E-content for Programme: M.Sc. Zoology (Semester-II)

Core Course (CC-7): Biochemistry

Unit V: Principles of Histology and Histochemistry
5.2 General principles of staining and types of dyes

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Staining

General principle of staining:

To bring out the outlines and structures more distinct by imparting color, and therefore contrast to specific tissue or cell constituents with their surroundings, to facilitate the histological analysis.

Staining may be attributed to:

(a) Chemical reactions
(b) Physical adsorptions or absorption and
(c) Physico-chemical processes.

Stain or dye:

Any chemical substance which imparts colour and contrast to the tissue.

Classification: Stains or Dyes

Dyes are classified into two main groups (according to source):

Natural dyes: Ex- Haematoxylin (extracted from the heartwood of the tree Haematoxylum), Carmine (derived from female cochineal bug), Orcein (a vegetable dye extract).

Synthetic dyes: These are derived from hydrocarbon benzene.

Components of a synthetic dye:

Chromogen group: Any group that makes an organic compound coloured is called a chromophore. Benzene ring and chromophore is collectively known as chromogen.

Auxochrome group: To turn a colored compound into a dye requires the addition of an ionizable group that will allow binding to the tissues. Such binding groups are called auxochromes.

Classification: Stains or Dyes (Cont'd…)

Non-dye constituents of staining solutions:

Mordants:
Chemicals which are required to bring about the staining reaction are called mordants. Basic mordant reacts with acidic stains and acidic mordant reacts with basic stains. Ex- Phenol in carbol-fuchsin (Ziehl–Neelsen technique) and iodine in the Gram staining method.

Accentuators:
These are group of substances which do not participate in the staining reaction, but accelerates the staining reaction by increasing the selectivity or staining power of the dyes. Effect of this group is due to the change in pH of staining solutions. Ex- Phenol in carbol-fuchsin and in carbol thionin, KOH in Mehtylene blue.

Leuco compounds:
Conversion of a dye into a colourless compound by the destruction of its chromophore. Ex- leucofuchsin used in PAS stain.

Classification: Stains or Dyes (Cont'd…)

According to affinity, dyes are classified as

•**Basic (cationic) dyes:** Basic dyes are cationic and stain anionic or acidic groups such as carboxylates, sulphates and phosphates. Ex- Methylene blue, Safranin, Malachite green, Basic fuschin, Crystal violet.

•**Acidic (anionic) dyes:** Acidic dyes are anionic and stain cationic or basic groups in tissues such as amino groups. Ex- Eosin, Nigrosin, India ink.

•**Neutral dyes:** Neutral dyes are simply compounds of basic and acid dyes. Ex- Giemsa stain, Leishman's stains.

•**Amphoteric dyes:** Amphoteric dyes also have both anionic and cationic groups, but these are on the same ion. These dyes stain both acidic and basic component of the cell at suitable pH. In acidic pH, it behaves like acidic dye and in basic pH, it behaves like basic dye.

Methods of staining

Direct staining:
A process of giving color to the sections by using aqueous or alcoholic dye solution.

Indirect staining:
A process whereby the action of the dye is intensified by adding another agent; either mordant or accentuator.

Regressive staining:
Tissue is initially overstained and then partially decolorized until desired intensity of color is obtained.

Progressive staining:
Tissue is stained for specific periods of time or until it retains the desired intensity of coloring of the different tissue elements.

Methods of staining (Cont'd…)

Counterstaining:
Application of different stains to bring out contrast and background to the staining of the structural components to be demonstrated.

Vital staining:
Selective staining of living cell constituents, demonstrating cytoplasmic structure by intake of the dye particle.

Supravital staining:
Method used to stain living cells immediately after removal from the living body.

Metallic impregnation:
In this process, specific tissue elements are demonstrated, not by stains, but by colorless solutions of metallic salts which are reduced by the tissue, producing an opaque deposit on the surface of the tissue.

Factors affecting staining

- Type and thickness of the section
- Fixation
- Temperature
- pH
- Presence of impurities
- Ripening of staining solutions

References:
- *Bancroft's Theory and Practice of Histological Techniques*
- *Handbook of histopathological and histochemical techniques: C.F.A. Culling*