Pigments and minerals

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Classification of pigments

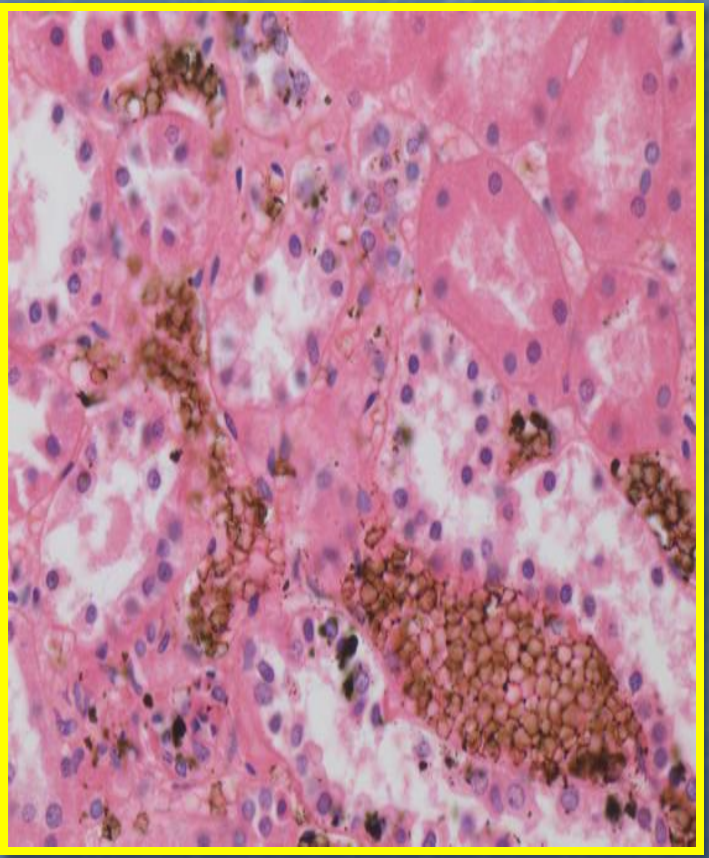
1. Artefact pigments
   * usually as a result of fixation eg formalin pigment
2. Exogenous pigments
   * pigments or minerals that are formed externally and taken into the body eg coal dust, copper
3. Endogenous pigments
   * pigments that are formed within the body
     i. haematogenous – haemosiderin, bile
     ii. non-haematogenous – melanin, lipofuschin

1. Artefact pigments

- Most commonly as a result of fixation
- Normally lie on top of tissues and not within cells
  - Formalin
  - Mercury
  - Chrome
  - Picrates

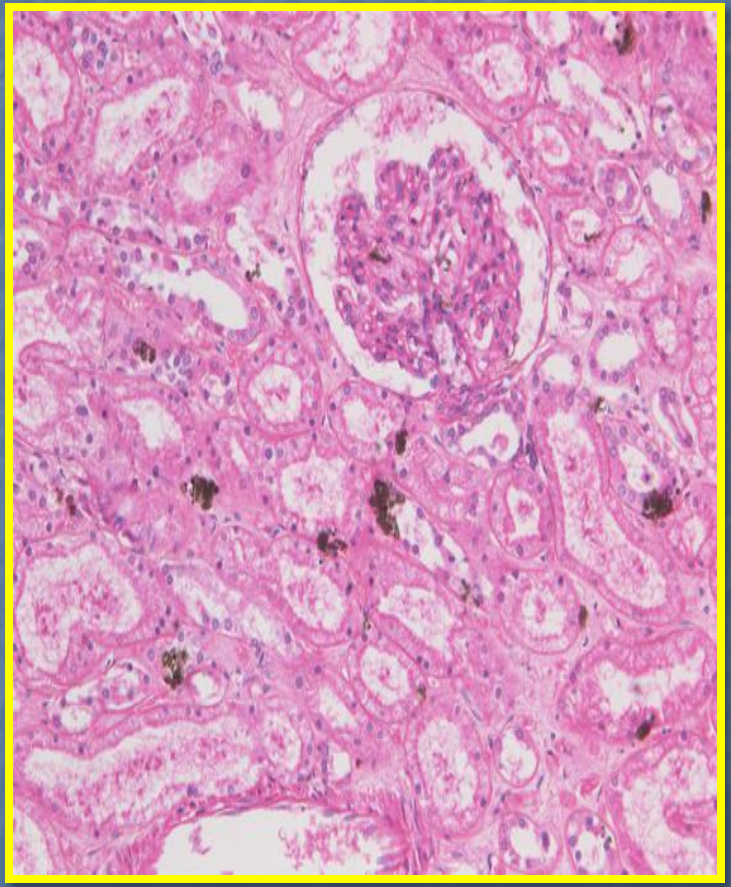
Formalin pigment

- Result of fixation of tissue in acid formalin
- Also called acid haematin
- Resistant to strong acids
- Usually associated with blood-containing tissues
- Can be removed from sections using alcoholic picric acid



Mercury pigment

- Occurs with mercury-containing fixatives such as Zenker's and Heidenhain's Susa
- Can be removed from sections using iodine followed by sodium thiosulphate (Hypo) solution



Chrome and picric acid pigments

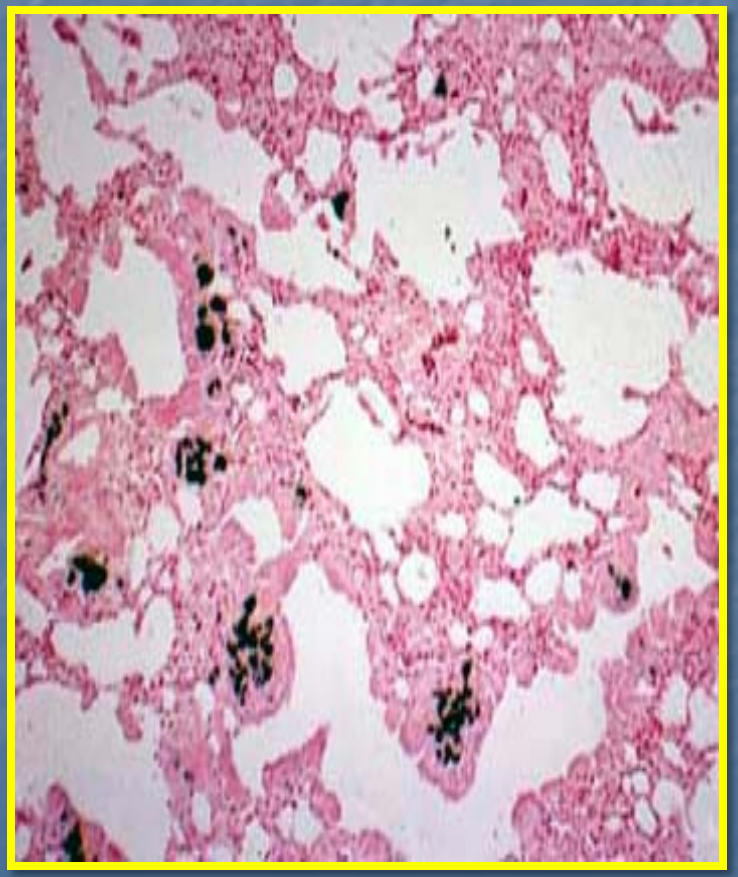
- Chrome pigment
- Consequence of fixation in solutions containing potassium dichromate eg Zenker's fixative
- Can be removed from sections by thoroughly washing in water prior to dehydration

- Picrate pigment
- Consequence of fixation in solutions containing picric acid eg Bouin's fixative
- Can be removed with saturated aqueous lithium carbonate

2. Exogenous pigments

Pigments that are formed externally and taken into the body

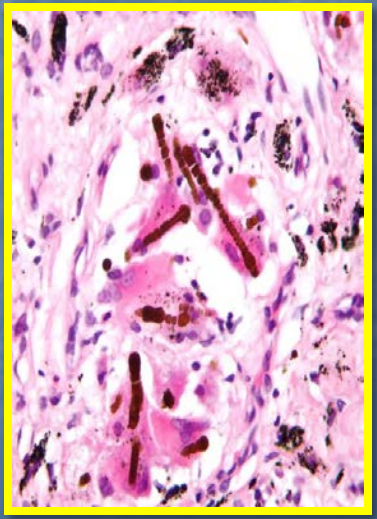
- Carbon is the most common as a result of accumulation from inhaled pollution, coal dust and cigarette smoke
- It resists bleaching and is insoluble in acid



Exogenous pigments

- Tattoo
- Found in skin and associated lymph nodes

- Asbestos
- Become coated with iron in the lungs (bodies) and stain positive with Perl's



Minerals

- Metallic and non-metallic ions necessary for growth and other bodily functions
- Calcium, ferrous and ferric iron, copper, phosphate and carbonate are the most common
- Some can be deposited pathologically in tissue eg gold, silver, copper and lead
- Minerals in tissue can be studied using the technique of microincineration

Microincineration

- Study inorganic substances found in tissue
- Cut paraffin sections as usual and mount on high temperature glass slides
- Slides are incinerated up to 650°C and then allowed to cool
- Slides are removed from oven and coverslipped with glycerol mountant
- Slides are examined under the microscope

Minerals

- Calcium
- Abnormal deposits can be associated with atherosclerosis, sarcoid, TB and some tumours
- Also found in joints in chondrocalcinosis
- Demonstrate with von Kossa silver method

Stain chemistry

Von Kossa method (calcium)

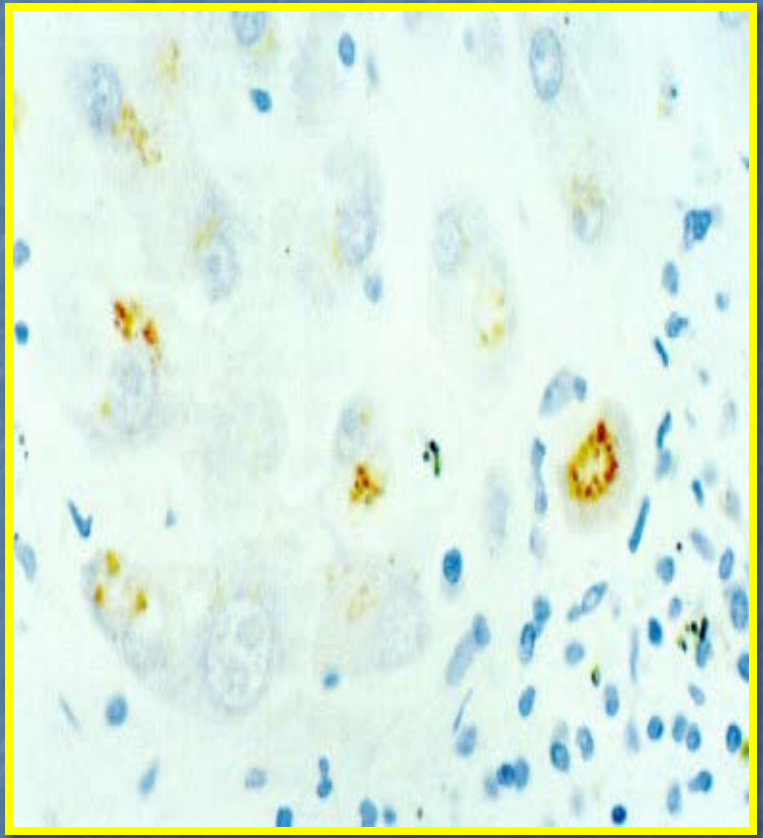
This is an indirect way of detecting calcium where silver nitrate replaces the carbonate anions of calcium salts (silver substitution method). Sunlight reduces the silver salt to visible metallic silver, and unreduced silver is removed by sodium thiosulphate

Rhodanine method (copper)

Rhodanine demonstrates the protein to which the copper binds rather than the copper itself. Therefore, although considered more sensitive, rhodanine may be less specific and false-positive results may be obtained

Minerals

- Copper
- Accumulates in liver and other organs in Wilson's disease
- Also found in primary biliary cirrhosis and other liver disorders
- Stain with rhodanine (or rubeanic acid)



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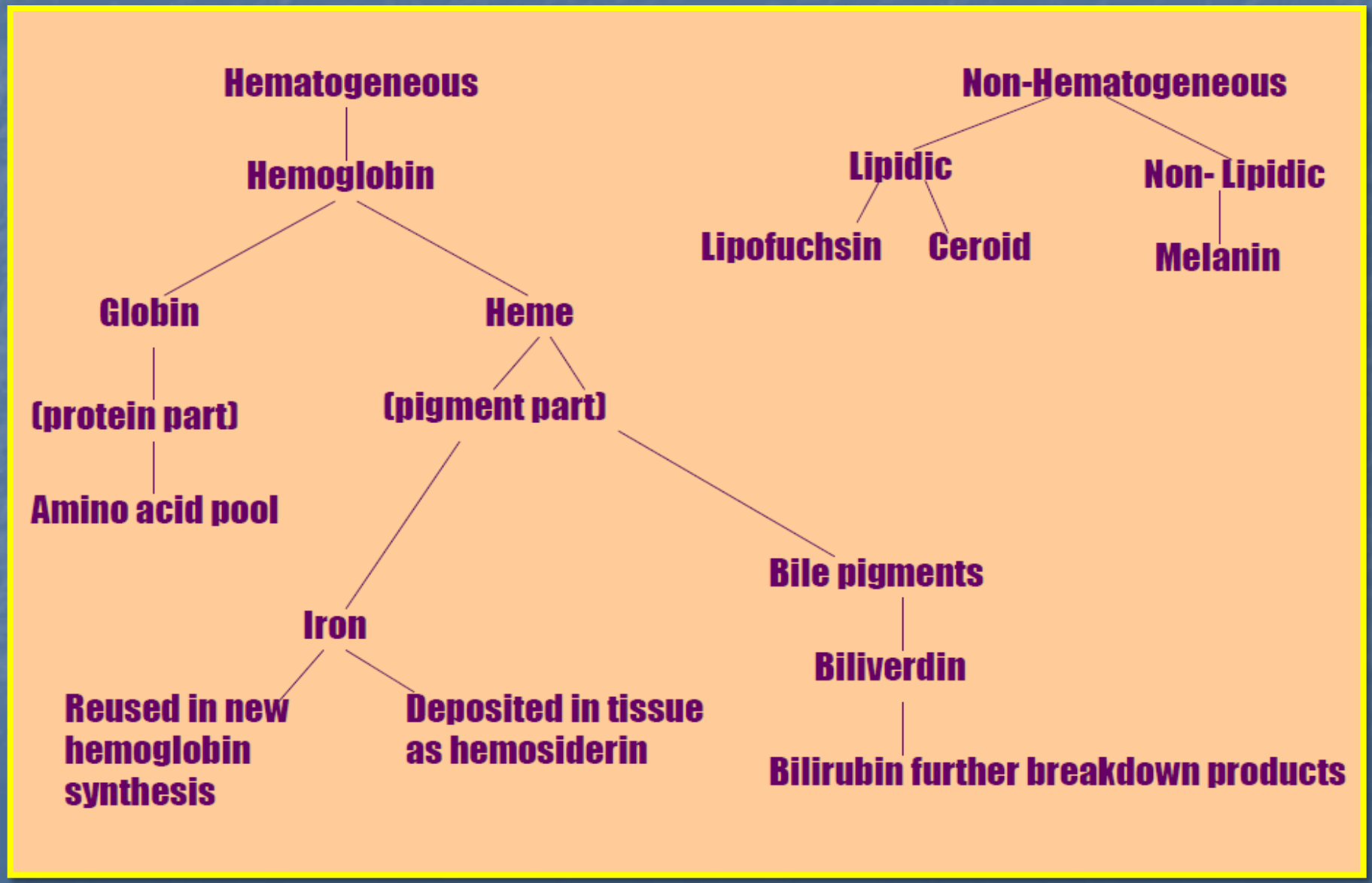
3. Endogenous pigments

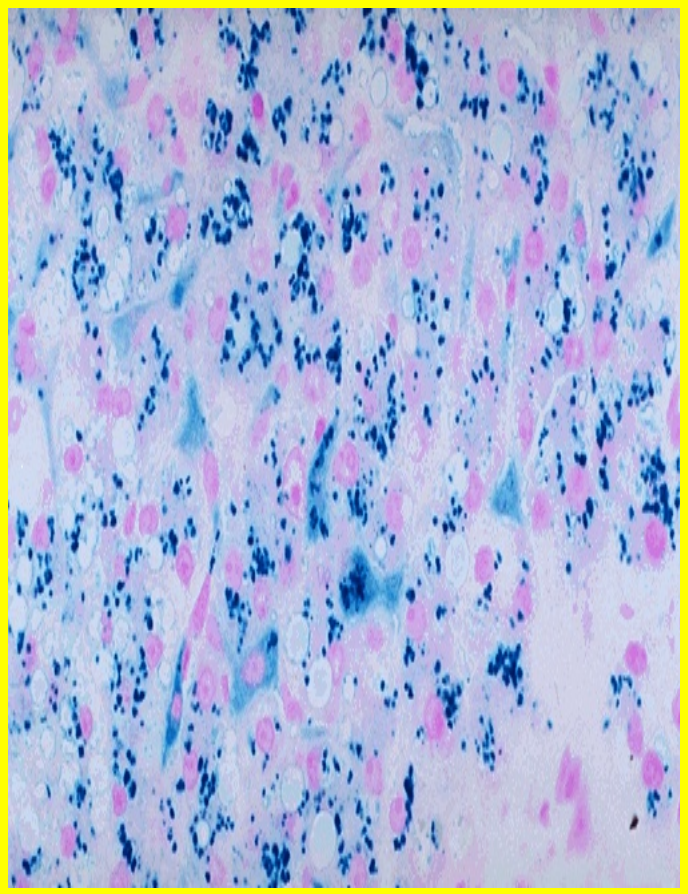


Haematogenous pigments

- Endogenous pigments derived from blood
- Main ones are haemoglobin, iron, haemosiderin, haematoidin and bile pigments
- Haemoglobin breaks down into two parts: globin and haem (iron-containing pigment part)
- The haem portion further splits into iron (haemosiderin) and bile pigments (biliverdin)
- Haematoidin (similar to bilirubin) is formed in tissue as a result of haemorrhage

Haematogenous pigments

- Haemosiderin
- Stored as ferric iron in bone marrow and spleen
- Can be demonstrated using Perl's prussian blue stain which detects ferric iron
- Excessive absorption of dietary iron causes haemochromatosis
- Ferrous iron is demonstrated by Turnbull's blue



Stain chemistry

Perl's stain – ferric iron

Ferric iron is released from haemosiderin with hydrochloric acid, forming ferric chloride. The iron reacts with potassium ferrocyanide to form ferric ferrocyanide (Prussian blue)

Turnbull's blue stain – ferrous iron

Treatment with acid solution of potassium ferricyanide. Any ferrous iron present reacts to form an insoluble bright blue pigment called Turnbull's blue (ferrous ferricyanide)

Schmorl's technique

Reducing substances present in tissue reduce the ferric ions (Fe^{3+}) present in the staining solution to ferrous ions (Fe^{2+}) which immediately combine with ferricyanide present in the staining solution to form an insoluble precipitate of ferrous ferricyanide (Turnbull's blue)

Haematogenous pigments

- Bile and bilirubin
- Biliverdin also results from destruction of RBCs
- Biliverdin (green) is transported to the liver
- Undergoes reduction to bilirubin (yellow-brown)
- Can be demonstrated by Hall's method which uses Fouchet's reagent

Stain chemistry

Hall's method (bile and bilirubin)

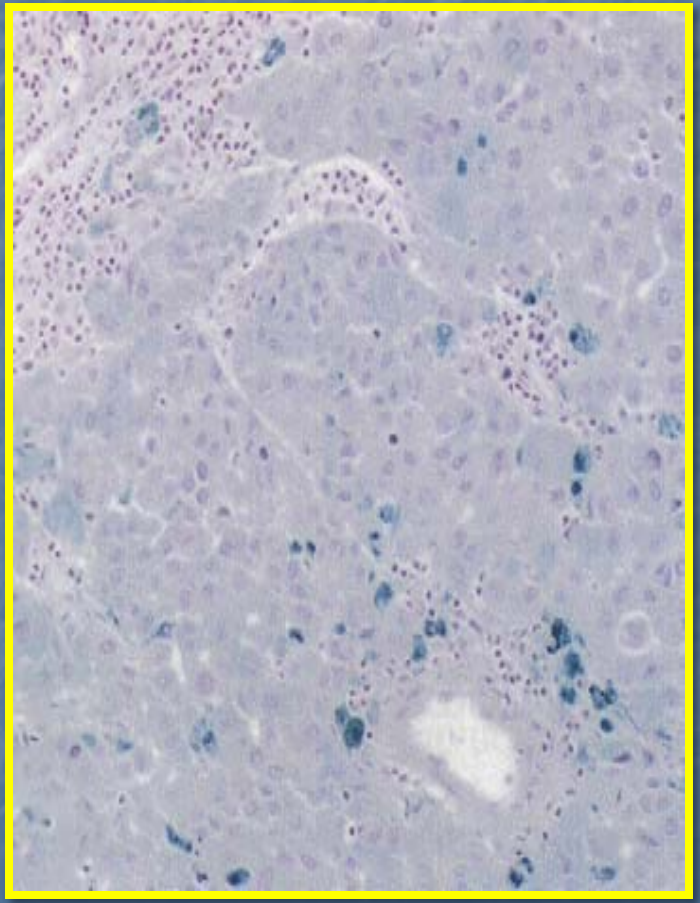
A specific and easily identifiable green colour develops when bilirubin is oxidized to biliverdin in an acid medium. This oxidation reaction is rapidly accomplished by ferric chloride in trichloroacetic acid medium (Fouchet's reagent). Biliverdin is then stained emerald green by acid fuchsin in van Gieson solution

Non-haematogenous pigments

- Endogenous pigments not derived from blood
- There are two types of non-haematogenous pigment
- Lipidic (lipofuschin and ceroid)
- Non-lipidic (melanin)

Lipidic pigments

- Lipofuschin
- Wear and tear pigment, usually found in the heart and liver
- Stain with Schmorl's stain. Also use oil red O, aldehyde fuchsin, Sudan black B and PAS
- Ceroid stains similar but can be differentiated by staining positive with ZN



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Non-lipidic pigments

- Melanin is the most important and is found in skin, hair, retina and parts of the CNS
- Melanin can be bleached in the tissue section by treatment with hydrogen peroxide or potassium permanganate and oxalic acid
- Pathologically, melanin is found in the cells of malignant melanomas and various benign naevi
- Melanin reduces silver nitrate to metallic silver in the Masson Fontana method (argentaffin)

Stain chemistry

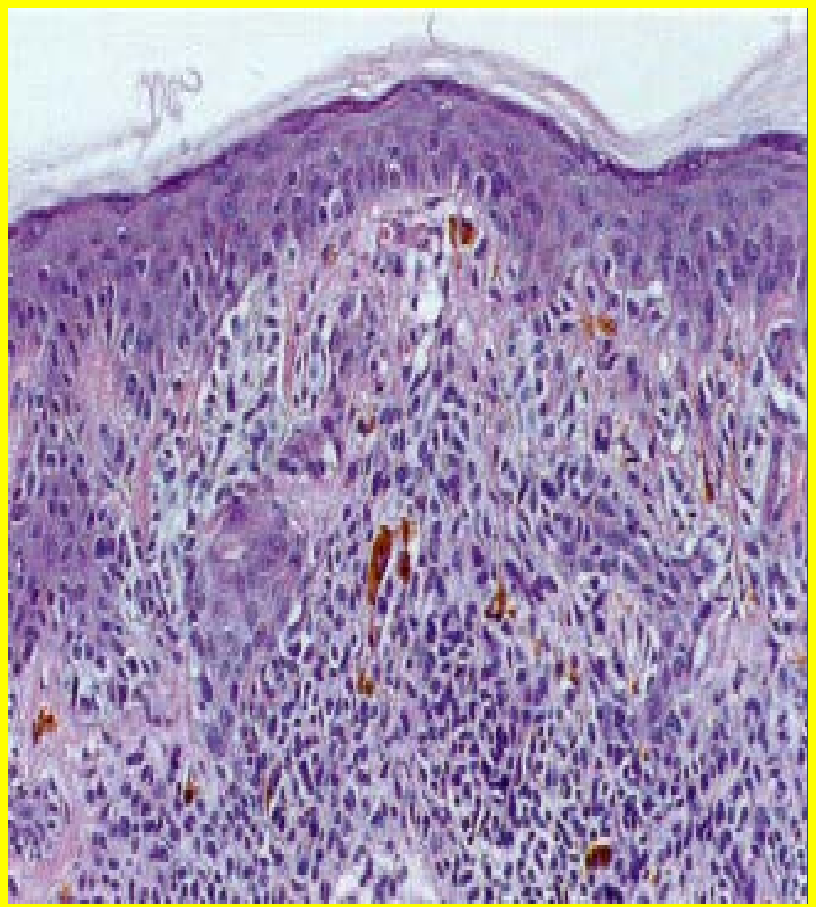
Masson Fontana and Methenamine silver (argentaffin)

Certain tissue components such as melanin possess the ability to bind silver ions from a silver solution and to reduce it to visible metallic silver without the need for a separate reducing agent

Grimelius stain (argyrophil)

Certain tissue components such as reticulin have the ability to bind silver ions from solution but no inherent ability to reduce the silver to its visible metallic form. An external or chemical reducer is used for this purpose. These tissue components are referred to as argyrophil

Melanin



Endogenous deposits

- Urates
- Uric acid crystals deposited in tissues or around joints in gout
- Demonstrated with methenamine silver
- Birefringent under polarizing microscope