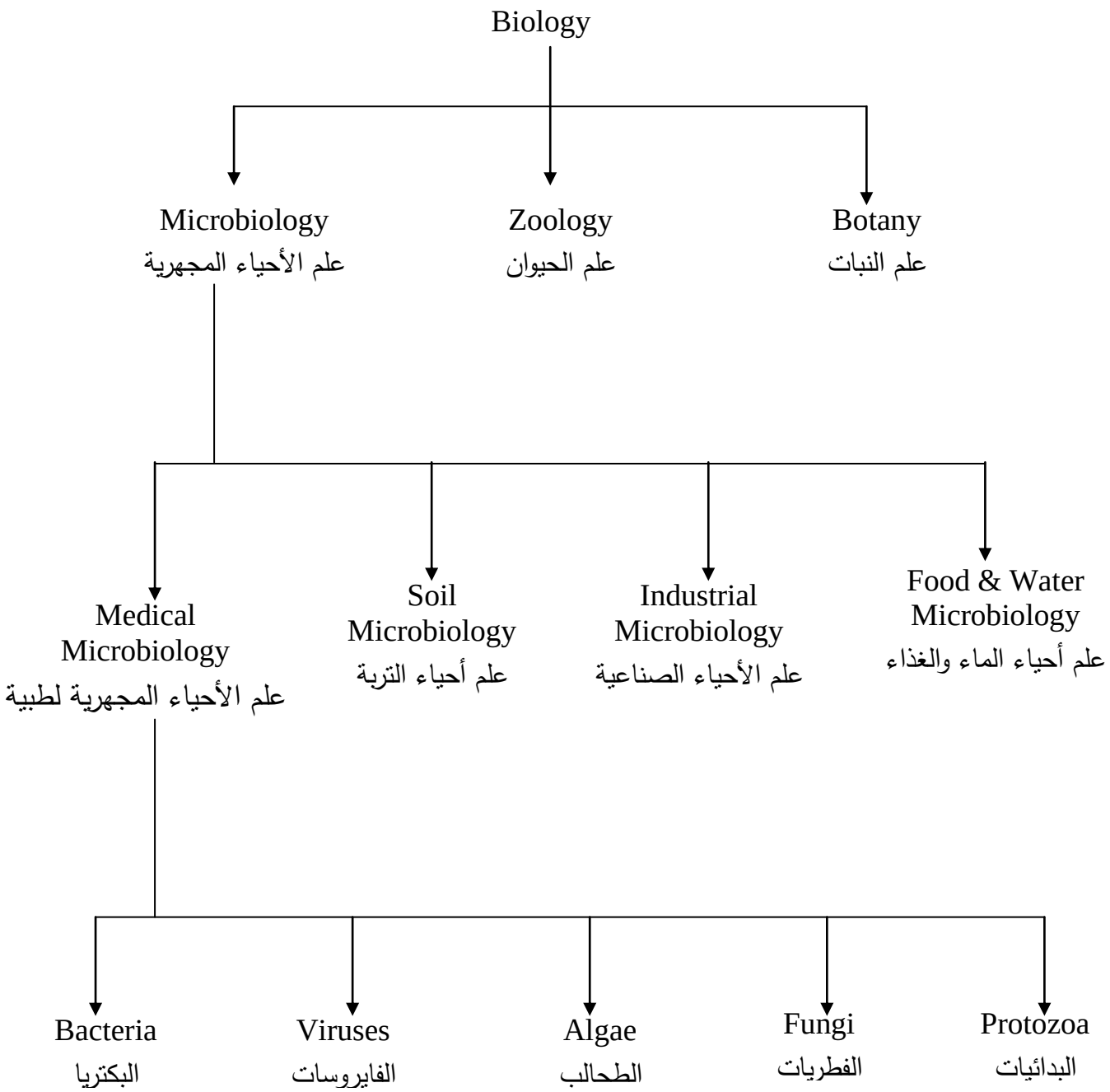
القسم : تقنيات الاشعة

البايولوجي الطبي

مدرسة المادة: د.آلاء سعيد


Biology
Microbiology
علم الأحياء المجهرية
Zoology
علم الحيوان
Botany
علم النبات
Medical Microbiology
علم الأحياء المجهرية لطبية
Soil Microbiology
علم أحياء التربة
Industrial Microbiology
علم الأحياء الصناعية
Food & Water Microbiology
علم أحياء الماء والغذاء
Bacteria
البكتريا
Viruses
الفايروسات
Algae
الطحالب
Fungi
الفطريات
Protozoa
البدائيات

INTRODUCTION

Biology

Is the science which study the life of living individuals and their environment.

The first scientist who used the word (cell) is Robert Hooke in 1665. in 1835 said Du Jardin that the contents of the cell are main substance in the life. Then Schleiden mentioned in 1839 that all developed plants are collection of cells. In 1840 said Purkinje of all the contents of the cell name "Protoplasm".

Then we improved our information about the cell, specially after the development of ordinary microscope and electronic microscope, then our study became a main branch of biology which named cytology.

Cell

It is a built unit and function in a living individuals.

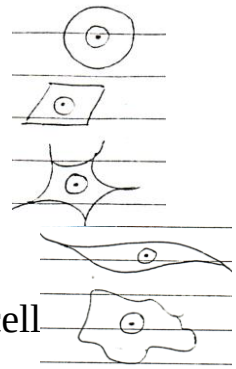
Eukaryotic cell	**Prokaryotic cell**
1- It has a nucleus covered with nuclear envelope. 2- Cell division by i. mitosis . ii. meiosis. 3- Example : i. Fungi. ii. Algae except blue green	1- It isn't have a nucleus, but it has a nuclear liquid. 2- Cell division by i. Amitosis. 3- Example : 1. Eubacteria. 2. Blue green algae.

algae. iii. Protozoa . iv. Plasmodium . 4- Have many chromosomes. 5- Large. 6- Have nucleolus .	4- Have one filament chromosome. 5- Small. 6- Have no nucleolus .

An Animal Cells

Shape : there are several shapes of the cells :

1. Circular cell
2. Cubic cell
3. Star cell
4. Spindle cell
5. unorganized cell



Size :

1- Micro cell : We cannot seen it by naked eye, just with the microscope .

Ex : blood cell .

2- Macro cell : We can seen it by naked eye.

Ex: The egg of the chicken .

General Structure

- **Cell wall :** it surrounds the contents of the cell it consist of two layers of phosphoric lipids. We can seen it by the electronic

microscope only. The cell wall do organizing of water transportation between inside the cell and outside.

- **Cytoplasm :** it is a living part of the cell it lies out of the nucleus and covered with cytoplasmic membrane. It consists of : water 80%, proteins 15%, lipids, sugars and salts 5%.

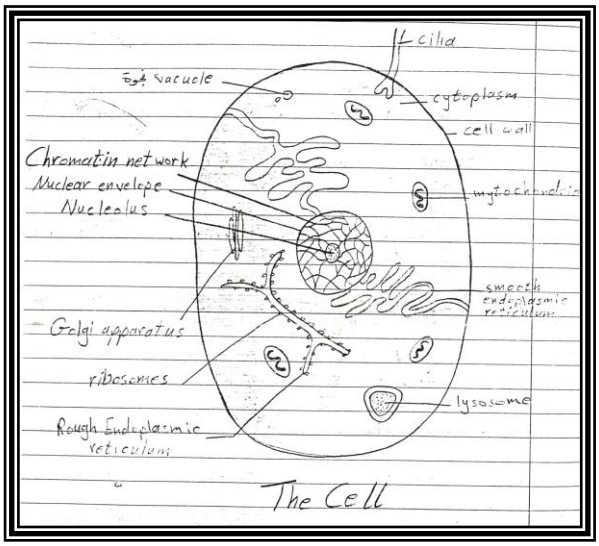
Cytoplasm has many contents, they are :

1. Nucleus : it surrounds by the nuclear envelope and it contains of :
   i. Nucleoplasm .
   ii. Nucleolus : it contains of Ribonucleic acid (RNA) and protein.
   Nucleolus function is : built Ribosomal RNA(r-RNA) to making Ribosomes.
   iii. Chromatin network.
   iv. Chromosomes : to carry Deoxyribonucleic Acid (DNA)
2. Endoplasmic reticulum :
   It has two types :-
   a. Rough Endoplasmic reticulum : it named that because it contains of Ribosomes.
   b. Smooth Endoplasmic reticulum .
3. Mitochondria : it is the center of providing the energy to the cell. It stores the energy as Adenosine Triphosphate (ATP), for that reason the main function of mitochondria is cellular respiration.
4. Golgi apparatus : it has an important benefit :
   a. built the complex sugars.
   b. Secretion the protein that will be leave the cell.

c. Secretion complex sugars, proteins, several hormones and enzymes.

5. Lysosomes : It can digest large particles like proteins and nucleic acids to smaller units.

- **cilia and Flagella** : There are moving fingers in monocellular individuals that are living in water area.



The Bacteria

Bacteria : They are microscopic , single organism – called prokaryotic. They are among the earliest known life form on the earth. Bacteria live in huge quantities in all environment of the

earth ,where they are found in the soil , oceans , rocks , arctic snow , glaciers , hot springs , radioactive waste and even in the depths of the earth crust , as well as some types of bacteria are found in living organisms such as plants and animals , as well as on or inside humans.

The importance of bacteria in life

Bacteria are one of the living organisms that live around us at all time and that also have a great impact on our daily lives , despite being organisms that cannot be seen with the naked eye. The human body contains balanced amounts of beneficial and harmful bacteria , the beneficial ones protect the human body and accrue to it with many benefits , while the harmful ones , if they exceed their normal rates , they causes some diseases. We will mention the benefits and harms of bacteria.

The benefits of bacteria to human

1. Bacteria help break down food and maintain.
2. Bacteria are used in food production to yogurt and cheese .
3. It is used to produce antibiotics.
4. It is involved in production of some vitamins ,such as vitamin B and vitamin K .
5. It is involved in the production of insulin, which is used to treat diabetes .

Bacterial Damage

1.It causes infection with some infection , such as : skin infection , pneumonia , meningitis , tuberculosis and anthrax .

2.Exposed foods are damaged and spoiled.

3. Cause teeth decay .

4. It sometime cause food poisoning .

Morphology of Bacteria

Size of bacteria : Most of bacteria are so small that their size is measured in micron.

1 micron (μ) or micrometer (μm) = $\frac{1}{1000}$ of millimeter.

1 millimicron (mμ) or nanometer (nm) = $\frac{1}{1000}$ of micron.

1 angstrom units (A^{o}) = 0.1 of nanometer.

Generally cocci are about 1μ in diameter and bacilli are 2 - 10 μ length and 0.2 - 0.5 μ in width. Obviously bacteria can be visualized only under magnification.

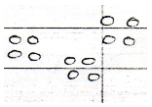
Shape of bacteria : on the basis of shape, bacteria are classified as under :

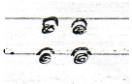
1. cocci

a. Staphylococci : They are spherical. One the basis of arrangement of individual organisms, they are described as staphylococci (clusters like bunches of grapes) 

b. Streptococci : Arranged in chain. 

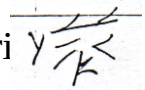
c. Diplococci : forming pairs. 

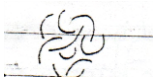
d. Tetrads : Arranged in groups of four packets. 

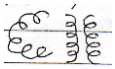
e. Sarcina : Arranged in groups of eight cells. 

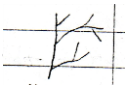
2. Bacilli : The cylindrical or rod shaped organisms are called bacilli. Their length may approximate the width of the organisms.

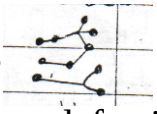
   Ex : Brucella. 

3. Chinese letter : this arrangement is seen in corynebacteri 

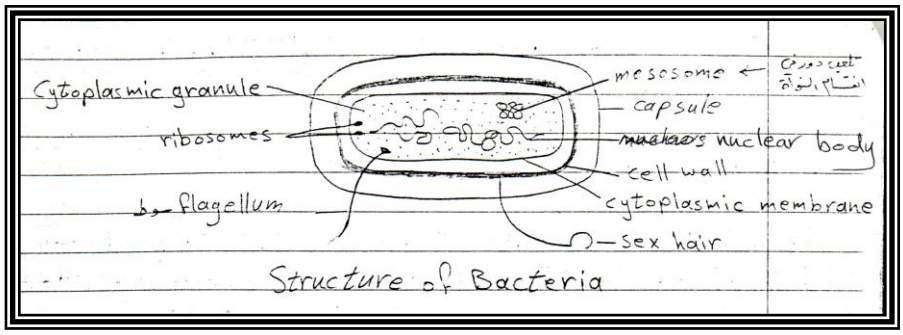
4. Vibrio : They are curved rods and derive the name from their characteristic vibratory. 

5. Spirochaete : They are relatively longer, thin, flexible organisms having several coils. 

6. Actinomycetes : they are branching bacteria 

7. Mycoplasma : they are organisms which, lack cell wall. They are round or oval bodies with interlacing filaments 

Many a time cell wall synthesis become defective either spontaneously or as a result of drugs. Ex : In presence of penicillin bacteria lose their distinctive shape. Such organism are called protoplast, spheroplast.



Bacterial Anatomy

The outermost layer consist of two components :

a. Rigid cell wall.
b. Cytoplasmic membrane.

Inside this there is protoplasm comprising of the cytoplasm, Cytoplasm inclusions such as ribosomes, mesosomes, granules, and nuclear body. The cell may be enclosed in a viscid layer which may be loose slime layer or organized as a capsule. A part from this some bacteria carry filamentous appendages protruding from cell surface : the flagella, organ of locomotion.

The important structure of bacterial cell is found under electronic microscope and described below :

*** Slime Layer :**

Some bacteria secrete viscid substance which may diffuse out into surrounding media or remain outside cell wall. This viscid carbohydrate material is called slime layer.

Bacteria secreting large amount of slime produce mucoid growth on agar with stringy consistency. Slime has little affinity for basic dye and so not visible in Gram stain.

Capsule

It is gelatinous secretion of bacteria which gets organized as a coat around cell wall, it is known as capsule. It may be composed of complex polysaccharides. Capsule have no affinity for dyes and so they are not seen in stained preparations.

Demonstration of Capsule

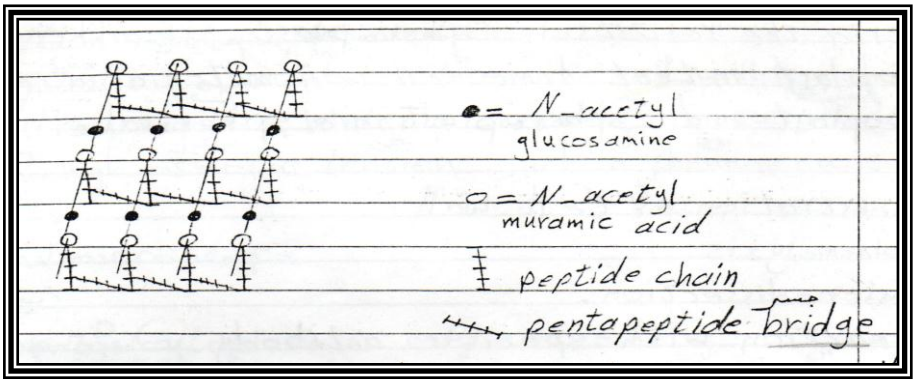
b. Negative staining with India ink : in this producer, bacterial bodies and spaces in between are filled with India ink and capsule is seen as hollow around the cell.
c. Special capsule staining technique using copper salt
d. Serological methods : if suspension of capsulated bacterium is mixed with its specific anti-capsular, examine under microscope, capsule becomes prominent and appears swollen due to increase in rate activity.

Functions of capsule :

1. Protection against deleterious agents. Ex: lytic enzymes.
2. contribute to the virulence of pathogenic bacteria by inhibiting phagocytosis.

*** Cell wall :**

The cell wall is the, outermost supporting layer which protect the internal structure. It is about 10-25nm. In thickness and shares 20-30% of dry weight of the cells.



Chemical structure of cell wall

Cell wall is composed of mucopeptide, scaffolding formed by N-acetyl glucosamine and N-acetyl muramic acid molecules alternating in chain cross linked by peptide chain. Cell wall antigenes of Gram negative organisms act as endotoxin. A comparison of cell walls of gram positive and Gram negative bacteria is as under :

	Gram Positive	**Gram Negative**
1. Thickness	15-23 mμ	10-15 mμ
2. Variety of amino acids.	few	several
3. Aromatic and sulfur	absent	present
4. Lipids.	low 2-4%	high 15-20%
5. Teichoic acid.	present	absent

Cell wall synthesis may be inhibited or interfered by many factors. Lysozyme enzyme present in many tissue fluid cause lysis of bacteria. They act by splitting cell wall mucopeptide linkages. When lysozyme acts on gram positive organism in hypertonic solution a protoplast in formed consisting of cytoplasmic membrane and contents with Gram negative bacteria the results in spheroplast. It differs from protoplast in that some cell wall material in retained Protoplast and spheroplast are spherical shape.

Demonstration of cell wall

1. Plasmolysis.
2. Microdissection.
3. Reaction with specific antibody .
4. Mechanical rupture of cell.
5. Differential staining procedure .
6. Electronic microscope .

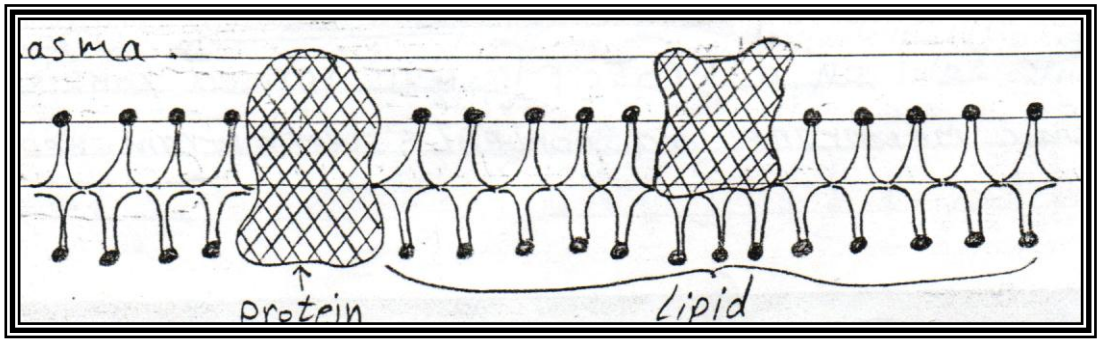
Functions of cell wall

1. Protection of internal structure (supporting layer) .
2. gives shape to the cell.
3. confers rigidity and ductility (Mucopeptide).
4. Role in division of bacteria.
5. offers resistance to harmful effect of environment.

Cytoplasmic membrane

It is thin semi-permeable membrane which lies just beneath the cell wall. It is 5-10 nm in width. Electron microscope shows the presence of three layers constituting a unit membrane structure.

Chemically the membrane consists of lipoprotein with small amount of carbohydrate. Sterol are absent except in mycoplasma.



Demonstration of cytoplasmic membrane

1. The separation of membrane from the cell wall is achieved readily in Gram negative bacteria when they are suspended in medium of high osmotic tension. Such a phenomena is called plasmolysis.
2. Electron microscope

Functions of cytoplasmic membrane

1. It controls inflow and outflow of metabolites to and from protoplast.
2. Presence in the membrane of specific enzyme plays important role in passage through membrane.

***Cytoplasm**

The bacterial cytoplasm is suspension of organic and inorganic solutes in viscous watery solution.

It doesn't exhibit protoplasmic streaming (Internal mobility) and it lacks endoplasmic reticulum or mitochondria. It contains ribosomes, mesosomes, inclusions and vacuoles.

*** Ribosomes**

These are ribonucleoprotein granules measuring 100-200A^{o} units in diameter their sedimentation coefficient is 70 Svedberg units. The 70s ribosome is composed of two smaller units of 50s and 30s.

Function of Ribosomes

They are the sites of protein synthesis.

Polysomes

They are groups of ribosomes linked together like beads of chain by messenger RNA.

*** Mesosomes**

They are vesicular, convoluted or multilaminated structures formed as invaginations of plasma membrane into the cytoplasm. They are more prominent in Gram positive bacteria.

Functions of Mesosomes

1. They are the sites of respiratory enzymes in bacteria.
2. Coordinate nuclear and cytoplasmic division .

Intracytoplasmic inclusions

(a) **Volutin granules:** They are highly refractive , basophilic bodies consisting of polymetaphosphate.

Demonstration of Volutin granules : Special staining techniques such as Albert or Neisser, demonstrate the granules more clearly. They are characteristically present in diphtheria bacilli.

Function of Volutin granules : They are considered to represent a reserve of energy and phosphate for cell metabolism.

(b) Polysaccharide granules : may be demonstrated by staining with iodine. They appear to be storage product.

(c) Lipid inclusion : Again storage product and demonstrated with fat soluble dyes such as Sudan black.

(d) Vacuoles : They are fluid containing cavities separated from cytoplasm by a membrane. Their function and significance is uncertain.

Nucleus:

It is a long filament of DNA tightly coiled inside the cytoplasm. The bacterial nucleus is not surrounded by nuclear membrane. They don't have nucleolus. Nucleus can't be demonstrated-under direct light microscope. They appear as oval or elongated bodies, generally one per cell.

The genome consists of a single molecule of double stranded DNA arranged in the form of circle. It may open under certain conditions to form long chain about 1000 μ in length. Genes are arranged along the length of chromosome in fixed order and bear hereditary characters.

Bacteria may sometimes have extranuclear genetic material these are called plasmid or episomes. They may be transmitted to daughter cell.

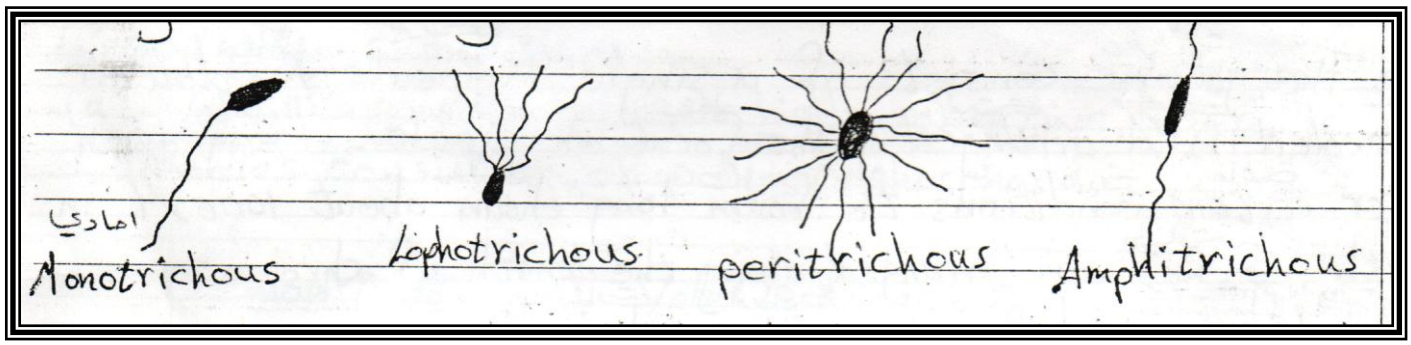
a. During binary fission.
b. By conjugation.
c. Through agency of bacterial phages.

Plasmids are not essential for the life of cell. They may confer certain properties like toxigenicity and drug resistance.

Flagella

These are long, contractile filamentous appendages known as flagella. They are organ of locomotion. e.g. *E coli, Salmonella, Vibrio, Pseudomonas* etc. They are extremely thin (0.05 μ or less), helical shaped, structure of uniform diameter throughout their length. Each flagellum originate in a spherical body (basal granule) located just inside cell wall. They are antigenic and are composed of protein, celled flagellin which has properties of fibrous protein kerasin and myosin.

The number and arrangement of flagella are characteristic of each bacteria. Flagella may be arranged on bacterial body in following manner :



Monotrichate : One flagellum at one end of the organism. e.g. *vibrio, pseudomonas, spirillum* etc.

Amphitrichate : One flagellum at both the poles. e.g. *Alcaligenes faecalis.*

Lophotrichate : A tuft of flagella at the end. e.g. pseudomonas

Peritrichate: Several flagella present all over the surface of bacteria. e.g. bacterial motility.

Demonstration of flagella

1. Dark ground microscope.
2. Special staining techniques in which their thickness is increased by mordanting.
3. Electron microscope.

Fimbriae : They are filamentous short, thin, straight hair like appendage (0.5µ long and less than 10nm thick). They are also called pili. They project from cell surface, as straight filaments. They are best developed in freshly isolated strains and in liquid culture. They tend to disappear following subcultures on solid media.

Functions of Fimbriae :

1. Organ of adhesion.
2. Conjugation tube through which genetic materials is transferred from donor to recipient cell.
3. They are antigenic.

Spores

They are highly resistant dormant state of bacteria found in certain genera. e.g. bacilli and clostridium. They are not destroyed by ordinary methods of boiling for several hours. They are killed when autoclaved at (15 lbs/square inch) pressure at 121^{o}C for 20 minute.

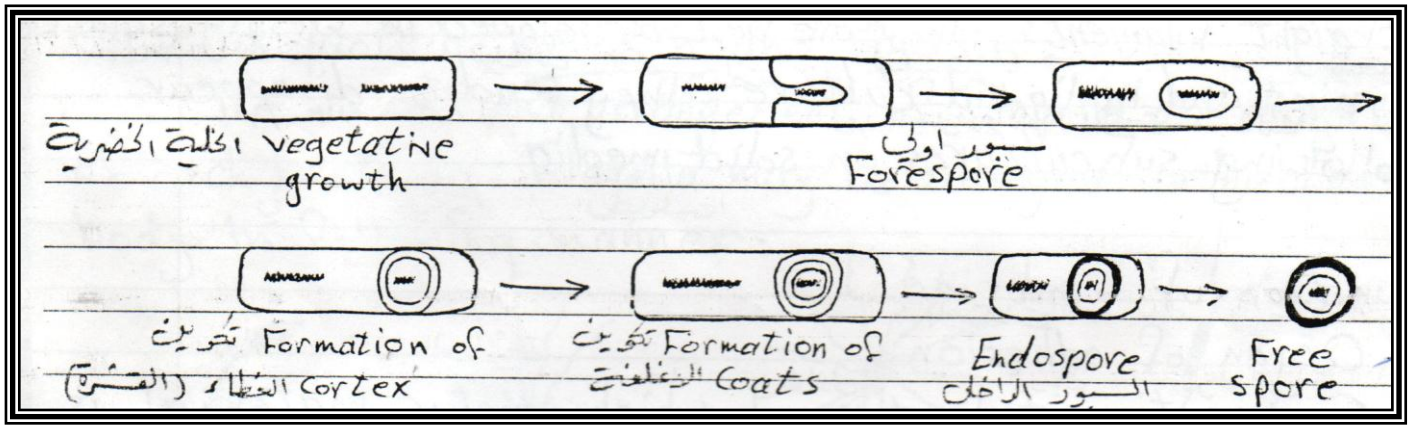
The spores are characterized by the presence of 5% to 20% dipicolinic acid which is not found in vegetative cell and by their

high calcium content. Spores of different species of bacteria are antigenically distinguishable. Spores are highly refractile and require special staining for demonstration. e.g.(1) Modified Ziehl Neelsen method,(2) Gram stain,(3) Moller stain.

Functions of spores :

They make survival of organism possible under unfavourable conditions like dry state. Spores are resistant to heat, drying, freezing and toxic chemicals.

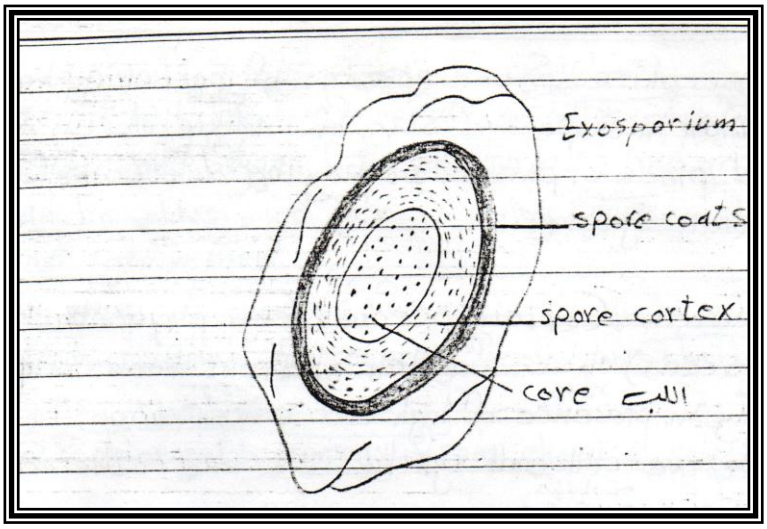
formation of Spores



Exact stimulus for sporulation is not known. Perhaps it is related to depletions of exogenous nutrient. Sporulation is initiated by appearance of clear area near one end of cell which gradually becomes more opaque to form forespore. The fully developed spore has at its core nuclear body surrounded by spore wall, a delicate

membrane (future cell wall). Outside this is spore cortex which in turn is enclosed by multilayered spore coat.

Some spores have an additional outer covering called exosporium having ridges and grooves.



Types of bacterial spores:-

1. Central bulging .
2. Central not bulging.
3. Sub terminal bulging.
4. Sub terminal not bulging .
5. Terminal spherical .
6. Terminal oval.

Pleomorphism

Some species of bacteria show great variation in shape and size of undivided cell. This is called pleomorphism. It may be due to defective cell wall synthesis.

Involution

Certain species (e.g. plaque bacilli, gonococcus) show swollen aberrant forms in aging culture especially in presence of high salt concentration. It may be due to defective cell wall synthesis or due to the activity of autolytic enzymes.

L. Forms

The name L. Form is after the Lister institute, London where swollen and aberrant morphological forms from the culture of *Streptobacillus moniliformis* was studies. They are in several species of bacteria developing that either spontaneously or a presence of penicillin like agent that interfere with cell wall synthesis.

NUTRITIONAL REQUIREMENT OF BACTERIA

Bacteria may require adequate nutrition of optimum pH, temperature and oxygen for multiplication and growth. Bacteria can be classified into following types on the basis of nutritional requirement.

1. On the basis of energy sources.
   (a) Phototrophic which get energy from photochemical reactions.
   (b) Chemotrophic gets energy from chemical reactions.
2. On the basis of their ability to synthesize essential metabolites.
   (a) Autotrophic : These are the organisms in which all essential metabolites are synthesized from inorganic sources. They use carbon dioxide as the main source of carbon and simple inorganic salts. e.g.: nitrates, nitrites ammonium sulphate, phosphates etc.
   (b) Heterotrophic : Here some of the essential metabolites are not synthesized. Organic compounds e.g. protein, peptones, amino acids, vitamins and growth factor are supplied from outside. Most of the bacteria producing disease in man are heterotrophic.

The other nutritional requirement are as under :

1. Minerals : These are sodium, potassium, magnesium, calcium, iron, chlorine, zinc, copper, iodine and strontium in traces. These are essential for physiological activities of bacteria.
2. Gas requirements:

(a) Oxygen : the capacity of bacteria to grow in the presence of oxygen and to utilize it depends on possession of a cytochrome oxidase system

- Aerobes : the aerobe organisms grow only in the presence of oxygen. E.g. pseudomonadaceae, *bacillus, nitrobacter, sarcina,…* etc.
- Facultative anaerobes : They are the organisms that can live with or without oxygen. e.g. *vibrio, E. coli, salmonella, shigella* and *staphylococcus*. The micro-aerophilic organism grow well with relatively small quantities of oxygen e.g. *Haemophilus*
- Obligate anaerobes : the strict anaerobes multiply only in the absence of oxygen. e.g. *bacteroides, clostridium.*

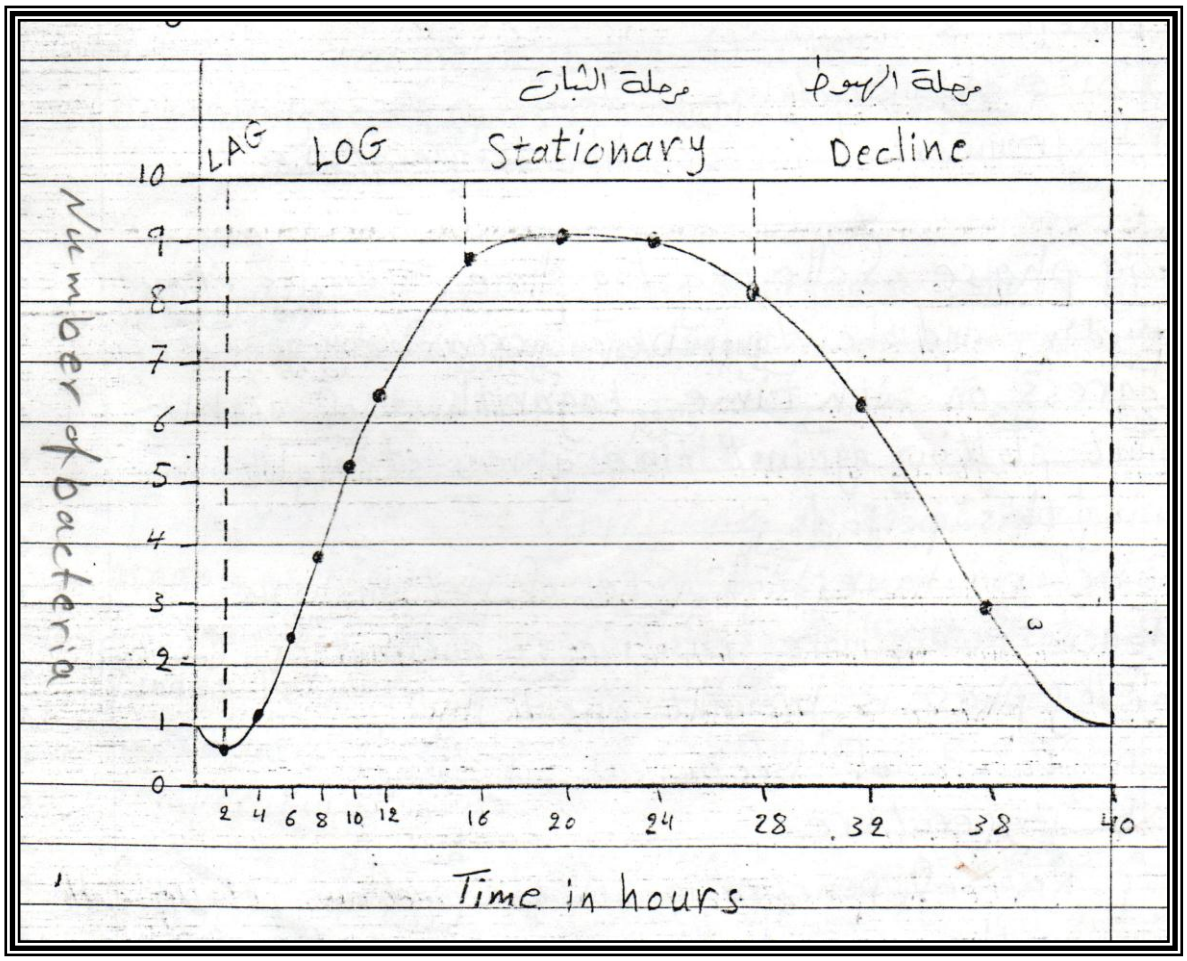
(b) Carbon dioxide : the metabolic activities of some organisms like *Neisseria*, *Brucella abortus* are greatly enhanced by the presence of extra amount of carbon dioxide in atmospheric air.

3. Moisture : Bacteria require water for their growth. Desiccation may kill most of bacteria.
4. Necessary nutritional requirement : Most often the necessary growth factors are vitamins. The requirement of growth factors differ widely in various bacteria.

Growth Curve

When organism are cultured in appropriate fluid media there would be increase in the size of bacteria without any multiplication for some time. This is followed by multiplication and increase in numbers of bacteria to the extent that media look turbid

to the naked eye (log phase). After some time growth rate become stationary and later on declines. counting of bacteria at different periods after inoculation and then events of sequences are represented on a graph which is called growth curve.



Growth Curve

Lag phase : During this phase there occurs

1. Increase in size of cell.
2. Increase in metabolic rate
3. Adaptation to new environment and necessary enzyme and intermediate metabolites are built up for multiplication to proceed.

The length of lag phase depends upon :

(a) Type of bacteria.

(b) Better the medium, shorter the lag phase.

(c) The phase of culture from which inoculation in taken.

(d) Size of inoculum.

(e) Environmental factors like temperature.

*** Log phase:** following lag phase. The cells start dividing and their number increase by geometric progress on with time logarithms of viable count plotting against time give straight line during this period.

(i) Bacteria have high rate of metabolism.

(ii) Bacteria are more sensitive to antibiotics. Control of log phase is brought about by :

   a. Nature of bacteria.
   b. Temperature
   c. Rate of penetration of the medium. It depends on the concentration of material in the medium.

*** Stationary phase**

After sometime a stage comes when rate of multiplication and death becomes almost equal. It may be due to ;

(a) Depletion of nutrient.

(b) Accumulation of toxic products. Sporulation may occur during this stage.

*** Decline phase**

During this phase population decreases due to death of cells. Factors responsible, for the phase are :

(i) Nutritional exhaustion.

(ii) Toxic accumulation.

(iii) Autolytic enzymes. Involution is common in phase of decline.

Survival phase :

When most organisms have died, a few survive for several months or years.

Factors influencing growth

1. Temperature : The temperature range at which an organism grows best is called optimum temperature. In human, parasitic organisms, optimum temperature ranges between 30°C and 37°C. There are three groups of bacteria as regards the temperature of growth
   - Psychrophilic : These are the organisms growing between 0°C to 25°C. They are mostly soil and water bacteria.
   - Mesophilic : They grow between 20°C and 45°C. This group includes bacteria producing disease.
   - Thermophilic : Some organisms grow between 50°C and 60°C. e.g. bacillus and algae.
2. Hydrogen ion concentration : most of pathogenic bacteria grow best at pH. 7.2-7.6. however lactobacilli grow at acidic pH while *Vibrio cholerae* grow at alkaline pH.
3. Moisture : Water in quite essential for the growth of bacteria. Organism like *Neisseria gonorrhoeae* and *Treponema pallidum* die almost at once on drying.

However *Mycobacterium tuberculosis* and *Staphylococcus aureus* survive for quite a long time even on drying.

4. Osmotic pressure : Bacteria are usually resistant to changes of osmotic pressure. However 0.5% sodium chloride is added to almost all culture media to make environment isotonic.
5. Light : darkness usually favorable for the growth and viability of all the organism. Direct light exposure shorten the survival of bacteria. Photochromogenic mycobacteria form pigment on

exposure to light. Organism are sensitive to ultraviolet and other radiations.

6. Mechanical and sonic stress: Bacteria have tough cell walls. Vigorous shaking and exposure to ultra sonic vibration may cause rupture or disintegration of cell wall.

Reproduction

Bacteria divide by binary fission. The individual cell grows in size, almost double its original size. Now critical nuclear cytoplasmic ratio and initiation of process of cell division.

The sequence of cell division includes

a. Formation of initiator of chromosome replicator.
b. Chromosome duplication.
c. Separation of chromosomes.
d. Formation of septa and cell division.

Generation gap :

Time required for bacterium to give rise to two daughter cell under optimum condition is called generation gap of :

(a) Coliforms bacteria is 20 minute.

(b) *Mycobacterium tuberculosis* is 20 hours.

(c) *Leprae bacillus* is 20 days.

GENETICS

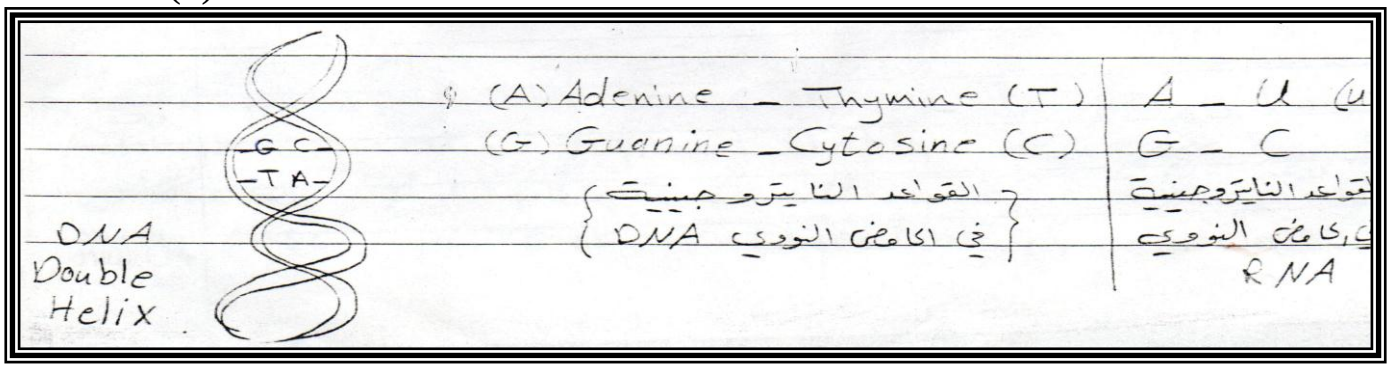
It is a science which study the similarity and differences for the following generations.

Mendel known with his important methods in genetics. He fixed an important symbols in genetics.

1. Dominant character (capital letter).
2. Recessive character (small letter).
3. every character has two letters, if it was pure, the two letters was similar and if it was hybrid, the two letter was different.

e.g. pure red plant RR
hybrid red plant Rr
white plant rr

4. When we make a mix or married, we use the following symboles
   (a) x : mix
   (b) ♀ : female
   (c) ♂ : male
   (d) P_1 : original parents
   (e) G_1: gametes of original parents
   (f) F_1 : filial generation
   (g) F_2 : Second filial generation.
   (h) % : Mendelian ratios



First rule (One Character)

Q/ Mixed tall plant with short plant. All the product plants was tall.

a- what are the genetic symbols for parents and new generation?

b- If you let the filial generation to self pollination, what are the genetic symbols for the new plants ? If you know the tall is a dominant character on the short character.

a- Tall plant X Short plant

TT tt P1

T t G1

Tt F1

100% hybrid tall plants

b- hybrid tall plant X hybrid tall plant

Tt Tt P2

T t T t G2

TT , Tt Tt , tt F2

75% tall plants 25% short plants

50% hybrid tall

3 : 1

Dominant recessive

Second rule (two characters):

Q/ pollinated plant have under red flowers with other plant have white top flowers. If we let the plants to self pollination. What are the products ? and what are the symbols for filial and second filial generations , if you know the under red flowers is a dominate characters on the top white flowers ?

Under red flowers X top white flowers

RRAA rr aa p1

RA ra G1

RrAa F1

100% hybrid under red flowers.

Hybrid under red flowers X hybrid under red flowers

RrAa RrAa

RA , Ra , rA , ra RA , Ra , rA , ra

$\frac{9}{16}$ under red

♀ \ ♂	RA	Ra	rA	ra
RA	RRAA	RRAa	RrAA	RrAa
Ra	RRAa	RRaa	RrAa	Rraa
rA	RrAA	RrAa	rrAA	rrAa
ra	RrAa	Rraa	rrAa	rraa

F2

$\frac{3}{16}$ top red

$\frac{3}{16}$ under white

$\frac{1}{16}$ top white

Media for bacterial growth

Culture media gives artificial environment simulating natural conditions necessary for growth of bacterial. The basic requirement of culture media are :

1. Energy source.
2. Carbon source.
3. Nitrogen source.
4. Salts like sulphates, phosphates, chlorides, carbonate sodium, potassium, magnesium, calcium and copper.
5. Satisfactory pH 7.2 - 7.6
6. Adequate oxidation reduction potential.
7. Growth factor like tryptophan for *S. typhi*, glutathione for gonococci X and V factors for *haemophilus*.

The characters : as of culture medium are :

1. Must give a satisfactory growth from single inoculum.
2. Should give rapid growth
3. Should be easy to grow
4. Should be reasonably cheap.
5. Should be easily reproducible
6. Should enable to demonstrate all characteristic in which we are interested.

Media used for obtaining the growth of bacteria

a. Fluid media : they are used as enrichment media before plating on solid media. They are not suitable the isolation of organism in pure culture. We can't study colony character as well.

   Examples of fluid media are nutrient broth, peptone

Types of liquid media :

1. Broth
2. Infusion broth
3. Digest broth
4. Meat extract broth
5. Peptone
6. Yeast extract

b. Solid media : They are used to study colonies of individual bacteria. They are essential for isolation of organism in pure form.

Agar : It is important constituent of solid media. It is complex polysaccharide obtained from sea plants (Algae Gelidium species). It melt at 80 - 100^{o}C and solidifies at 35 - 42ºC. It doesn't provided any nutrition to the bacteria. It acts only as solidifying agent. It is not metabolized by any pathogenic bacteria .

Gelatin : It is protein prepared by hydrolysis of collagen with boiling water. It melts at 37^{o}C. it forms transparent gel below 25^{o}C. the main use of gelatin is to test the ability of bacteria to liquefy it.

This feature is important for the identification and classifications of bacteria.

Classification of media

A : 1. Solid media .

2. Liquid media .

3. Semi-solid media .

B: 1. simple media .

2. Synthetic media .

3. Complex mdia .

4. Simplified media .

5. Special media .

Special media are further divided as under :

1. Enriched media.
2. Selective media .
3. Differential media.
4. Sugar media .
5. Transport media.

C: Aerobic media and anaerobic media.

Sterilization and Disinfection:

Sterilization: is a process by which articles are become free from all microorganisms either in vegetative or spore state.

Bactericidal agents: The agents which have ability to kill bacteria.

Bacteriostatic agents: Agents which only prevent multiplication of bacteria so bacteria remain alive.

Factors which effect sterilization:

1- The number of microorganism which to be sterilized.
2- The temperature degree which used.
3- Time of sterilization, if the temperature increase the time is reduce and vice versa.
4- Type of microorganism.

e.g: * Syphilis bacteria need 43°c for 10min.
* Hepatitis virus need 60°c for 10min.
* clostridium need 100°c for 10min.

5- The nature of the compound which have to be sterilized.

Sterilization methods:

*There are two methods:

A- Physical methods:

1- sun light.

2- Drying.

3- Heat:

I- Dry heat:

a- Red heat.
b- Flaming.
c- Incineration.
d- Hot air oven.

II- Moist heat:

a- Temperature below…100°c.
b- Temp. at…100°c.
c- Temp. above…100°c.
d- Tyndallization.

B- Chemical methods.

A- Physical methods:

1- sun light:

It is the bactericidal activity. The action is due to. Ultraviolet rays.

e.g: Water in tanks, river and lakes.

2- Drying:

Drying in air has deleterious effect on many bacteria. Spores are unaffected by drying.

3- Heat:

a- Dry heat.

b- moist heat.

1- dry heat:

a- Red Heat: It is used to sterilize metalic objects by holding them in flam till they became red hot.

e.g: Inoculating wire loop, needle, forceps.

b- Flaming: The article is passed over flam without allowing it to be red hot.

e.g: Mouth of culture tubes, cotton, wool plugs and glass slides and media in petri dish.

c- Incineration: This is excellent method for rapidly destroying infected material.

e.g: Dressing, pathological, material.

d- Hot air oven: Sterilization by hot air oven require temperature 160°c for one hour or 180°c for1/2hour. It is the best sterilizing method.

e.g: Glass syringes, petri dishes, test tubes, pipettes, flask, cotton swabs…etc.

2- Moist Heat:

a- Temperature below 100°c: (Pasteurization of milk).

Temperature employed is either 63°c for 30 minutes (Holder method) or 72°c for (15-20) seconds, (flash method). Organisms like *Mycobacterium*, *Salmonella* and *Brucella* are killed under this temperature.

b- Temp at 100°c: (Boiling)

Most of vegetative form of bacteria, fungi and viruses are killed between (50- 70 °c) in short time. There for needle and instrument boiling in water at 100c between (10-20) minute.

c- Temp. above 100°c: (By autoclave)

Material for sterilization are exposed to 121°c for (15-20) minute , pressure 15 l b / square inch.

This method sterilized material by steam under pressure it is the best method for sterilizing material like culture media, cotton, surgical instruments, dressing, syringes…etc.

d- Tyndallization: some culture media which contain sugar and gelatin media cannot state above high level of temperature, so the material is exposed to steam atmospheric pressure for (30)min. on (3) successive days.

1- first day: ⟶ steaming kill all vegetative form of bacteria.

2- second day:⟶ steaming kill the germinated spore.

3- Third day: ⟶steaming kill all bacteria if it remained.

4- Radiation :-

A- Non Ionizing radiation.

B- Ionizing radiation.

A- Non ionizing radiation:

1- Ultraviolet radiation ⟶ sterilizing bacteria air, water and contaminated surface. This method used for producing bacterial and Viral Vaccines.

2- Infra red radiation: ⟶ sterilizing a large number of syringes in short time.

B- Ionizing radiation: Like X-ray Radiation causes changing in cell:

1- Denaturation of protein.

2- Damage to DNA.

3-Inhibition of DNA replication.

5- Filtration :-

Used for sterilizing liquid which damage by heat such as serum and antibiotic solution.

B- Chemical methods:

* Antiseptic: It is a Chemical substance that prevents growth either by inhibition or destruction microorganisms and used for topical application to living tissues.

e.g: 70% alcohol, savlon, Halogens (iodine) used for skin (chlorine) combines with water to sterilizing water.

*Disinfection: A process of destruction of pathogenic organisms which giving rise to infection.

e.g: Phenol, formaldehyde.

*Phenol: Used in 3% solution for sterilizing surgical instruments.

*Formaldehyde: Used in sterilizing bacteria Vaccine.

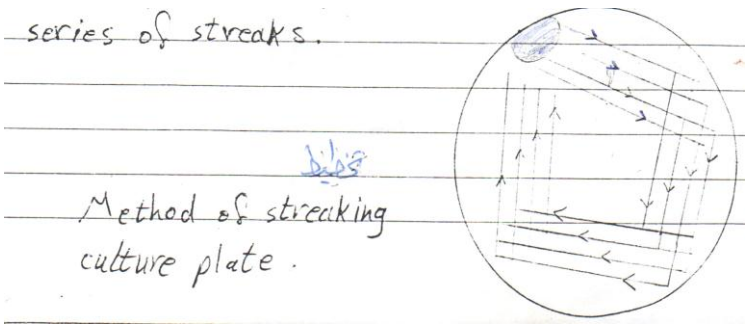
Culture techniques

In clinical laboratory indications for culture are :

a. Isolation of bacteria in pure culture.
b. To demonstrate their properties.
c. To obtain sufficient pure growth for preparation of antigen and for other tests.
d. For type isolate by method like bacteriophage and bacteriocin susceptibility.
e. To determine sensitivity to antibiotics.
f. To estimate viable count
g. To maintain stock culture

Method of culture

Streak culture (Surface plating) is the method routinely employed for the isolation of bacteria in pure culture. A platinum loop with 2.5 long wire and loop at one end with diameter 2 mm is charged with specimen to be cultured and is placed on the surface dried plate of solid media towards peripheral area. The inoculums is spread thinly over the plate in series of parallel lines in different on the plate. On incubation we may find confluent growth at the site of primary inoculums. Well separated colonies are obtained over the final series of streaks.



Liquid Culture :

In tube, bottle or flask may be inoculated by touching with a charged loop. Liquid culture are preferred when large and quick yield is required. The major disadvantage of liquid culture is that it doesn't provide pure from mixed inocula.

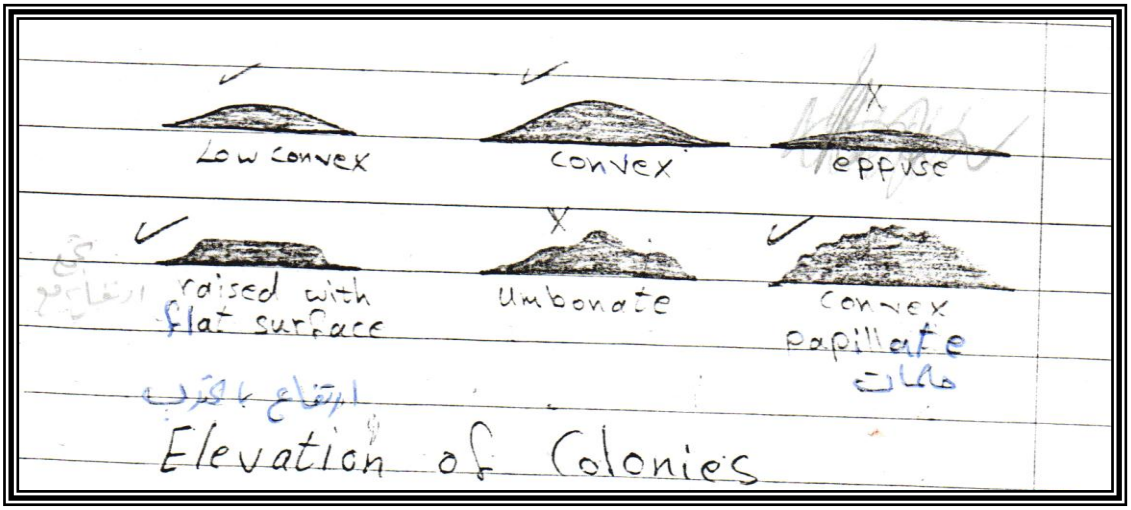
Description of colonies of bacteria

Shape : circular, irregular, radiate

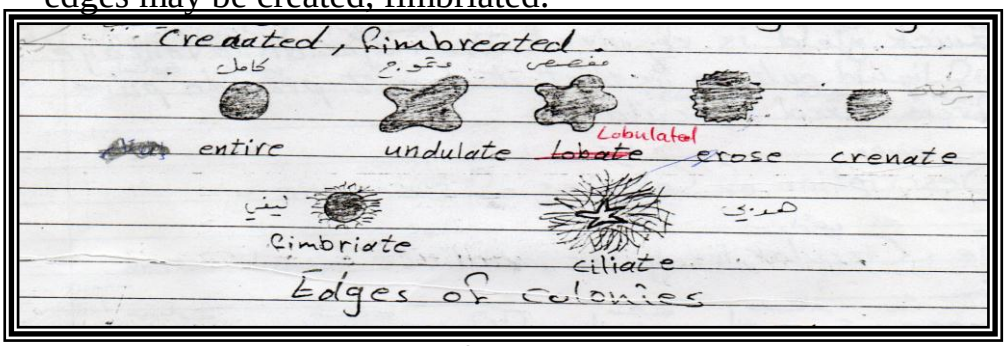
Surfaces : smooth, rough, fine coarsely granular, papillate .

Size : Surface of colonies are measured in millimeter they are 2-3µm. If very small then 0.50-1 µm

Elevation: raised, low convex, dome, umbilicate. Some bacteria produce, spreading growth, e.g. *proteus* , *clostridia*.



Edges : Mostly edges are entire e.g. : *E. coli*, *Staphylococcus*. Sometimes edges may be created, fimbriated.



Colour : Some organism may produce pigmented colonies. e.g. *staphylococci, Pseudomonas*.

Opacity : Colonies on nutrient agar may be transparent, translucent or opaque.

Consistency : colonies may be hard or firm e.g. *Mycobacterium tuberculosis*, friable and membranous e.g. *B. subtilis*. Mostly they are soft e.g. *E. coli*

Changes in the medium : Some organisms produce beta type of hemolysis around the colony e.g. *staphylococcus* and *streptococcus*. Few bacteria, produce soluble pigment that diffuses into the medium e.g. *pseudomonas*.

Emulsifiability : Growth of bacteria like *E. coli., Salmonella* is easily emulsifiable where as growth of *N. catarrhalis* is not emulsifiable and form granules.

Growth in liquid media is described as :

1. Turbidity
2. Deposit : Growth of *Streptococcus pyogenes* is characterized by deposit at the bottom of tube.
3. Surface growth : Surface growth is related to aerobic nature of organism.
4. Colour changes : Some organisms produce water soluble, pigment which after diffusion change the colour of medium e.g. : *Pseudomonas*.

Methods for Anaerobic culture :

Obligate anaerobes grow only is absence of free oxygen. These bacteria lack mechanism of oxidation through respiratory enzymes like cytochrome oxidase, catalase and peroxidase resulting in H_2O_2 accumulation. This H_2O_2 is toxic for the growth of anaerobic bacteria.

INFECTION

Infection : the lodgement and multiplication of organism in the tissue of host constitute infection.

Classification of infection

1. Primary infection : Initial infection with organism in host constitute primary infection.
2. Reinfection : Subsequent infection by same organism in a host in called reinfection.
3. Secondary infection : when in a host whose resistance is lowered by preexisting infectious disease a new organism may set up an infection.
4. Focal infection : It is a condition where due to infection at localised sited like appendix and tonsil general effects are produced.
5. Cross infection : when a patient suffering from a disease and new infection is set up from another host or external source.
6. Nosocomial infection : Cross infections occurring in hospital is called nosocomial infection.
7. Subclinical infection : Is one where clinical affects are not apparent.

- Saprophytes : They are free living organisms which live on decaying organic matter. They fail to multiply on living tissue and so not important in infections disease.
- Parasites : Are organism that can establish themselves pathogens are those which are capable of producing disease in a host. On the contrary commensal microbes can live in a host without causing any disease.

Source of infection in man :

1. Man : Man is himself a common source of infection from a patient or carrier. Healthy carrier is a person harboring pathogenic organism without causing any disease to him. A convalescent carrier is one who has recovered from disease but continues to harbor the pathogen in his body.
2. Animals : Infections diseases transmitted from animals to man are called zoonosis. Zoonosis may be bacterial (e.g., plague from rat) rickettsial (e.g. murine typhus from rodent), viral (e.g., rabies from dog), protozoal (e.g., leishmaniasis from dogs), heliminthic (e.g., hydatid cyst from dogs) , fungal. (zoophilic dermatophytes from cats and dogs).
3. Insects : The disease caused by insects are called arthropod borne disease. Insects that transmit infection are called vector. Transmission may be mechanical (transmission of dysentery or typhoid bacilli by house fly) these are called mechanical vector. They are called biological vector if pathogens multiplies in the body of vector, e.g., *Anopheles mosquito* in malaria.
4. Some vector may act as reservoir host.

5. Soil : Soil may serve as some of parasite infection like roundworm and hookworm . Spores of tetanus bacilli remain viable in soil for along time.
6. Water : Cholera vibrio, infective hepatitis virus may be found in water.

7. Food : Contaminated food may be a source of infection. Presence of pathogens in food may be due to external contamination (e.g., food poisoning by staphylococcus).

Methods of transmission of infection :

1. Contact : Syphilis, gonorrhea, trachoma.
2. Inhalation : Influenza, tuberculosis.
3. Infection : Cholera (water) food poisoning (food) dysentery (hand borne).
4. Inoculation : Tetanus (infection) rabies (dog), arbovirus (insects), serum hepatitis (injection).
5. Insects : Act as mechanical vector (dysentery and typhoid by house fly) or biological vector (malaria) of infectious disease .
6. Congenital : Congenital syphilis, toxoplasma disease.
7. Laboratory infection : Infection may be transmitted during procedure like, injection if proper care is not taken.

Virulence is the sum of the following factor :

(A)- **Invasiveness** : It is the ability of organism to spread in a host tissue after establishing infection.

(B)- Toxigenicity : Bacteria produce two types of toxins **:**

a- Exotoxin : It has following characters :

1. Heat labile proteins.

2. Diffuse readily into the surrounding medium.

3. Highly potent, e.g., 3kg botulinum can kill all the inhabitant of world where as 1 mg of tetanus toxin is sufficient to kill million guinea pigs.

4. They are generally formed by gram positive organism except *shigella*, *cholera, vibrio* and *E. coli*

5. Exotoxin are specifically neutralized by antitoxin.

6. Can be separated from culture by filtration.

7. Action is enzymatic.

8. It has specific tissue affinity.

9. It is highly antigenic.

10. Specific pharmacological effects for each exotoxin .

11. Can be toxoid.

b- Endotoxin : It has following characters :

1. Proteins polysaccharide lipid complex heat stable.

2. Forms part of cell wall and will not diffuse into the medium.

3. Obtained only by cell lysis.

4. They have no enzymatic action.

5. Effect is non specific action common to all endotoxin.

6. No specific tissue affinity.

7. Active only in large doses 5 - 25 mg.

8. Weakly antigenic .

9. Neutralization by antibody ineffective.

10. Can't be toxoid.

11. Produce in Gram negative bacteria.

c- Communicability : This is ability of parasite to spread the survival and distribution of organism in a community. Highly virulent organism may not exhibit a high degree of communicability due to rapid lethal

effect on hosts. Infections in which pathogen is shed in secretions as in respiratory and intestinal diseases are highly communicable.

d- Other bacterial products :

1. Coagulase : Which prevents phagocytosis by forming fibrin barrier around bacteria.

2. Fibrinolysin promotes the spread of infection by breaking down the fibrin barrier in tissues .

3. Hyaluronidase split hyaluronic acid, thus facilitating spread of infection along tissue spaces.

4. Hemolysin is produced by some organism capable of destroying erythrocytes.

e- Bacterial appendages :

Bacterial appendages :

Capsulated bacteria like *pneumococcus* will stand phagocytosis. Surface antigen like K. antigen of *E. coli* resist phagocytosis and lytic activity of complement.

f- Infecting dose :

To minimum infecting dose (MID) or minimum lethal dose (MLD) is the minimum number of organisms required to produce clinical evidence of infection or death of susceptible animal.

g- Route of infection :

Vibrio cholerae is ineffective orally. No effect when it is introduced subcutaneously. Streptococci can initiate infection whatever be the mode of entry. They also differ in ability to produce damage to different organs in different species.

Antimicrobial Therapy

Chemotherapeutic agents :

These are the agents which have lethal or inhibitory effect on the microbes responsible, but it therapeutic concentration have little or no toxic action on the tissues.

However these agents used in chemotherapy are of very diverse chemical structure. They can be divided into two categories.

a- Relatively simple compounds obtained by laboratory synthesis, e.g., sulfonamides.

b- Antibiotics are the substances produced by living organisms and which are active against other living organisms.

Most of them are produced by soil Actinomycetes . Antibacterial agents are divided into two classes on the bases of type of action they exhibit against bacteria :

1. Bacteriostatic drugs : are drugs which in the concentration attainable in the body, only inhibit bacterial growth, e.g., Chloramphenicol.
2. Bactericidal drugs : are the drugs which kill the bacteria by virtue of their rapid lethal action. E.g., Penicillin. Bactericidal drugs are more effective therapeutic agents than bacteriostatic drugs.

Mode of action : The problem can be considered from two aspects :-

A. Identification of site of action of drug.

B. Its precise mechanism of action.

Site of action : There are four major sites of action :-

1. Inhibition of synthesis of cell wall. E.g., Penicillin.
2. Damage to the permeability of the cytoplasmic membrane.
3. Inhibition of protein synthesis.
4. Inhibition of nucleic acid synthesis.

Antibiotics :

Antibiotics : are substance produced by the some living microorganisms which inhibit other microorganisms. E.g., Penicillin.

Antibiotic

Bactericidal	**Bacteriostatic**
Killing the microbes completely	Non-killing
e.g. Penicillin, streptomycin	e.g. tetracycline

Antibiotic

Narrow spectrum	**Broad spectrum**
Against single microbe	Against many number of microbes.
e.g. Penicillin	

Penicillin discovered by Alexander in 1929.

Mode of action of antibiotic on microbes :

1. Destruction of cell wall synthesis.
2. Destruction of protein synthesis.

Sensitive antibiotic (S)

Resistant (R)

Intermediate sensitive antibiotic (MS)

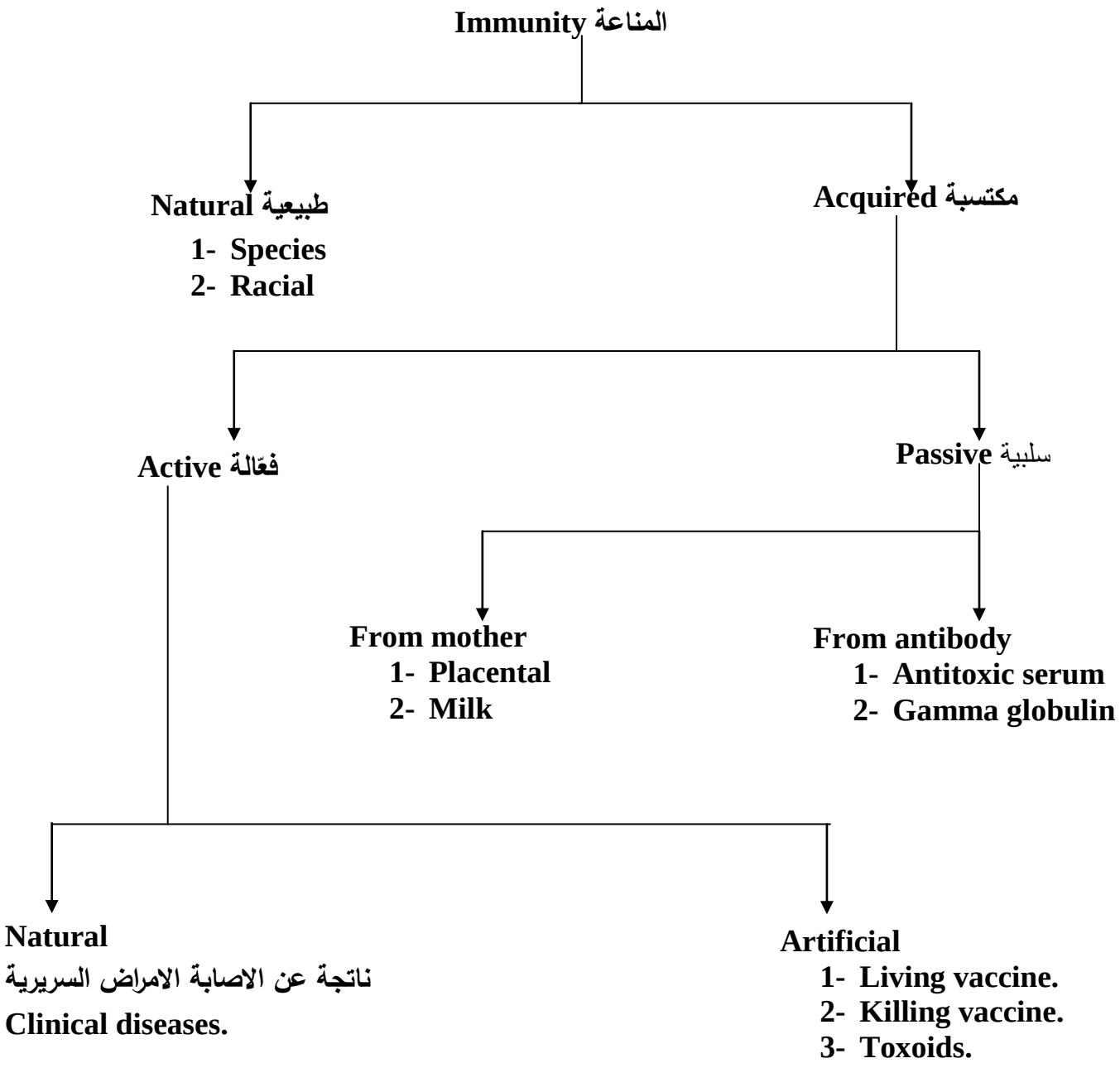
Sensitive in media which is not more than 3 mm. intermediate is more than 3 mm. resistant which is no zone of inhibition or zone reduce measure 2 mm or less.

Laboratory uses of antibiotics:

1. They may be incorporated as selective agents in culture media.
2. They are used for the control of bacterial contamination in tissue cultures used for virus isolation.
3. The pattern of sensitivity of an organism to a battery of antibiotics constitute simple method of typing which is of considerable epidemiological value.

Some antibiotics in current use :

1. Antibiotics mainly active against gram positive bacteria (Penicillin).
2. Antibiotics active against gram negative bacteria (Polymyxins).
3. Antibiotics active against both gram positive and gram negative bacteria (Tetracycline).
4. Antibiotics active against fungi (Griseofulvin).



Natural Immunity: This is basic immunity which may be genetically passed on from one generation to other generation. It dose not depend on prior contacts with microorganisms. It may be non specific when indicates a degree of resistance to all infections.

Acquired immunity : The immunity acquired during the life time of an individual is known as acquired immunity.

Acquired immunity differs from natural immunity in following respects :

1. It is not inherent in the body but is acquired during life.
2. It is specific for a single type of microorganism.

Local immunity : It has importance in infections which are either localized or where it is operative in combating infection at the site of primary entry of pathogen, e.g., in influenza immunization with killed vaccine elicit humoral antibody response but antibody titre in respiratory secretion is not high enough to prevent infection.

Difference between active and passive immunity :

1. Active produced actively by host, immune system.
2. Induced by infection or by contacts with immunogen e.g., vaccines.
3. Afford durable and effective protection
4. Immunity effective after lag phase.
5. Immunological memory present.
6. Negative phase may occur
7. Not applicable to immuno deficient hosts
8. Used for prophylaxis to increase resistance of body.
9. Both cell mediated and humoral immunity take part
10. No inheritance of immunity.

Passive immunity:

1. Received passively by the host. No participation by host's immune system.
2. Conferred by introduction of ready made antibodies.
3. Temporary and less effective protection
4. Immunity effective immediately
5. No immunological memory

6. No negative phase
7. Applicable to immuno deficient hosts.
8. Used for treatment of acute infection
9. Exclusively humoral immunity
10. May be inherited from mother.

Phagocytosis : Some cells of human body are phagocyte bacterial cells.

Parasitology

Parasitology : Is the science which deals with study of parasites, it is a branch of biology.

Parasite : Is the organism that adapted it self to existence live in or on other organism (host)

Parasitism : Is the relationship between two organisms in which one of them is benefit (parasite) and the other host is harmfully effected.

Types of parasites :

1. Ectoparasite : that parasite which live out side the body of the host.
2. Endoparasite : That live parasite inside the body of it's host.

Host : Is the organism that harbour the parasite and provided it with food.

Kinds of hosts : Some parasites require more than one host to complete it's life cycle, other complete life cycle in the same host.

1. Final (definitive) host : Is that host which harbour the adult or the sexually mature reproducing stage of life parasite.
2. Intermediate host : Is that host which harbour the larval stage of the parasite, some parasites require more than one intermediate host to complete its life cycle.

Life cycle : Is the different development stages of the parasite to complete one generation.

Habitat : Is the place in which the parasite live or found in the host body or human body.

Pathogenic parasite : Is that parasite which causes harmful to the host (infected).

Commensal parasite : Is that parasite which are not causes harmful to the host (not infected)

Pathology : The damage which caused by the parasite to its infected host.

Mode of infection :

1. Oral - route.
2. Skin - route.
3. Sexually.

Disease : The harmful which causes by parasite .

Methods of parasite diagnosis :

1. Stool examination.
2. Blood examination.
3. Urine examination.
4. Tissue examination.
5. Sputum examination .

Treatment : include the drugs which effected on the parasite .

Phylum protozoa

Include four classes :

1. Class : Sarcodina : e.g., *Entamoeba histolytica* .
2. Class : Mastigophora e.g.. *Giardia lamblia* .
3. Class : Ciliophora : e.g., *Balantidium coli* .
4. Class : Sporozoa : e.g., *Plasmodium vivax* .

Entamoeba histolytica

Phylum : Protozoa .

Class : Sarcodina .

Disease : Amoebic dysentery .

Hosts : Final host human .

Intermediate : host No .

Habitat : In large intestine .

Infective stage : mature cyst with 4 nuclei .

Mode of infection : Oral route by ingestion-mature cyst with contaminated foods or drinks .

Diagnostic sample : Stool examination .

Diagnostic stages : Trophozoite cyst .

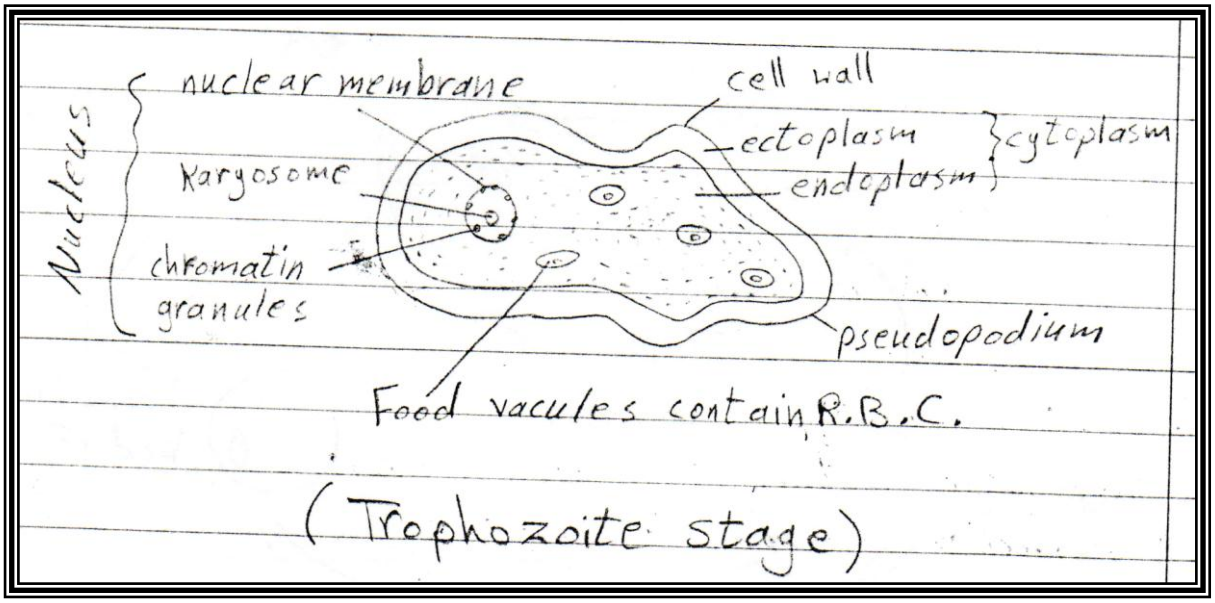
Treatment : Flagyl .

Morphology : have 2 stages :

1. Trophozoite stage .

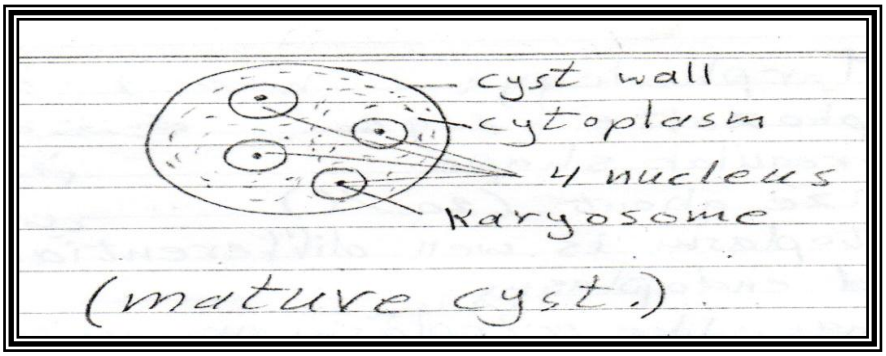
- Irregular shape .
- Size about (20 μ) .

- Cytoplasm is well differentiated into ectoplasm and endoplasm.
- Finger like ectoplasm process called pseudopodium.
- One spherical nucleus or single spherical nucleus with small central karyosomes and many chromatin granules.
- Number of food vacuoles in the cytoplasm containing R.B.C, .



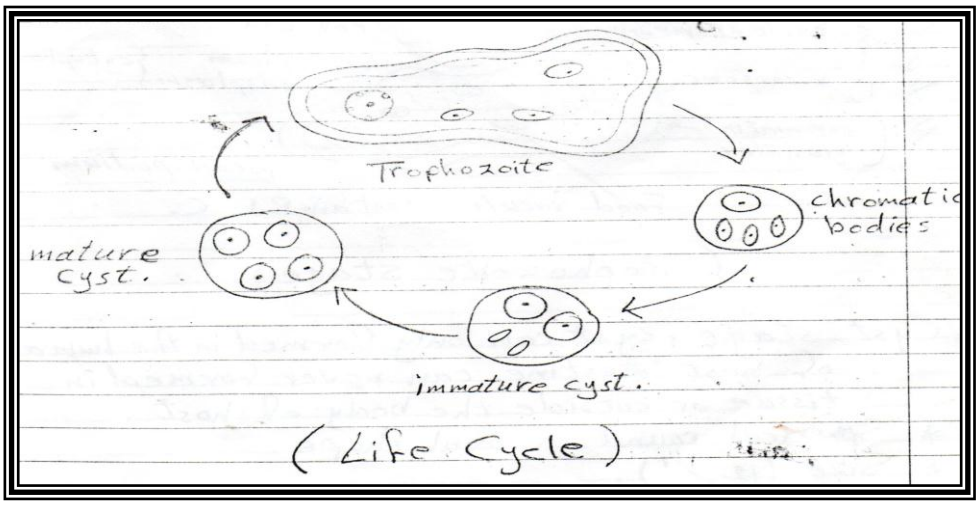
2. cyst stage : cyst can only formed in the human of host intestine can never formed in tissue or outside the body of host.

- Spherical round or oval shape .
- Size (12 μ).
- Contain : one – two – three - four (mature cyst).



Life cycle :

Trophozoite and cyst in large intestine → pass out with stool → mature cyst ingestion with food and water → trophozoite and cyst in the large intestine.



Intestinal Flagellates

Giardia lamblia

Phylum : Prtozoa .

Class : Mastigophora, species : lambilia

Diseases : Giardiasis, lambliasis .

Host : Final host human.

Intermediate host : No.

Habitat : upper part of small intestine .

Infective stage: mature cyst with 4 nuclei.

Mode of infection : oral-route (by ingestion mature cyst which contaminated food and drinks) .

Diagnostic sample : stool .

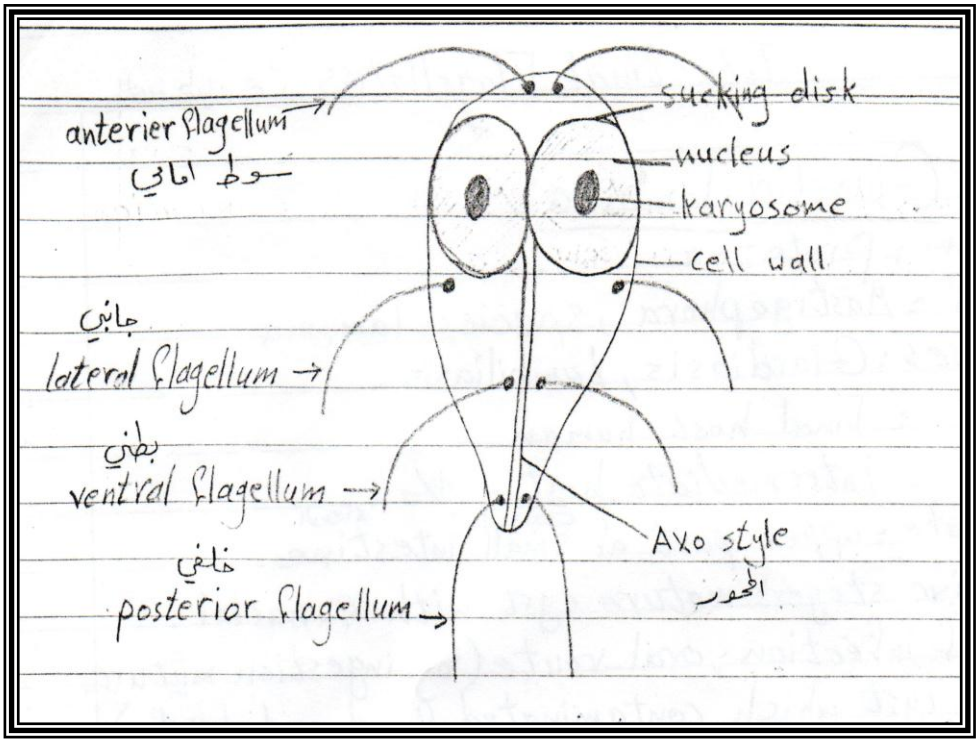
Diagnostic : Trophozoite and cyst.

Treatment : Flagyl.

Morphology

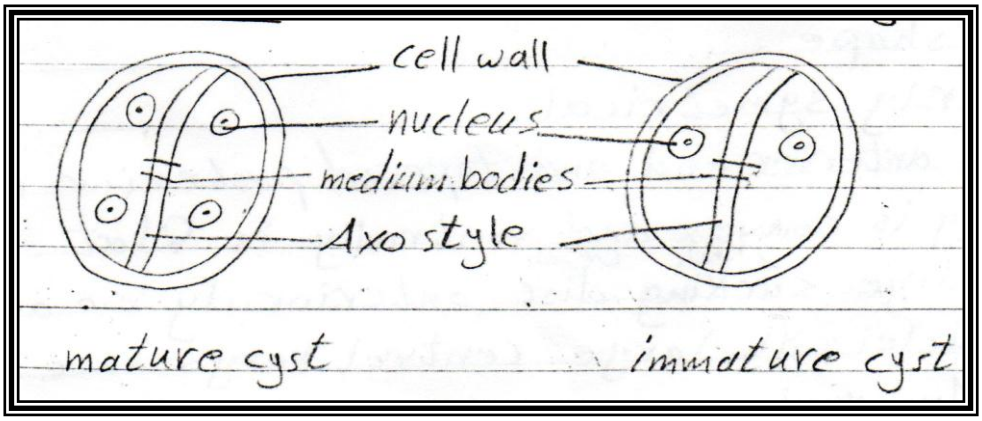
A/ Trophozoite stage

1. Pear-shape.
2. Billaterly symetrical.
3. Broad anterior end and tapered posterior end.
4. Dorsally is convex and ventrally is flat.
5. Two large sucking disc anteriorly.
6. Two nuclei with large central karyosome.
7. Two Axostyles .
8. Two median bodies.
9. 4 pairs of flagella.



B/ Cyst stage :

1. Oval shape.
2. Well define double cyst wall .
3. 2 or 4 nuclei
4. Found in diarrhonic stool and immature cyst of *G. lamblia* in normal stool in large number.



Life cycle

Cyst forming in human in small intestine pass with feces

ingested by new host → Excitation in small intestine → Trophozoite →

multiply by binary fission → cyst forming in human in small intestine.

Diagnosis

Iodine

Stool → direct smear → Microscopic examination reveal Trophozoite -----→ cyst.

Normal saline

Phylum : Protozoa .

Class : Ciliophora .

Genus : *Balantidium* .

This class includes those protozoal parasites which move by means of Cilia.

Cilia which are usually shorter than flagella and which usually cover the whole body of the protozoa.

The genus *Balantidium* is the only genus of the above class which contains a species pathogenic to man, namely *Balantidium coli*. This Ciliate can be obtained for study from the bind gut of newly killed pigs in the abattoir, a similar one occurs in the frog's rectum.

Habitat : Man is rarely infected with this parasite, which has an uncysted form, sometimes referred to as the Trophozoite, and an encysted form. The Trophozoite is found in the large institute for man and the pig. The cysts are formed in the intestine and are not formed outside the body.

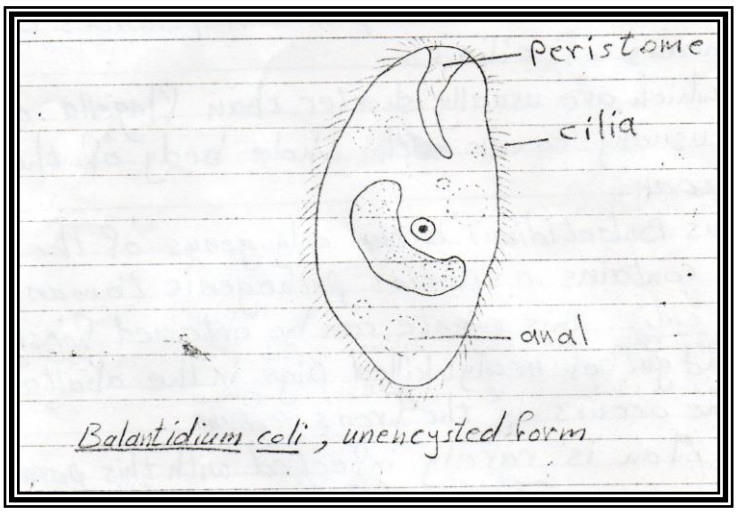
When man or pig is infected, either the uncysted or encysted form may pass out of the intestine in the feces. As in infections with *Entamoeba histolytica*, which causes dysentery in *B . coli* infections the uncysted form occurs in man's feces in acute cases of

Balantidial dysentery, and the cyst in chronic cases, or both may be present in the substance conditions.

Morphological characters

(a) Uncysted form : The Trophozoite.

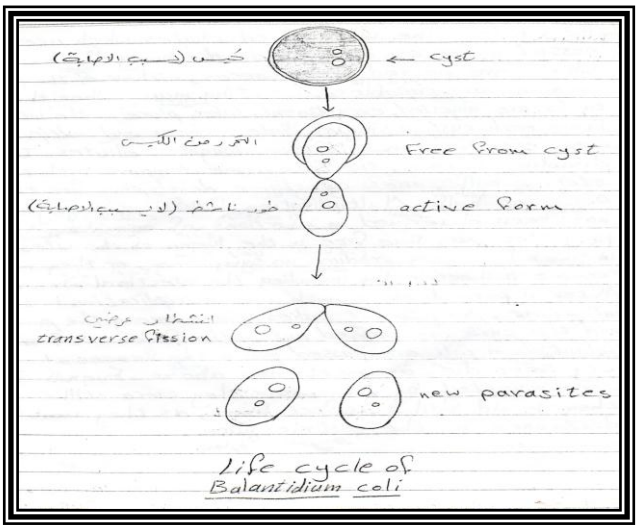
The body is oval and the size is very variable, from about 30-300μ in length, by 20-120 μ in breadth. At the anterior end is a depression called the peristome, which leads into the mouth, there is no intestine but at the posterior end, is a depression called the anal.



(b) Encysted form :

Cysts are formed inside the body and are found in the feces.

The cyst is more or less circular in outline, measuring about 50μ in diameter and contains a single parasite from which the cilia and food vacuoles have disappeared.



Life cycle :

The uncysted ciliates which are passed in feces die outside the body. The cyst, which are passed in the feces, either of the pig or man, are capable of infecting man. When the cysts are ingested, excystment takes place in the intestine. From each cyst a single ciliate escapes and multiplies by transverse fission into two daughter ciliates. In dividing forms, the cytostome remains with the anterior half, a new cytostome developing for the posterior half. Multiplication following conjugation has been observed in infections of animals. The parasites may live free in the lumen of the intestine, in which case they produce no symptoms, or they may become pathogenic by invading the intestinal wall.

After a period of growth and multiplication in the intestine, encystment takes place among the parasites, and the cyst are passed out in the feces. If uncysted *Balantidium coli* are passed out in the feces they will very soon die, as mentioned above. Even if they were ingested by man while they were still a live they would fail to infect him, as they would be destroyed by the gastric juice.

Phylum **:** Protozoa

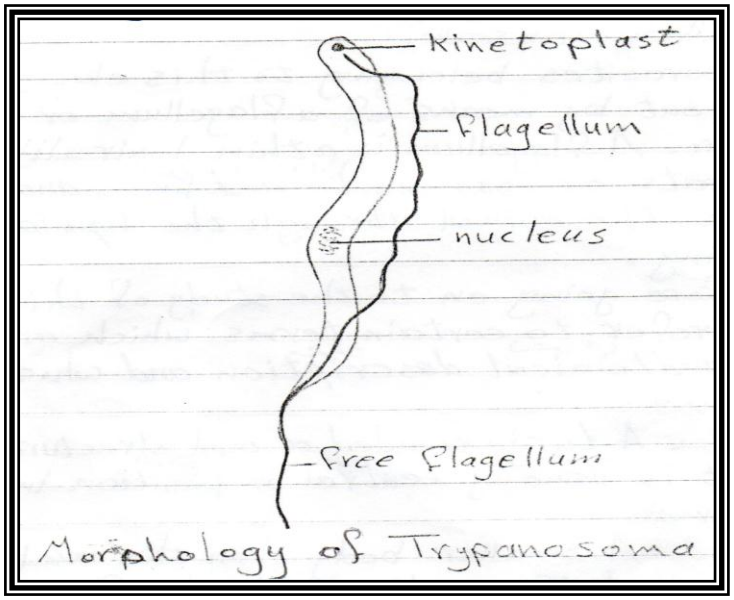
Class : Zoomastigophorea

Genus : Trypanosoma

In parasites belonging to this class, movement is carried out by means of a flagellum or a number of flagella. A flagellum is a thin hair-like organelle which beasts or waves to and fro, and either pulls or pushed the animal through the liquid in which it is living.

Before going on to the study of this genera, we shall refer to certain terms which are frequently used in anatomical description and which may be defined here :

1. Nucleus : A large rounded or oval structure, readily stained, it is usually central in position in the body of the animal.
2. Kinetoplast : A smaller body than the nucleus and round or rod-shaped from this body the flagellum appears to take origin.
3. Flagellum : A thin hair-Like structure which arises at the kinetoplast, the flagellum may terminate at the anterior end of the parasite, so that there is no "free" portion, or a part may project beyond the anterior end of the body as a "free" flagellum.
4. Body : In the flagellated stage, the body is narrow, flat and often curved, in the non-flagellated stage it is spherical or avoid. The cytoplasm may contain granules and vacuoles of various sizes.
5. Undulating membrane : This structure which consists of ectoplasm extending outwards from the body to surround the flagellum as it runs alongside.

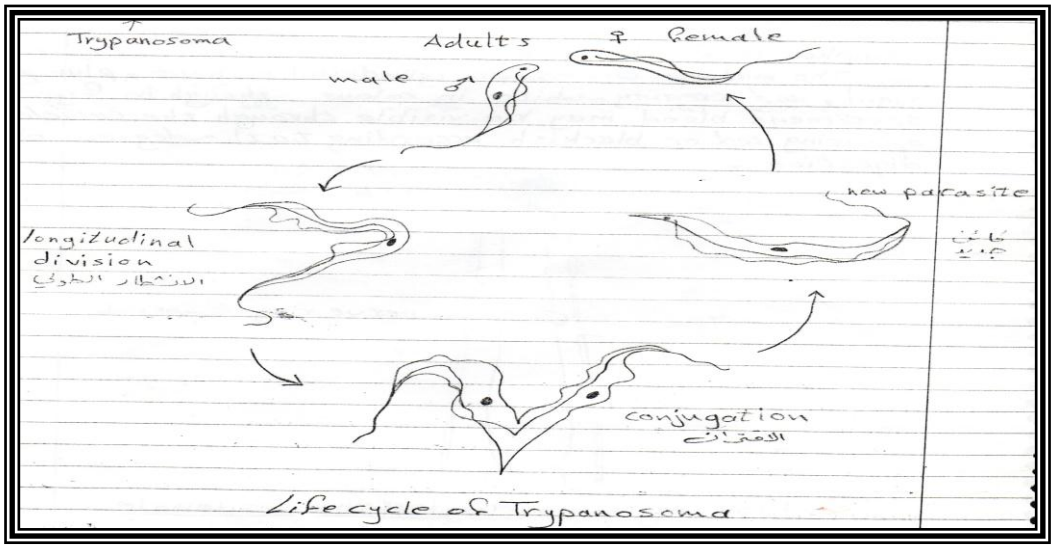


Morphology of Trypanosoma

Life cycle :

The metacyclic trypomastigotes, introduced into the body of man with the salivary fluid by the fly in biting, multiply locally, by longitudinal fission, at the site of inoculation, and they also enter the lymphatics and the blood stream, and increase so as to produce symptoms after a period of ten days or more. If parasites are numerous, all stages of longitudinal division may be found in the blood, some trypomastigotes having only the kinetoplast divided, some having the nucleus also divided, and some having, in addition, a double flagellum.

The movement of the trypomastigotes is fiarly active, but they do not travel across the microscope field as does. T. lewisi.



Phylum : Nematoda

Class : Nematoda

Genus : a ncylostoma

Species : duodenale

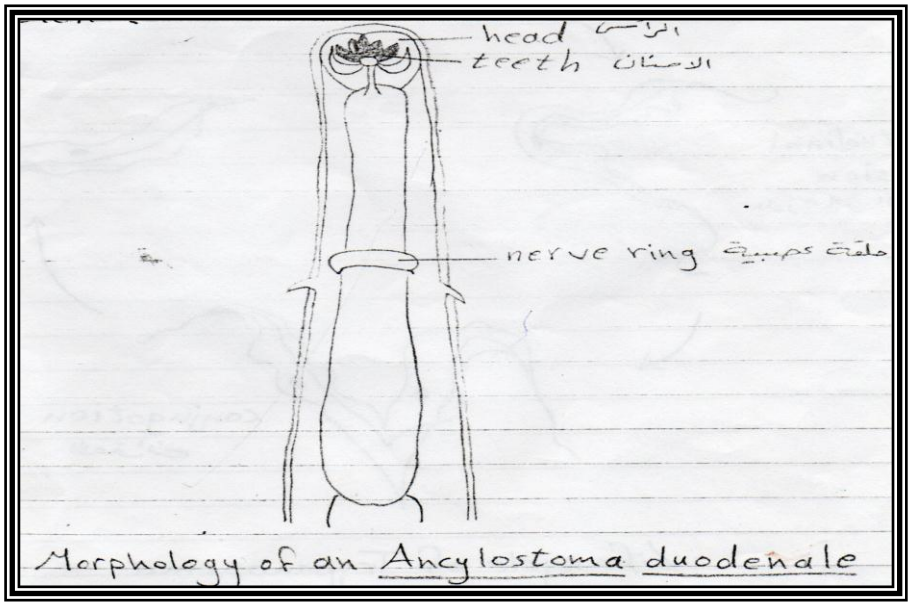
Hook worms

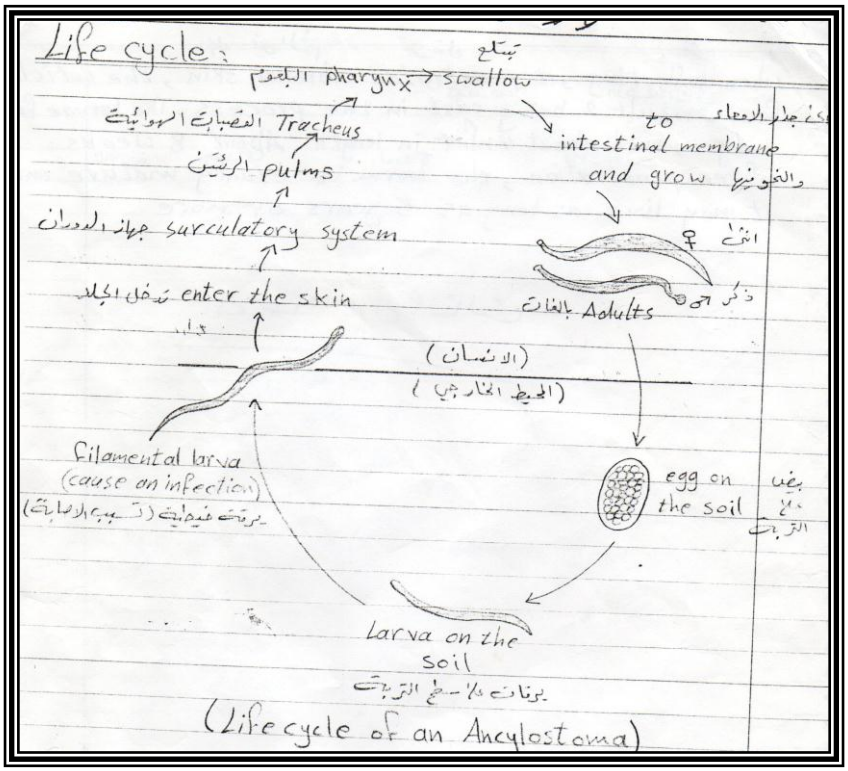
Ancylostoma duodenale

It causes acute anemia

Morphological characters

The mature worms are cylindrical in shapae, plump, rigid, and creamy-white in colour, though in fresh specimens blood may be visible through the cuticle, appearing red or blackish according to the degree of digestion.





Life cycle

The development of the egg takes place as follows : a larva hatches out of the egg in 24-48 hours in the presence of damp soil, warmth and sufficient oxygen. The newly hatched larva (250μ in length). The larva, now infective but still enclosed in the old skin, is very active and does not feed. At this stage it is rapidly killed by freezing, desiccation, direct sunlight or certain chemicals. It may live for several months in the surface of soil and when infecting it penetrates unbroken skin, the cuticle from month 2 being cast in the process. The larvae feed and grow until about 4mm in length. Abut 8 weeks after penetration the worm is sexually mature and it may live as long as 5 years or more.