Carbohydrates are the most abundant and one of the four essential macromolecules, required for the survival of living beings. Structurally, these are polyhydroxy aldehydes or ketones. Carbohydrates are divided into three classes depending upon the number of forming units (aldehyde or ketone), which are *monosaccharides*, *oligosaccharides*, and *polysaccharides*.
These carbohydrates have extensive roles to perform inside the living organism. Monosaccharides, the simplest unit of carbohydrates, including glucose, which acts as an energy source, and amino sugars are the structural part of oligosaccharides and polysaccharides. Disaccharides like sucrose and maltose are used as sweetener and sucrose also acts as a major source of energy in plants. Polysaccharides provide mechanical support to cells in different organisms and they also help in energy storage.

Histochemical demonstration of Carbohydrates

Carbohydrates which can be demonstrated by histochemical methods are polysaccharides. Polysaccharides are polymers of more than ten monosaccharides units. There are two types of polysaccharides: *homopolysaccharides* and *heteropolysaccharides*. Homopolysaccharides contain a single type of monomeric units (example: starch, glycogen, cellulose, and chitin), while heteropolysaccharides contain two or more kinds of monomeric units (example: glycosaminoglycans and peptidoglycan).

Demonstration of Homopolysaccharide

1. Starch

Starch is a branched polymer of D-glucose units. It is a mixture of amylose and amylopectin. They are the storage form of polysaccharides in plants. The presence of starch in tissues can be determined by an iodine test.

- **Iodine Test**

Principle: Reaction of iodine with the amylose in starch results in the formation of a polyiodide chain which gives deep blue color.
Materials required: Sample tissue, Iodine-potassium solution (add 0.2g iodine in 2% potassium iodide solution), distilled water, glycerin jelly, and slides.
Procedure:

- Place the section of a sample tissue in iodine-potassium for 2 minutes.
- Rinse the section with distilled water.

- Mount in glycerine jelly.[8]

Observation: You will observe deep blue or blue-black colored starch granules in the tissue section.

2. Glycogen

In animals, glycogen is the major storage form. It is a highly branched polymer of D-glucose units and is mostly found in the liver and the muscles.

- **Carmine Method**
  Principle: Carminic acid reacts with the hydroxyl group of glycogen (formation of hydrogen bonding) that results in red color glycogen.
  Materials required: Sample tissue, Hematoxylin crystal, Ferric chloride, concentrated HCl, Carmine, potassium carbonate, potassium chloride, ammonium hydroxide, absolute alcohol, methanol, distilled water, and slides.
  Reagents preparation
  Weigert's Iron Hematoxylin:
  *Solution A***:**0 gm hematoxylin crystals in 100 ml of 90% alcohol;
  Solution B: Add 4 ml Ferric chloride in 95 ml distilled water followed by 1 ml of concentrated HC.

For Weigert's Iron Hematoxylin solution, mix solution A and B in equal parts for use.
Carmine solution (stock): Add 2.0 gm carmine, 1.0 gm potassium carbonate, 5.0 gm potassium chloride in 60 ml distilled water. Boil solution for 5 minutes; cool it down; add 20 ml of 28% ammonium hydroxide; you can store it in the refrigerator.
Carmine working solution: Mix 10 ml carmine solution, 15 ml 28% ammonium hydroxide, and methyl alcohol.
Differential solution: mix 20 ml absolute alcohol, 10 ml methanol, and 25 distilled water.

Procedure:

- Deparaffinize the section containing slide and hydrate it by using distilled water.
- Put the slide in Weigert's iron Hematoxylin for 1 minute.
- Wash the slide under running water.
- Rinse the slide with 0.5 % HCl followed by 70% alcohol for 10 seconds.
- Wash the slide under running water for 5 minutes.
- Rinse the slide with distilled water.
- Put the slide in a working carmine solution for 30 minutes.

- Transfer the slide in differentiating solution for 3 seconds.
- Rinse the slide in 70% alcohol.
- Dehydrate the slides in graded alcohol.
- Clear the slide in xylene and mount in synthetic resin.[5]

Observation: You will observe glycogen granules in pink to red color.

1. **Periodic acid-thiocarbohydrazide-silver proteinate reaction**
   Principle: Reduction of osmium tetroxide and silver salts occurs when thiocarbohydrazide is added to the carbonyl solution.
   Materials required: Sample tissue, Paraperiodic acid (H_3IO_6), Crystalline thiocarbohydrazide (TCH), acetic acid, Triple distilled water, protargol silver proteinate, and columbia jar.
   Procedure:

- Oxidize the tissue section with H_3IO_6 and then wash it with distilled water.
- In 1% w/v TCH (dissolved in 10% v/v acetic acid), incubate the section at room temperature for 5 minutes.
- Rinse the section with 5 % and 1 % v/v acetic acid.
- Wash the section with distilled water for 3 minutes.
- Pipette out 10 ml of triple distilled water (before using, sprinkle 100 mg protargol silver proteinate without stirring) in columbia jar.
- Heat the staining solution at 50 °C for 5 minutes and put the section in it for 20 minutes.
- Blot the section with filter paper and then wash it with distilled water.[2]

Observation: You will observe silver granules of glycogen in the tissue.