Stains

'**Stain**' is the common name for the dye or pigment. In general, stains of synthetic nature are called ***dyes***, whereas those extracted from animals or plants are known as ***pigments***.

I-Classification of stains

Stains can be classified according to many considerations including:

A. Origin

1- *Animal Origin*: For example, **carmine** is prepared from the body of an insect.

2- *Plant Origin*: As, **hematoxylin** is prepared from a special Mexican tree.

3- *Synthetic*: For example, **eosin**.

B. Mode of Action

1- Physical Staining

This type of stains is **physically combined** with its corresponding structure. For example, **Sudan III** is a physical stain consisting of a powder usually **dissolved in** alcohol, **but is more soluble in** fats. Therefore, when it comes near fats, it leaves the alcohol to be dissolved in fats giving rise to an orange-yellow color. Such special affinity of the used dye to certain parts of the stained tissue is known as '**Elective Staining**'.

2- Chemical Staining

Whereas the stain is **chemically reacted** with its corresponding structure (c.f. **Feulgen reaction**). Chemical stains are, in fact, neutral salts, each with an **acid** and a **basic radical**.

According to the color-inducing radical, chemical stains can be classified to

a. Acid Stains

- In acid stains, the color is given by the **acid radical** (e.g. **eosin**).
- Substances having the affinity for acid stains are known as **acidophilic** (e.g. **cytoplasm**).

b. Basic Stains

- In basic stains, the color is given by the **basic radical** (e.g. basic fuchsin, hematoxylin).
- Substances having the affinity for basic stains are called **basophilic** (e.g. DNA).

c. Neutral Stains:

- These stains are able to provide two different colors at the same time whereas each of their **anion and cation** can produce a different color.

- Such neutral stains are widely employed for staining of blood cells.

C. End Color of the stained Content

1- *Orthochromatic Stains:* These react with the cell content giving rise to the **same color** of the stain (e.g. staining of cytoplasm by methylene blue).

2- *Metachromatic Stains*: Whereas the reaction between the stain and the content results in a **new color** different from that of the stain itself (e.g. staining of starch by iodine solution). This reaction is called '**Metachromasia**'.

II- Staining Techniques

These can be categorized according to:

A. Number of the used stains:

1- *Monochromatic (simple) Staining:* Whereas **one dye** is used.

2- *Polychromatic Staining:* Whereas **many dyes** are used. Accordingly, different structures become stained in different colors.

This may be applied through:

a) *Staining in succession*: Whereas the stains are used **one after the other**. In double and multiple staining methods, two and several stains are used respectively.

b) *Staining by a mixture of dyes*: Whereas the staining takes place **at the same time**. For example, the trichrome stain involves three types of stains.

B. Nature of the Stained Material

1- *Staining in toto:* This means the staining of the **entire** mass of the tissue.

2- *Section staining:* Whereas the specimen is **firstly sectioned** and then sections are stained.

3- *Vital Staining:* Vital stains are those which can **diffuse through the living (or fresh) tissues** and stain them with no harmful effects. Therefore, they must be '**Non-toxic**'.

a) Intravital Stains: These can stain living cells **inside** the living body (i.e. ***in vivo***).

b) Supravital Stains: These can stain living (or fresh) cells **outside** the body (i.e. ***in vitro***).

C. Applied Method

1- ***Regressive (Retrogressive) Method:*** Whereas **overstaining** of sections was firstly applied then followed by **removal of excess** stain until the desired intensity is reached (e.g. staining by crystal violet in **paraffin technique**).

2- ***Progressive Method:*** Whereas sections are **stained gradually** until they reach the right intensity (e.g. staining by basic fuchsin in **Feulgen technique**).

3- ***Staining through the use of a Mordant:*** Whereas treatment of the tissue with a special mordant is needed to allow the **color of the stain to be more fixed** to this tissue (e.g. iodine solution for crystal violet in bacterial Gram staining and paraffin technique).

4- ***Special Methods:*** As those applied for some types of microscopy (e.g. **fluorescent** and **electron microscopy**).

III- Selected Techniques for Cytological Studies

A. Feulgen Squash Technique (for mitotic division)

The Feulgen squash technique has become firmly established in cytological research.

Procedure:

a. Root tips were ***cut*** and ***fixed*** in acetic–alcohol (1:3) for 15 min, then ***stored*** in 70% alcohol.

b. ***Wash*** the used roots in few changes of distilled water for 5 min.

c. ***Hydrolyze*** in **1N HCl at 60 ˚C** for 6-12 min. ***Wash*** in distilled water to ***get rid*** of the acid.

d. ***Stain*** for 2 hr (**1/2 hr in lab**) in leucobasic fuchsin, which is prepared as follows

i. 1 g of basic fuchsin was dissolved in 200 ml boiling distilled water; shake well, cool to 50 ˚C and then filter.

ii. To the filtrate add 30 ml of 1N HCl and 3 g of potassium metabisulphite ($K_2S_2O_5$) and then allow bleaching for 24 hr in tightly stopper bottle in the dark.

iii. Shake, filter and store in the dark.

e. ***Squash* in 45% acetic acid** as follows

i. The meristematic region, as indicated by the ***deeply stained part*** of the root tip, was cut into thin slices and put on a clean slide in a drop of 45% acetic acid.

ii. The coverslip was placed on the material ***by gentle tapping***, followed by applying pressure under several thicknesses of blotting paper allowing ***no movement of the coverslip*.**

iii. The material was ***spread into one layer*** of flattened cells.

N.B.

i. ***Optimum hydrolysis*** releases aldehyde groups from the deoxypentose sugar of DNA which, in turn, react with the staining reagent giving rise to the maximum results of the purple dye.

ii. ***Excess hydrolysis*** produces a negative reaction (as indicated by the faint color). This is due to its destructive effect on the structure of the nucleic acid.

iii. On the other hand, ***less hydrolysis*** produces the same result due to the less liberated aldehydic groups.

iv. It should be recalled that acid hydrolysis *is sufficient to remove RNA* **not** *DNA*. Thus, the reaction *is positive* in nucleus *and negative* in nucleoli and cytoplasm.

Permanent Preparations can be made as follows

1. Invert slide and cover downwards in a Petri-dish containing 10% acetic acid until cover separates. Quick and proper separation can be made by using liquid nitrogen or solid CO_2 (dry ice).
2. Pass both slide and cover in a series of acetic-alcohol at ratios of 1:1, 1:3 and 1:9 for 2 min at each step, then they were placed in absolute alcohol for 5 min.
3. Slide and its cover were passed in a series of alcohol-xylol at ratios of 3:1, 1:1 and 1:3, then in xylol, respectively for 10 min. at each step.
4. Recombine slide and cover by mounting in Canada balsam.

B. Acetocarmine Technique (for meiotic division)

1. Preparation of the stain

Dissolve 1g carmine in 100 ml of boiling 45% acetic acid, cool, filter and store in refrigerator.

2. Procedure

a. ***Flower buds*** were collected and fixed in ***Farmer's fluid*** (acetic-alcohol solution in ratio of 1:3) for 24h then ***kept*** in 70% alcohol.

b. By the use of ***steel needles***, content of ***proper anther*** were ***squeezed*** on a slide in few drops of the stain and a cover glass was lowered on the contents.

c. ***Contents of anthers*** were ***smeared*** into a thin film by pressing the slide gently between different thicknesses of filter paper

d. Slides were ***heated gently***, and ***quickly***, several times over an alcohol flame (***avoid boiling***)

e. ***Drain*** excess stain, ***examine*** anther contents (***acetocarmine smears***) with a microscope and ***seal*** the edges of the cover glass with paraffin wax (or nail polish) then ***store*** in a refrigerator.

The ***color improves*** in few days, reaches ***maximum*** intensity, and then begins to deteriorate.