HISTOPATHOLOGY
CARBOHYDRATES (CHO)
By;
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INTRODUCTION

- The word carbohydrates was coined more than 100 years ago to describe a large group of compounds of the general formula; \( C_n(H_2)O_n \).
- Carbohydrates are involved in a wide range of cellular functions including proteins folding, cell adhesion, enzyme activity, and immune recognition.
- The techniques of carbohydrates demonstration yield a valuable diagnosis findings which help in the characterizing various pathological conditions such neoplasia, inflammation, autoimmune disorders and infectious diseases.
The classification of carbohydrates is a complicated subject due in part to the numerous classifications schemes or systems used in the past.

Some classification based upon the reaction of various dyes or dyes under different conditions of ionic strength or pH with tissue components.

Now more broad and generalized categorization is applied in which the classification is based upon; (1) structure of the monosaccharide moieties within the molecule.
The structure of the polysaccharide components, as well as, the structure or nature of molecules attached to the polysaccharide.

In addition some carbohydrates – containing molecules (i.e. mucins) are classified in part based upon genetic or molecular criteria.
Cn(H2)On

Simple Cn(H2)On

Monosaccharides

Oligosaccharides

Polysaccharides

Proteoglycans

Mucins

Glycoproteins

Glycoconjugates
Simple
$C_n(H_2)O_n$

Monosaccharaides
(Glucose, Mannose, Galactose)

Oligosaccharides
(Sucrose, Maltose)

Polysaccharides
(Glycogen, Starch)
Glycoconjugates

Mucins
(Neutral mucins, sialomucins, sulfomucins).

Connective tissue glycoconjugates
(Proteoglycans)
(Proteoglycans, hyaluronic acid).

Other glycoproteins
{Membrane proteins (receptors, cell adhesion molecules), blood group antigens}.

Glycolipids
(Cerebrosides, gangliosides).
Monosaccharides

- The most basic or simple form of carbohydrates, they are of the empirical formula $(\text{CH}_2\text{O})_n$, where $n$ is the value between 3 – 9.
- The high number of hydroxyl (OH) groups present on the monosaccharide renders most of them extremely water soluble.
- Monosaccharides within a tissue specimen are lost during fixation and tissue processing.
- As a result, they are not easily demonstrated with the most histochemical techniques.
POLYSACCHARIDES

- Is a larger molecules composed of multiple monosaccharaides joined by (glycosidic linkage).
- The $\alpha$ 1-4 glycosidic linkage of glucose units is the predominant linkage in the polysaccharides starch and glycogen.
- Starch and glycogen differ only in size and branching structure.
- Glycogen is the only polysaccharides found in animals that frequently evaluated by histochemical techniques.
- Glycogen serves as a major form of stored energy reserves in the human.
There are a number of disease processes in which histochemical assessment of glycogen content or accumulation may be of value diagnostically.

In most of these disorders the liver shows massive accumulation of glycogen.

In some diseases glycogen accumulation also is observed in skeletal and cardiac muscle.

Histochemical detection of glycogen also may prove helpful in the differential diagnosis of several malignancies such as Seminoma, Rhabdomyosarcoma, Ewig's tumours.
**CONNECTIVE TISSUE GLYCONJUGATES (PROTEOGLYCAN)**

- These molecules are large glycoconjugate complexes that are found in high concentration within the extracellular matrix of connective tissue.

- The carbohydrate components of proteoglycans are known as glycosaminoglycans, they are large polysaccharide polymers that are covalently bound to the protein core of proteoglycan.

- The glycosaminoglycans contain high concentration of acidic monosaccharides which contain a sulfate ester linkage or a carboxyl group.
Several pathological conditions involve accumulation of glycosaminoglycans,

Mucopolysaccharidoses are group of genetic disorders that result from a deficiency of one or more of the enzymes that are involved in the degradation of heparin sulfate and dermatin sulfate,

This deficiency will results in abnormal accumulation of glycosaminoglycans in connective tissue as well as cell types such as neurons, histiocytes and macrophage.
Glycosaminoglycans and proteoglycans are expressed by a number of different sarcomas.

Hyaluronic acid and chondroitin sulfate may be found in high concentration in myxoid chondrosarcomas as well as the myxoid variants of liposarcoma and malignant fibrous histiocytoma.

In addition proteoglycans may be observed in the stromal component of the sarcomas as well as carcinomas.
Mucins consist of polysaccharides chains covalently linked to a protein core.

The carbohydrates component is attached via an O-glycosidic linkage to serine or threonine.

Mucins are categorized into functionally distinct families (muc1, muc2, muc3, etc.).

The carbohydrate content of mucin may account for up to 90% of its molecular weight, the polysaccharide chains of the mucins vary from neutral or weakly acidic to strongly acidic sulfomucins.

The neutral mucins contain a high content of uncharged monosaccharides such as mannose.
Neutral mucins are found in high concentration in the surface epithelial of the gastric mucosa, Brunner’s glands of the duodenum and in the prostatic epithelial.

The function of the mucins varies in part upon the tissue location of the mucin-producing cell as well as the mucin type.

In most cases the secreted mucins provide lubrication and protection for the secreting cells or tissues in the immediate area.

Detection of mucins in a tumour may be a valuable clue in the identification of malignancy.
Malignancies derived from simple epithelial tissues (carcinomas), frequently contain detectable mucin, in contrast melanomas, lymphomas and sarcomas rarely exhibit significant levels of mucins.

Determining the type of mucin (neutral, acidic), may be helpful in evaluating neoplastic changes within a tissue.

The detection of acid or sulfomucins within the gastric mucosa may be aid in the detection and characterization of intestinal metaplasia.
Fixation of carbohydrates

- The selection of an appropriate fixative for the histochemical detection of carbohydrates depends largely on the type of carbohydrates to be demonstrated.
- Due to the aqueous solubility of glycogen we should avoid aqueous based fixatives.
- Glycogen loss during formalin fixation usually does not compromise the ability to detect glycogen with technique such as the periodic acid-Schiff (PAS) method.
- Alcoholic formalins are superior fixatives for glycogen preservation.
Rossman’s fluid, alcoholic formalin with picric acid also has been recommended for glycogen fixation.

Mercuric chloride containing fixatives such as Zenker’s-acetic acid or Susa’s fluid are not recommended for glycogen fixation.

The fixation requirements for the mucins and proteoglycans are less stringent than for glycogen.

Formalin or alcoholic formalin fixation is adequate for the preservation of mucins.
DEMONSTRATION OF CARBOHYDRATES

1. The periodic acid-Schiff (PAS) technique

- It is the most versatile and widely used technique for the demonstration of carbohydrates.
- From diagnostic perspective PAS is one of the more useful and valuable special stains used in the pathology laboratory.
- The PAS technique may aid in the differential diagnosis of tumours.
- The PAS can be used to detect fungal diseases in tissue section.
Mechanism of the PAS technique:

- The PAS technique is based upon the reactivity of the free aldehyde groups within carbohydrates with Schiff reagent to form a bright red magenta end product.

- The first step is oxidation of the hydroxyl groups attached to adjacent carbon atoms (1.2 glycols) within the carbohydrates by dilute solution of (0.5 – 1.0%) periodic acid (HIO₄) for (5-10) minutes.

- The result is the formation of two free aldehyde groups and the cleavage of the adjoining carbon-to-carbon bond.
The aldehyde groups which result will react with the Schiff reagent to give the magenta colour.
2. Alcian blue

**Standard alcian blue technique:**

- Alcian blue is cationic dye.
- The cationic group bond via electrostatic linkages with polyanionic molecules within tissues.
- Alcian blue used mainly to demonstrate mucins.
- Alcian blue dye can be used in many different methods e.g. 1. Alcian blue with different pH level, 2. Alcian blue with varying electrolyte concentration, 3. Combined alcian blue – PAS.
3. Mucicarmine

- This technique is one of the oldest histochemical methods for the visualization of mucins.
- It is mainly used for acidic mucins.
- The carmine complex with it is positive charge will attract the polyanionic molecules such as sialomucins and sulfomucins in the section.
- Because the mucicarmine technique is specific for the mucins of epithelial origin, this technique may be useful for the identification of adenocarcinomas of the GIT.
4. Colloidal iron technique

- This technique is for the detection of mucopolysaccharides.
- This technique is based upon the reaction of ferric cations in a colloidal ferric oxide solution for the negatively charged carboxyl and sulfate group of acid mucins and proteoglycans.
- The tissue–bound ferric ions subsequently are visualized by treatment with potassium ferrocyanide to form bright blue deposits of ferric ferro-cyanide (Prussian blue).
5. Metachromatic methods

Metachromasia may be defined as the staining of tissue or tissue components such that the colour of the tissue – bound dye complex differs significantly from the colour of the original dye complex to give a marked contrast in colour.

Methylene blue, Azure A, and toluidine blue are cationic dyes that typically stain tissue blue, under conditions of metachromasia these dyes stain tissue components purple – red (the use of such dyes to identify mucins and proteoglycans is one of the oldest of the histochemical techniques for CHO).
The end