Special Staining (I)
Carbohydrates
1- PERIODIC ACID SCHIFF'S (PAS)

- **Purpose:** Glycogen is present in liver, kidney, skeletal and cardiac muscle.

- The **PAS** stain is used to demonstrate neutral polysaccharides that are present in basement membranes, and mucus substances secreted from the epithelia of various organs.

- A routine stain for: liver and kidney biopsies.
**Principle:** The PAS stain is a histochemical reaction in that the periodic acid oxidizes the carbon to carbon bond forming aldehydes which react to the fuchsin-sulfurous acid which form the magenta color.

**Results:**

Glycogen: magenta (red)
2- Alcian Blue  pH 2.5  
Acid Mucopolysaccharides

**PURPOSE:** Alcian blue stains acid mucus substances and acidic mucins.

- Excessive amounts of non-sulfated acidic mucus substances are seen in *mesotheliomas*, certain amounts occur normally in blood vessel walls but increase in early lesions of atherosclerosis.
**PRINCIPLE:** Alcian blue is a group of polyvalent basic dyes that are water soluble. The blue color is due to the presence of copper in the molecule.

- Alcian blue stains both sulfated and carboxylated acid mucopolysaccharides and sulfated and carboxylated glycoproteins.

- It is believed to form salt linkages with the acid groups of acid mucopolysaccharides.

**RESULTS:**

Acid mucins/mucus substances: blue
3- Alcian Blue-PAS (PAb)

- **Purpose:** To differentiate between neutral and acidic mucus substances.

- **Routine stain for G.I. biopsies.**

- **Results:**

  Acid mucus substances: **blue**

  Neutral polysaccharides: **magenta**
Acid mucus substances: blue
Neutral polysaccharides: magenta
4- Mucicarmine Stain - Mucin

**PURPOSE:** To stain acid mucopolysaccharides (mucin), which is a secretion produced by a variety of epithelial cells and connective tissue cells.

- Excess mucin is secreted by epithelial cells in certain inflammations and in certain intestinal carcinomas.
- The mucicarmine technique is also useful in determining the site of a primary tumor in that finding mucin positive tumor cells.
**Principle:** aluminum is believed to form a chelation complex with the carmine, changing the molecule to a positive charge allowing it to bind with the acid substrates of low density such as mucins.

**Results:**
- Mucin: **deep rose**
- Nuclei: **black**
- Other tissue elements: **yellow**
Mucin: deep rose
Nuclei: black
Other tissue elements: yellow
Nucleic Acids
Methyl Green Pyronin Stain

- **PURPOSE:** The nuclear chromatin in the nucleus is composed of nucleoproteins.

- The two types of nucleic acids are deoxyribonucleic acid (DNA), found in the nucleus, and ribonucleic acid (RNA), found in the nucleolus and cytoplasm.

- DNA functions in cell heredity, and synthesis of RNA.

- RNA functions principally in protein synthesis.
• **Principle:** This stain demonstrates RNA and DNA. DNA stains with methyl green, RNA is colored red with pyronine.

• **RESULTS:**

  DNA: blue-green to green

  RNA: pink to red
Methyl Green Pyronin Stain

DNA: blue-green to green
RNA: pink to red
Feulgen stain

- A selective cytochemical reaction for DNA in which sections or cells are first hydrolyzed with hydrochloric acid to produce apurinic acid and then are stained with Schiff reagent to produce **magenta-stained nuclei**.

- Generally the concentration of DNA in nucleoli and mitochondria is too low to permit detection by this stain.
Lipids
Oil Red O

• **Purpose:** To demonstrate fat or lipids in *fresh frozen sections*.

• Fat occurring in an abnormal place, such as fatty emboli, and tumors arising from fat cells (liposarcomas) can be differentiated from other types of tumors.

• **Principle:** Staining with oil-soluble dyes is based on the greater solubility of the dye in the lipoid substances than in the usual hydroalcoholic dye solvents.

• **Results:**

  Fat: red  

  Nuclei: blue
Oil Red O

Fat: red
Nuclei: blue
Sudan Black

- **Purpose:** For the demonstration of fat using *fresh frozen sections* (cryostat sections).

- **Principle:** Sudan Black is slightly basic dye and will combine with acidic groups in compound lipids, thus staining phospholipids also.

- **Results:**
  - **Fat:** blue-black
  - **Nuclei:** red