Notes

16

PROCEDURES FOR DNA, RNA AND MITOCHONDRIA DEMONSTRATION

16.1 INTRODUCTION

Nucleoproteins are combinations of basic proteins and nucleic acids. The two nucleic acids are deoxyribonucleic acid (DNA), which is mainly found in nucleus and ribonucleic acid (RNA) which is located in the cytoplasm of cells, mainly in the ribosomes. Both DNA and RNA molecules consist of alternate sugar and phosphate groups with a nitrogenous base being attached to each sugar group. The sugar in DNA is deoxyribose and in RNA it is ribose. The demonstration of nucleic acid depends upon either the reaction of dyes with the phosphate groups or the production of aldehydes from the sugars.

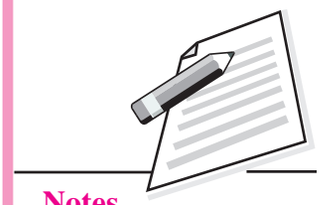
OBJECTIVES

After reading this lesson, you will be able to:

- explain the methods used in demonstrating nucleic acids
- describe the techniques and principles of the methods used.

16.2 DNA

The demonstration of DNA is either by Feulgen technique (which demonstrates the sugar deoxyribose) or the methyl green-pyronin technique (where the phosphates combine with basic dye methyl green at acidic pH). It can also be demonstrated by fluorescent methods using acridine orange, but is considered



less reliable than the above mentioned methods. The definitive and most sensitive technique is in situ hybridization.

16.3 FEULGEN TECHNIQUE

This technique involves mild acid hydrolysis with 1M hydrochloric acid at 60°C to break the purine-deoxyribose bond, the resulting exposed aldehydes are then reacted with Schiff's reagent to stain the DNA red-purple in color.

Feulgen nuclear reaction for DNA

Fixation: Not critical but do not use Bouin's fixative

Solutions

(a) 1 M hydrochloric acid

Hydrochloric acid (conc.)	8.5 ml
Distilled water	91.5 ml

(b) Schiff reagent

(c) Bisulfite solution

10% potassium metabisulfite	5 ml
1M hydrochloric acid	5 ml
Distilled water	90 ml

1. Bring all sections to water.
2. Rinse sections in 1M HCl at room temperature.
3. Place sections in 1M HCl at 60°C
4. Rinse in 1M HCl at room temperature, 1 minute.
5. Transfer sections to Schiff's reagent, 45 minutes.
6. Rinse sections in bisulfate solution, 2 minutes, repeating twice again.
7. Rinse well in distilled water.
8. Counterstain if required in 1% light green, 2 minutes.
9. Wash in water.
10. Dehydrate through alcohols to xylene and mount.

Results

DNA	red-purple
Cytoplasm	green

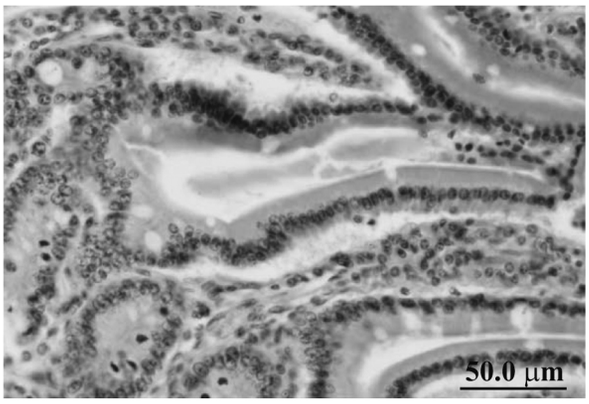


Fig. 16.1: Mouse small intestine stained with Feulgen's reaction and fast green counterstain. DNA is stained a magenta color; the cytoplasm is stained a uniform green .

16.4 RNA

The method of choice for demonstrating RNA is the methyl green-pyronin technique.

Methyl green-pyronin

Methyl green is an impure dye containing methyl violet. When methyl violet has been removed by washing with chloroform, the pure methyl green appears and is specific for DNA. Both dyes are cationinc, when used in combination methyl green binds preferentially and specifically to DNA, and pyronin binds RNA.

Methyl green-pyroninmethod for RNA

Fixation: Carnoy preferred, but formalin acceptable.

Staining Solution: Methyl green pyronin Y

2% methyl green (chloroform washed)	9ml
2% pyronin Y	4 ml
Acetate buffer pH 4.8	23 ml
Glycerol	14 ml

Mix well before use.

Method

1. Take sections down to water.
2. Rinse in acetate buffer pH 4.8.
3. Place in methyl green-pyronin Y solution for 25 min.
4. Rinse in buffer.

5. Blot dry.
6. Rinse in 93% ethanol, then in absolute ethanol.
7. Rinse in xylene and mount.

Results

DNA	green-blue
RNA	red

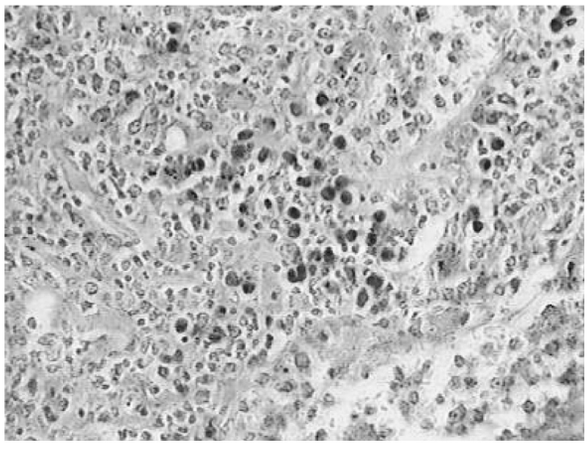


Fig. 16.2: R.N.A. – (notably in plasma cell cytoplasm) – magenta. D.N.A. – green or purplish green.

16.5 MITOCHONDRIA

Mitochondria are the cytoplasmic organelle found in variable numbers in all animal cells. Large number of mitochondria in the cells can change the appearance of cells. Mitochondria are considered the 'power houses' of the cell as many of the energy producing biochemical reactions like oxidative phosphorylation and Krebs cycle activity takes place in mitochondria. Mitochondria can be demonstrated by electron microscopy, enzyme histochemistry and histological methods however electron microscopy is the most satisfactory method. Histopathological methods such as Altman's technique for mitochondria is simple and useful for demonstration of mitochondria.

Altman's technique for mitochondria

Fixation

Champy's fluid is usually recommended, Helly's fluid works equally as well

Aniline-acid fuchsin – saturated solution of acid fuchsin in 5% aniline in distilled water.

Notes

Differentiator 1

Saturated alcohalic picric acid	10 ml
30% alcohol	40 ml

Differentiator 2

Saturated alcohalic picric acid	5 ml
30% alcohol	40 ml

Method

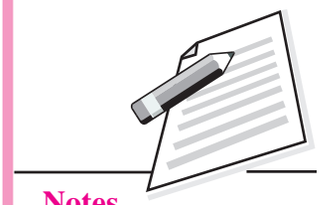
1. Take sections down to water.
2. Flood sections with aniline-acid fuchsin solution.
3. Gently heat the slide until steam rises and leave for 5 min.
4. Rinse in tap water.
5. Differentiate in solution 1 until the excess red stain is removed.
6. Completely differentiate in solution 2, controlling microscopically.
7. Dehydrate rapidly in two changes of absolute alcohal.
8. Clear in xylene and mount in DPX.

Results

Mitochondria	red
RBC and nuclei	red
Background tissue	yellow

INTEXT QUESTIONS 16.1

1. Deoxyribonucleic acid is found in .....
2. Ribonucleic acid is located in ..... of cells
3. Definite and most sensitive technique is .....
4. ..... should not be used as fixation for DNA
5. ..... technique is the method of choice for demonstrating RNA
6. ..... is the preferred fixation for RNA demonstration
7. ..... is used for demonstration of mitochondria
8. In Methyl green pyronin technique the DNA appears ..... and RNA appears .....



WHAT HAVE YOU LEARNT

- Nucleoproteins are combination of basic proteins and nucleic acids
- Two nucleic acids are deoxyribonucleic acid found in nucleus and ribonucleic acid found in cytoplasm of cells
- Demonstration of nucleic acid depends upon either the reaction of dyes with phosphate groups or production of aldehydes from the sugars
- DNA is demonstrated by Feulgen technique and RNA by Methyl green pyronin technique the most definite and sensitive technique is in-situ hybridization
- Carnoy is preferred as fixative in methyl green pyronin technique for RNA demonstration
- Mitochondria is demonstrated by Altman's techniques and is best visualized by electron microscopy

TERMINAL QUESTIONS

1. What are the components of nucleic acids?
2. What are the procedures used to detect DNA and RNA?
3. Describe the method used to detect mitochondria.

ANSWERS TO INTEXT QUESTIONS

16.1

1. Nucleus
2. Cytoplasm
3. In-situ hybridization
4. Bouin's fixation
5. Methyl green pyronin
6. Carnoy
7. Altman's technique
8. Green-blue & Red