Notes

12

METACHROMATIC STAINING

12.1 INTRODUCTION

There are certain basic dyes belonging to aniline group that will differentiate particular tissue components by giving them a different color to that of original dye. The phenomenon is known as metachromasia.

OBJECTIVES

After reading this lesson, you will be able to:

- define metachromasia
- describe the process metachromasia
- know common metachromatic dyes
- describe the factors enhancing metachromasia.

The tissue element reacting in this manner are said to be exhibiting metachromasia.

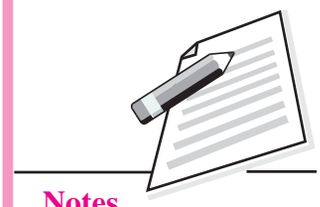
- The generally accepted explanation of this phenomenon is that change in color is due to polymerization.
- Sulfated substances are highly metachromatic e.g. Mast cell granules.
- Mast cells contain Heparin which is highly sulfated.

Some of the common metachromatic dyes are:

- Methylene blue, Methyl violent
- Thionin, Crystal violet
- Toluidine blue

12.2 METACHROMASIA

Metachromasia takes place when certain negatively charged groups on the tissue react with cationic dyes. On polymerization the original colour of the dye changes to another colour (eg mast cell stain pink with toluidine blue).



Metachromatic Staining

Thionin and toluidine blue dyes are commonly used for quick staining of frozen selection using their metachromatic property to stain nucleus and cytoplasm differently.

Metachromasia is enhanced when intermolecular distances are reduced.

Factors which enhance metachromasia are

1. Increasing concentration of dye.
2. Decreasing temperature.
3. pH
4. Water a polar solvent, contributes to the efficiency of van der Waal's forces by which the molecules are held together.

In tissues, where there is a high concentration of anions e.g. in sulphated mucopolysaccharides, the cationic dye molecules may be held in such close proximity to one another that van der Waal's forces can exert their influence and cause the dye to polymerize. Consequently the colour changes from blue to red.

Tissue components often demonstrated by metachromatic stains:

- Amyloid material, Mast cell granules
- Mucin Cartilage

Amyloid Stain -Various stains are used to demonstrate amyloid

12.3 CRYSTAL VIOLET STAIN FOR AMYLOID

Aim: To demonstrate amyloid in tissue sections.

Principle: Amyloid (a glycoprotein) exhibits metachromasia in tissue sections when stained with crystal violet and other cationic dyes.

Control: ositive control.

Reagents

Crystal violet solution

Stock solution

- Crystal violet 14gm
- 95% alcohol 100ml

Working solution

- Stock solution 10ml

- Distilled water 300ml
- Concentrated hydrochloric acid 1ml

Procedure

- Deparaffinize and bring the sections to water.
- Put working crystal violet solution for 1 to 2 minutes and check under microscope.
- Rinse in tap water.
- Mount in water or in water soluble media.
- Put on the coverslip seal the edges with nail polish (Do not let it dry.)

Result

- Amyloid purple violet
- Other tissues blue

12.4 CONGO-RED STAIN FOR AMYLOID

Aim: To demontrate amyloid in tissues.

Principle: Diazo dye attaches itself to amyloid fibrils. The union is affected by H bonds between the OH groups of amyloid and amino side groups of the dye. Congo red dye forms non-polar hydrogen bonds with amyloid. The green birefringence of congo red stained amyloid by polarized light is considered diagnostic of amyloid.

Control: Known positive tissue

Reagents

Congo red solution

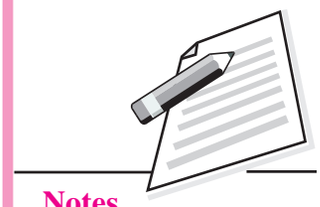
- Congo red 1.0gm
- Distilled water 100ml

Saturated solution of Lithium Carbonate

- Lithium carbonate 1.3gm
- Distilled water 100ml

Procedure

- Bring section to water.



- Pour congo red solution for 20 minutes.
- Pour off the solution and cover the slide with lithium carbonate for 1.5 minutes to differentiate.
- Wash with water.
- Counter-stain with hematoxyline for 5 minutes.
- Differentiate with 1% acid alcohol.
- Wash in running tap water.
- Dehydrate, clear in xylene and mount in DPX.

Result

- Amyloid — bright red which gives apple green birefringence in polarized light.
- Nuclei — blue
- Other structures — unstained to yellow

Notes

1. Sections must be cut at 8 to 10 microns for birefringence
2. Solution must be filtered through glass wool, not paper filters for birefringence to occur
3. Tissue fixed in solutions other than formalin may display false positive birefringence

INTEXT QUESTIONS 12.1

1. ..... & ..... dyes are commonly used for quick staining of frozen section
2. Metachromasia is enhanced when ..... are reduced
3. Tissues demonstrated by metachromatic stain are ....., ... & .....
4. ..... & ..... alcohol is used as fixation in crystal violet stain
5. ..... and ..... are used for demonstrating amyloid in tissues

WHAT HAVE YOU LEARNT

- Basic dye belonging to aniline group differentiates particular tissue components by staining them a different colour to that of original dye by phenomenon known as metachromasia
- Metachromasia occurs due to polymerization
- Methylene blue, methyl violet, thionin, crystal violet and toluidine blue are the metachromatic dyes
- Thionin and toluidine blue dyes are commonly used for quick staining of frozen section
- Metachromasia is enhanced when intermolecular distance are reduced
- Increasing concentration of dye, decreasing temperature, pH, water enhance metachromasia
- Amyloid material, mast cell granules, and mucin cartilages are demonstrated by metachromatic stains
- Crystal violet, cango red stain are used for demonstrating amyloids
- In metachromatic stain amyloid appear pink and the surrounding tissues stain purple
- Crystal violet stain and congo-red stain are used to demonstrate amyloid in tissues.

TERMINAL QUESTIONS

1. What are the dyes used in metachromasia?
2. What are the factors which enhance metachromasia?
3. Explain the procedure of metachromasia.
4. Explain briefly stains used for demonstrating amyloid in tissues.

ANSWERS TO INTEXT QUESTIONS

12.1

1. Thionin and toluidine
2. Intermolecular distance
3. Amyloid material, Mast cell granules & Mucin Cartilage
4. Carnoy's and absolute
5. Crystal violet, congo – red stain.