

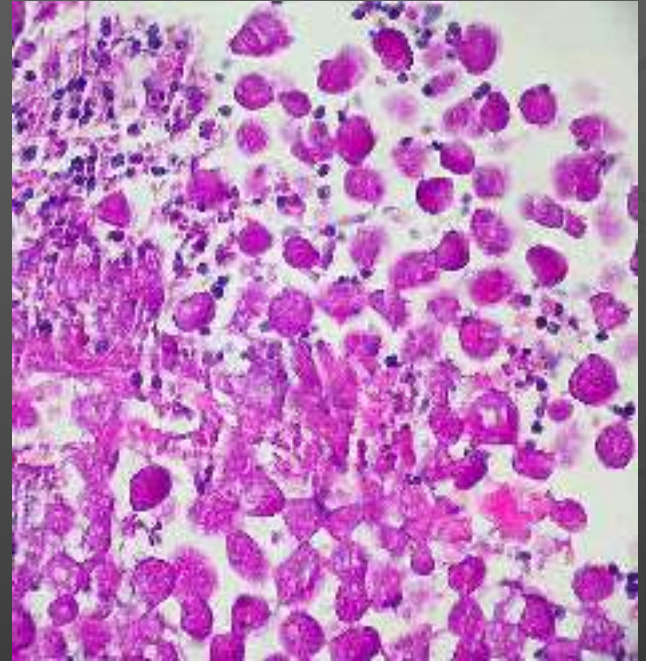
# SPECIAL STAINS IN HISTOPATHOLOGY



DR RAZANA MOHD ALI

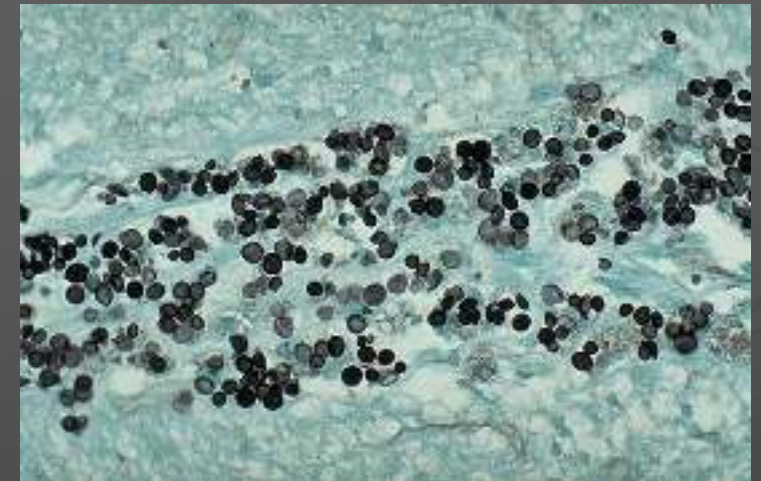
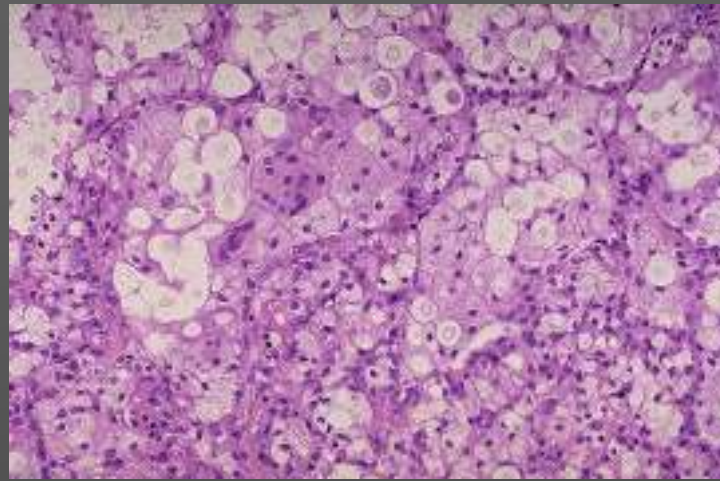
# SPECIAL STAINS IN HISTOLOGY

- **STAINS FOR MICROORGANISM**
- **CONNECTIVE TISSUE STAINS**
- **STAINS FOR PIGMENTS AND MINERAL**





# INTRODUCTION

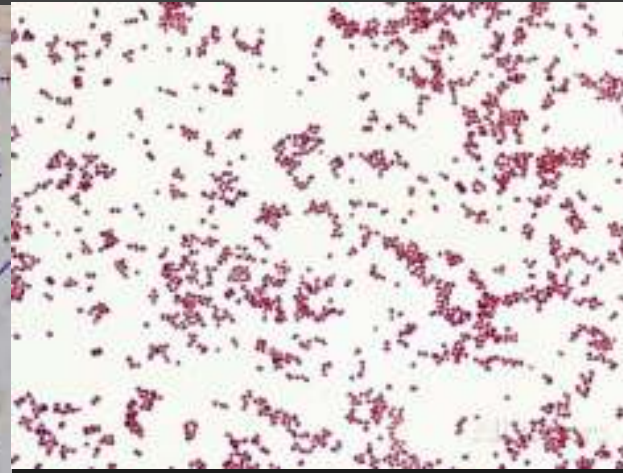
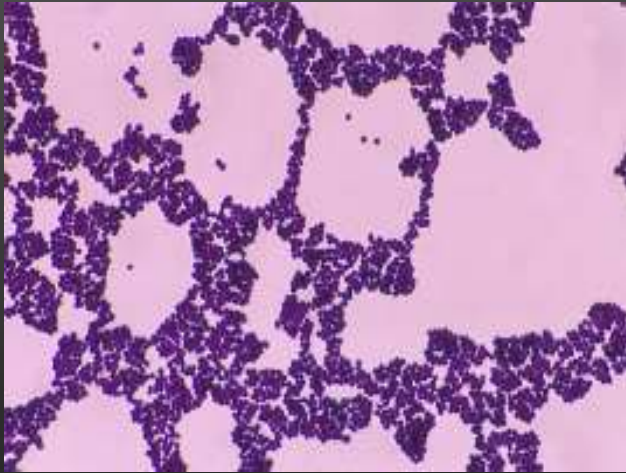


**cryptococcus**

- Most infectious agents are rendered harmless by direct exposure to formal saline (fixative).
- Standard fixation process should be sufficient to kill microorganisms.
- H&E stains may stain many organisms. Some require special techniques to demonstrate their presence.
  - Reason: small size, hydrophobic, weakly charged (mycobacteria, spirochaetes and cryptococci)
- Microorganism can also be detected through immunohistochemistry.

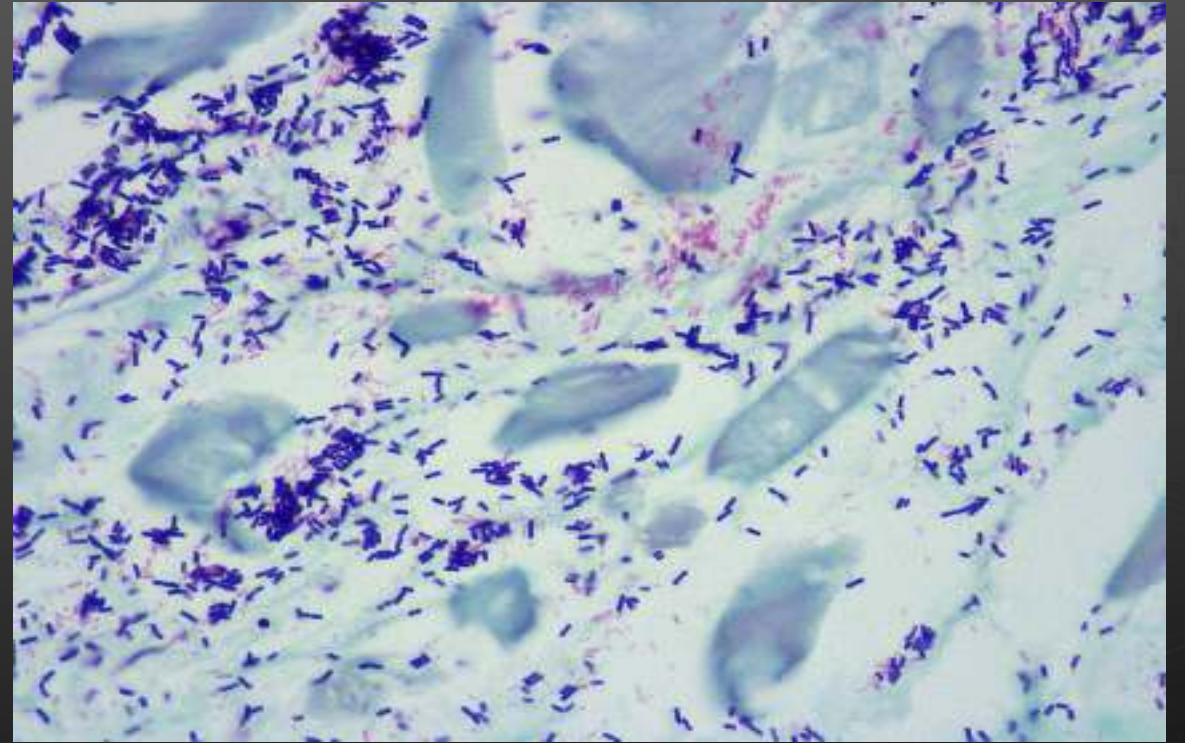
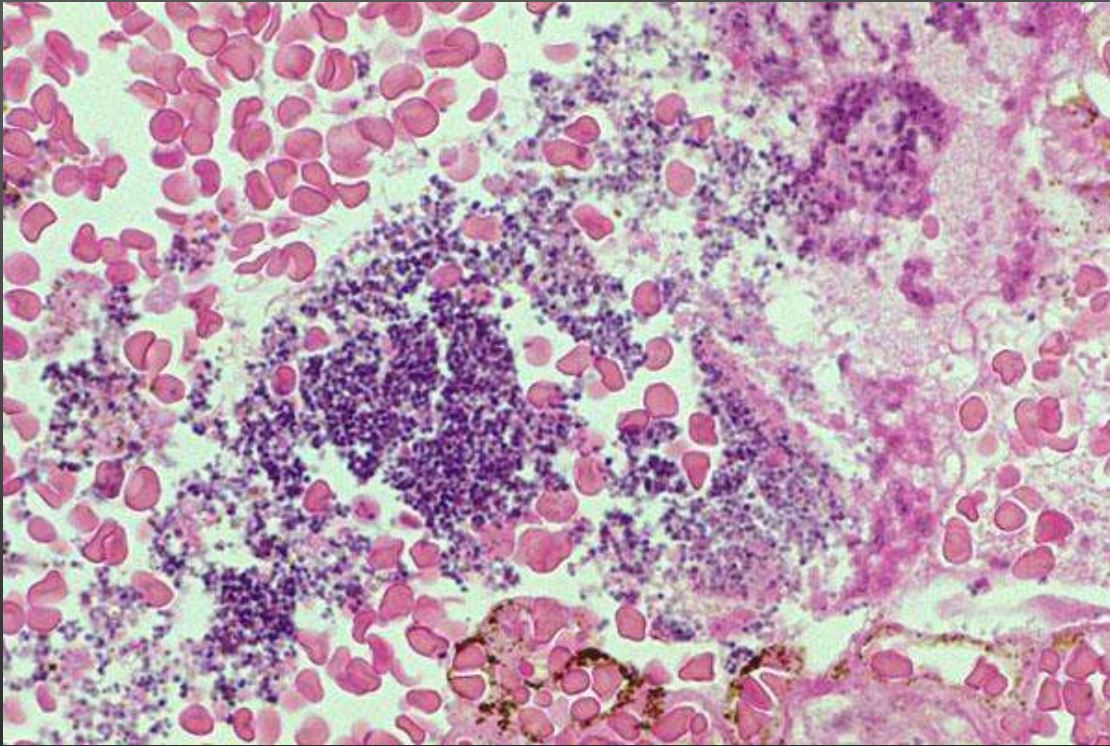
# STAINS FOR MICROORGANISM

STAIN	COMPONENTS STAIN	POSSIBLE USES	
GRAM	GM POSITIVE – BLUE /PURPLE GM NEGATIVE – RED NUCLEI – RED OTHER TISSUE – VARIABLE, YELLOW	Identification of BACTERIA, ACTINOMYCETES, NOCARDIA, AMOEBIASIS	<ul style="list-style-type: none"> <li>Gram control should have both gram positive and negative.</li> </ul>



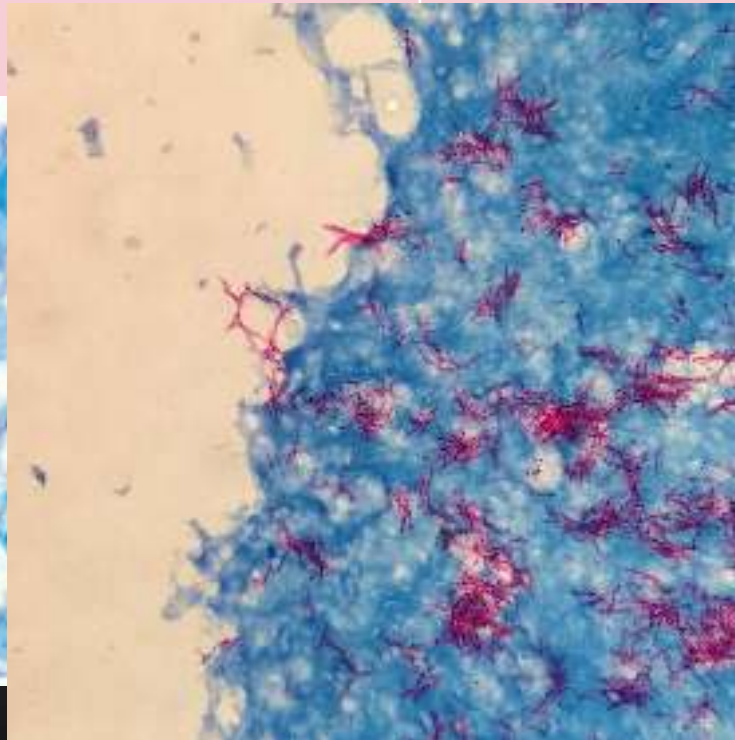
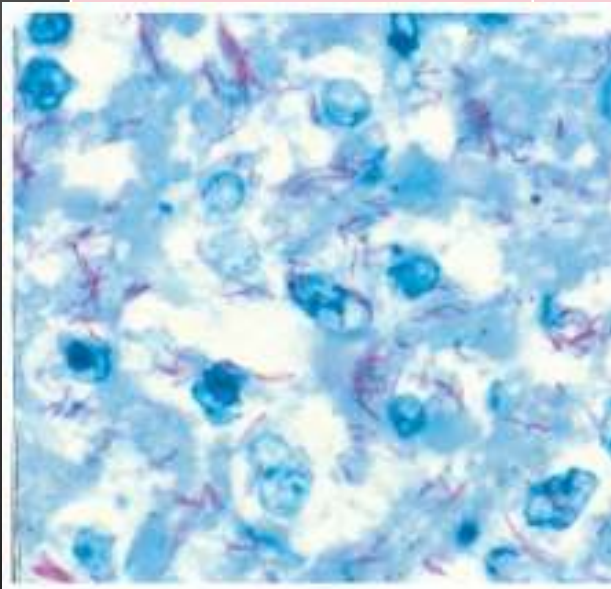


# BACTERIA -H&E VS GRAM STAIN



# STAINS FOR MICROORGANISM

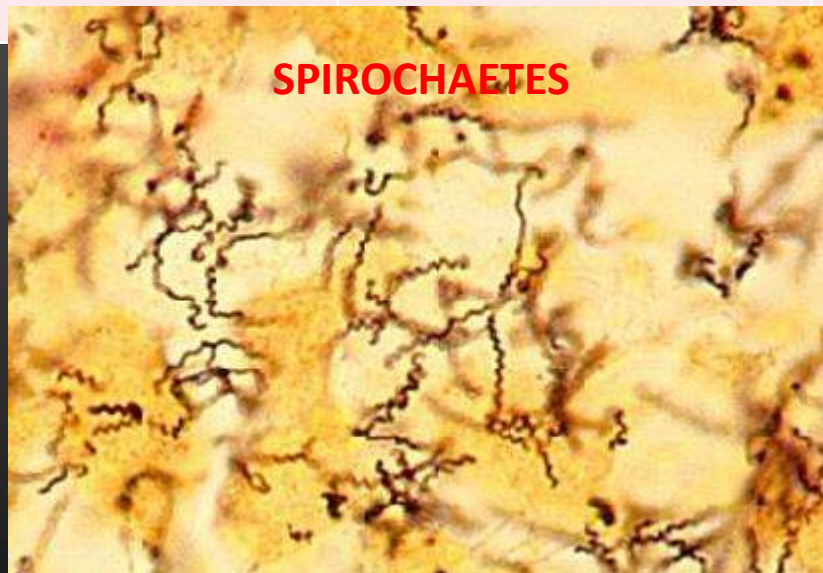
STAINS	COMPONENTS STAIN	POSSIBLE USES	
ACID FAST BACILLI STAINS (ZIEHL NEELSEN , KINYOUN (COLD ZN)) WADE FITE	MYCOBACTERIUM– BRIGHT RED AND BEADED NOCARDIA –PINK TISSUE – PALE BLUE	Identification for Mycobacterium -tuberculi -leprae	<ul style="list-style-type: none"> <li>• Mycobacterium are difficult to demonstrate by gram stain → they possess a capsule containing long chain fatty acid (<b>mycolic acid</b>) → makes them <b>hydrophobic</b>.</li> <li>• The fatty capsule resists removal of stain by acid and alcohol solution (acid and alcohol fastness)</li> <li>• They are PAS positive due to the carbohydrate content in their cell walls (esp if large amount are present)</li> <li>• Acid fastness can be destroyed by decalcification using strong acid -&gt; therefore formic acid is recommended</li> </ul>





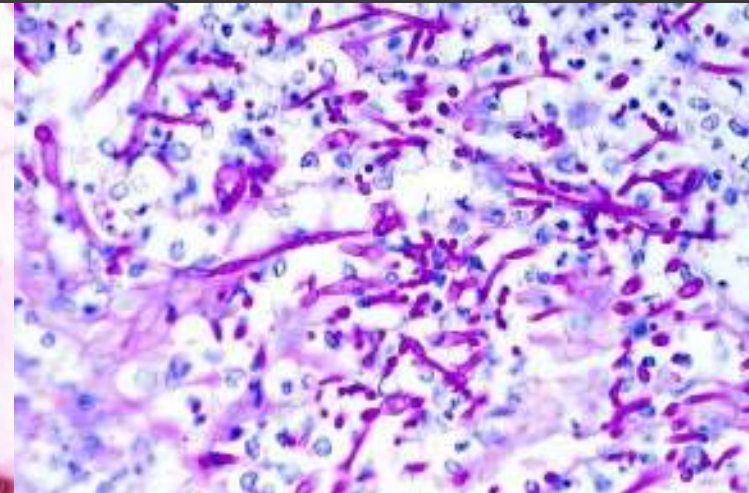
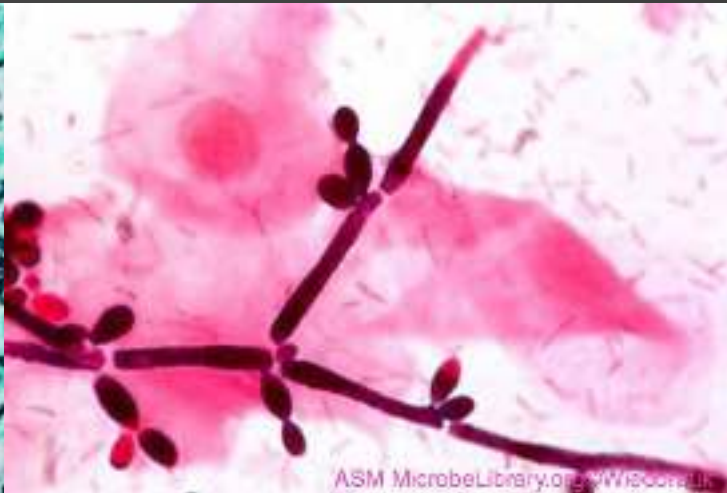
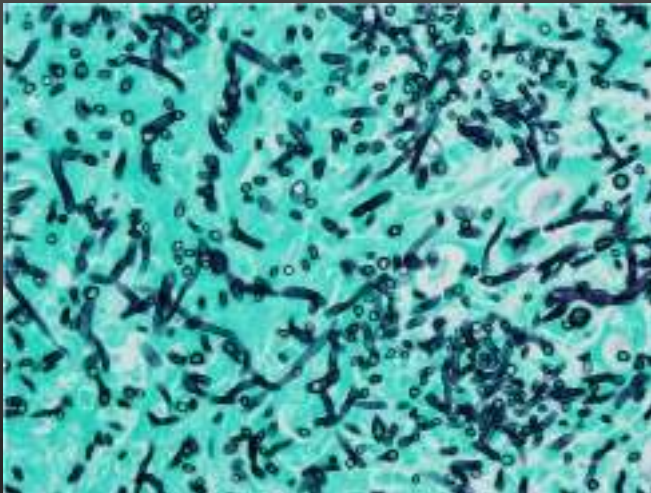
# STAINS FOR MICROORGANISM

STAIN	COMPONENTS STAIN	POSSIBLE USES	
WARTHIN STARRY	SPIROCHAETES - BLACK OTHER BACTERIA (eg H PYLORI) –BLACK TISSUE PALE <b>YELLOW</b> TO LIGHT <b>BROWN</b>	Identification of spirochaetes, H pylori	Eg. of spirochaetes (gram-negative, motile, spiral bacteria with endocellular flagella) Treponema pallidum causing syphilis Leptospira interrogans causing leptospirosis



# STAINS FOR MICROORGANISM

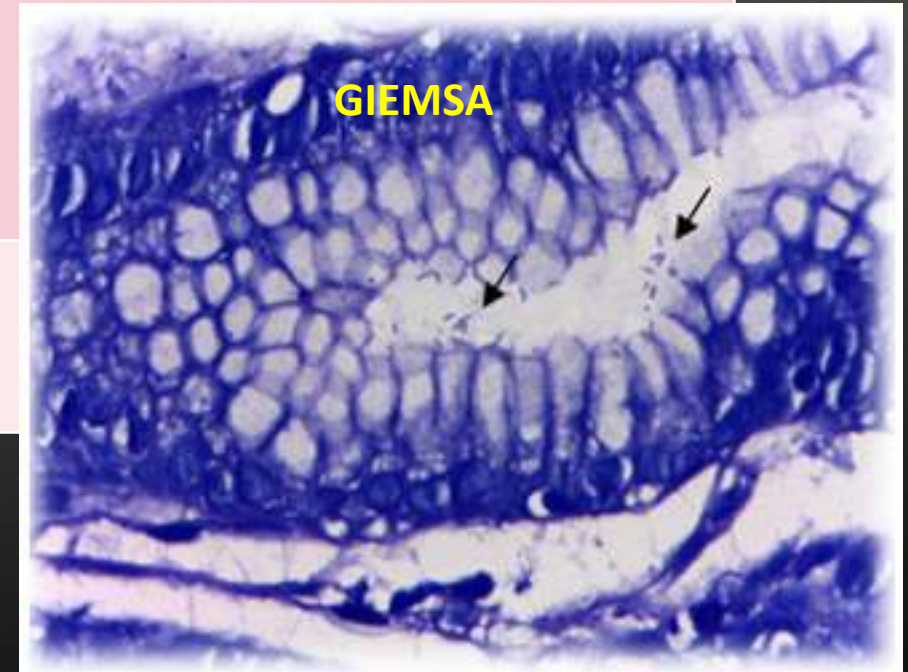
	STAINS	COMPONENTS STAIN	
FUNGI	1. SILVER STAINS (GROCOTT METHENAMINE SILVER NITRATE-GMS)	FUNGI (BLACK) MUCIN (TAUPE TO GRAY) TISSUE – GREEN	<ul style="list-style-type: none"> <li>Seen fairly well in H&amp;E, but are demonstrated well with GMS and PAS. (yeasts, hyphae and spores)</li> <li>Fungal cell walls are rich in polysaccharides which can be converted by oxidation to dialdehydes.</li> <li>Dialdehydes are then detected by silver solution.</li> <li>In suspected fungal infections</li> </ul>
	2. PAS	FUNGI CELL WALL - MAGENTA	



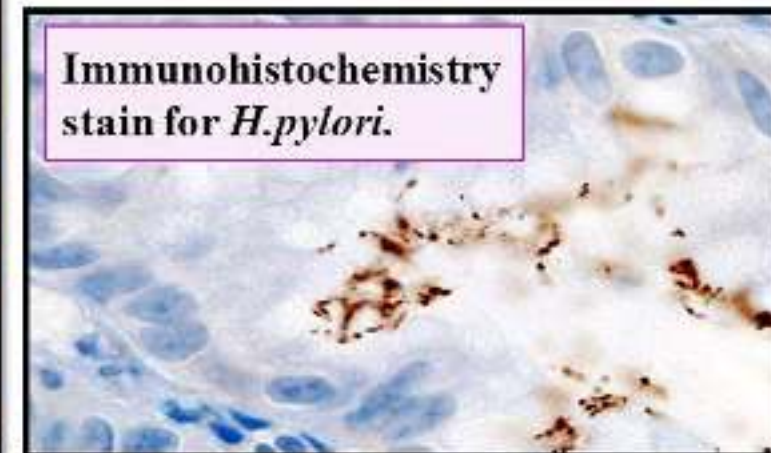
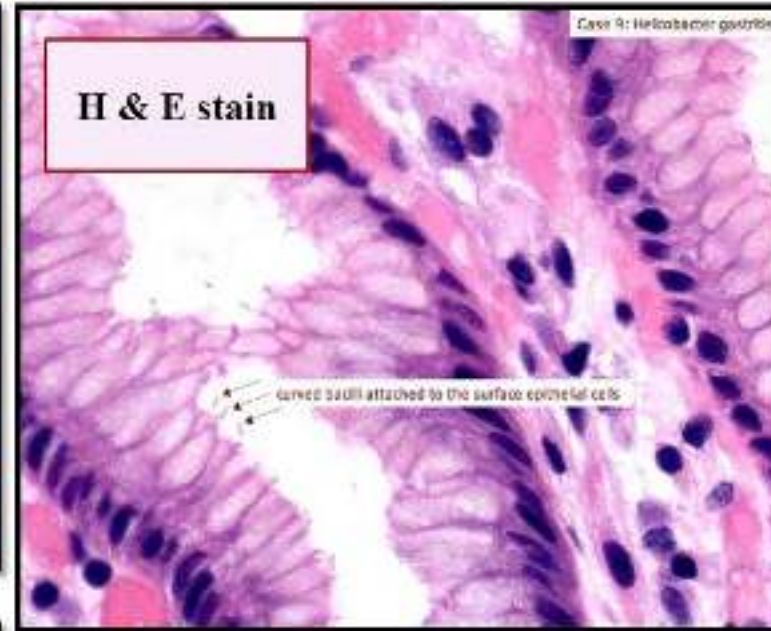


# STAINS FOR MICROORGANISM

ORGANISM	STAINS	COMPONENTS STAIN	
HELICOBACTER PYLORI	GIEMSA, TOLUIDINE BLUE, WARTHIN STARRY, CRESYL VIOLET ACETATE (CVA)	GIEMSA HELICOBACTER AND NUCLEI – BLUE BACKGROUND -SHADES OF BLUE VIOLET	<ul style="list-style-type: none"> <li>A spiral vibrio organism causing chronic gastritis.</li> <li>Can be identified by H&amp;E.</li> </ul>
	DIFF QUIK	H PYLORI –DARK BLUE OTHER BACTERIA – BLUE NUCLEI – DARK BLUE	



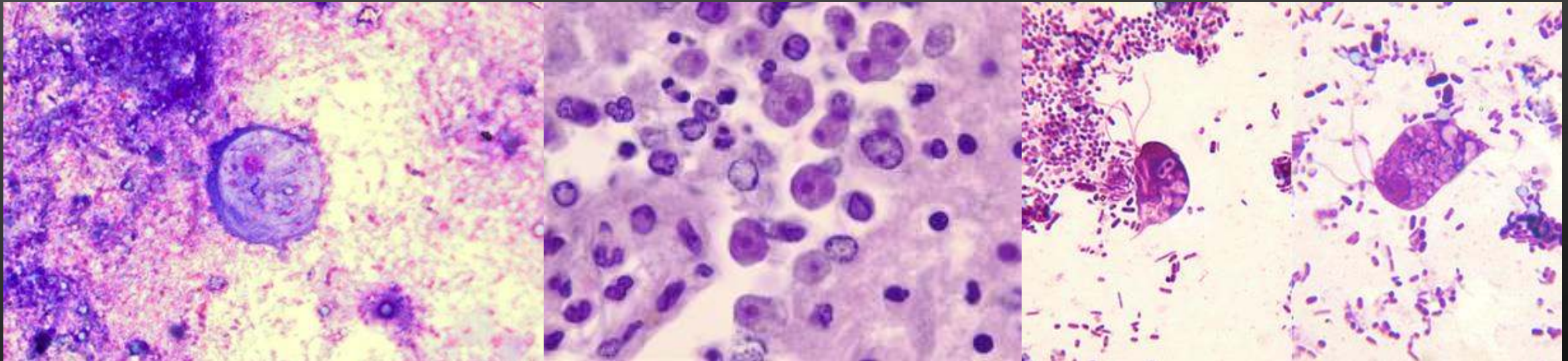
# HELICOBACTER PYLORI





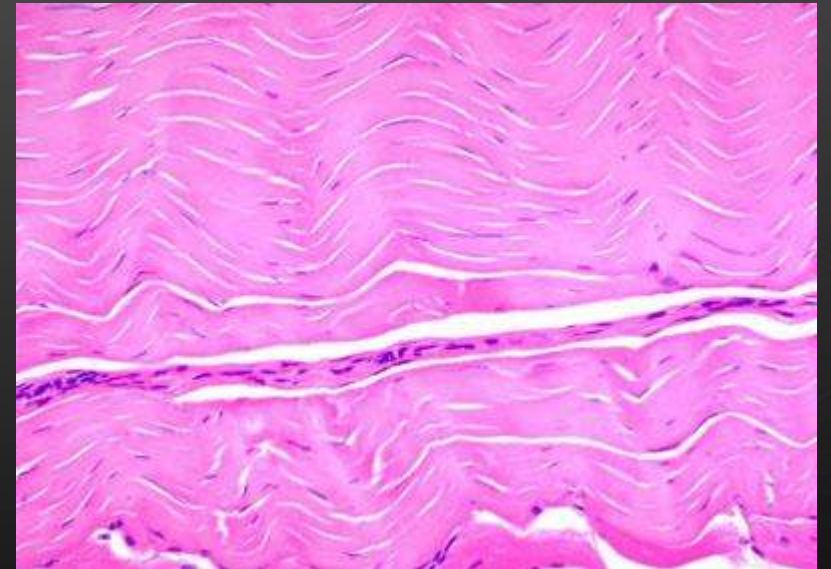
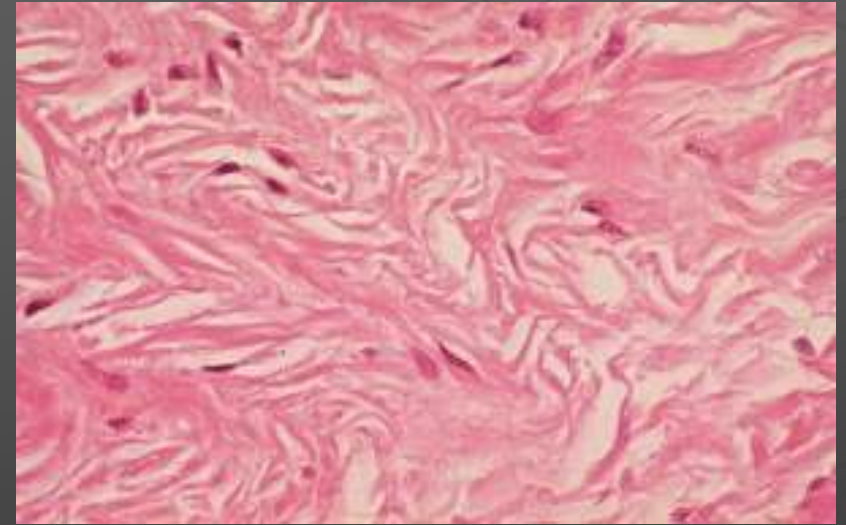
# STAINS FOR MICROORGANISM

STAIN	COMPONENTS STAIN	POSSIBLE USES	
GIEMSA	PROTOZOAN – BLUE NUCLEI- RED VIOLET BACKGROUND – PINK PALE BLUE	Identification of protozoa such as Entamoeba histolytica, Giardia lamblia which causes gastroenteritis	Trophozoites of entameoba contains small nucleus and ingested red blood cells PAS positive - Magenta



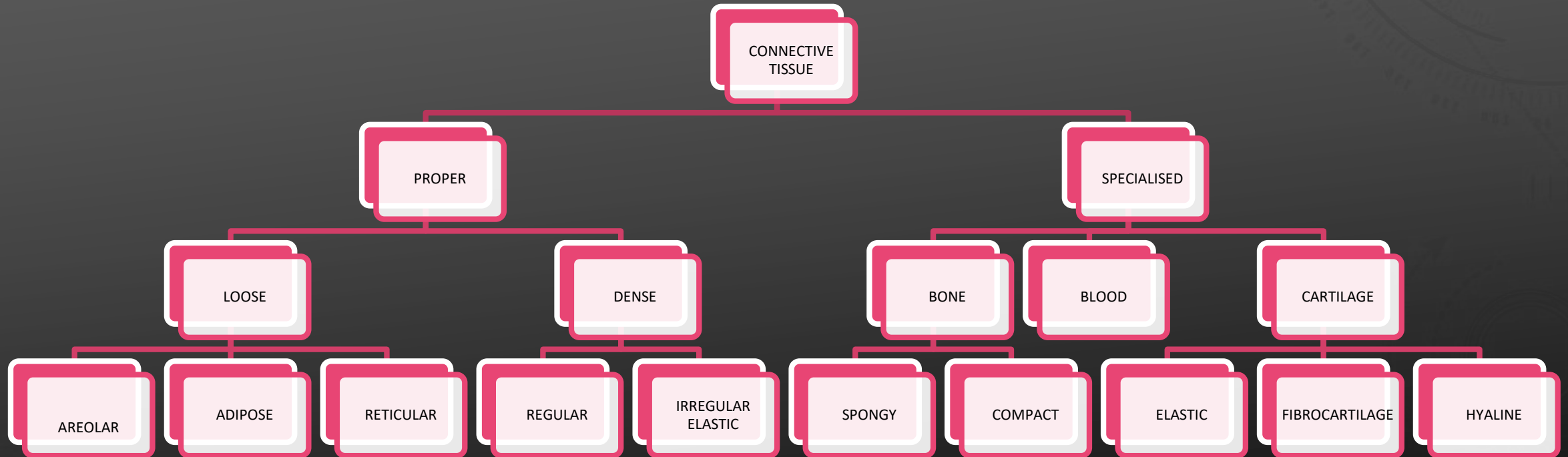
# CONNECTIVE TISSUE STAINS

- Connective tissue – in latin – to bind
- Function – to connect together and provide support to other tissues of the body
- Consists of **cellular portion in a surrounding framework of non cellular substance.**
- Cellular portion consists of **fibroblasts**, mast cells, histiocytes, adipose cells, reticular cells, osteocytes, chondrocytes etc
- Intercellular substance consists of amorphous material (mucopolysaccharides) and formed elements (**collagen, reticular fibers, elastic fibers**)



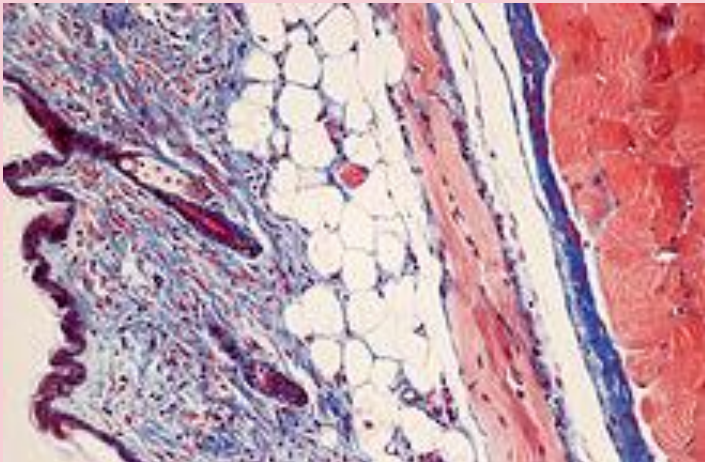


# CONNECTIVE TISSUE



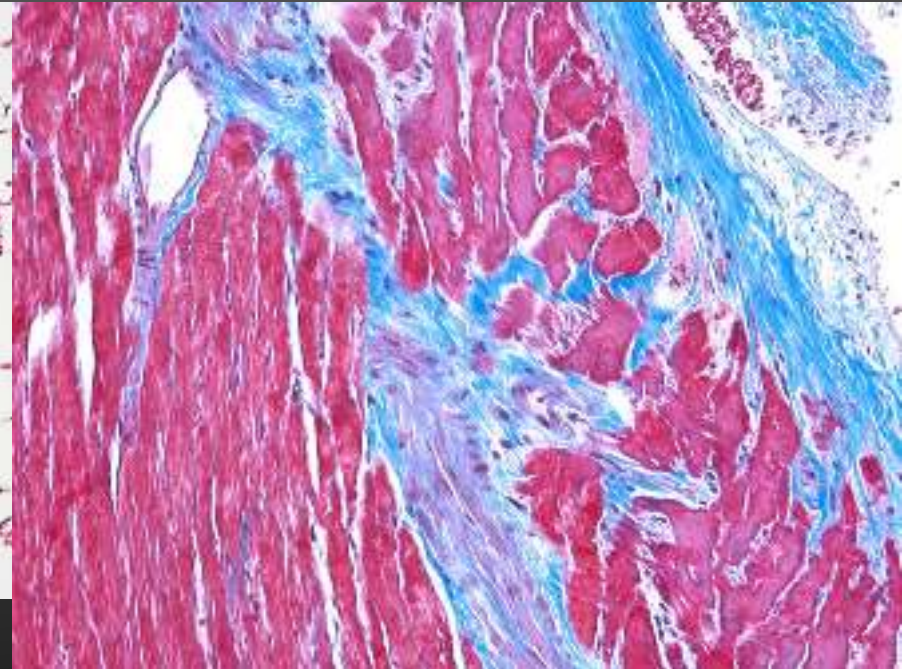
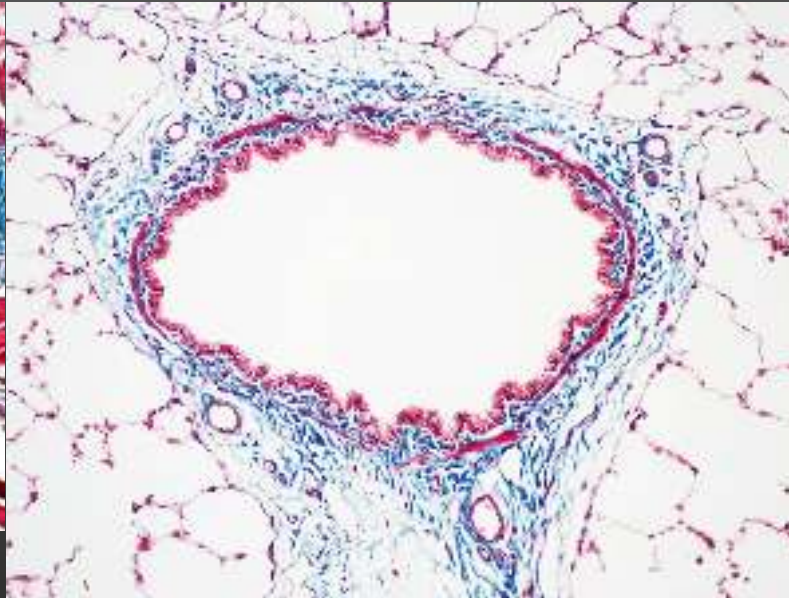
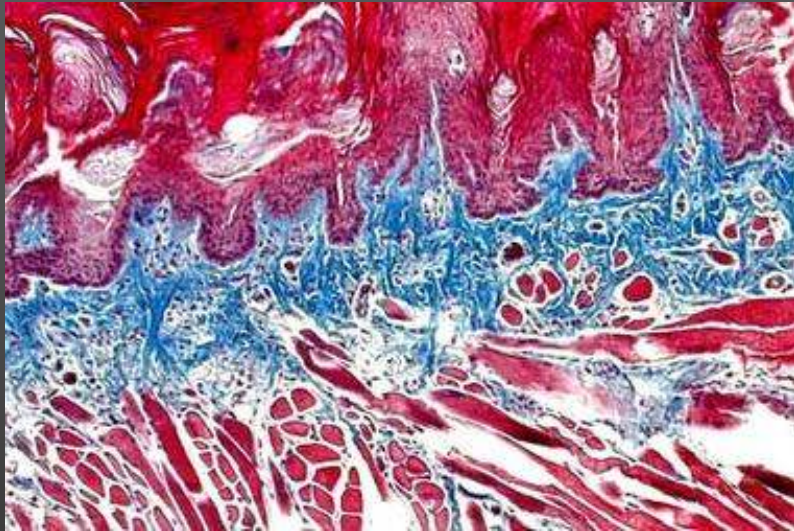
# CONNECTIVE TISSUE STAINS

SUBSTANCE	STAINS	COMPONENT STAINS	POSSIBLE USES
COLLAGEN	MASSON TRICHROME	COLLAGEN – BLUE /GREEN MUSCLE – RED RETICULIN – BLUE GREEN FIBRIN - RED	<ul style="list-style-type: none"> <li>• <b>Trichrome stains</b> – three colours, for selective demonstration of muscle, collagen fibers, fibrin and erythrocytes.</li> <li>• Factors affecting trichrome staining:               <ol style="list-style-type: none"> <li>1. <b>Tissue permeability and dye molecular size</b> <ul style="list-style-type: none"> <li>-When the protein component of a tissue is exposed to a fixative agent an insoluble protein network is formed.</li> <li>-Different proteins will form network with different physical features.</li> <li>-The <b>structure and density of protein network may relate directly to the staining reactions of the tissue components</b>.</li> </ul> </li> <li>Eg: smaller dye molecules will stain any 3 tissue types, however larger dye molecules will penetrate only collagen leaving muscle and erythrocytes unstained.</li> <li>2. <b>Heat</b> <ul style="list-style-type: none"> <li>-Increase rate of staining and penetration by larger dye molecules</li> </ul> </li> <li>3. <b>pH</b> <ul style="list-style-type: none"> <li>-Low pH 1.5 to 3.0 is required to achieve adequate and even staining.</li> </ul> </li> </ol> </li> </ul>





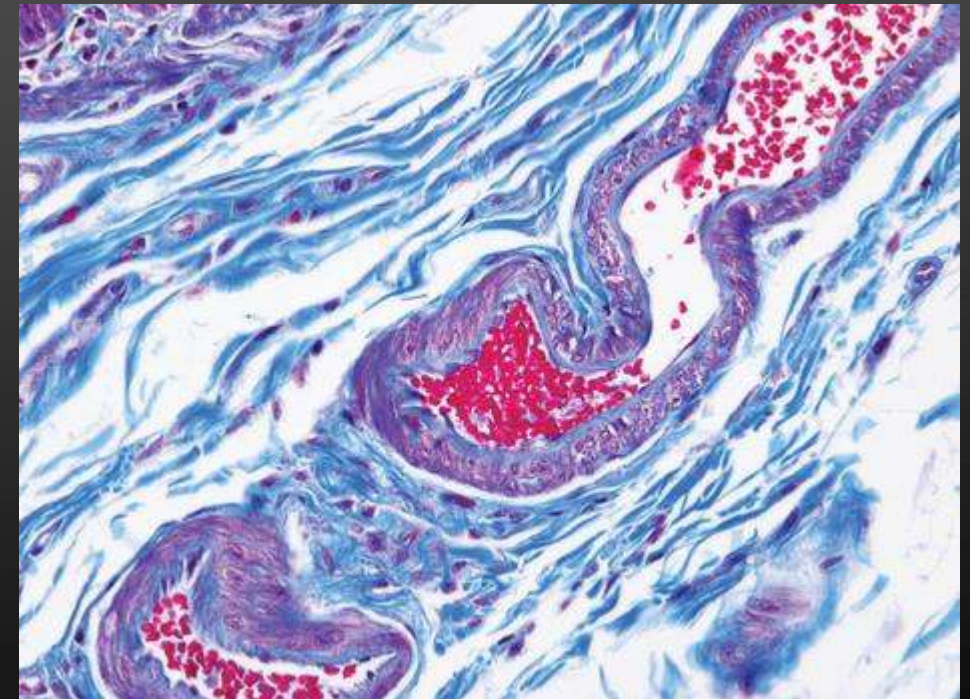
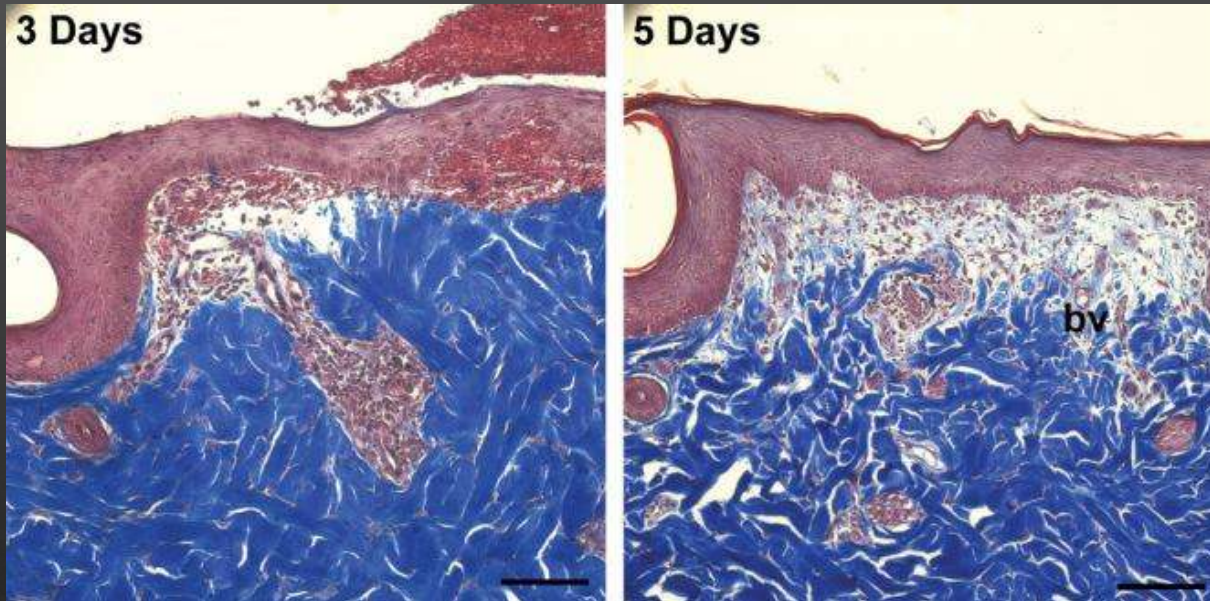
# MASSON TRICHROME





# INDICATIONS

MASSON TRICHROME	DIFFERENTIATE COLLAGEN AND MUSCLE IN TUMOURS
	IDENTIFY AN INCREASE IN COLLAGENOUS TISSUE
	INDICATE FIBROSIS IN LIVER CIRRHOSIS
	INDICATE FIBROSIS IN PYELONEPHRITIS





# MASSON TRICHROME SCORING – IN RESEARCH

**Table 2: Parameters assessed to calculate healing score**

Number	Histological Parameter
1	Amount of granulation tissue (profound-1, moderate-2, scanty-3, absent-4)
2	Inflammatory infiltrate (plenty-1, moderate-2, a few-3)
3	Collagen fiber orientation (vertical-1, mixed-2, horizontal-3)
4	Pattern of collagen (reticular-1, mixed-2, fascicle-3)
5	Amount of early collagen (profound-1, moderate-2, minimal-3, absent-4)
6	Amount of mature collagen (profound-1, moderate-2, minimal-3)

Number 1-4: H and E, Number 5-6: Masson's trichrome stain, old collagen fibers take deep blue color and the new collagen fibers stain light blue

**Table 4: Parameters of histologic assessment of wound**

Semi-quantitative method	Quantitative method
Wound reepithelialization: migration of keratinocytes, bridging of cells, keratinization	Polymorphonuclear leucocytes/ tissue macrophages ratio
Inflammatory cells: absence/ presence (mild/moderate/marked)	Percentage of reepithelialization
Fibroblasts: absence/presence (mild/moderate/marked)	Area of the granulation tissue
New vessels: absence/presence (mild/moderate/marked)	-
Collagen: absence/presence (mild/moderate/marked)	-

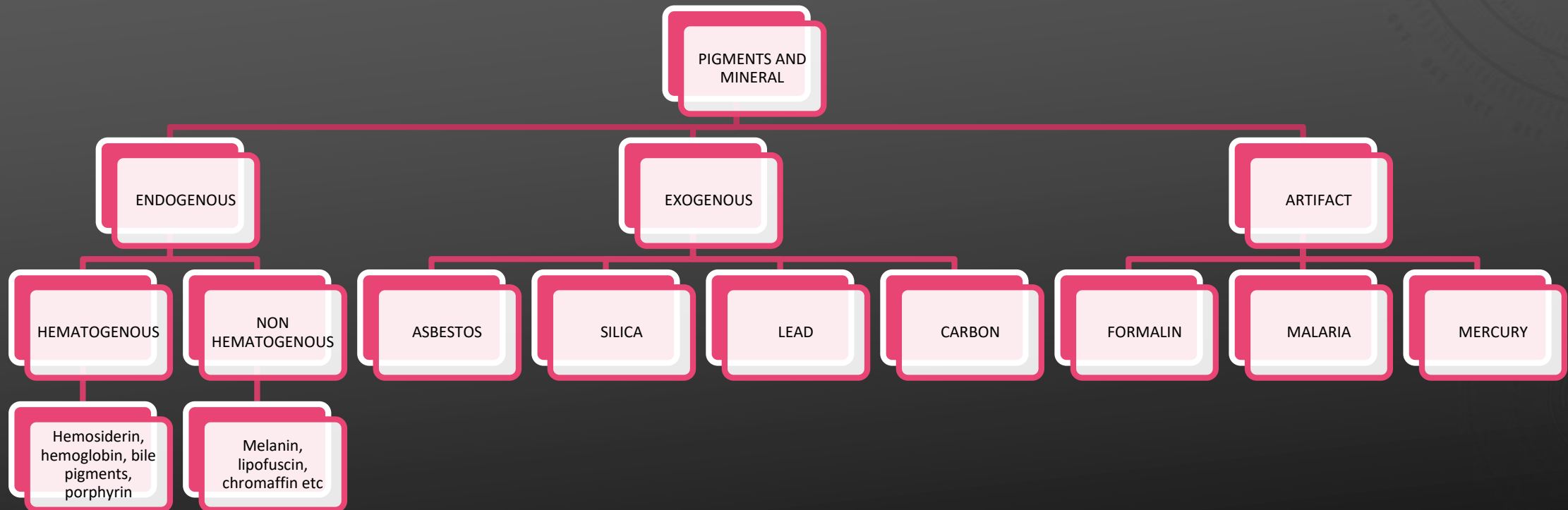
# CONNECTIVE TISSUE STAINS

SUBSTANCE	STAIN	COMPONENT STAINS	
ELASTIC FIBERS	VERHOEFF VAN GIESON	COLLAGEN – RED MUSCLE, ELASTIN, RETICULIN – YELLOW NUCLEI – BLUE BLACK	Elastic fibers in large vessels - aorta
RETICULIN FIBERS	RETICULIN STAIN	RETICULIN FIBERS - BLACK	Demonstrates reticular fibers and basement membrane material





# STAINS FOR PIGMENTS AND MINERAL

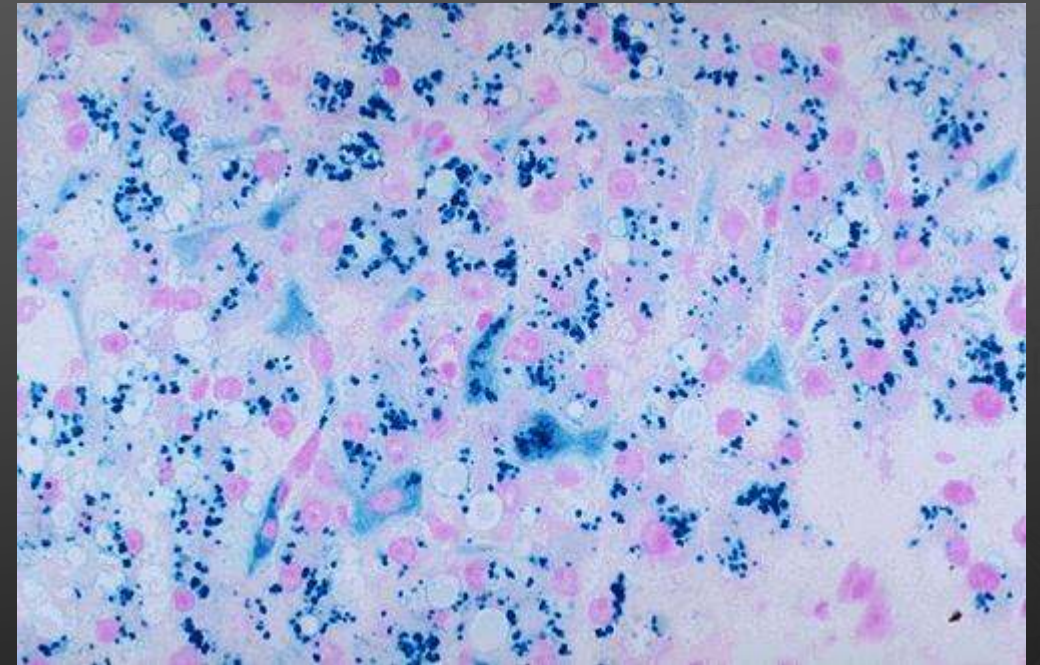
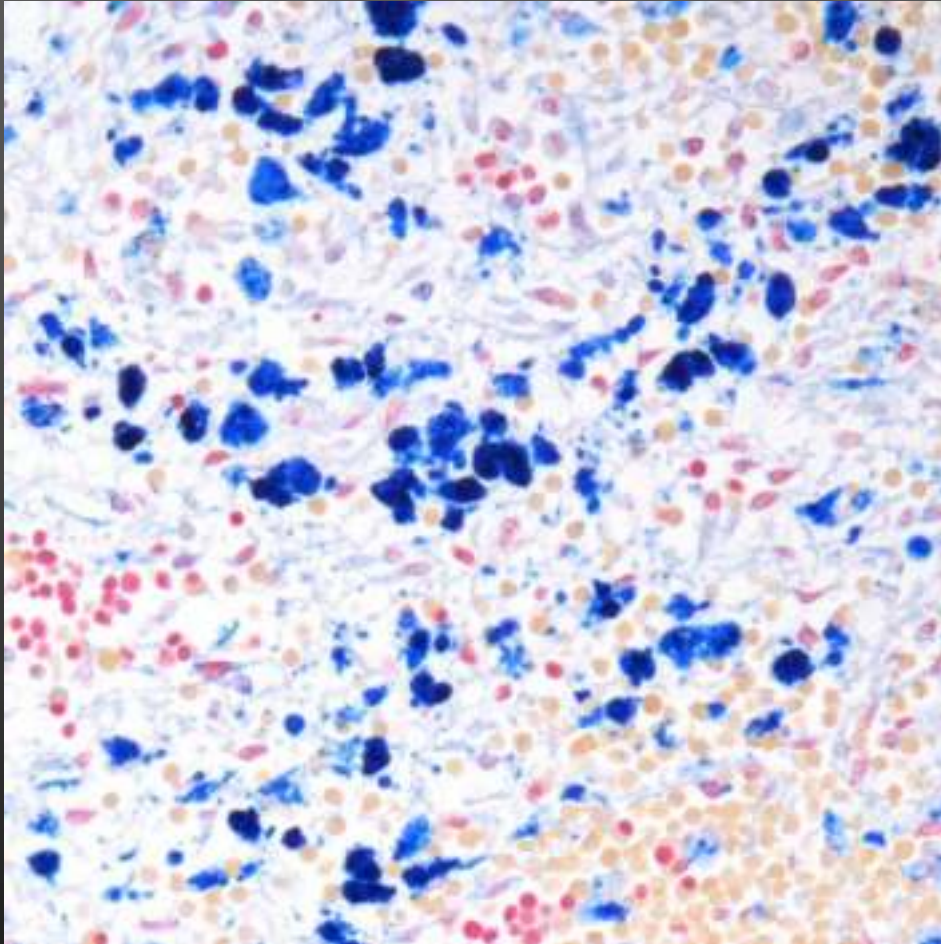


# STAINS FOR PIGMENTS AND MINERAL

	STAINS	COMPONENTS STAIN	USAGE
HEMOSIDERIN	PERLS PRUSSIAN BLUE REACTION	FERRIC IRON – BLUE NUCLEI – RED BACKGROUND	<ul style="list-style-type: none"><li>• Breakdown product of hemosiderin composed of ferric iron and protein.</li><li>- Seen as yellow brown granules</li><li>- Unmasking of ferric iron in hydroxide form by dilute HCl</li><li>- <b>Prussian blue reaction</b> – (ferric hydroxide + potassium ferrocyanide=ferric ferrocyanide (insoluble blue compound))</li><li>- In Hb and myoglobin – iron is tightly bound within protein complex- cannot be demonstrated by using traditional technique</li><li>- *Best positive control – postmortem lung tissue containing good amount of iron positive macrophages.</li></ul> <p>Bone marrow (iron stores, myelodysplasia), liver (hemochromatosis)</p>



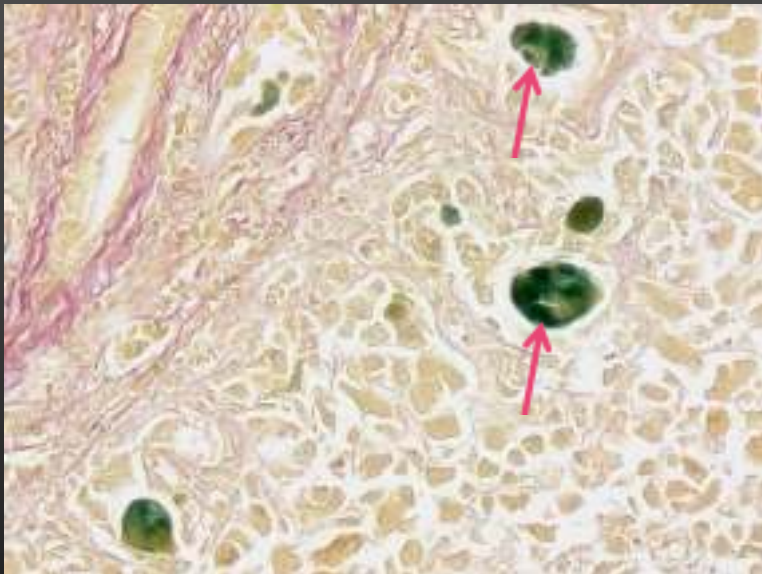
# PRUSSIAN BLUE



A Prussian blue iron stain demonstrates the blue granules of hemosiderin in hepatocytes and Kupffer cells in liver.

# STAINS FOR PIGMENTS AND MINERAL

	STAINS	COMPONENTS STAIN	
BILE	MODIFIED FOUCHET'S	BILE-EMERALD BLUE GREEN MUSCLE- YELLOW COLLAGEN - RED	<ul style="list-style-type: none"><li>• Distinguishing bile pigment from lipofuscin.</li><li>• Both appear yellow brown on H&amp;E.</li><li>• Pigment → converted to green colour of biliverdin and blue cholecyanin by the oxidative action of the ferric chloride in the presence of trichloroacetate</li></ul>

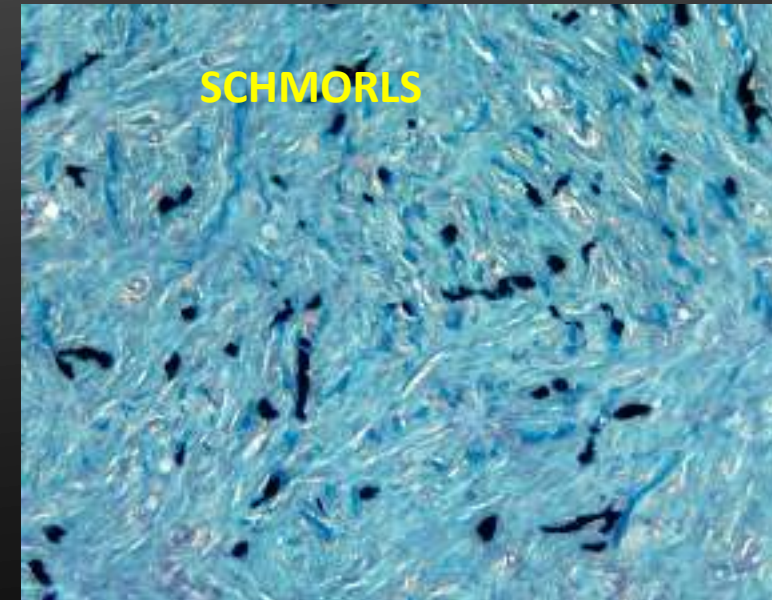
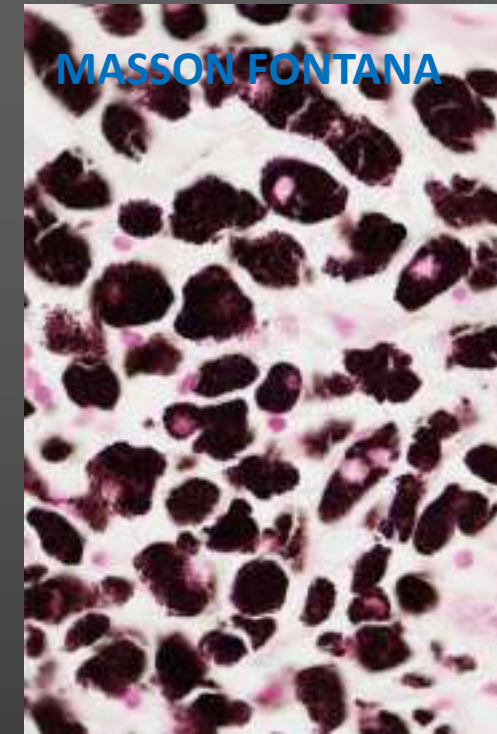
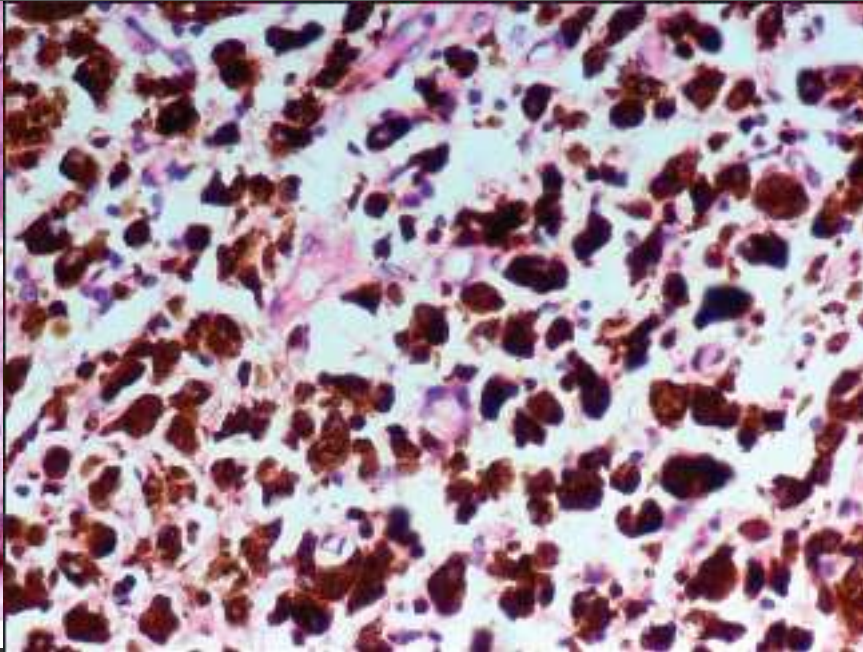




# MASSON FONTANA

MELANIN	1. MASSON FONTANA	MELANIN, ARGENTAFFIN GRANULES, CHROMAFFIN GRANULES, SOME LIPOFUSCIN – BLACK NUCLEI –RED	<ul style="list-style-type: none"> <li>• Normally occurs as light brown to black granules in hair, skin, eyes, substantia nigra</li> <li>• <b>Melanin are blackened by acid silver nitrate solution.</b></li> <li>• Melanin reduces ferricyanide to ferrocyanide with production of prussion blue in the presence of ferric salts</li> <li>• Pigments identification of melanin in melanomas and secretory granules in neuroendocrine tumours</li> </ul>
	2. SCHMORLS REACTION	MELANIN – DARK BLUE NUCLEI - RED	

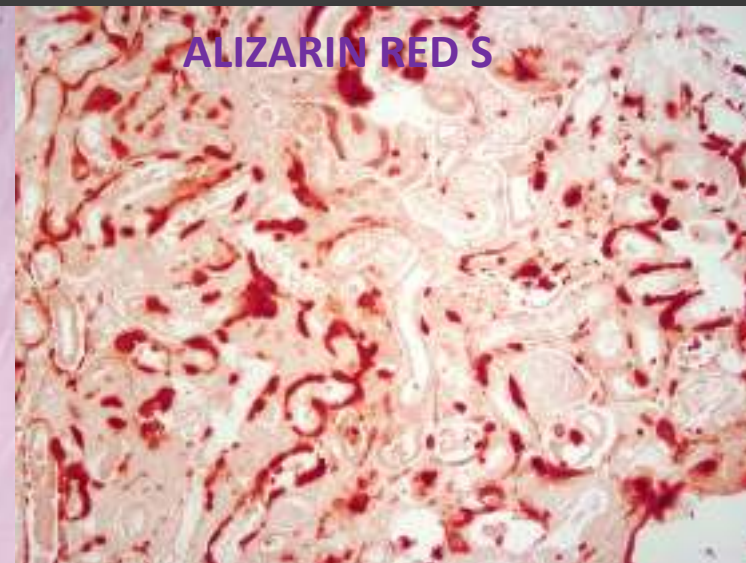
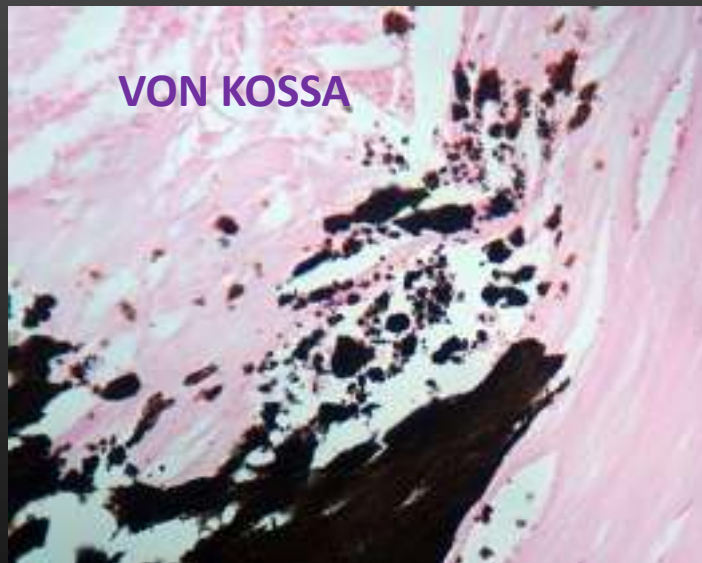
# MELANIN





# STAINS FOR PIGMENTS AND MINERAL

	STAIN	COMPONENT STAINS	
CALCIUM	VON KOSSA	MINERALISED BONE (CALCIUM) – BLACK OSTEOID - RED TISSUE – RED	<ul style="list-style-type: none"> <li>• Demonstration of phosphate and carbonate radicals with calcium in tissues</li> <li>• ID of michaelis guttman bodies in malakoplakia</li> <li>• Not specific as melanin will also reduce silver to give a black deposits.</li> </ul>
	ALIZARIN RED S	CALCIUM – ORANGE-RED	



# CONCLUSION

- SPECIAL STAINS ENHANCE DETECTION AND LOCALISATION OF INDIVIDUAL TISSUE COMPONENT



