Protein

- Major component in tissue , made up of amino acid.
- Can occur either alone or combined with other substance (lipoprotein. Mucoprotein , nucleoprotein)
- Identification of the above compounds is based on the lipid or muco poly saccharide or nucleic acid.

Demonstration of Nucleic Acid

- *General Notes:*
- *1. Fixation*

Nucleic acids are best preserved in alcoholic and acidic fixatives e.g. Carnoy s fluid

Low temperature fixation in neutral buffered formalin give acceptable result

Decalcification:

- 1. Strong inorganic acid must be avoided
- 2. organic acid permit acceptable result .

DNA DEMONSTRATION

- DNA demonstrated by ;
- 1-Feulgen technique
- 2-Fluorescent method
- 3-Gallocyanin chrome-alum technique
- 4-Methyl green- Pyronin
- 5-In situ hybridization

Feulgen reaction

Mild acid hydrolysis , employing 1M HCL at 60c is used to break the purines -deoxyribose bond .the resulting aldehydes are then demonstrated by Schiff's reagent (red – purple color)

- The ribose- purines bond is un affected by hydrolysis and the RNA is not demonstrated
- Feulgen reaction used in conjunction with micro-dinstomitry to study cancer (DNA content in certain lymphoma is inverse to the tumor prognosis)

Feulgen stain

- Generally the concentration of DNA in nucleoli and mitochondria is too low to permit detection by this stain

- Can be useful to quantify amount of DNA (by using spectrophotmetry of Feulgen stained tissue)
- Phospholipid (plasmal reaction) may give feulgen reaction in cryostat technique but not in paraffin tech

FEULGEN-NAPHTHOIC ACID-HYDRAZIDE METOHD

- This technique can used as control method for standard feulgen reaction. The sections are hydrolyzed in 1 M Hcl as in the feulgen reaction .

- The aldehydes produced by this hydrolysis are coupled with 2-hydroxy-3- naphthoic acid which in turn is coupled with diazonium salt salt Fast Blue B.