, to free it from sulphate of potassa, and then dried. When dry it is to be boiled in coal-tar naptha, to extract a brown

History of Dyes con.

- Waldeyer1863 – Hematoxylin used in Histology and Cytology



Hematoxylin

- Natural day from logwood tree (Mexico) called Hematoxylon campechianum Linnaeus
- Hematoxylon is derived from Greek, haimatodec (blood like) and xylon (wood)

Tissue –Dye Reactions

- Both chemical and physical reactions occur such as
  - **Simple absorption** such as the Oil Red O stain for lipids.
  - **Adsorption** such as in colloid dyes
  - **Electrostatic** attraction as seen in acidic and basic dyes.
  - **Van der Waal Forces** such as hydrogen bonding, covalent bonding and hydrophobic bonding may all be involved. e.g. Alum Hematoxylin staining the nuclei

Factors influencing dye uptake

- Mordants
  - Substance that causes certain staining reactions to take place by forming a link between the tissue and the stain. The link is referred as lake. Without it, dye is not capable of binding to and staining the tissue.

    e.g. Ammonium and Potassium alum for hematoxylin.
- pH of the dye
  - e.g. Alcian Blue at different pH stain different mucins.
- Concentration of the dye
  - can stain quicker up to a point at which all dye uptake sites have been utilized.
- Type of fixative used
  - Formalin fixation enhances basic dye uptake. Mercuric chloride enhances acid dye uptake.
- Temperature
  - increase in temperature usually increases the rate of staining.

Classification of Stains

Basic Days (Basophilic stain)

These are cationic dyes and stain acidic components of the cell, nuclei, basophilic granules or bacteria

nuclear stain special affinity for nuclei

ex. Hematoxylin

Acid Days (Acidophilic stain)

These are anionic dyes and stain many cytoplasmic elements that tend to be basic and eosinophilic granules

cytoplasmic stain - affect the cytoplasm of the cell

ex. Eosin.

H&E - hematoxylin and eosin is the most used combination of stained for routine histology

Staining of paraffin section

- Slides with paraffin sections must have the paraffin removed for staining

1. Deparaffinize in hot air oven 10-15 min
2. Place slides in Xylene for 10 minutes
3. Next a second change of xylene for 10 minutes
4. Slides are then rehydrated through a grades series of alcohols to distilled water
5. The slides are then placed in hematoxylin for 3 to 5 minutes
6. Next place the slides in 70% ethanol for 2 to 5 minutes

Staining of paraffin section con.

6. Counter stain with Eosin for 2 to 5 minutes
7. Rinse off excess Eosin
8. Dehydrate the section in 95% and absolute Alcohol 2 changes (2minutes each).
9. Clear in Xylene 3 changes (2 minutes each)
10. Add a small drop of mounting medium to the slide and finally add a cover slip
11. Allow to dry before examining

Video

Causes of poor quality of staining

1. Poor or inadequate fixation of tissue.
2. Over or under-ripened Haematoxylin.
3. Overused or worked out Haematoxylin.
4. Over or under differentiation of haematoxylin
5. Insufficient blueing following differentiation.
6. Failure to wash blueing agent out of section before counter staining with eosin (especially when ammonia is used).
7. Insufficient differentiation of eosin during washing or dehydration.
8. Insufficient dehydration and clearing of sections.
9. Contamination of stains.

- http://staff.ksu.edu.sa/sites/default/files/28829_2010_conn14_h_e_staining_gill_0.pdf
- http://staff.ksu.edu.sa/sites/default/files/08066_12may10_webchapter17_0.pdf