Histochemical Staining of Carbohydrates

The tissues sections need to be fixed before they can be stained to detect the presence of carbohydrates.

Fixation: The selection of fixative depends on the type of carbohydrate that is to be demonstrated. As a general principle, fixation of glycogen is more difficult in comparison to other glycoconjugates such as mucins and proteoglycans.

For fixation of glycogen Rossman`s fluid which is alcoholic formalin with picric acid is used. In case of glycoconjugates such as mucins and proteoglycans formalin or alcoholic formalin can be used for fixation.

The Periodic Acid – Schiff (PAS) Technique: This is the most widely used method for the histochemical staining of carbohydrates. This technique was first used by McManus in 1946 to demonstrate mucin.

Mechanism of staining:

A. Basic steps in staining:

The hydroxyl groups attached to adjacent carbons are called as 1,2-glycols. First step in the staining is to oxidize these hydroxyl groups into aldehydes while breaking the bond between the adjacent carbons.

This is done by treating the sections with 0.5-1% solution of periodic acid (HIO_4) for 5-10 minutes.

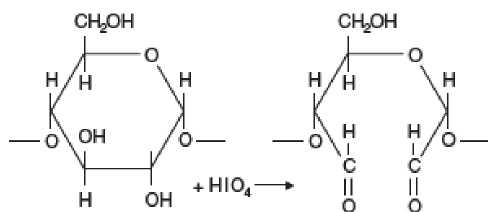

Figure: Periodic acid oxidation of glucose unit within glycogen.

This oxidized form is now made to react with the Schiff`s reagent for 15 minutes and then given aqueous rinse. After this the carbohydrate containing parts of the section develop a deep red/magenta color.

Section is observed after mounting using microscope.

B. Schiff`s reagent and its chemistry:

This is prepared using basic fuschin. Basic fuschin is not a dye but a mixture of triarylmethane dyes such as pararosaniline, rosaniline and new fuschin. To produce Schiff`s reagent basic fuschin and sodium metabisulfite ($Na_2S_20_5$) are dissolved in dilute HCl. This leads to production of sulfur dioxide and it reacts with basic fuschin to add a sulfonic group to the central carbon of the triarylmethane molecule.

Due to this reduction quinoid configuration within triarylmethane is lost and so is magenta color. The free amino groups of the triarylmethane react with additional equivalents of the sulfur dioxide. This leads to formation of the Schiff`s reagent shown in the figure below.

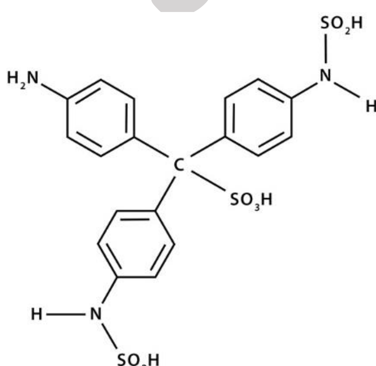

Figure: Schiff`s reagent

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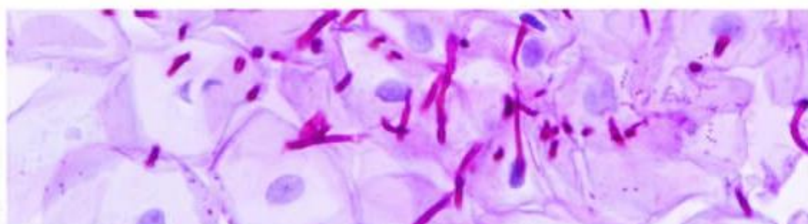
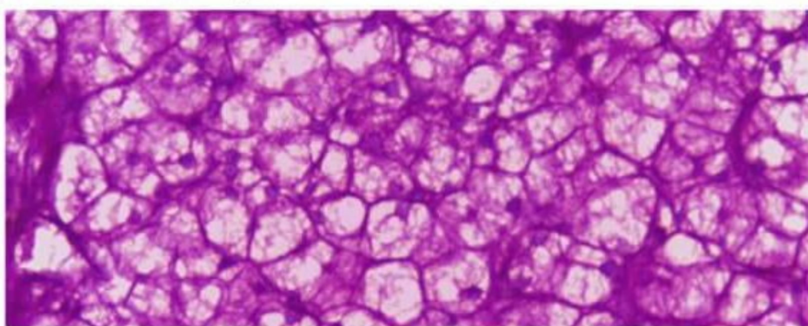
Now, this Schiff` reagent reacts with the free aldehyde groups generated due to the action of periodic acid. Initial monosaccharide - Schiff reagent conjugate is colorless and represent an intermediate reaction. When the section is given aqueous rinse, loosely bound sulfonate at central carbon is removed and it re-establishes the quinoid structure of the triarylmethane molecules.

Due to this reaction deep red/magenta color develops at the sites where monosaccharide – Schiff conjugate is deposited in the section.

Limitation: Monosaccharides that lack1,2 glycols or contain hydroxyl groups in an ester or glycosidic linkage can`t be oxidized using periodic acid. Thus, they can`t be detected using PAS technique.



Esophageal candidiasis, PAS stain



Liver in glycogen storage disease, PAS stain

Figure: Tissue sections stained using PAS method.

Histochemical Staining of Nucleic Acids

These methods are based on the fact that DNA and RNA have negatively charged phosphates in their backbone. Thus they can be stained using basic dyes such as hematoxylin, methyl green or pyronin etc. This is called as basophilia and is mainly based on electrostatic interactions.

We generally do not prefer to use hematoxylin because it stains glycosaminoglycans and other anionic complexes along with DNA and RNA.

Before staining, the sections must be fixed and when going for nucleic acid staining fixation is done using Carnoy`s fluid.

Feulgen staining:

This method was developed by Feulgen and Rossenbeck in 1924 and is a standard method for demonstrating DNA. It does so by demonstration of the sugar deoxyribose.

Mild acid treatment is given to the section using 1M HCl at $60^{\circ}C$. This breaks the purine-deoxyribose bond. The exposed aldehydic groups are now demonstrated using Schiff`s reagent.

After this the section is subjected to series of rinse steps and then counterstained with 1% light green.

DNA appears red-purple and cytoplasm appears green.

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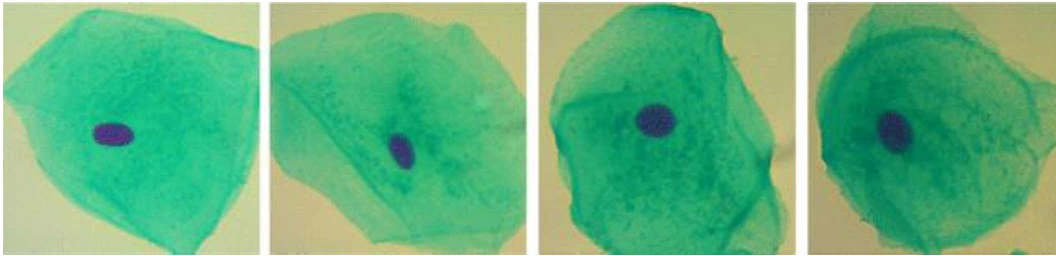

Figure: Buccal epithelium cells stained with Feulgen and light green viewed at 1000X.

Methyl green-pyronin:

This is method of choice for demonstration of the RNA in sections.

Methyl green-pyronin contains two different dyes i.e. methyl green and pyronin Y which are dissolved separately and then mixed in acetic acid buffered solution to generate the functional dye.

When used in combination both the dyes are cationic and methyl green preferentially binds to the DNA leaving pyronin to bind with the RNA.

Methyl green shows specificity for the DNA due to spatial alignment of NH_2 groups in the dye with phosphate groups in the DNA backbone and this arrangement is not possible in RNA.

Also pyronin does not show this spatial affinity for DNA and stains any negatively charged tissue component including RNA. Since DNA is occupied by methyl green it will not be stained.

DNA appears green-blue while RNA appears red.

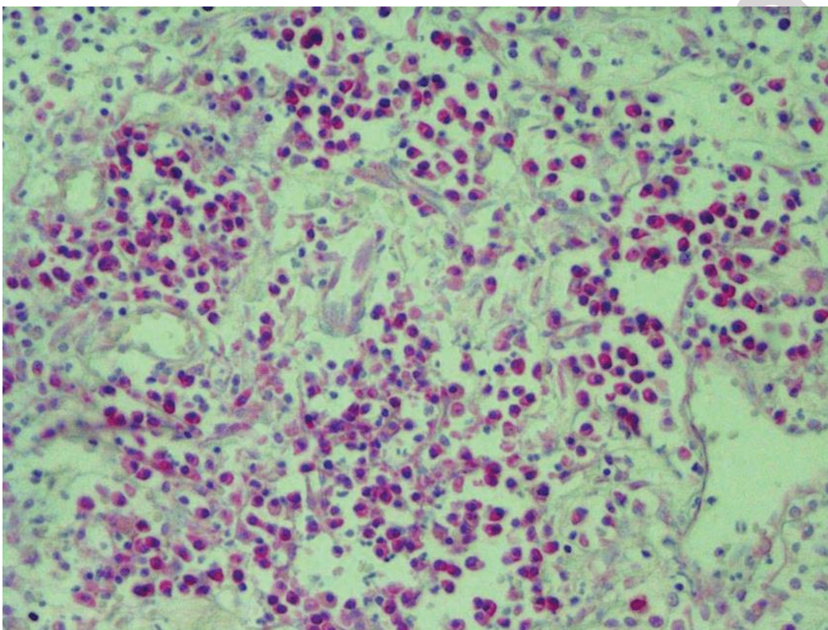

Figure: Tissue section showing plasma cells stained using methyl green pyronin method.

DNA is stained blue while RNA is stained red.

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Histochemical Staining of Lipids

This is done using Sudan family of dyes, mainly Sudan black B. This family is collection of lipophilic diazo dyes and are also called as lysochrome dyes.

Examples of the dyes from the family are Sudan II, Sudan III, Sudan IV, Oil Red O and Sudan Black B.

Sudan Black B is a slightly basic dye that can combine with acidic groups in the lipid compounds. Thus, it can be used for staining the phospholipids, lipoproteins and triglycerides. Since it binds to membranes it will stain the organelles that are part of the endomembrane system.

The section is fixed in formal calcium or can be unfixed. As a matter of general principle for Sudan Black B staining unfixed sections are preferred. It is because fixation enhances the staining of phospholipids and it is undesirable in general uses.

The section is rinsed using 70% ethanol and then stained using Sudan Black B prepared in 70% ethanol for up to 2 hours. After this section is rinsed with 70% ethanol and water respectively and counterstained using Kernechtrot for 2-5 minutes.

This is the standard procedure for Sudan Black B staining and it stains unsaturated esters and triglycerides blue – black.

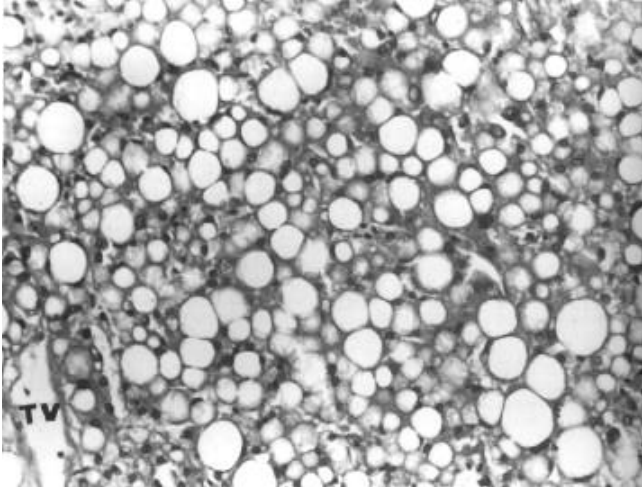

Figure: Section stained using Sudan Black B.

Pratap Singh

Histochemical Staining of Proteins

This staining was first described by Yasuma and Ichikawa in 1953. This reaction is based on oxidation of the primary amino groups in the proteins using ninhydrin. The result is that protein bound aldehyde groups are generated and these are stained using rosaniline.

The sample is fixed in Cornoy`s fluid and the for best results 1% ninhydrin is prepared in 70% ethanol which is saturated with $NaHCO_3$. The tissue section shows purple-pink stain for the protein rich parts.

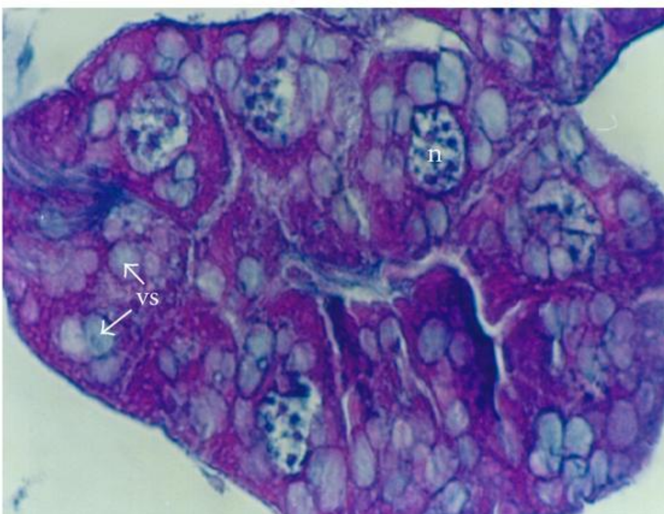

Figure: Section showing staining with Ninhydrin-Schiff method in secretory cells. The protein rich regions are stained purple-pink.