Histopathological Diagnosis

PREPARATION OF HISTOLOGICAL SPECIMENS

STAINING

- **What kinds of histological stains are there?**

- Most cells are colourless and transparent, and therefore histological sections have to be stained in some way to make the cells visible.
- The techniques used can either be non-specific, staining most of the cells in much the same way, or specific, selectively staining particular chemical groupings or molecules within cells or tissues.
- Staining usually works by using a dye, that stains some of the cells components a bright colour, together with a counterstain that stains the rest of the cell a different colour.

STAINING

- **When paraffin sections** are mounted on slides and viewed down a microscope, they appear colourless and offer little in the way of information.
- In order to improve contrast, staining the sections with dyes imparts colour to both cells and tissues.
- However, the principal staining technique (and the gold standard) used in histological diagnosis is the haematoxylin and eosin (H&E) stain.

STAINING

- **Basophilic and acidophilic staining.**

- **Acidic** dyes react with cationic or basic components in cells.

Proteins and other components in the cytoplasm are **basic**, and will bind to **acidic** dyes.

Another way of saying this is that cytoplasmic proteins are **acidophilic** (acid liking - i.e. bind to acidic dyes).

- **Basic** dyes react with anionic or **acidic** components in cells. **Nucleic acids** are **acidic,** and therefore bind to **basic dyes.**

Another way of saying this is that nucleic acids are **basophilic (basic liking).**

STAINING

- **Haematoxylin**

This is a natural product that is extracted from the heartwood of the logwood tree Haematoxylon campechianum which is native to Central America and the West Indies.

- Haematoxylin is relatively colourless and without modifications has little value as a biological stain.

STAINING

- **Haematoxylin**

- Haematoxylins with high aluminium to haematein ratios will stain sections slowly until selective colouration of nuclear chromatin is achieved. This is called **progressive staining**.
- Conversely, solutions with lower chemical ratios will over stain all tissue components more rapidly. As a result, the desired nuclear staining intensity is selected by differentiating the sections in a dilute acid-alcohol mixture, thereby removing excess metal-dye complexes from the sections. This selective method is termed **regressive staining**.

STAINING

Haematoxylin

There are many alum haematoxylins available such as those of Carazzi, Cole, Delafield, Ehrlich, Gill, Lillie, Mallory and Masson**.** However, two of the most common haematoxylins are those of Mayer (a progressive stain) and Harris (a regressive stain) and their compositions are shown below:

MAYER		HARRIS
Haematoxylin	DYE	Haematoxylin
Sodium iodate	OXIDISER	Mercuric oxide
Ammonium alum	MORDANT	Ammonium/potassium alum
Citric acid	ACIDIFIER	Acetic acid
Chloral hydrate	STABILIZER	Absolute alcohol
Water		Water
PROGRESSIVE STAIN		REGRESSIVE STAIN

STAINING

- **Haematoxylin**

The process shown below of converting the initially red soluble aluminium-haemalum ions to a final blue insoluble form is termed **‘blueing’.**

- Other than aluminium, there are several other metal ions that may be used with haematoxylin.

What can haematoxylin demonstrate?

- Haematoxylin solutions are able to demonstrate many tissue structures of which examples are:

  Cartilage and cement lines of bone

  Collagen and reticulin

  Elastic tissue

  Fatty acids

  Lead and copper

  Muscle striations

  Myelin

  Nerve cells and fibres

- Haematoxylin can be considered as a basic dye (general formula for basic dyes is:dye+ Cl-).
- Haemotoxylin is actually a dye called hematein (obtained from the log-wood tree) used in combination with aluminium ions (Al^{3+}).
- It is used to stain acidic (or basophilic) structures a purplish blue. (Haematoxylin is not strictly a basic dye, but it is used with a 'mordant' that makes this stain act as a basic dye.
- The mordant (aluminium salts) binds to the tissue, and then haematoxylin binds to the mordant, forming a tissue-mordant-haematoxylin linkage.)

EOSIN

- Eosin is an acidic dye: it is negatively charged (general formula for acidic dyes is: Na+dye-). It stains basic (or acidophilic) structures red or pink. This is also sometimes termed 'eosinophilic'.
- Thus the cytoplasm is stained pink in the picture below, by H&E staining.

What structures are stained purple (basophilic)?

- DNA (heterochromatin and the nucleolus) in the nucleus, and RNA in ribosomes and in the rough endoplasmic reticulum are both acidic, and so haemotoxylin binds to them and stains them purple.
- Some extracellular materials (i.e. carbohydrates in cartilage) are also basophilic.

What structures are stained pink (eosinophilic or acidophilic)?

- Most proteins in the cytoplasm are basic, and so eosin binds to these proteins and stains them pink.
- This includes cytoplasmic filaments in muscle cells, intracellular membranes, and extracellular fibres.

Histological Stains other than H&E

- For basic dyes, the reaction of the anionic groups of cells (these include the phosphate groups of nucleic acids, sulphate groups of glycosoaminoglycans, and carboxyl groups of proteins) depends on the pH at which they are used.
- For acidic dyes, the dye in question can often in addition be selective for particular acidophilic components. I.e. a technique called the Mallory staining technique uses three acidic dyes: aniline blue, acid fuschin and orange G, which selectively stain collagen, cytoplasm and red blood cells respectively.