CYTOCHEMISTRY

- Two types of nucleic acids – DNA & RNA
- Consist of nitrogeneous bases (purines & pyrimidines), ribose sugar, and phosphate group.
- Formed of purine and pyrimidine bases.
- Purines – adenine & guanine
- Pyrimidine – thymine & cytosine
- DNA contains deoxy ribose sugar and RNA contains ribose sugar.

- Both DNA & RNA are precipitable from alkaline solutions by hydrochloric acid and ribonucleic acid is also precipitated by acetic acid.

- Both form salts with alkaline earths or heavy metals and on hydrolysis they yield phosphoric acid, purine and pyrimidine bases and a carbohydrate or carbohydrate derivative.

- Histochemical methods for their demonstration in the tissue have been based on reactions for all these constituents.

General reactions for Nucleic Acids

1.Reaction for organic phosphate (Serra and Queiroz Lopes,1945)

- Serra & his co-author demonstrated the phosphate radical in the DNA of the nucleus by hydrolysis and subsequent fixation of the released PO4 groups with ammonium molybdate.

- The resulting phosphomolybdate was reduced to a blue compound with benzidine instead of the more usually employed stannous chloride.

- They employed either enzymatic hydrolysis with nucleases or prolonged hydrolysis with N-HCl.

- The method is not very suitable for general use in histochemistry but serves to demonstrate the presence of PO4 in the chromosomes.

2. Reactions Specific For Deoxyribonucleic Acid

a)The Fuelgen (Fuelgen Schiff) reaction

- The reaction was introduced by Fuelgen and Rossenbeck (1924) as a specific test for thymonucleic acid (DNA).

- The reaction depends on the treatment of fixed tissues by mild hydrolysis (with N-HCl at 60ºC) which Fuelgen showed could release aldehyde groups from the deoxyribose of DNA.

- Following hydrolysis the tissues are washed and transferred to a solution of Schiff's reagent (fuscin-sulphurous acid) which reacts with the exposed aldehyde group to produce a purple dye in the nuclear chromatin alone.

- The chemical explanation of the reaction is, the gentle acid hydrolysis would transform deoxyribose into ω-hydroxy laevulinic aldehyde this labile aldehyde was responsible for the purple colour given with Schiff's reagent in the Fuelgen test.

- Acid hydrolysis first breaks the sugar linkages engaged in polymeric bonding and secondly ruptures the glycoside linkages between the purine bases.

- The deoxyribose compound thus revealed are described as being attached through PO4 linkages in the main nucleic acid chain where they are firmly held in the furanose form(5 membered ring), with exposed aldehyde residue.

The ribose sugars (present in RNA) with an OH at carbon atom 2, are not hydrolysed by normal HCl and therefore do not react in Fuelgen test. Therefore the Fuelgen test is specific for DNA.

DETECTION OF CARBOHYDRATES

Periodic acid – Schiff's reaction for carbohydrates detection

- Periodic acid–Schiff (PAS) is a staining method used to detect polysaccharides such as glycogen, and mucosubstances such as glycoproteins, glycolipids and mucins in tissues.

- The reaction of periodic acid oxidizes the vicinal diols in these sugars, usually breaking up the bond between two adjacent carbons not involved in the glycosidic linkage or ring closure in the ring of the monosaccharide units that are parts of the long polysaccharides, and creating a pair of aldehydes at the two free tips of each broken monosaccharide ring.

- The oxidation condition has to be sufficiently regulated so as to not oxidize the aldehydes further.

- PAS method works by exposing the tissue to periodic acid.
- Periodic acid acts as oxidizing agent which oxidizes compounds having free hydroxyl group (-OH group) or amino/alkylamine group resulting in dialdehydes.
- These dialdehydes when exposed to Schiff’s reagent, an insoluble magenta colored complex is formed. A suitable basic stain is often used as a counterstain.

DETECTION OF PROTEINS

MERCURY BROMOPHENOL METHOD

- This method was introduced by Durrum (1950) for the demonstration of proteins.
- Mercury of the stain react with acid group of protein especially hydroxyl, carbonyl, and phosphoric acid parts.
- It has selective action towards SH groups.

- Thus mercury remains bound with nucleopreoteins.

- Other proteins and some SH groups gives an intense blue colour. The solubility of Hg-S bond is greater than that of the bond between Hg and any other group of proteins.

- The sharp and intense staining of protein permits good differentiation of structures often difficult to observe, such as cilia, spindle elements, regions of spindle fiber attachment to chromosomes and "lamp brush" chromosomes.

- The procedure is specific for proteins and those peptides which are not removed in the washing procedure.

- The preparations stained by this procedure follow the Beer and Lambert Laws in microspectrophotometric measurements. The absorption maximum is at 610 millimicrons.

- Basic proteins bind the dye under the conditions of the method even when Hg is omitted.

- Other proteins bind the dye by coupling through Hg. As expected, structures containing basic proteins show enhanced staining after removal of the nucleic acid.

DETECTION OF LIPIDS

SUDAN BLACK B STAIN FOR LIPIDS

- Sudan Black B ($C_{29}H_{24}N_6$) is a nonfluorescent, relatively thermostable lysochrome (fat-soluble dye) diazo dye used for staining of neutral triglycerides and lipids on frozen sections and some lipoproteins on paraffin sections.

- Due to the invariable presence of triglycerides in tissue fat, this dye can be effectively used for the histochemical demonstration of lipids.

- However no chemical reaction is involved in this histochemical technique and the dye simply dissolve in lipid part of the tissue.

- The characteristic blue black colour is retained only where it remain dissolved in lipids. Colour from any other part get washed off when treated in alcohol.