S. S. College, Jehanabad

Department: Zoology

Class: M.Sc. Semester II

Subject: Zoology

Topic: Histochemical localization of glycogen

Mode of teaching: Google classroom & WhatsApp

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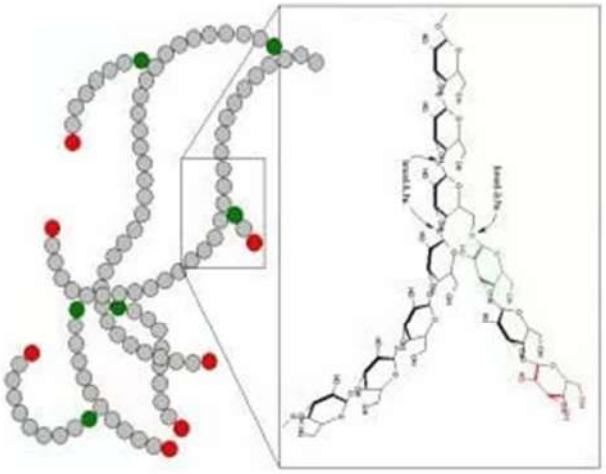
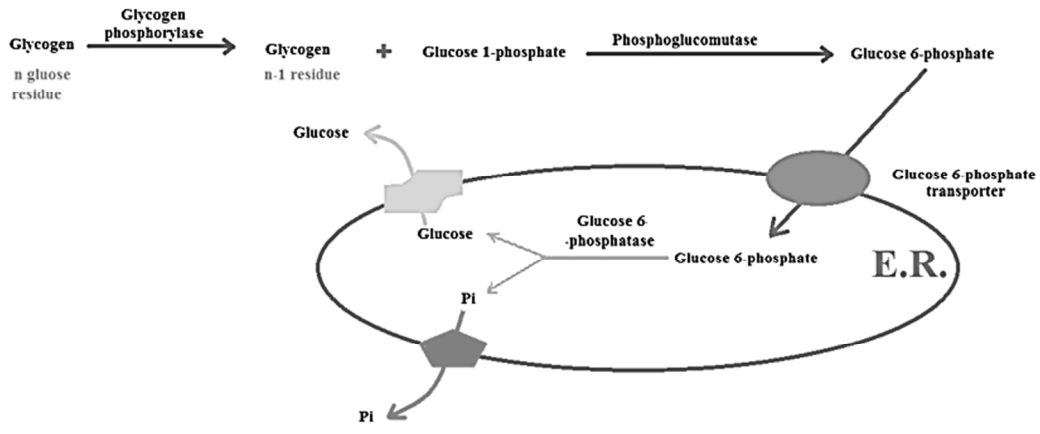
HISTOCHEMICAL LOCALIZATION OF GLYCOGEN

Glycogen is large, branched polysaccharide that is the main storage from of glucose in animals and humans. It is mainly stored in the liver and skeletal muscle. The concentration of glycogen is higher in liver than the muscle. It is estimated to be 10% versus 2% by weight, but more glycogen is stored in skeletal muscle overall because of its much greater mass. Glycogen is formed from glucose following Cori cycle or lactic acid cycle during rest[1] and by insulin hormone secreted by β cells of islets of Langerhans in the pancreas through the process of glycogenesis in which glucose molecules are added to form glycogen chain, and it is readily become available in the form of glucose the need of energy through the process of glycogenolysis, which is actually breakdown of the glycogen molecules into glucose upon activation with glucagon secreted form α cells of islets of Langerhans and epinephrine or adrenaline secreted by adrenal gland primarily in the liver. In addition to liver and skeletal muscles, glycogen is also present in skin, parathyroid gland, cardiac muscle, kidney, uterus, and intestine (Horne & Magee, . It is also an important form of glucose storage in fungi and bacteria.



As described above, it is a branched polymer of glucose in which glucose residues are linked linearly by α-1,4 glycosidic bonds, and approximately at every ten residues, a chain of glucose residues branches off via α-1,6 glycosidic linkages (represented by dark circle inside the chain, while dark circle at the end represent the non-reducing ends of the chain). Glycogen is hydrated with 3 to 4 parts water and forms granules in the cytoplasm that are 10 – 40nm in diameter. The protein glycogenin, which is involved in glycogen synthesis, is located at the core of each glycogen granule. It is an analogue of starch, which is the main form of glucose storage in most of the plants, but starch has fewer branches and is less compact than glycogen.



[1] The Cori cycle named after its discoverer, Carl Ferdinand Cori and Gerty Cori, is a metabolic pathway in which lactate produced by anaerobic glycolysis in muscles is transported to the liver and converted to glucose, which then return to the muscles and is cyclically metabolized.

Glycogen can be localized in the tissue specimen by going through Periodic Schiff's reagent test which is popularly known as PAS reaction, or Carmine method.

Periodic Schiff's reagent test (PAS reaction)

Principles

The PAS stain is widely used for the demonstration of glycogen. Tissue sections are first oxidized by periodic acid. The oxidative process results in the formation of aldehyde groupings through carbon-to-carbon bond cleavage. Free hydroxyl groups should be present for oxidation to take place. Oxidation is completed when it reaches the aldehyde stage. The aldehyde groups are detected by the Schiff reagent. A colorless, unstable dialdehyde compound is formed and then transformed to the colored final product by restoration of the quinoid chromophoric grouping. The PAS stain with diastase or -amylase digestion has histochemical specificity for glycogen. Skeletal muscle normally contains glycogen and is often recommended as a positive control tissue.

Specimen required

Generally, skeletal muscles or section of intestine is utilized going through the process of tissue fixation, processing and microtomy or sectioning into thin pieces. Occasionally, snap frozen human striated muscle is also used when needed.

Methods

For going to histochemical localization of glycogen in a tissue specimen, tissue processing process such as selection of tissue, fixation and optimal tissue processing through dehydration of tissue specimen and later sectioning of thin pieces is needed after paraffin embedding of a tissue specimen before fixation in a standard fixative solution. For the best results, different fixatives for different types of carbohydrate are recommended. Though, for glycogen localization, fixation with Bouin's fixative is largely used, other fixatives are also used as described in table given below.

- Formaldehyde containing fixative
  (*good if sample tissue source is liver, but no reasonable results will be obtained with muscle cell or placenta.)*
- Carnoy's fixative prepared in a fume hood.
- Bouin's fixative at 4 °C which contains picric acid, formaldehyde, and acetic acid.
  (streaming artifacts can be observed)
- For better results, instead of freezing, Lison's "Gendre fluid" at -73 °C which contains ethanol, formaldehyde, picric acid, and acetic acid can be used.

Technique

- Sections of 4 or 5µM are cut in a microtome and gently put on a slide.

- Tissue section is then spread with the help of a water bath top.
- Spread section is then processed to make ready for the staining

Equipment

- Slides
- 22mm square coverslips
- Ceramic staining rack
- Columbia staining dish
- Columbia staining jar
- Forceps
- Latex globes

Reagents

- Absolute alcohol (100% ethanol) – Flammable; store at room temp in a flammable cabinet.
- Glacial Acetic Acid – Corrosive; store at room temperature.
- Amylase - store at room temperature.
- Chloroform – Flammable; store at room temp in a flammable cabinet.
- Periodic Acid - store at room temperature
- Permount – Flammable
- Reagent alcohol
- ACS – histological flammable, toxic & teratogenic; store at room temp in flammable cabinet.
- Schiff Reagent - store at room temperature.
- Xylenes – Flammable; store room temp in flammable cabinet.

Solution

I. Carnoy's Fixative – store at room temp, prepare in a fume hood.

- Alcohol, 100 % 60 ml
- Chloroform 30 ml
- Glacial acetic acid 10 ml

2. Periodic Acid Solution – 0.5 % (w/v) – prepared freshly for each stain

- Periodic acid 50 mg dissolved in deionized water 10 ml

3. Alcohol 50 %

- Reagent alcohol ~50 ml
- Deionized water ~50 ml

4. Alcohol 70 %

- Reagent alcohol ~70 ml
- Deionized water ~30 ml

5. Alcohol 80 %

- Reagent alcohol ~80 ml
- Deionized water ~20 ml

6. Alcohol 95 %

- Reagent alcohol ~95 ml
- Deionized water ~ 5 ml

Procedure

1. Place the coverslip with section in a columbia staining dish.
2. Add Carnoy's fixative to dish for 10 minutes.
3. Rinse very carefully with several exchanges of deionized water. Sections may wash off!!
4. Add Periodic Acid solution to staining dish for 10 minutes.
5. Rinse very carefully with several exchanges of deionized water. Sections may wash off!!
6. Add Schiff Reagent for 5 minutes.
7. Carefully wash with three exchanges of tap or deionized water. Sections may wash off!!
8. Dehydrate in ascending alcohol solutions (50%, 70%, 80%, 95% x 2, 100% x 2) in columbia staining jars.
9. Clear with xylene (3 - 4×) also in columbia staining jar.
10. Mount coverslip on to a labeled glass slide with Permount or some other suitable organic mounting medium.

Results

Glycogen, neutral mucosubstances, basement membranes, collagen fibers, glycolipids and phospholipids will be demonstrated as pink to red to purple color. If diastase or -amylase is used for a negative control, the glycogen deposits are removed leaving only the plasma membrane staining pink. The two major types of fibers are usually distinguished by different intensity of staining.

Carmine method

Principle

Carminic acid reacts with the hydroxyl group of glycogen (formation of hydrogen bonding) that results in red color glycogen.

Equipment

- Slides
- One 22mm square coverslips
- Ceramic staining rack
- Columbia staining dish
- Columbia staining jar
- Forceps
- Latex globes

Reagents

- Hematoxylin crystal
- Ferric chlorid
- Concentrated HCl
- Carmine
- Potassium carbonate
- Potassium chloride
- Ammonium hydroxide
- Absolute alcohol
- Methanol
- Distilled water

Solution

1. Eigert's Iron Hematoxylin solution

- *Solution A* – 10 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 HCl.

For Weigert's Iron Hematoxylin solution, mix solution A and B in equal parts for use.

2. 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
- Store it in the refrigerator.

3. Carmine working solution – Mix 10 ml carmine solution, 15 ml 28% ammonium hydroxide, and methyl, and then mix alcohol.

4. Differential solution – mix 20 ml absolute alcohol, 10 ml methanol, and 25 distilled water.

5. Alcohol 50 %

- Reagent alcohol ~50 ml
- Deionized water ~50 ml

6. Alcohol 70 %

- Reagent alcohol ~70 ml
- Deionized water ~30 ml

7. Alcohol 80 %

- Reagent alcohol ~80 ml
- Deionized water ~20 ml

8. Alcohol 95 %

- Reagent alcohol ~95 ml
- Deionized water ~ 5 ml

Procedure:

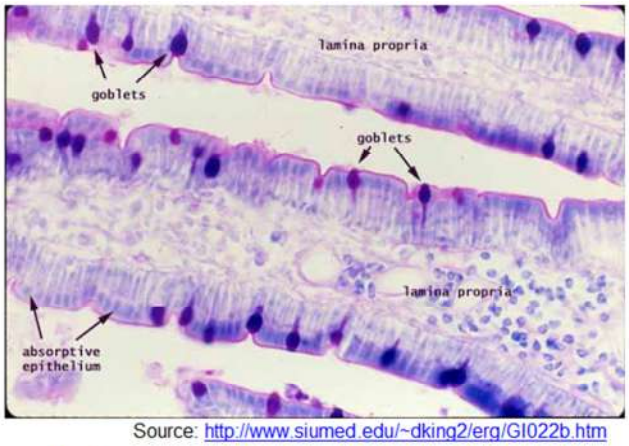
1. Deparaffinize the section containing slide and hydrate it by using distilled water.
2. Put the slide in Weigert's iron hematoxylin for 1 minute.
3. Wash the slide under running water.
4. Rinse the slide with 0.5 % HCl followed by 70% alcohol for 10 seconds.
5. Wash the slide under running water for 5 minutes.
6. Rinse the slide with distilled water.
7. Put the slide in a working carmine solution for 30 minutes.
8. Transfer the slide in differentiating solution for 3 seconds.
9. Rinse the slide in 70% alcohol.
10. Dehydrate the slides in graded alcohol.
11. Clear the slide in xylene and mount in synthetic resin.

Observation: Glycogen granules are observed in pink to red color as seen in figure above.

References

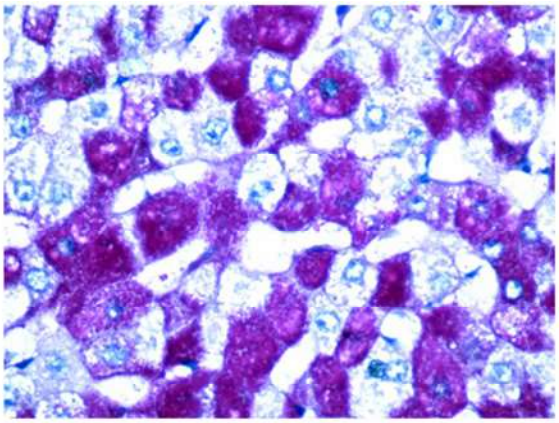
1. Thompson, Samuel W. Selected histochemical and histopathological methods. Charles C. Thomas, Springfield, IL, 1966.
2. Sheehan, D.C. and Hrapchak, B.B. Theory and practice of histotechnology. 2nd Edition; Battelle Memorail Institute, Columbus, OH, 1987.
3. Anjali Singh. Histochemical techniques to demonstrate carbohydrates – polysaccharides. https://conductscience.com/histochemical-techniques-to-demonstrate-carbohydrates-polysaccharides/.

Figures



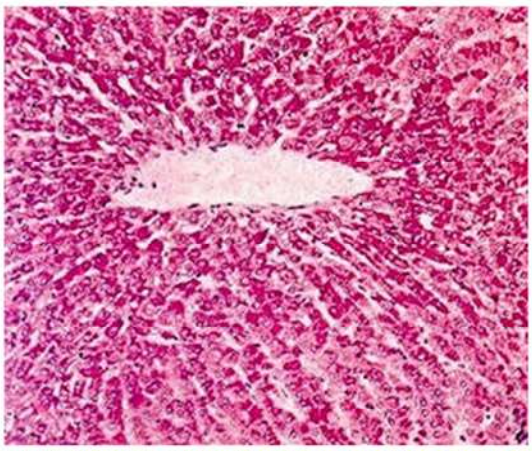
Source: http://www.siumed.edu/~dking2/erg/GI022b.htm

PAS staining of a section of small intestine



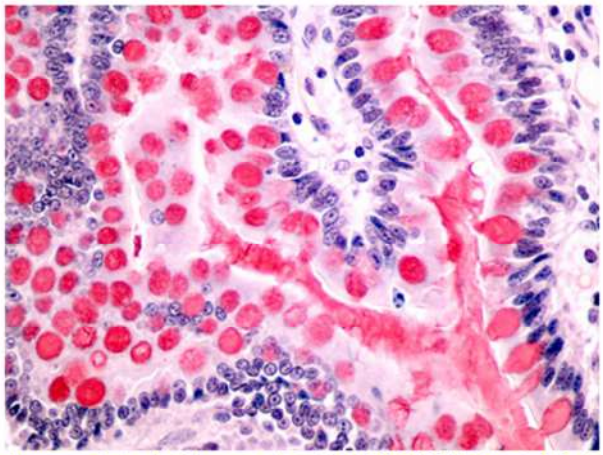
PAS staining of a section of liver

PAS-positive is stained with dark pink to purple colour



Source: http://www.ihcworld.com/royellis/gallery/glycogen.htm

Carmine staining of a section of liver



https://www.vetmed.wisc.edu/lab/wp-content/uploads/sites/32/2019/06/hMucicarmine.1.40x.jpg

Carmine staining of a section from intestine

Glycogen is stained with light to dark pink colour with Carmine staining