HYSTOLOGICAL STAINIGS

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-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.

-CONTRAST IS OBTAINED WITH SPECIFIC DYES USED IN HISTOLOGY WHICH HAVE DIFFERENT AFFINITY TOWARDS THE TISSUE STRUCTURES

-HYSTOLOGICAL DYES CANE BE OF ANIMAL ORIGIN, VEGETAL ORIGIN OR SYNTHETIC (anilines)

DYES : usually they are organic aromatic compounds containing a **CHROMOPHORE** (visible light 400-800 mm) and an **AUXOCHROME**.

Chromophore usually has functional groups that give colour

CHROMOPHORE GROUPS: *CARBOXYL (C=O), AZO (-N=N-), NITROUS (-N=O), NITRO (–NO2), ethylene (C=C)……*

THE AUXOCHROME INCRESES THE INTENSITY OF THE CHROMOPHORE.

An auxochrome is a functional group of atoms attached to the chromophore which modifies the ability of the chromophore to absorb light, altering the wavelength or intensity of the absorption. AUXOCHROME is usually an ionizable group , linked to the Chromophore by a covalent bond.

The auxochrome:

- confers water solubility to the dye
- is responsible of its ionizability
- confers stability through the formation of saline bonds with proteins or other tissue component

Depending on the charge in solution, the auxochrome can be:

- acidic (where ionizing as anion)
- basic (where ionizing as cation)

That feature groups histologic dyes into acid and basic dyes.

Auxochrome

- **Acidic auxochromes**:
  - sulphonic group (-SO3H the most frequent)
  - carboxyl group (-COOH)
  - hydroxyl group (-OH)
- **Basic auxochromes**:
  - amino group(-NH2) and derivatives
  - metals (in lacquers)
- Dyes are usually sold as salts:
  - **Acidic:** sodium salts, sometimes K^{+}, Ca^{++} or $NH4^{+}$
  - **Basic:** Cl^{-}, sometimes acetates or sulphates

DYES 2

Can be divided according to charge:

BASIC	*-CATIONS WITH POSITIVE CHARGE*
ACIDIC	*-ANIONS WITH NEGATIVE CHARGE*
NEUTRAL	*-BOTH NEGATIVE AND POSITIVE CHARGES*
AMPHOTERIC	*-CHARGE DEPENDS ON pH*
INDIFFENT	*-WITHOUT CHARGE*

INDIRECT STAINING

-THE STAINING IS POSSIBLE ONLY AFTER A PRETREATMENT WITH A SPECIFIC SUBSTANCES , CALLED MORDANT DYE, THAT CAN COMBINE TO THE DYE

THE PROCESS IS CALLED ***MORDANTING***

-IF DYE AND MORDANT ARE USE SIMULTANEOUSLY WE OBTAIN A ***LACQUER***

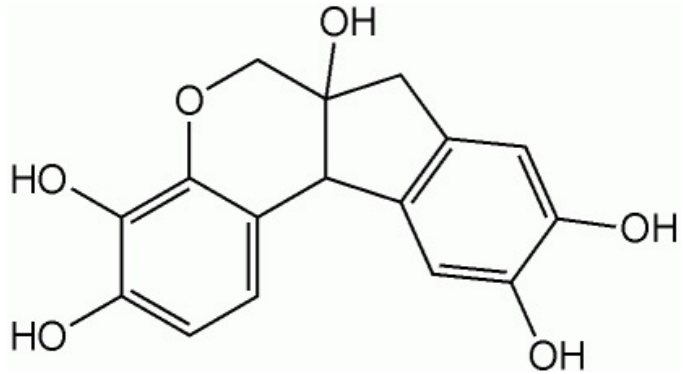
-MORDANTS ARE OXYDANT SUBSTANCES SUCH AS ACID AND METALLIC SALTS (CHROMIC ACID, CORROSIVE SUBLIMATE), PICRIC ACID, PHENOL,, OSMIUM TETROXIDE AND ALUME ($KAl(SO_4)_2 \bullet 12\ H_2O$)

DIFFERENTIATIATION STEP

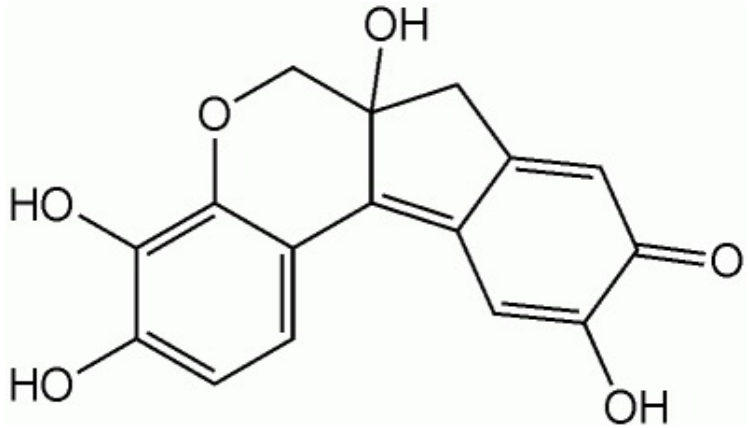
TO RINSE THE DYE, AND TO FIX THE SPECIFIC STAIN

H&E 1

Haematoxylin can be considered as a basic dye. Haemotoxylin is a vegetal dye. The dye is actually hematein used in combination with aluminium ions (Al3+). It is used to stain acidic (or basophilic) structures into purplish blue. Haematoxylin is not strictly a basic dye, but it is used with a 'mordant' that makes this stain to 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. Thus the nucleus is stained purple. Haematoxylin is soluble in water and glycerol.



HAEMATOXYILIN



HEMATEIN

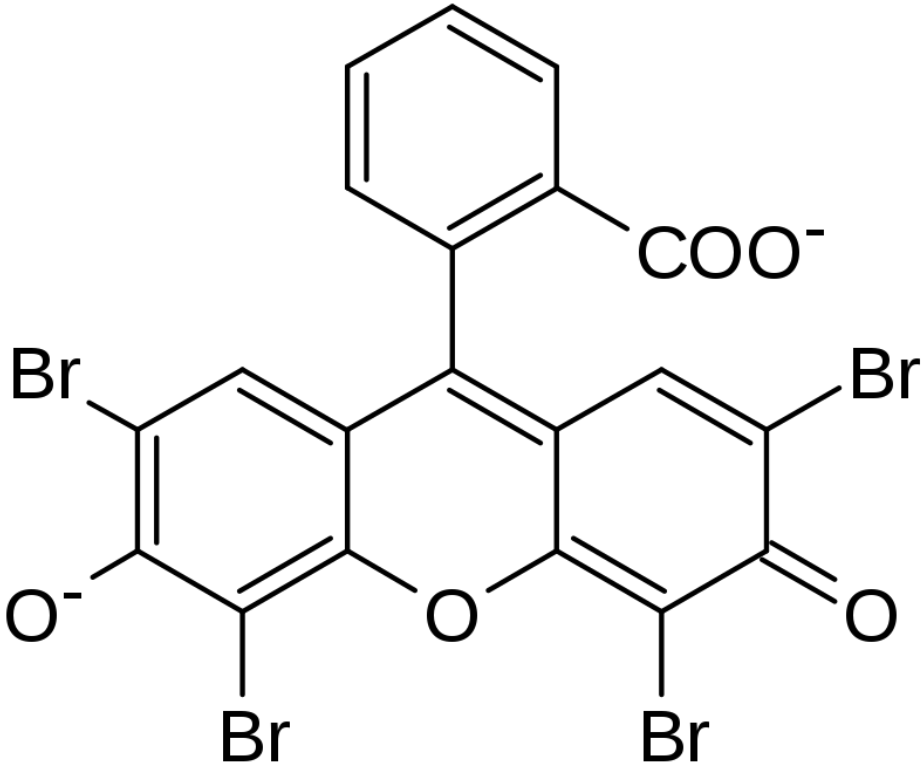
H&E 2

- *To obtain haematin, it is left to ripen in the air or oxidising substances (K ... permanganate) are added.*
- *It is used together with alum (mordant to form a lacquer).*
- *Depending on the oxidant and mordant used there are hematoxylins with different names (Carazzi, Mayer, Harry, ferric, ...)*
- *It is a basic dye that acts better in acidic environment and reacts with the phosphoric groups of nucleic acids, staining the nuclei in dark violet.*
- *The differentiation is made using tap water and it turns the color from violet to blue.*
- *Nuclei: blue (hematoxylin) Endoplasmic reticulum: blue (hematoxylin)*

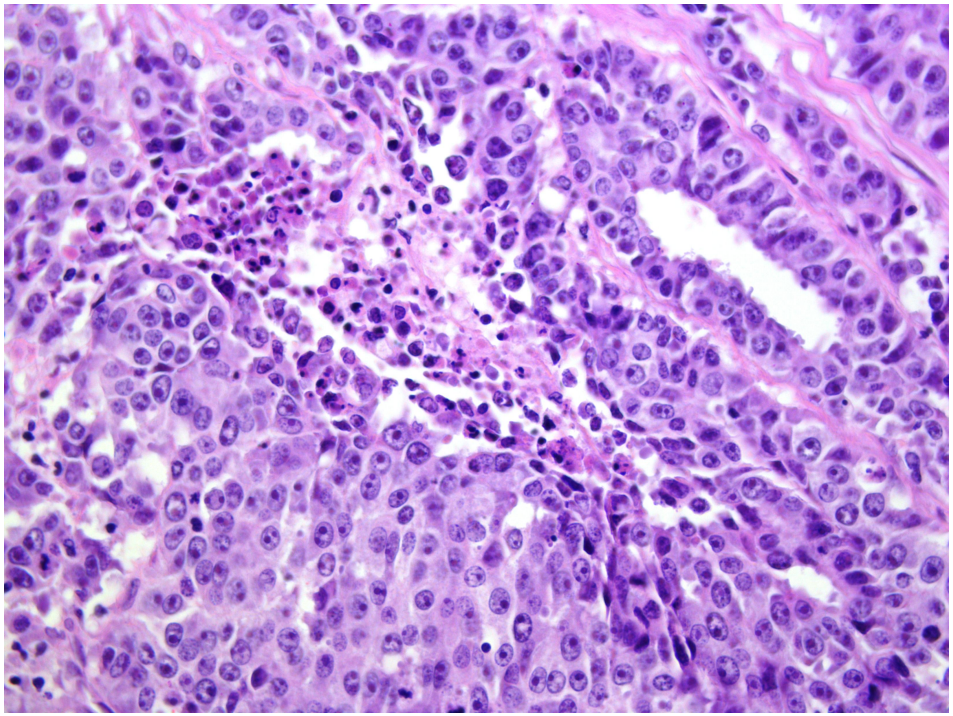
H&E 3

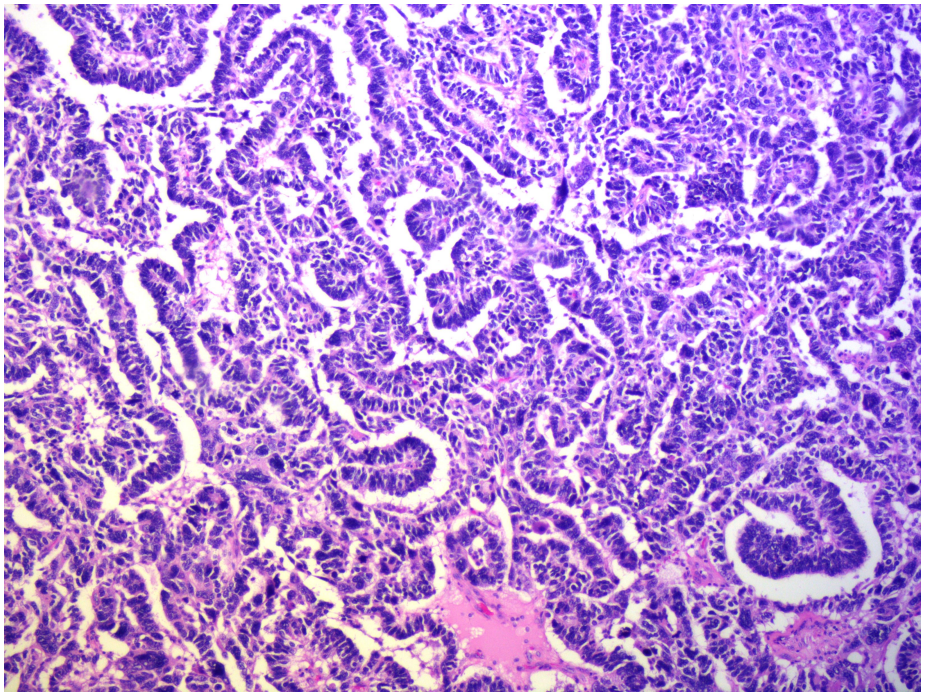
EOSIN:

- ***It is an artificial acid dye (different ones, the most common is yellow eosine).***
- ***It is soluble in water, it is used in 1% solution.***
- ***It stains cytoplasm, fibers and intercellular substance in pink more or less intensely.***
- ***Differentiation is made with 95% alcohol.***
- ***It stains Elastic, collagen and reticular fibers in pink.***



H&E STAIN





H&E staining

- *After section re-hydration:*

-Haematoxylin	*10 min*
-Differentiation-tap water	*30 min*
-Eosin	*1 min*
-Differentiation with Ethanol 95%	*rapidly*

MOUNTING

STAINING

-ETHANOL 70 °

-ETHANOL 95° ***DEHYDRATION***

-ETHANOL 100°

-ETHANOL 100°

-XYLENE ***CLEARING***

-XYLENE

MOUNTING

Canada Balsam OR Eukitt + slide coverslip

FROZEN TISSUES STAINING 1

Histological examination can be performed on frozen tissue sections.

The sections can be cut from:

a) Forminin-fixed tissues: before being frozen, tissue must be washed extensively with water.

b) Fresh tissues:

- ***a 2-3 mm thick sample is taken from the blood-washed tissue,***
- ***it is placed on a metal support***
- ***it is frozen for a few seconds on the liquid nitrogen vapors***
- ***it is immersed in liquid nitrogen for one minute***

FROZEN TISSUES STAINING 2

Sectioning is carried out by the use of a cryostate:

-Temperature regulation (if too cold the tissue is friable, if too high the tissue is soft)

-In the cutting area there is a teflon plate for collecting the tissue sections.

-A transfer from the teflon plate to a cold slide is carried out by placing one near the other. Adhesion and distension on the glass is carried out by the heat transferred from the fingertip.

-It dries quickly by shaking the slide in the air

-A quick fixation of 1 min in acetone or alcohol can be carried out.

CRYOSTAT:
Rotary Microtome
Cooling chamber

RAPID STAINING

H&E

-Concentrated Harry Haematoxylin 1 min.

-Hydrochloric acid 0.5% - Immerse slides 20 times

-Ammonia 2% in ethanol – Immerse slides 20 times

-Eosin 1 min.

-Differentiation in Ethanol 95% - rapidly

Dehydration

Clearing

Mounting