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Techniques in Histopathology

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CBSPD Dedicated to Education
CBS Publishers & Distributors Pvt Ltd

Chapter

9

Pigments

- Pigments: Definition and classification
- Formalin
- Mercury
- Malaria pigment
- Haemosiderin
- Melanin

Pigments are defined as "substances occurring in living tissue and having its own colour". There are different types of pigments present in the body in normal and pathological conditions. These pigments are classified in three groups:

1. Artefact pigments
   - Formalin pigment
   - Mercury pigment
   - Stain precipitate
   - Malaria pigment
   - Chrome deposit
2. Endogenous pigments
   - Haematogenous
     - Haemoglobin
     - Bile pigments
     - Haemosiderin
     - Porphyrins
   - Nonhaematogenous
     - Melanins
     - Chromaffin
     - Lipofuscins
     - Dubin-Johnson pigment
   - Endogenous minerals
     - Iron
     - Copper
     - Calcium
3. Exogenous pigments
   - Carbon
   - Asbestos
   - Silica

Some of the common pigments are described here.

FORMALIN

This brown-black pigment is produced when tissues, especially those containing large amount of blood, are fixed in acid solution of formaldehyde.

Removal of Formalin Pigment

1. Deparaffinise the section
2. Wash with absolute alcohol.
3. Place in closed container of saturated alcoholic picric acid solution for ½–2 hours depending upon the amount of pigment.
4. Wash with 95% alcohol.
5. Wash with distilled water.
   Now the sections are ready for staining.

Note: The problem of formalin pigment deposition can be reduced by the use of neutral buffered formalin.

MERCURY

Fixatives containing mercuric chloride may produce dark-brown extracellular deposits in the section.

Removal of Mercury Pigment

1. Bring the section to water.
2. Treat with Lugol's iodine for 5 minutes.
3. Wash with water.
4. Rinse with 5% sodium thiosulphate (hypo) to bleach.
5. Wash with tap water for ½ to 1 minute.
6. Wash with distilled water for ½ to 1 minute.

MALARIA PIGMENT

Malaria pigment is also picric acid soluble.

So, method of removal is same as for formalin pigment but the time required to remove malaria pigment may be longer than required for formalin pigment. The other difference is that malaria pigment is intracellular not extracellular.

HAEMOSIDERIN

Haemosiderin is an iron containing pigment that appears as golden-brown granules in routine H&E sections. Normally, a small amount is present in cells of reticuloendothelial system. Ferric form of iron in the form of ferric hydroxide is present in haemosiderin. Perls Prussian blue reaction method is used to demonstrate haemosiderin.

Perls Prussian Blue Reaction for Ferric Form of Iron and/or Haemosiderin

Reagents:

Acid ferrocyanide solution

1% aqueous potassium ferrocyanide	20 ml
2% aqueous hydrochloric acid	20 ml

Technique:

1. Bring sections to water.
2. Rinse in distilled water.
3. Transfer sections to freshly prepared acid ferrocyanide solution for 10–30 minutes.
4. Wash in distilled water.

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Technique:

1. Bring sections to water.
2. Rinse in distilled water.
3. Transfer sections to freshly prepared acid ferrocyanide solution for 10–30 minutes.
4. Wash in distilled water.

5. Counterstain with 0.5% aqueous neutral red.
6. Wash in distilled water immediately.
7. Dehydrate, clear and mount.

Results (Fig. 9.1):

Haemosiderin or ferric iron	blue
Nuclei	red

Note: Stain appropriate positive control section simultaneously.

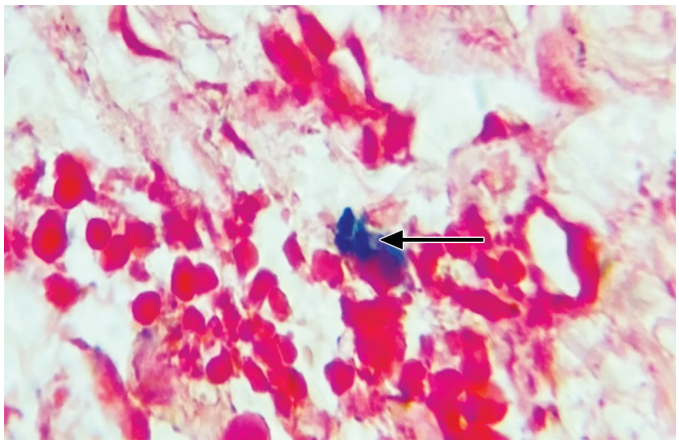

Fig. 9.1: Prussian blue stain for iron (arrow)

MELANIN

Melanin is a brown-black pigment present in normal skin and eye. It is also found in benign and malignant melanocytic tumours and other diseases of skin.

Masson-Fontana-Silver Method

Reagents:

Silver solution

10% aqueous silver nitrate	20 ml
Triple distilled water	20 ml

Concentrated ammonia

5% aqueous sodium thiosulphate

0.5% aqueous neutral red

Take aqueous silver nitrate in a flask. Add conc. ammonia drop by drop to silver nitrate solution until the precipitate is almost dissolved leaving only a slight opalescence. Add distilled water and filter the solution in a dark bottle.

Notes:

- Preferably silver solution should be prepared fresh or can be kept in the refrigerator for few days.
- Store ammoniacal silver solution carefully because of its explosive hazards.

Technique:

1. Bring solution to water and wash thoroughly.
2. Place the sections in a Coplin jar containing silver solution and keep in dark for 12–18 hours or overnight.
3. Wash in several changes of distilled water.
4. Treat sections with 5% aqueous sodium thiosulfate for 1–2 minutes.
5. Wash in running tap water for 2–3 minutes.
6. Counterstain with 0.5% aqueous neutral red for 5 minutes.
7. Rinse in distilled water.
8. Dehydrate, clear and mount.

Results (Fig. 9.2):

Melanin	black
Nuclei	red

Note: Clean the glassware thoroughly as silver solution may react with residue left in the glassware.

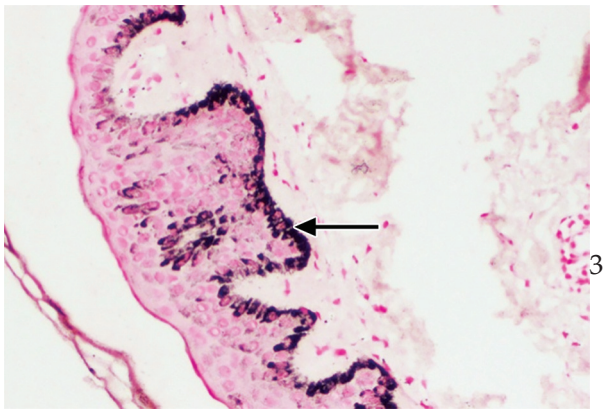

Fig. 9.2: **Masson-Fontana stain** for melanin (arrow)

Melanin Bleach

Melanin removal is important to study cellular details in tissue that are heavily pigmented with melanin.

Reagents:

Potassium permanganate solution:

Potassium permanganate	0.15 g
0.3% sulphuric acid	50 ml

(To be prepared fresh every time)

1% oxalic acid

Technique:

1. Deparaffinize the section
2. Hydrate and bring section to distilled water.

3. Treat the section with potassium permanganate for 2–4 hours.
4. Simultaneously put a control slide in distilled water till step 6.
5. Place the slides in 1% oxalic acid for 2–4 minutes or until colourless.
6. Wash in tap water.
7. Stain test and control both the slides with H&E stain.
8. Dehydrate, clear in xylene and mount.

Results:

If the pigment is melanin it gets bleached and will not be present in test slide