By Dr. Vijay Kumar

Topic : staining

Staining is the process of colouring the cells, tissues, microscopic organisms or their parts by certain coloured organic aromatic substances which have a special aptitude for being retained by the tissue elements.

~~Not only~~ A large number of organic and inorganic substances ~~are used~~ act as colouring agents, but their use depends upon the chemical nature of material, pH value of the fixative and the chemical activity of stain material.

Stains

The Cytological stains are solution of those aromatic organic compounds which impart colour to the objects. These have two kinds of active chemical groups ~~xxx~~ namely -

(I) Chromophoric groups

These impart colour to the dyes (stains) and includes carboxyl group (-COOH), azo group (-N=N-) nitro group ($-NO_2$), indamin group (-N=) and Quinoid group () ek.

(II) The Autochromic groups

These provides the dye an ability to attach to the tissue or the material. These dissolve and dissociates in water.

Ex- Hydroxyl group (-OH)

On the basis of medium stains are classified as

(a) Basic stains

The chromophoric group of basic stains is cationic (basic) in nature and stain in alkaline medium only.

The basic stains are used to stain nucleus and chromosomes, particularly the nucleic acids.

Ex - Basic fuchsin, Crystal Violet, methyl green, methylene blue, safranin, azures & acridine red. (These combine with $-NH_2$ group.

(b) Acidic Stains

The chromophoric group of acidic stains is acidic in nature.

These Contains - Nitro ($-NO_2$) and Quinoid groups.

These combine with the Protein structure of the cell at a low pH by their hydroxy ($-OH$), carboxyl ($-COOH$) or sulfonic ($-HSO_4$) groups.

Ex- Picric acid, acid fuchsin, methyl blue, eosine, organge-G, Congo red, aniline blue.

(c) Neural strains

The neutral strains have the ~~protein~~ properties of acidic as well as that of basic stains.

Mechanism of staining

It is well known fact that Proteins, Certain Polysaccharides and nucleic acids have the property of ionisation. But the ionisation of proteins depends upon pH of the medium.

At pH values above isoelectric point, acid groups becomes ionised and below isoelectric point, all the basic groups dissociates. Thus, at a pH above isoelectric point, the proteins reacts with basic dyes and exhibit basophilic property.

At pH value below isoelectric point, the proteins react with basic dyes and exhibits acidophilic property.

The intensity of staining depends upon the degree of acidity or alkalinity of the medium.

The basophilic or acidophilic property of cell components also depends on the fixative used.

Acidophilic and Basophilic Tissues

The objects, tissues or cell components which are stained with acidic dyes are known as acidophilic, where as those stained with basic stains are basophilic.

Ex- The cytoplasm & its components are acidophilic, and Nucleus chromosomes & DNA are basophilic.

Metachromasia

Some basic dyes stain certain cell components with a colour totally different from their original colour. This property of stain is called metachromasia.

Ex- Toluidene blue, azure-A, Thionine.

These react with mucopolysaccharides, nucleic acids and certain acidic lipids and stain them in different colours.

Mordants and Lakes

Certain dyes can stain the proteins and the cell Cytoplasm only when their action is supplemented by some metals or metallic compounds, which are capable of combining both with proteins of cell and the dye. Such metals or metallic compounds are called mordants.

Ex- usually a double salts of Potassium or ammonium with aluminium or ferric Sulphate.

⇒ The combination of mordant and dye is called Lake

Ex- Ammonium Sulphate (iron alum) is the most common mordant used with haematoxylin and carmine.

Staining for Light Microscope

The selection of stain for the study of cells under light microscope depends upon the nature of materials to be studied, the type of fixative used, and chemical reactivity of the dye.

The concentration of dye and the temperature and pH at which it is most effective are also to be considered.

The Cytoplasmic proteins and carbohydrates are stained with acidic stains, while the nucleus Chromosomes etc are stained with basic dyes.

1. Staining of lipids & steroids

Sudan dyes, fluorescent dyes, aqueous Nile blue, rhodamine B or Phosphiene 3R.

2. Staining with Fluorescent dye

The most commonly used basic dyes are berberine, acridine organge & xellow etc.

Staining for Electron microscopy

Usually no stains are required for electron microscopy, because the image is photographed in black and white.

For obtaining better contrast following methods are used –

(a) **Heavy metal shadow casting**

The material is deposited with vapours of heavy metals like Platinum, chromium or uranium from one side and a shadow is formed on the other side. This is known as heavy metal shadow casting.

(b) **Negative staining method**

For studying Viruses and macromolecules, the specimen is embedded in a droplets of some electron dense medium like Phosphotungstate.

The dense material penetrates into all empty spaces between the macromolecules. These spaces filled with electron dense material appear dark, while the biological macromolecules appear electron transparent.

(c) **Electron staining**

The heavy atoms of osmium tetraoxide lead, uranyl etc are electron stains and combine selectively with certain regions of the cells and appear darker.

Vital staining

Vital staining is the staining of living cells and stains are used are called Vital stains.

The Commonly used Vital stains are Janus green-B, neutral red, methylene blue and trypan blue.

The Vital staining can be done by dissociating the living cells from the body and keeping them in stain (supra Vital staining) or by injecting of the dye into the living organisms (intra vital staining).

Vital staining demonstrates the cytoplasmic structure either –

(i) By Phagocytosis of dye particles and
(ii) By specific staining of the cellular components. Example staining of mitochondria by Janus green.

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