Lesson for P.G. 3rd Semester Zoology

Principle and Method for cytochemical demonstration of Nucleic acid by Methyl green method.

Nucleic acid, DNA molecule consists of a long unbranched chain which is made up of five carbon sugar (deoxyribose), alternating with phosphate group, a nitrogenous base and purine (adenine and guanin) and pyrimidine (thymine and cytosine). The chain is subdivided into units called nucleotides made up of phosphate - sugar - base. Watson and Crick proposed DNA model - the polynucleotide chain intertwined into a double helix held together by hydrogen bond.

In RNA, the sugar is ribose instead of deoxyribose and adenine, guanine, cytosine and uracil (in place of thymine) are the bases. They are linked by simple groups forming a polynucleotide chain.

F. Miescher 1869 experimented with nucleic ~~acid~~ of cells and isolated a substance at first named nuclein.

R. Feulgen 1914 made his first test on DNA in 1924 and describe the method of staining the nucleic acid, and stated DNA is located in chromosomes in the nucleus.

Fixation of Nucleic acid

A nuclear fixative like Carnoy, or formol saline are good. For smears of tissues, methyl alcohol or clarke's fixative are superior. When fixed in neutral buffered formalin at 4°C, DNA degradation by cell nuclease is prevented. (Tokuda ed. al 1990).

Clarke's fluid

It is prepared by mixing absolute alcohol 75.0 ml and glacial acetic acid 25.0 ml.

The penetration quality of this fixative is superior to others. It is good for cytoplasmic elements also.

When a decalcified tissue is demonstrated for nucleic acid, there is a chance of these nucleic acid being extracted since nucleic acids are extracted with acids.

DNA can be demonstrated by Feulgin method of Feulgen and Rossenbeck (1924), methyl green pyronin technique, fluorescent method using acridine orange.

Methyl Green Pyronin method for Nucleic acid.

(Elias, 1969)

Fixation — Carnoy

Reagent required

Methyl green
Acetate buffer (Walpole)
Pyronin.

Preparation of reagent

Solution 1

Methyl green 500 mg
Acetate buffer 100.0 ml
Pyronin G or Y 200 mg

Procedure

1. Slides is deparaffinize and hydrated in wa
2. Treated it with solution 1 for 1 hour at 37
3. Rinse it in cold distilled water.
4. Rinse it in butanol
5. Dehydrate in butanol giving 2 changes of 5 minutes each
6. Clear the mount.

Result

Nuclear and cytoplasmic basophilic substances are stained red.

RAJ NARAIN COLLEGE
Hajipur (VAISHALI) - 844101
(A Post Graduate Unit of B.R.A. Bihar University, Muzaffarpur)
Fax/Ph.No. : 06224-265512, Website : www.rncollegehajipur.com
Head
Deptt. of Zoology

LESSON FOR Part I (Zoology H)

विभागाध्यक्ष
जन्तु विज्ञान

Ref./पत्रांक : .....

Date/दिनांक : .....

Leishmania

Genus - Leishmania was created by Rossi 1908 word kalazar derived from Kalo - black, azar - disease

Phylum - Protozoa
Sub Phylum - Sarcomastigophora (Plasmodroma)
Super class - Mastigophora
class - Zoomastigophora
order - Kinetoplastida
Genus - Leishmania

Habit & Habitat

Leishmania is a pathogenic intracellular endoparasite of humans and other mammals, causing the three types of diseases in human.

(1) L. donovani causes kala-azar or Visceral Leishmaniasis in human being in India and other Asian Countries.

kala-azar is a chronic disease characterized by enlargment of liver, spleen, and by an irregular fever, anaemia & leucopenia

L. donovani has a digenetic life cycle (a) Its primary host is human being in which parasite lives in the endothelial cells of blood vessels and also enters cells of liver, spleen & bone marrow.

(b) Its secondary host or intermediate host and Vector is blood sucking sand fly Phlebotomus species (Ex. P. argentipes in India).

(2) L. tropica causes oriental sore or Delhiboils.

(3) L. brassiliensis causes muco-cutaneous American Leishmaniasis.

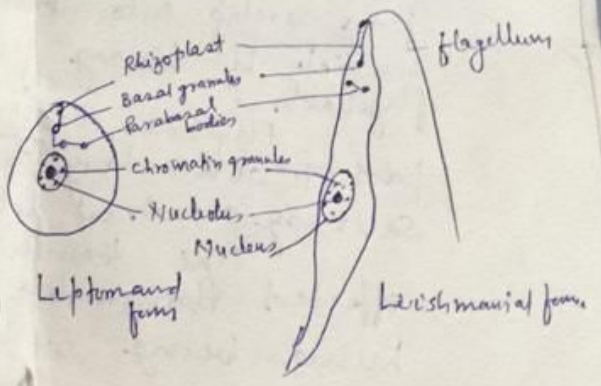
~~Genus~~

Morphology

L. donovani has two phases in the life cycle —

(a) one flagellar Leishmanial form (amastigote form). It is found in reticulo-endothelial cells of human beings and other

(b) Leptomonad form (Promastigote form) in the alimentary canal of sand fly



Leptomonad form

Leishmania is intracellular and found in the cells of reticulo-endothelial system of sandfly. The form is oval leptomonad forms 3 to 4 µm in diameter with a limiting membrane-like Kellich.

The cytoplasm contains an oval nucleus, rod-shaped a dot like kinetoplast and parabasal body.

- It reproduces asexually by binary fission.
- Flagellar promastigote form of L. donovani occurs in insect vector (sand flies). It is long slender spindle shaped measuring 15 to 20 µm in length and 1 to 2 µm in breadth.
- Single flagellum projects from the anterior end of body and do not form undulating membrane along the body.

Transmission

→ Amastigote forms of the pathogens are liberated in human blood stream as a result of the rupture of Reticulo-endothelial cells.

- In the blood stream, some of the free amastigote are phagocytosed by neutrophilic granulocytes and monocytes (macrophages).

In India the vector of blood sucking sand fly (Phlebotomus argentipes) draws these free amastigote form during its blood meal.

Inside the midgut of Sandfly these amastigote form develops into promastigote forms which again multiply by binary fission producing numerous flagellates.

Flagellates tend to spread forward to anterior part of alimentary canal (pharynx & buccal cavity, salivary glands are not infected).

The transmission of Pathogens is thereby effected through bite of infected Sand fly from human being to human being.

Transmission

L. donovani is transmitted by the bite of infective Sand fly Phlebotomus argentipes.

Pathogenicity

- L. donovani infection in man starts as a primary lesion in the skin.
- After 4-6 months the parasite spreads to the viscera with preference for spleen, liver & bonemarrow infecting the cells of the reticulo endothelial System.
- The harmful effects are due to the blockage of the RES and anaemia resulting from the invasion of the bone marrow.
→ At the later stage the skin may be infected and if the person suffering from this disease is left untreated death often results.