

Carbohydrates and mucins

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Carbohydrates

- Provide cells with energy by converting to glucose
- Excess glucose stored in liver and muscle as glycogen
- Residual unstored glycogen is turned into fat
- Carbohydrates comprise carbon, oxygen and hydrogen and include sugars, starch and cellulose
- Chemically they are ketones or aldehydes
- They are classified as follows:

Monosaccharides = one sugar unit (glucose, ribose, fructose)

Oligosaccharides = 2-10 sugar units (sucrose, lactose, maltose)

Polysaccharides = many sugar units (amylose, cellulose)

Classification of carbohydrates

1. Glycans

Group I - Neutral polysaccharides

2. Glycosaminoglycans (GAGs)

Group II - Acid mucopolysaccharides

3. Glycoproteins

Group III - Glycoproteins and Group IV - Glycolipids

1. Glycans

- Carbohydrates that contain glucose - eg glycogen, starch and cellulose
- Those that contain N-acetylglucosamine - eg chitin
- All PAS positive
- All negative with Alcian Blue and Mucicarmine stains

2. Glycosaminoglycans (GAGs)

- A. Carboxylated (COOH) - hyaluronic acid (found in connective tissue)
- B. Sulphated and carboxylated - chondroitin sulphates 4 and 6 (found in cartilage, blood vessels)
- C. Chondroitin sulphate B - dermatan sulphate (found in skin, connective tissues, aorta, lung)
- D. Heparin (found in mast cells and arteries)
- E. Sulphated only (found in aorta)

1. All polysaccharides in this group are acidic and attached to protein
2. Acid mucopolysaccharides are also called connective tissue mucins

3. Glycoproteins

- A. Sialomucin - found in submaxillary glands, small intestine and colonic mucins
- B. Sulphated only (COOH-free) - aorta
- C. Serum glycoproteins - blood group substances
- D. Sulphated and Carboxylated - sialoglycoproteins that contain sialic acid and sulphate - colonic mucins

Glycolipids

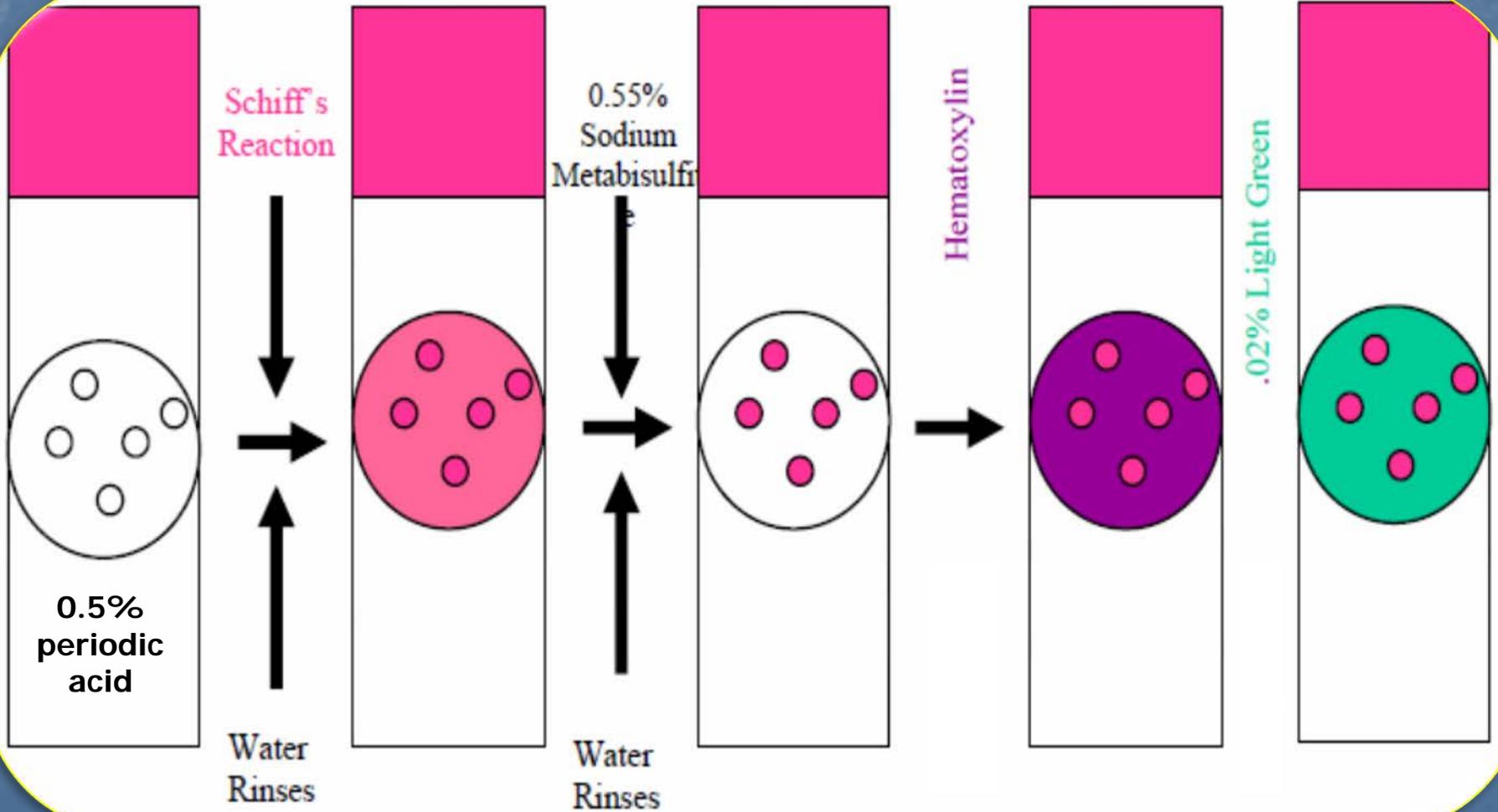
1. Cerebrosides: fatty residues bound to carboxyl COOH
2. Phosphatides: non-carbohydrate-containing lipids such as lecithin, cephalin and sphingomyelin

Staining methods for carbohydrates

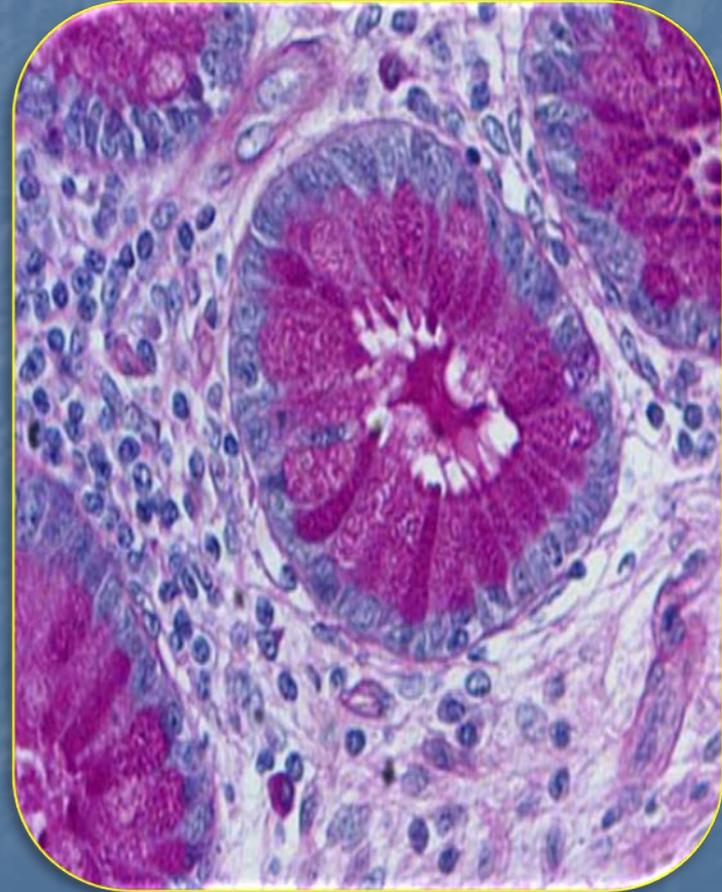
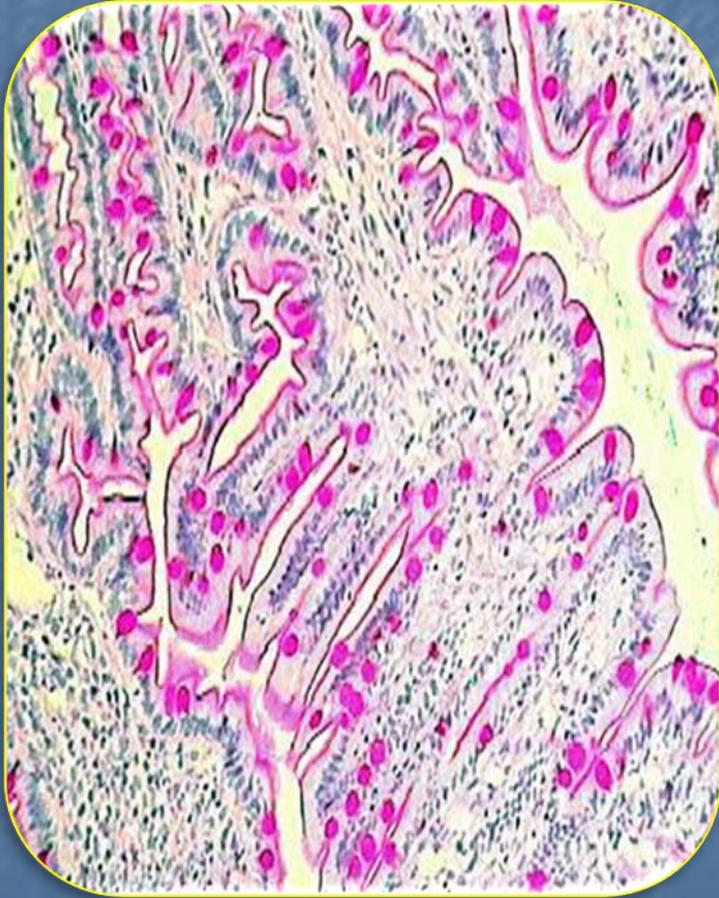
Periodic acid Schiff (PAS)

- Based on the oxidation of 1,2 glycol groups to aldehydes using periodic acid
- Aldehydes are detected using Schiff reagent
- The sugar units from which aldehydes can be formed by reaction with periodic acid are glucose, galactose, mannose, fucose and some sialic acids
- Glycogen and some mucins are PAS +
- Sites rich in GAGs (such as cartilage and mast cells) stain weakly or negative

Periodic acid Schiff (PAS)

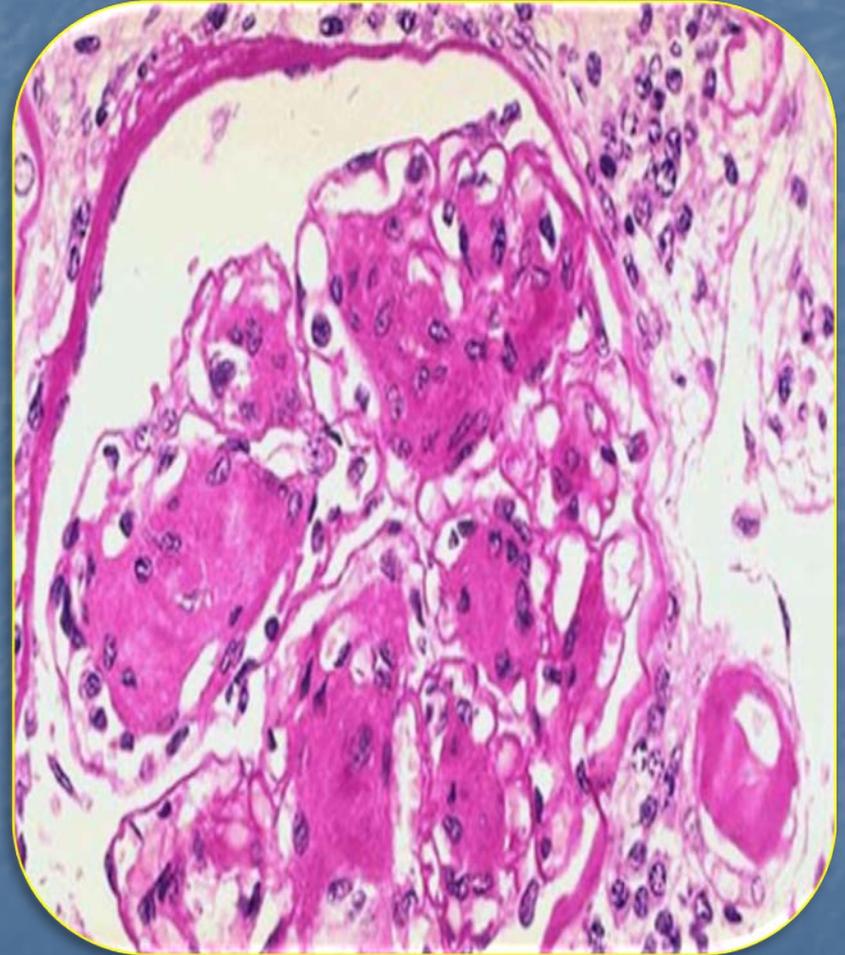


Periodic acid Schiff (PAS)

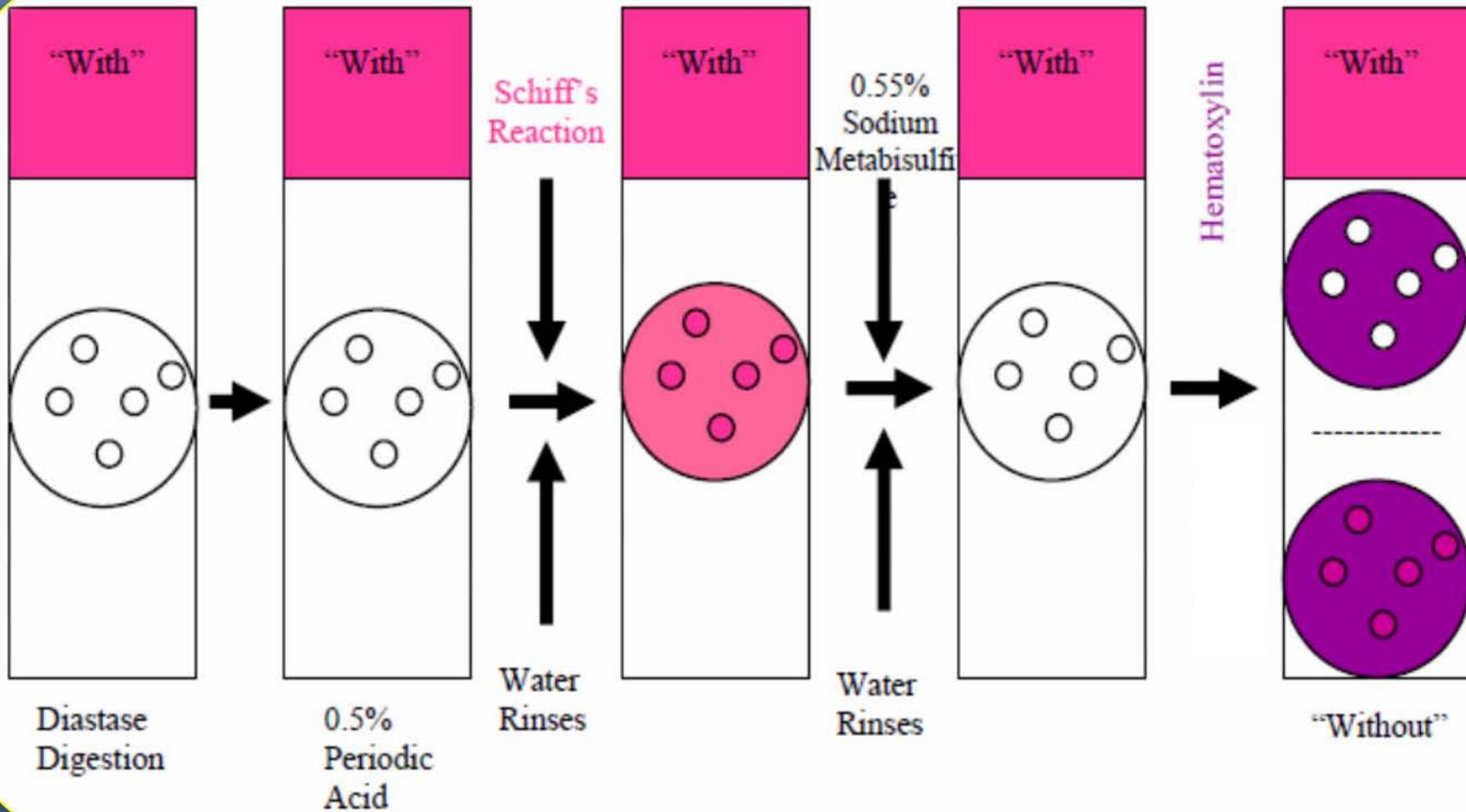


Periodic acid Schiff (PAS)

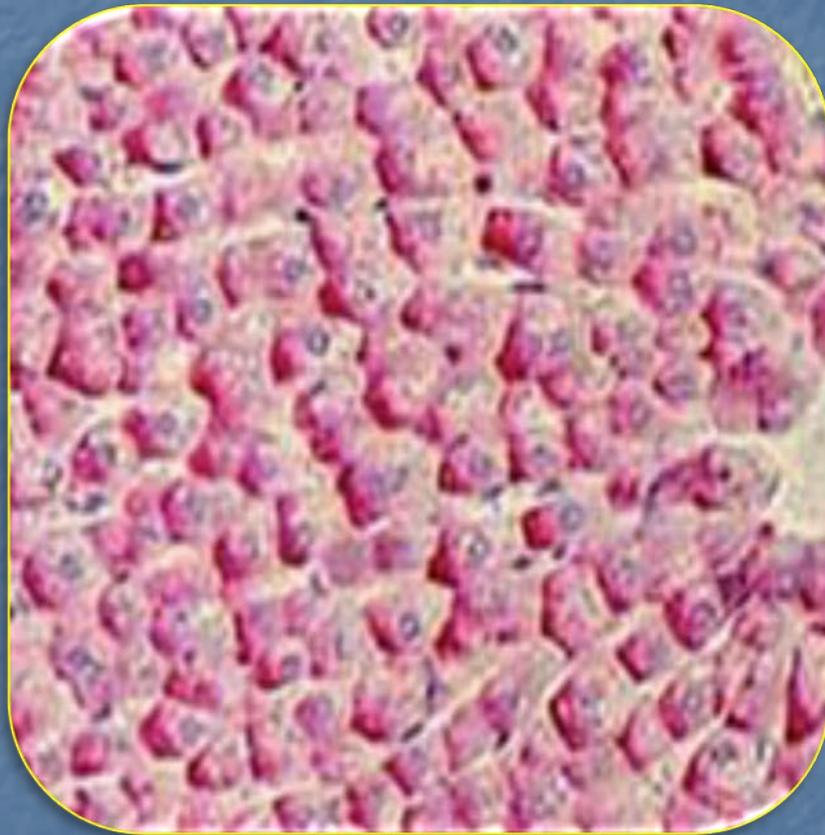
- High concentrations of hexose sugars, including glucose, in the collagen of basement membranes
- The collagen fibres of connective tissue stain pink with PAS
- Glomerular basement membranes become thickened and irregular in glomerulonephritis



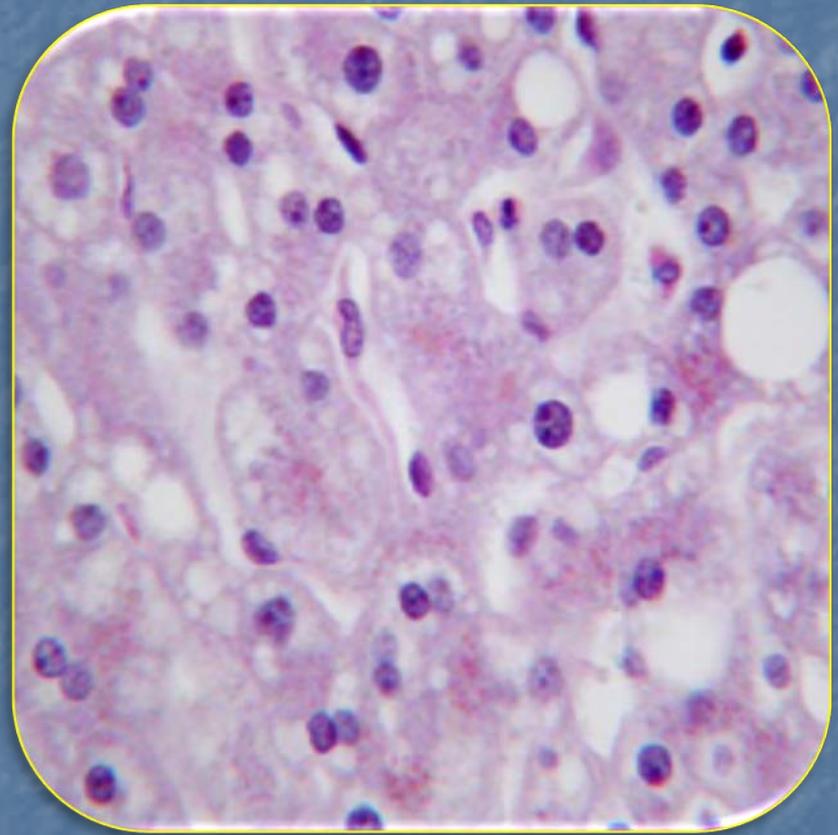
PAS - Diastase



Periodic acid Schiff - Diastase



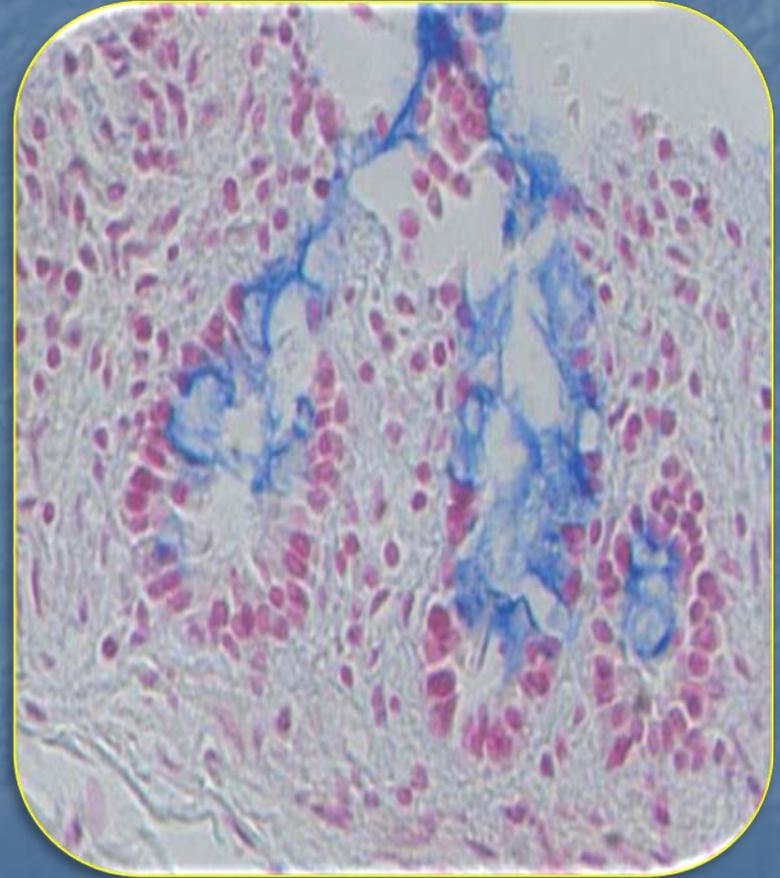
No digestion
PAS +



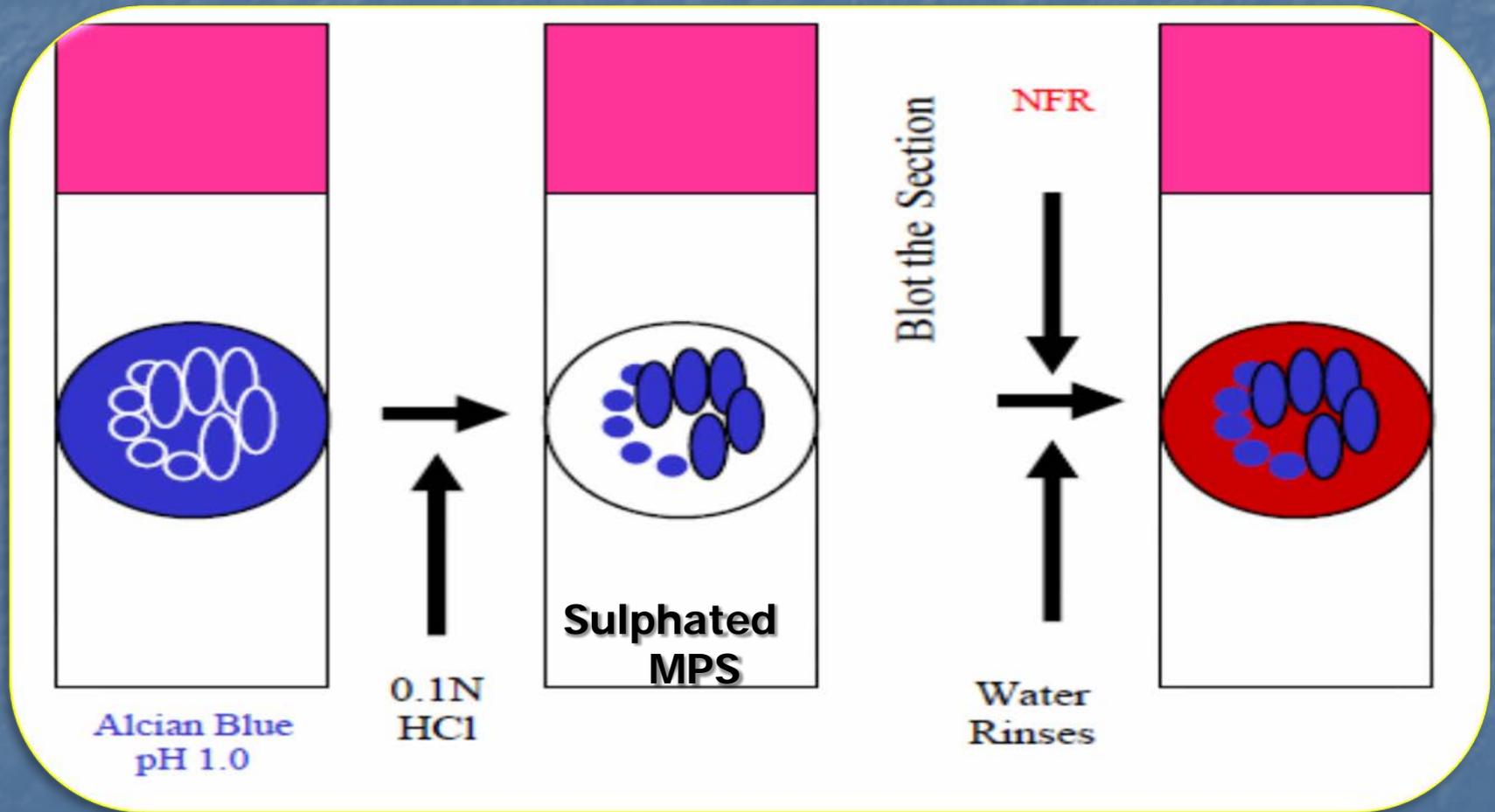
After digestion
PAS -

Alcian blue pH 1.0 (sulphated mucopolysaccharides)

- Alcian blue is a basic dye
- When used in a 0.1N hydrochloric acid (pH 1.0), alcian blue stains only sulphated acid muco-polysaccharides and glycoproteins
- Acid mucopolysaccharides that are carboxylated only will not stain

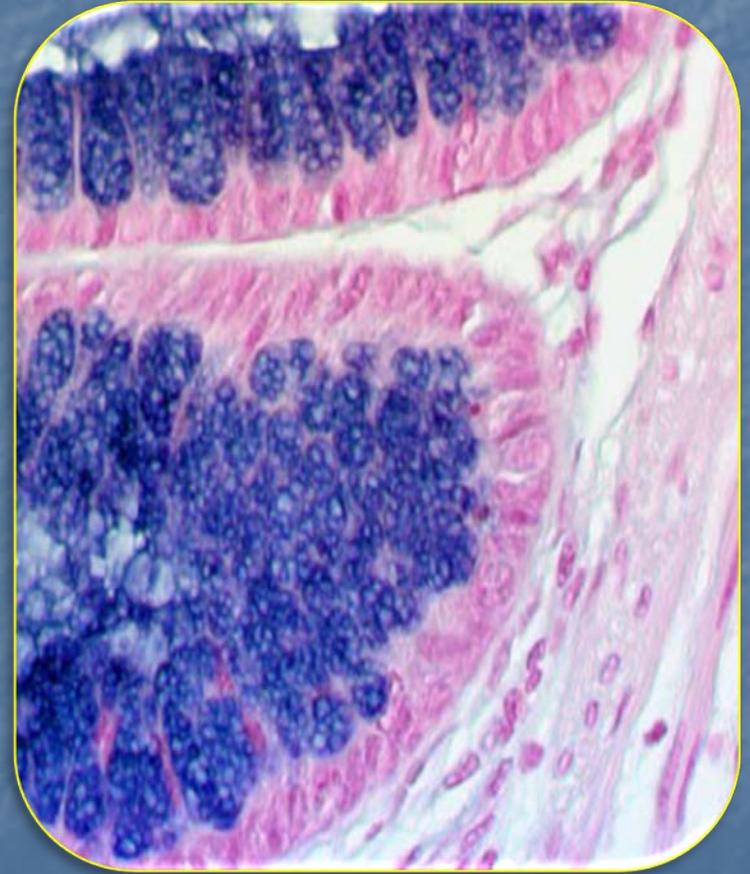


Alcian blue pH 1.0

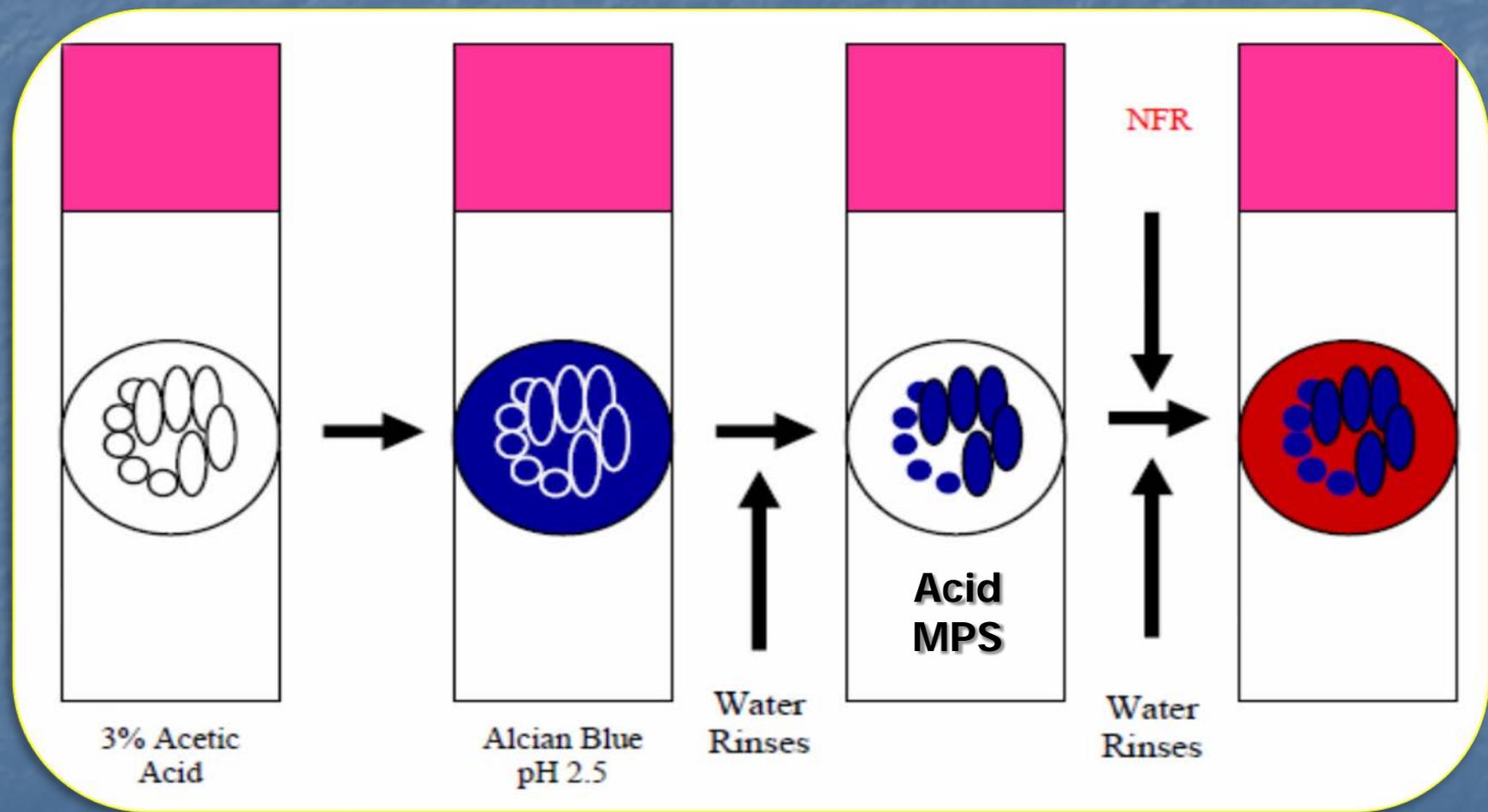


Alcian blue pH 2.5 (acid mucopolysaccharides)

- When used in a 3% acetic acid solution (pH 2.5), it stains sulphated and carboxylated acid mucopolysaccharides and glycoproteins



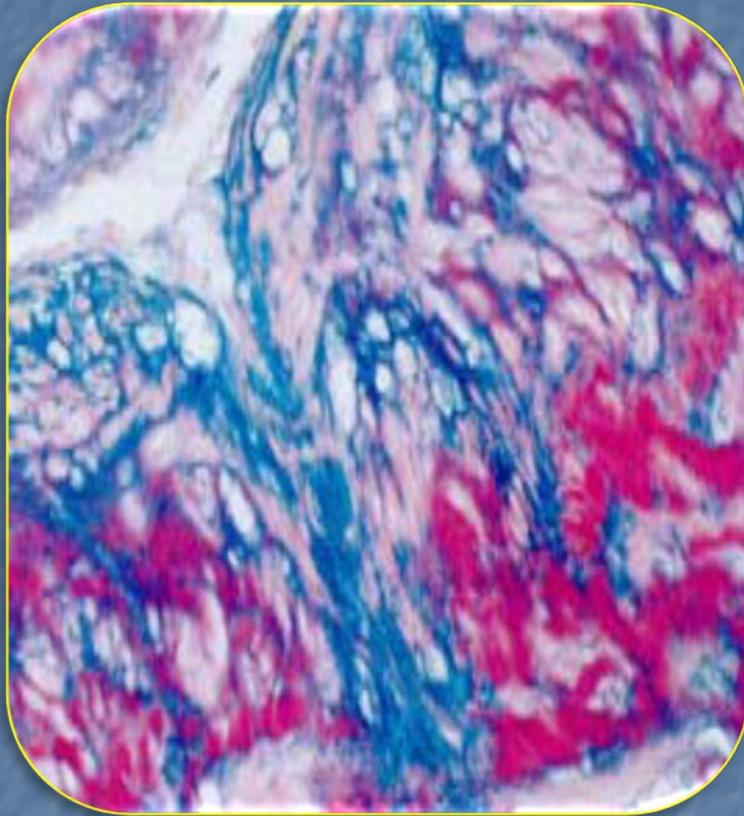
Alcian blue pH 2.5



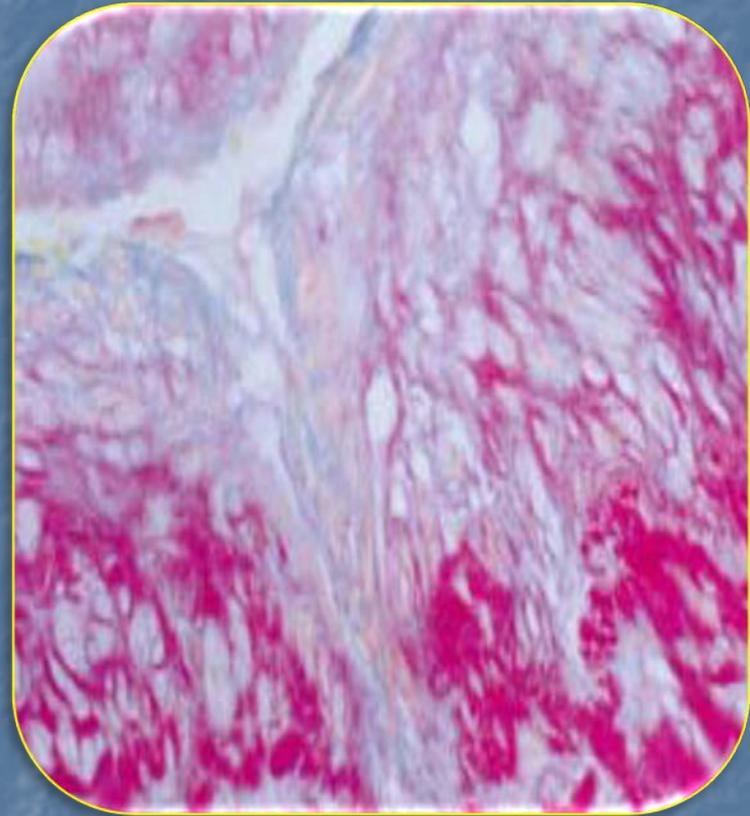
Alcian blue with hyaluronidase

- This stain differentiates between epithelial and connective tissue mucins
- Staining will disappear or be dramatically reduced when tissue sections containing hyaluronic acid, chondroitin sulphate A or chondroitin sulphate C (connective tissue mucins) are digested with testicular hyaluronidase
- Glycoproteins (epithelial mucins) will not be affected

Alcian blue with hyaluronidase



Without digestion -
Acid mucopolysaccharides
and sialomucins

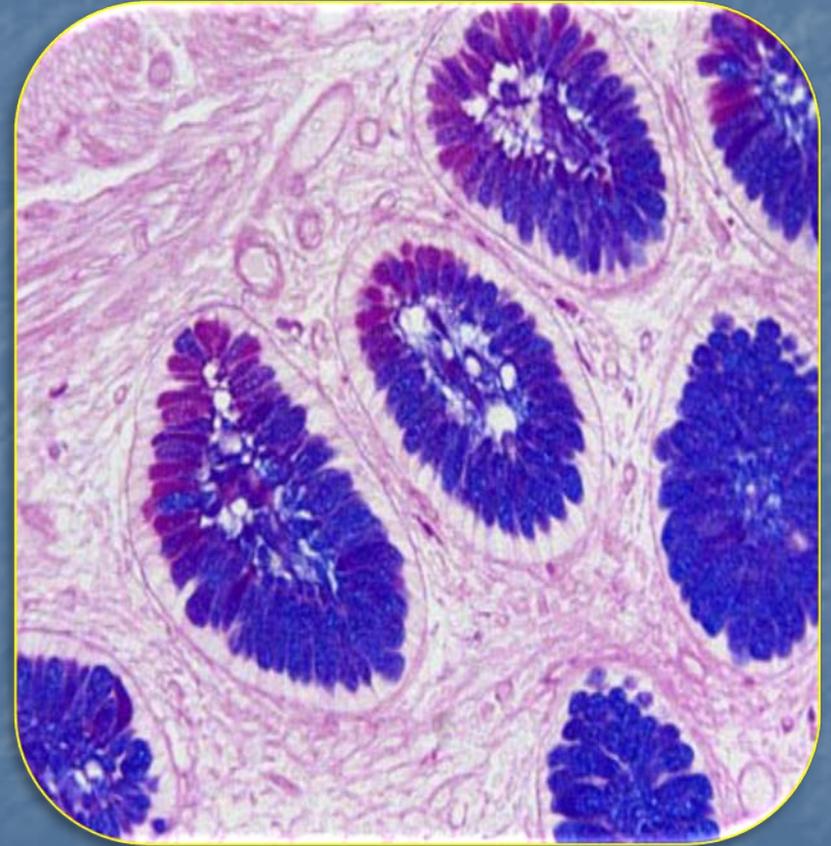
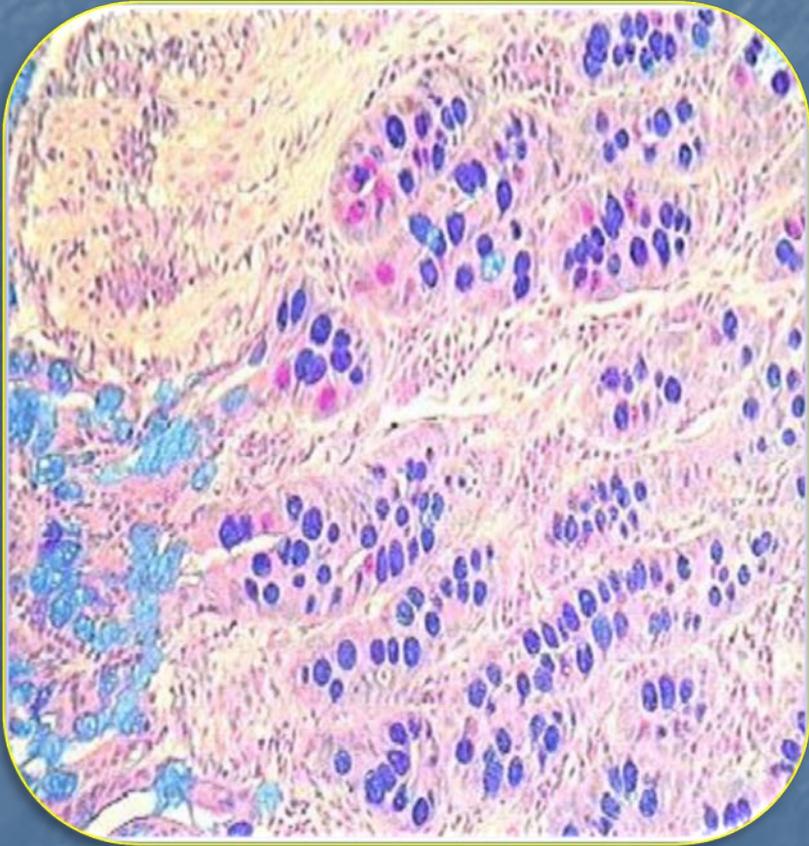


With digestion -
Loss of mucosubstances
containing hyaluronic acid

Alcian blue / PAS

- Differentiate between neutral and acidic mucosubstances
- Staining for the acidic mucosubstances will be done with the Alcian Blue pH 2.5 technique and the neutral mucosubstances by the PAS reaction
- Acid mucopolysaccharides - Blue
- Neutral polysaccharides - Magenta
- Other substances - Purple

Alcian blue / PAS



Alcian blue / PAS results

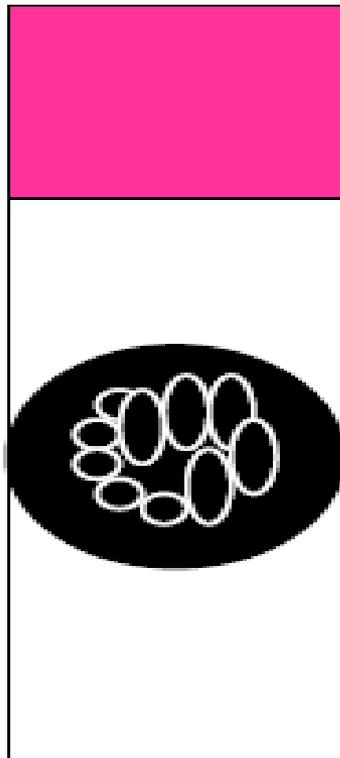
- Alcian blue performed before PAS
- Combined stain shows neutral mucin red (notably that of the oesophagus and stomach)
- Mucous cells of gastric glands have sialic acid mucins and stain purple with alcian blue pH 2.5 and PAS
- Secretory cells of the duodenum and jejunum are both neutral and sialic acid mucins and also stain purple
- Useful for Barrett's oesophagus - reflux of acid from the stomach causes the normal squamous epithelium to change to a columnar intestinal form with goblet cells containing stainable sialic acids

Mucicarmine

- Aluminum in the haematoxylin solution forms a chelating complex with carmine
- The resulting compound has a net positive (+) charge and attaches to the acid groups of mucins

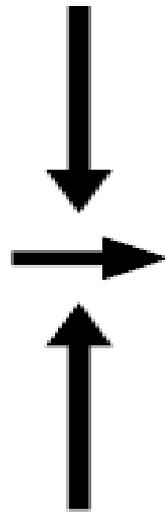


Mucicarmine

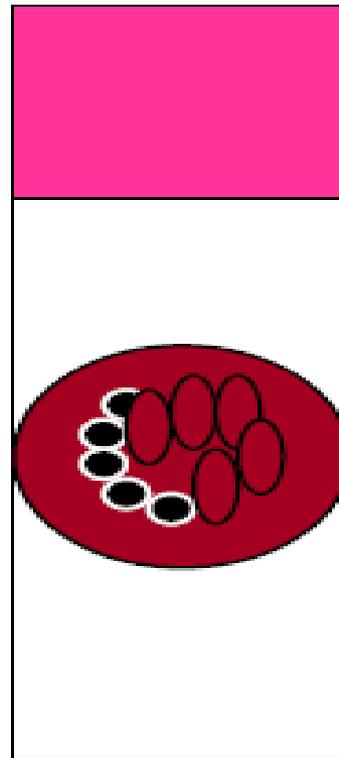


Wiegert's
Hematoxylin

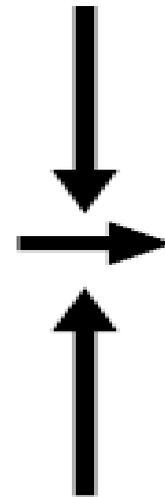
Mucin
Solution



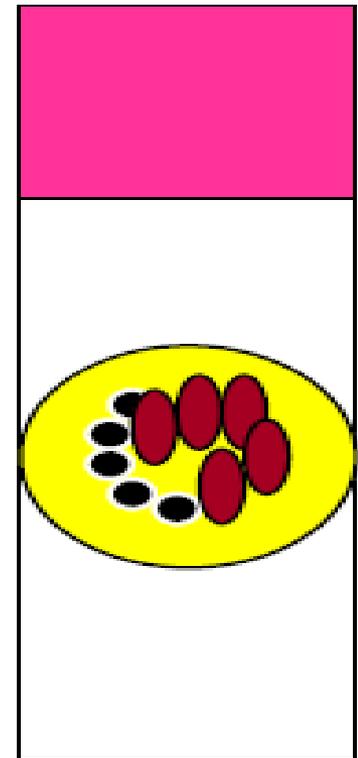
Water
Rinses



Metanil
Yellow

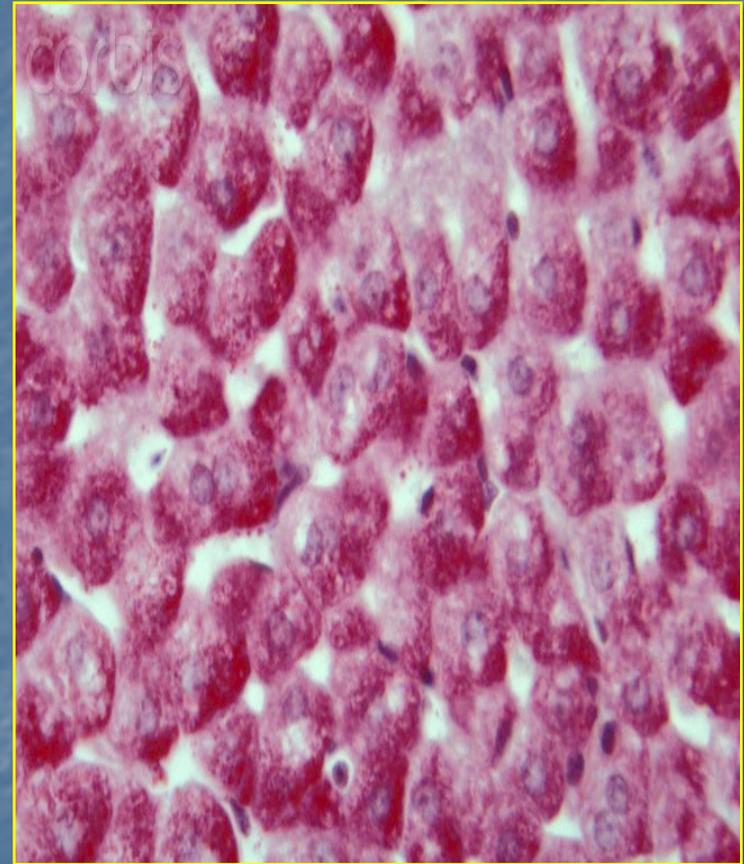


Water
Rinses



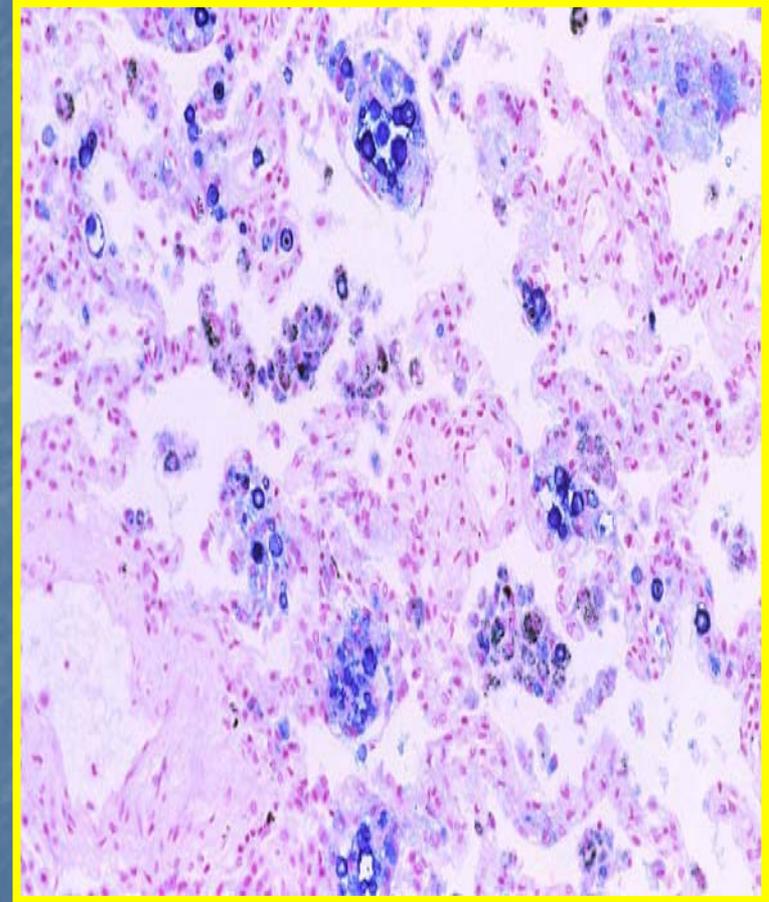
Best's carmine for glycogen

- ❑ An alkaline solution containing an aluminium complex of carminic acid in 20% methanol
- ❑ This technique is used only to detect glycogen
- ❑ The dye is held in contact with glycogen by hydrogen bonding to the numerous hydroxyl groups present



Colloidal iron

- Demonstrates sulphated and carboxylated mucopolysaccharides
- At low pH, colloidal ferric iron is absorbed by both mucosubstances
- After excess reagent is washed out, the Prussian blue detects the iron bound to the tissue



Grocott's methenamine silver

- The oxidant chromium trioxide over-oxidizes most of the carbohydrate content of connective tissue to yield carboxyl groups
- However it generates abundant aldehydes in the cell walls of fungal hyphae (chitin) and these are then stained with methenamine-silver nitrate (or the PAS)

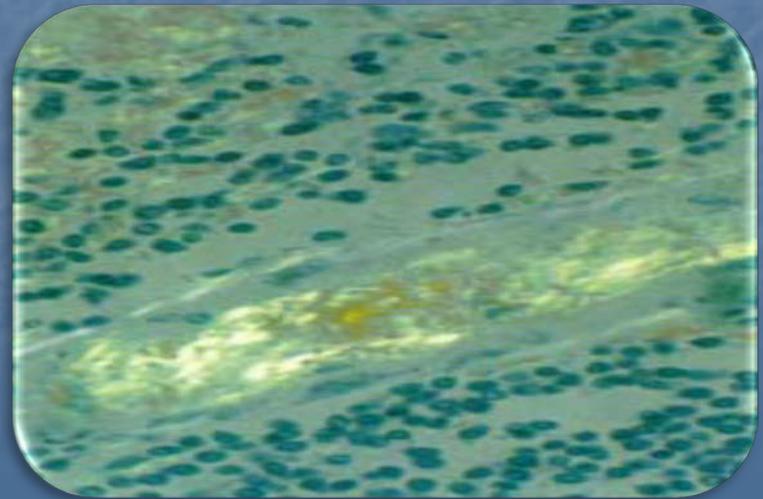
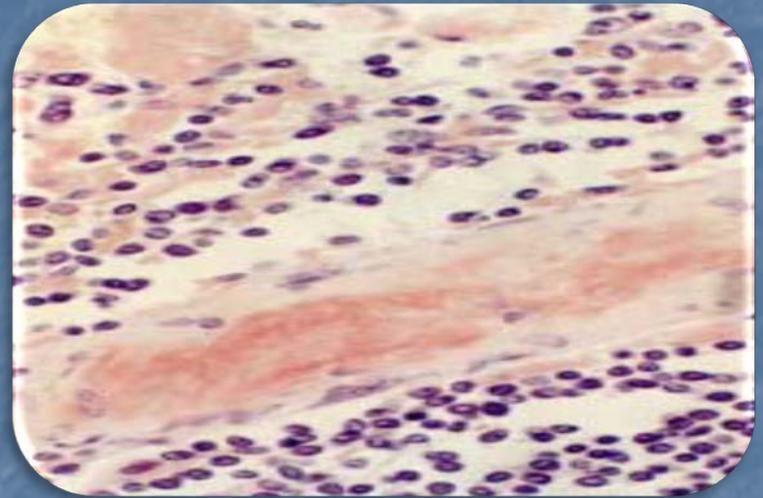


Amyloid

- A fibrillar protein that deposits in many tissues under certain pathologic conditions
- Amyloid contains 1-2% carbohydrate, mostly acid mucopolysaccharides
- Amyloidosis is a disease characterized by an amorphous, eosinophilic, extracellular deposit
- It gradually replaces cellular elements of vital organs and causes progressive loss of function and death

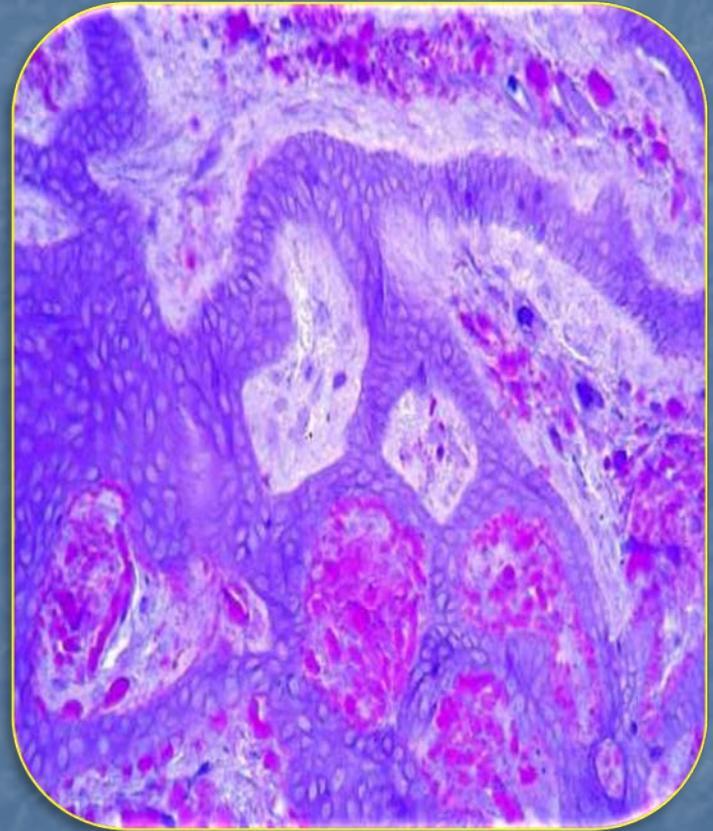
Alkaline Congo Red

- Amyloid chemically resembles cellulose and stains with Congo Red
- Using a polariser, green birefringence is most specific for amyloid demonstration



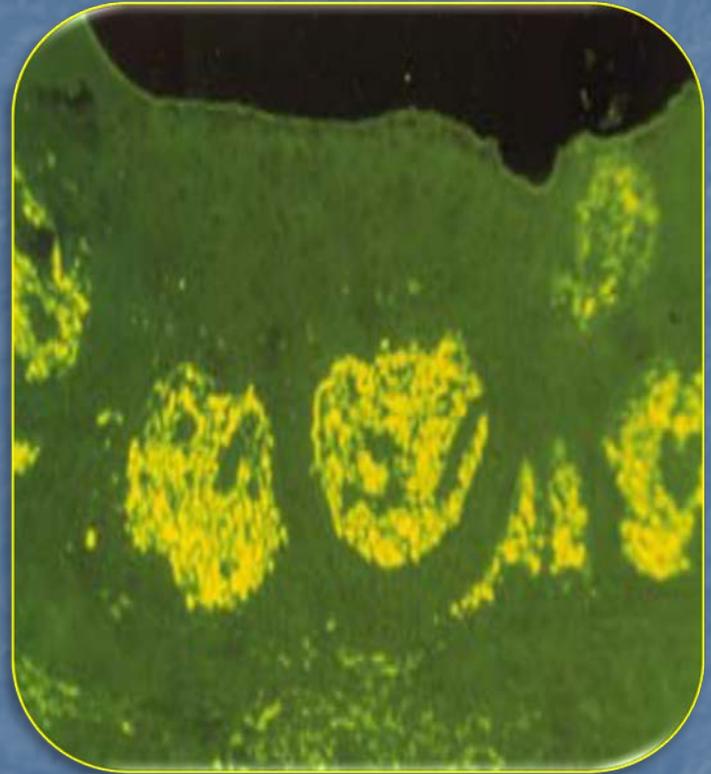
Methyl Violet

- Used for demonstration of amyloid, but not as specific as Congo Red
- Induces a metachromatic reaction by staining amyloid purple



Thioflavine T

- Thioflavine T is a fluorescent dye that attaches non-specifically to amyloid
- Background nuclear staining is quenched by staining with haemalum



Lectin histochemistry

- Proteins (mostly derived from plants) that resemble an antibody in having at least two sites that can bind to specific sugar receptors in a carbohydrate
- **Applications:**
- Show specific cell-types in organs such as the stomach and kidney
- Stain nerve cells and processes in the CNS + PNS
- Many uses in tumour biology and pathology

Lectins as histochemical agents

Group 1 - Affinity for glucose and mannose

Canavalia ensiformis (concanavalin A) - Con A

Galanthus nivalis (snowdrop lectin) - GNL

Group 2 - Affinity for N-acetylglucosamine

Lycopersicon esculentum (tomato lectin) - LEL or TL

Phytolacca Americana (pokeweed mitogen) - PAA or PWM

Group 3 - Affinity for galactose and N-acetylgalactosamine

Arachis hypogaea (peanut agglutinin) - PNA

Artocarpus integrifolia (jackfruit lectin) - Jac

Group 4 - Affinity for fucose

Anguilla Anguilla (eel lectin) - AAA

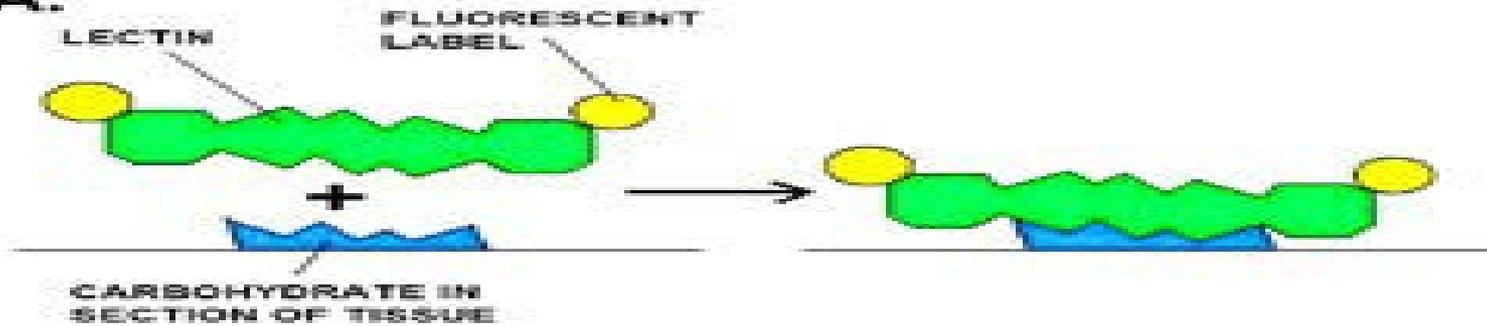
Ulex europaeus (gorse lectin) - UEA

Group 5 - Affinity for sialic and/or uronic acids

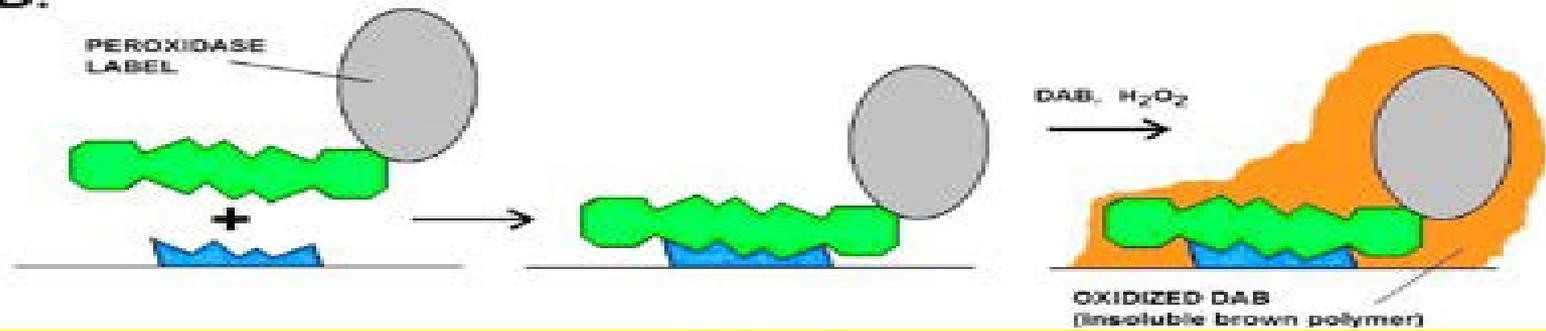
Aplysia depilans (sea-hare gonad lectin) - AGL, SHL

Limax flavus (slug lectin) - LFA

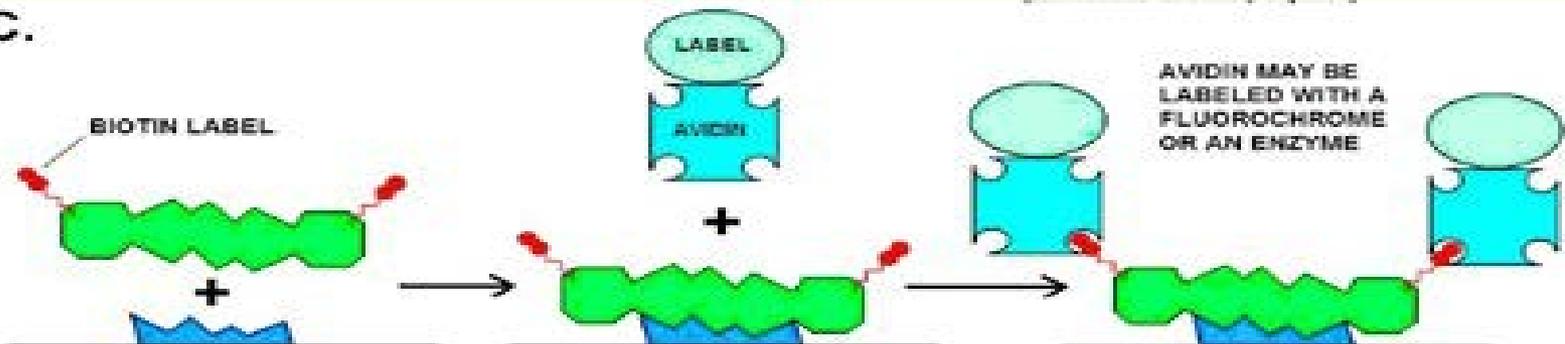
A.



B.



C.





Microglial cells in the brain, stained by virtue of their affinity for the lectin RCA-1 from *Ricinus communis*