Microbiology

Historical Preview

Microbiology : Is the study of organism too small to be seen clearly as individuals by the Unaided human eye , also it deals with structure , morphology , importance , control – etc .
It include : Bacteria , Fungi , Viruses , Algae and protozoa .
Microbiology dawn was in the end of 19th century , In general any organism has a diameter of less than 1 mm will be considered as microorganism [m-ms]
The very best human eyes fail to see objects less than 100 µm in diameter [bacteria :

1-5µm , virus : 0.25 µm , animal cell : 50 µm] [µm = $\frac{1}{1000}$ mm] .

Stage of microscope invention :

1-simple magnifying glasses [hand lenses] giving enlargement of 2 – 10 X .

2- exciting discovery made by Zaccharia Janssen by using a second lens which enlarge the image to about : 50 – 100 X . This is the basic principle of compound microscope.

3- Robert Hook : made and used a compound microscope with magnification possible 200 X to reveal bacteria , but he made no observation of them because he studied mainly opaque object in dry state by reflected light.

4- Antoni Van Leevenhock made a simple but powerful lenses that have a magnification up to 300 X which sufficient to visualize bacteria , Protozoa and other m – ms . He named them animalcules [infusora] which include : bacteria and Protozoa .

Size of microbial cells :

Viruses : 0.01 - 0.2 µm

Bacteria : 0.1 – 15 mm

Fungi : 2 µm – 20 cm

Algae : 1 µm – meters

Protozoa : 2 – 200 µm

Microorganism and the origin of life

The ancient knew nothing of microorganism or evolution . Many scientists and philosophers believed that some firms of life could arise spontaneously from nonliving matters and they called this process , spontaneous generation theory [S G t] In order to refute such theory Francesco redi filled two flasks with meat , one left open and the 2nd sealed , Maggots appeared only in open flask after flies entered the flask of laid there eggs , so he deified the S G t .

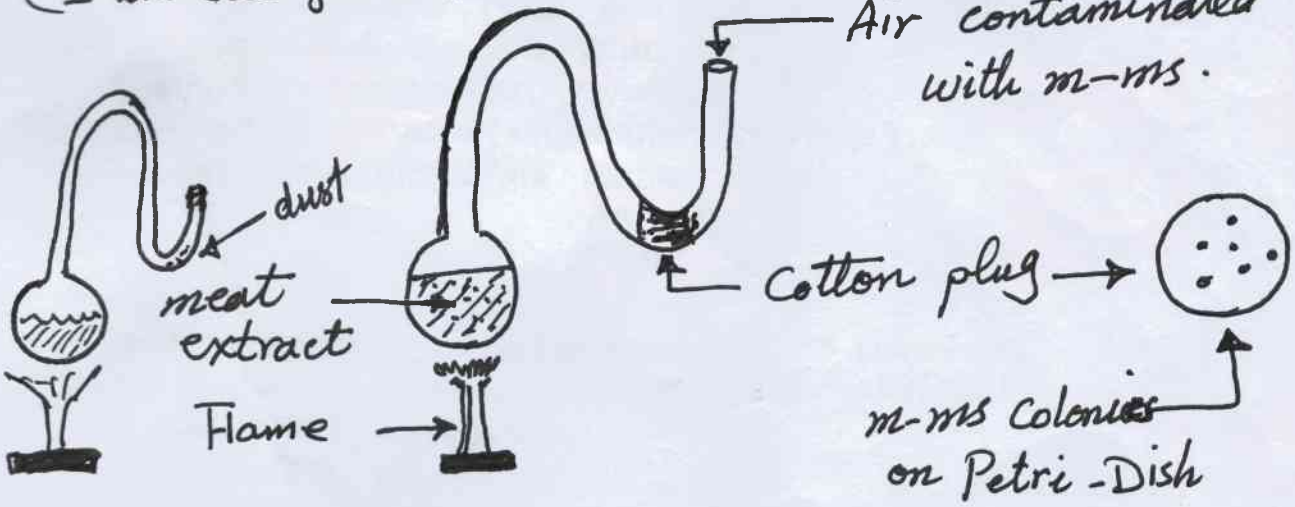
Louis Joblot suggested heating the 2 flasks and he noticed that the open one teemed with m – ms so he deified also S G T . But Needham believed in S G t because he noticed that m – ms appeared in both flask , Spallanzani suggested heating to kill m – ms but English scientist Tyndall did not agree with Spalanzani because heat did not kill all food m – ms , because m – ms found in 2 stages : 1-Heat-labile vegetative cells .

2- Heat – resistant bacterial spores

1n order to overcome such problem Tyndall suggested the discontinuous heating [Tyndallization] in which the extract heated three successive days very short period of time in order to promote the germination of bacterial spores which resist the heat first and second day of converted to vegetative cells on third day to be sensitive to the heat .

1822 – 1895 Louis Pasteur disproved the doctrine of STG and demonstrated that m – ms. are present in air attached to dust particles , he used flasks with long open necks with vertical bends in them [swan – necked – flasks] , the dust was caught by gravity in the bends of the neck and no life appeared in the infusions , Also Pasteur discovered that the wine did not spoil if it were held for some minutes at a temperature . between 50 and 60 C^1 this application gone rise to pasteurization, Pasteurization of milk is heating at $63C^0$ for 30 minute, 72^0C for 15 sec .in addition to his works Pasteur strongly proved that no living things created from non – living matter and the S.TG is wrong .

Pasteur's discover that bacteria are responsible for spoiling wine.
(I will Lead you to fame)



Robert Koch is the first real bacteriologist , he was the first scientist who used : -

1- Oil immersion in examining bacteria.
2- Using oil to enhance the microscopically vision .
3- He introduced a new and very important technology that is cultivation of m – ms on solid Media , he used 5-10% gelatin to prepare solid media , later agar was used for solidifying.
4- He figured that the bacteria have the ability to transform from a form to another (spore) in order to resist the unfavorable environmental conditions .
5- Using the staining methods to see the bacteria under microscope.
6- He discovered bacterial flagella after staining with a special stain .
7- Using the condenser .
8- Smearing the bacteria and fixing it on slide by heating.

Koch's four postulates hypothesis .

1-Infected animals should have the pathogen .
2- pathogens should cultivated in vitro .
3- The pathogen should shows the disease symptoms when injected in healthy animal .
4- possible reisolation of pathogen from experimental animal, which should be resemble to the origin pathogen .

The nonaproval of Koch's hypothesis :

1-When the pathogens are facultative and exist in healthy animals naturally.
2-When the experimental animal have immunity against the pathogen naturally.
3-When the disease caused by more than one pathogens.
4- When the pathogens not grow in vitro.

Location of m-ms in organism world :

1- Before the discovery of m-ms biologists classified all organisms in to two major kingdoms : animal kingdom [Animalia] , and plant kingdom [plantae] . the differentiation was based on : motility , photosynthesis, green color .
2- Haekel's system of three kingdoms based on evolutionary relationships, which are: Animalia , planta and protista which includes all microorganisms.
3- Five kingdoms systems by Whittaker :
   1- kingdom: monera (prokaryote) .
   2- Kingdom : Protista (unicellular Eukaryote) .
   3- Include three kingdoms : planta
   : Anemalia
   : Fungi

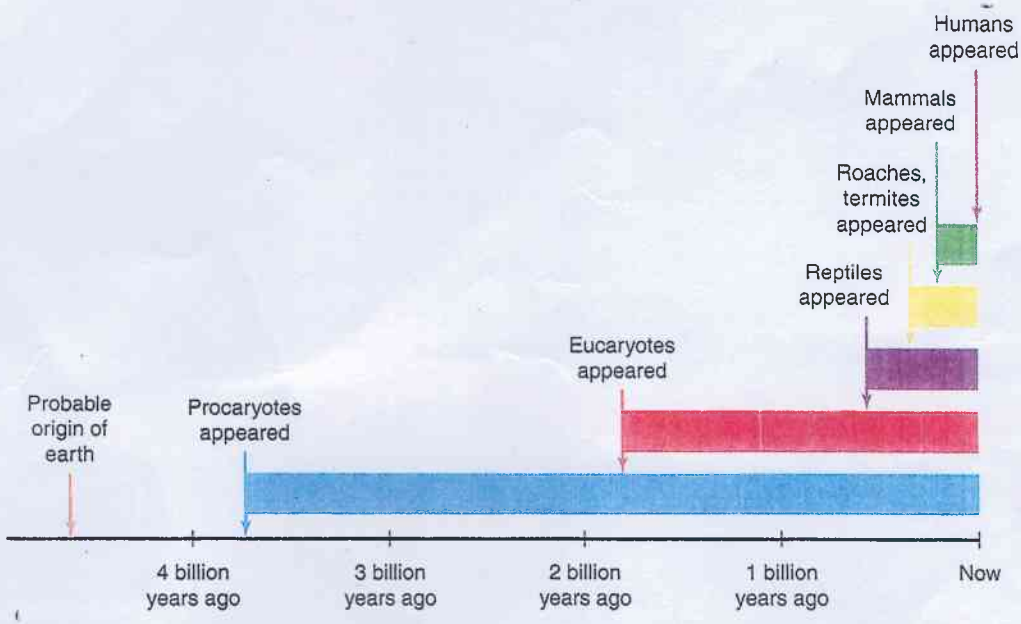
① monera – prokaryotic
② Protista – unicellular Eukariotic .
③ plant
④ animal
⑤ Fungi

4 – Carl Worse classification system: -
   1- Archaebacteria 2- Eubacteria 3- Eukaryote .
5 – The general system of classification of microorganisms :
   1- Eukaryotes Protista include : -
      a – Algae b – protozoa c – Fungi d – slime molds .
   2- prokaryotes :
      a – Eubacteria b – Archaebacteria C-Cyanobacteria .

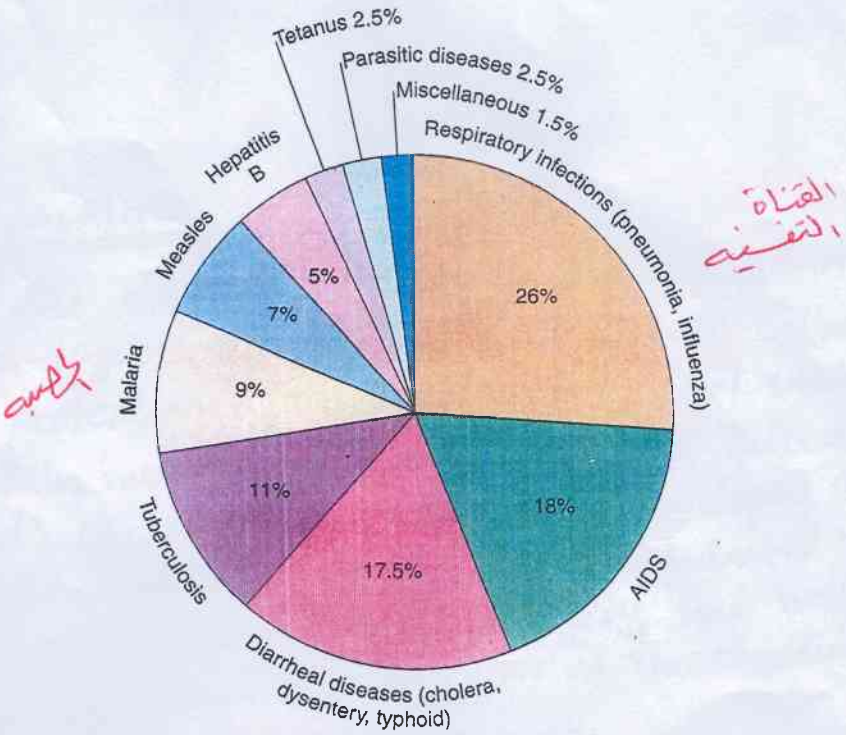
1- Eukargotic protista Algae, protozoa, fung slime mold.
1- Prokaryotic Eubacteria, Archaeba esia, Cyanobacteri

Pasteurization :

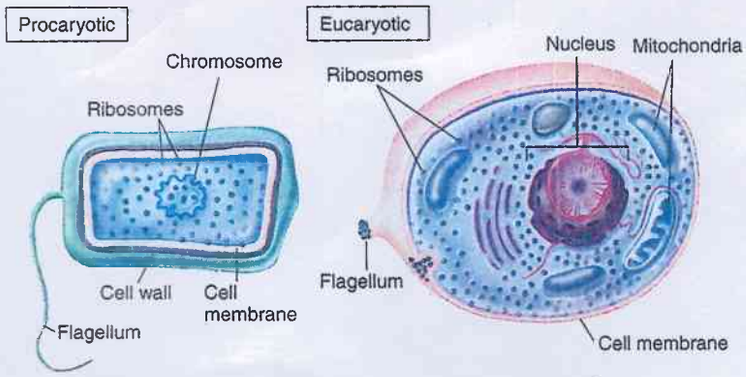
One of the most successful application of bacteriology . milk is raised to a temperature of either 63 – 66 C^0 for 30minuts or in the flash method to 72 C^0 for 15 second . milk so treated is not sterile but it is safe from contamination with viable Mycobacterium tuberculosis ,Brucellae, Campylobacter , Coxiella burneti and other Pathogenic vegetative bacteria .



Evolutionary timeline
Figure 1.1

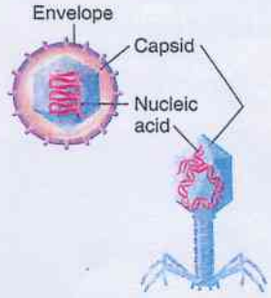


Worldwide infectious disease statistics
Figure 1.2



(a) Cell Types

Microbial cells are of the small, relatively simple procaryotic variety (left) or the larger, more complex eucaryotic type (right). (Not to scale)



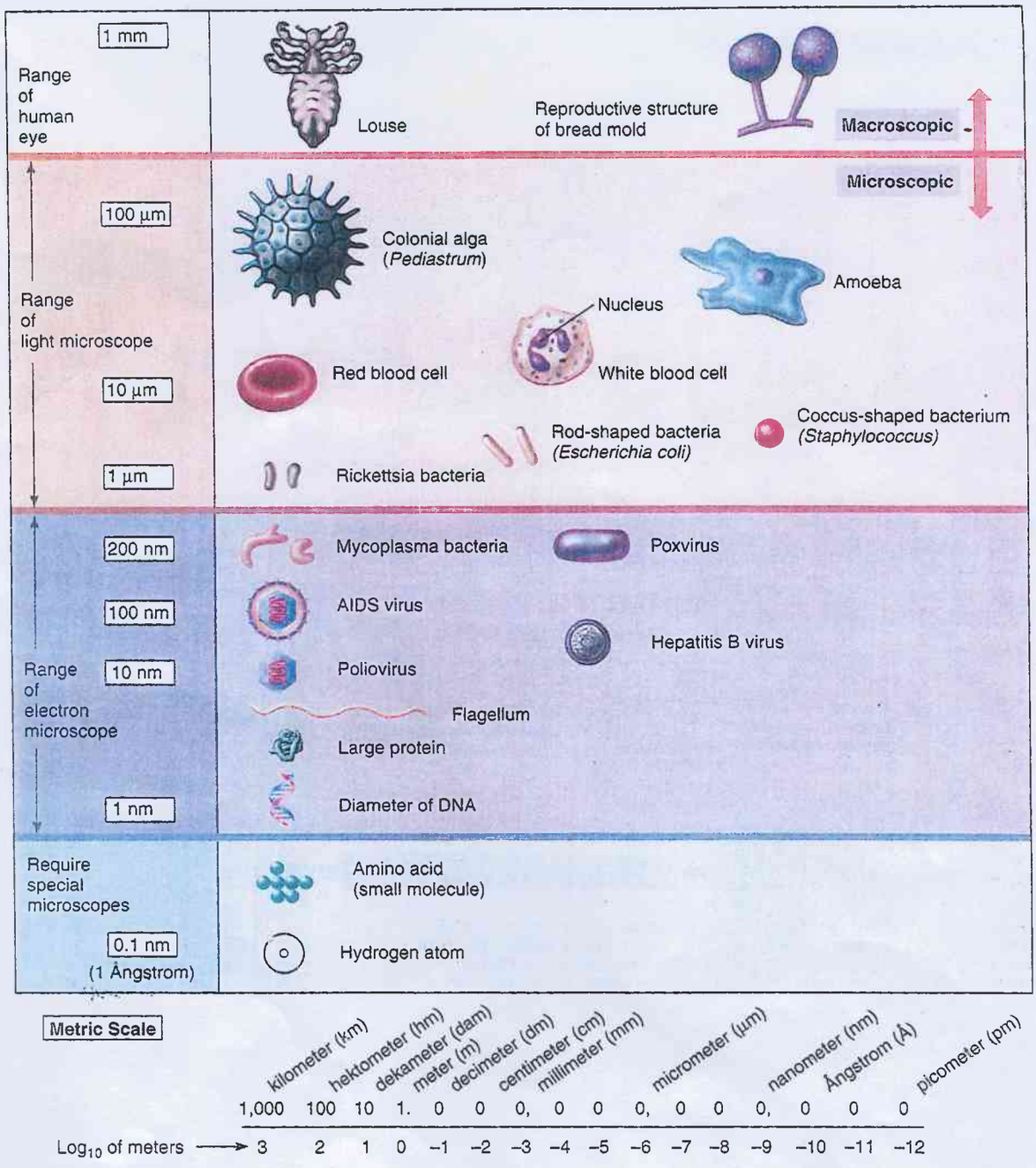
(b) Virus Types

Viruses are tiny particles, not cells, that consist of genetic material surrounded by a protective covering. Shown here are a human virus (top) and bacterial virus (bottom). (Not to scale

Cell structure
Figure 1.3

Jenner's Vaccine:

In 1796 the english physician Edward Jenner (1749-1823) tested the hypothesis that a mild disease called Cowpox provided protection against potentially fatal smallpox. After he intentionally inoculated a boy with pus collected from a milkmaid's cowpox lesion, the boy developed cowpox, which, of course, he survived. When Jenner then infected the boy with smallpoxe pus, he found that the boy had become immune to smallpox. He named the processes as Vaccination.



The size of things
Figure 1. 4

Bacteria : organization , structure , taxonomy

Bacteria are , a heterogeneous group of unicellular organisms , their cellular organization is described as prokaryotic (i.e. having a primitive nucleus) and differs from that of eukaryotes (plants , animals) . Some of the main differences are listed in Table 2.1.

Table 2.1 Differences between prokaryotic and eukaryotic cells

Property	prokaryotic cells	Eukaryotic cells
Chromosome number	one	Multiple
Nuclear membrane	Absent	present
Mitochondria	Absent	present

Genome . The most fundamental difference between bacteria and Eukaryotes is that the bacterial chromosome , or genome , is a single circular molecule of double-stranded DNA; there is no nuclear membrane. Bacteria may from time to time harbor other smaller circular DNA molecules or plasmids which code for certain non-essential functions.

STRUCTURE

Bacteria have a rigid wall which determines their shape – only plants amongst other forms of living organisms possess this.

Shape. Bacteria may be:

1. Spherical – cocci
2. Cylindrical – bacilli
3. Helical – spirochaetes

Arrangement depends on plane of successive cell divisions:

e.g. chains – streptococci ; clusters – staphylococci ; diplococci – pneumococci; angled pairs or palisades – corynebacteria .Grams stain divides bacteria into Gram-positive or Gram negative ,an important step in classification . The Gram-staining depending on the structure of the cell wall.

A diagram of a typical but composite bacterium is shown in figure 2.1. Bacteria are cells with a rigid cell wall which surrounds the protoplast ; this consists of a cytoplasmic membrane enclosing internal components and structures such as ribosomes and the bacterial chromosome.

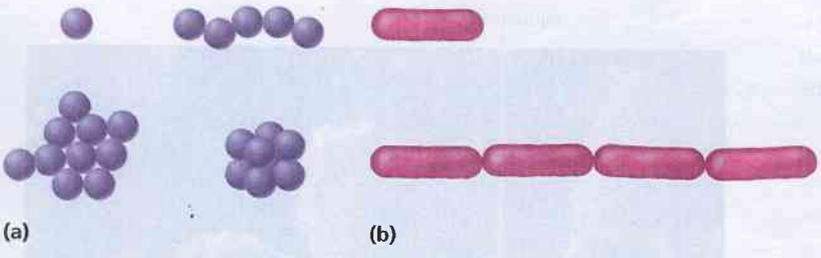


Figure **Bacterial shapes and arrangements.** **(a)** Spherical cocci may be in arrangements such as single, chains (streptococci), clusters (staphylococci), and cuboidal packets. **(b)** Rod-shaped bacilli may also be single or in arrangements such as chains.

(c) helical (spiral)

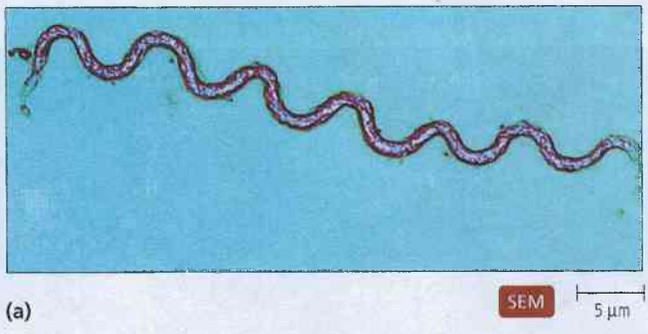
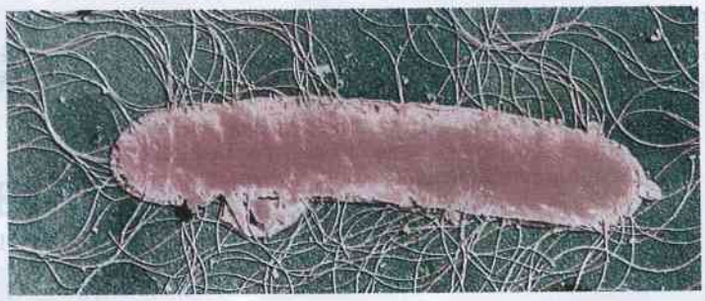
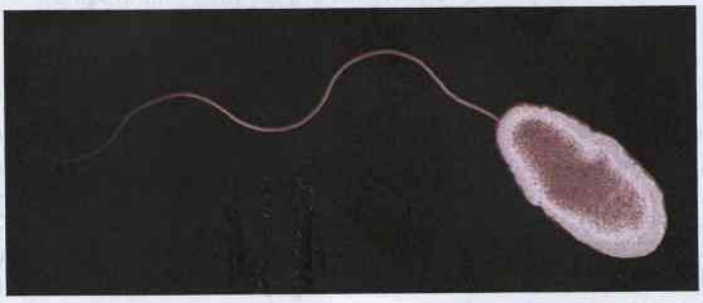
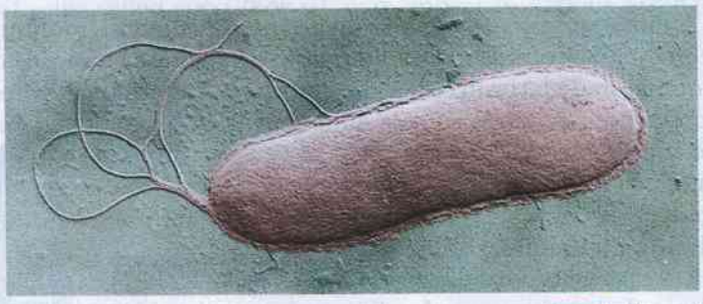


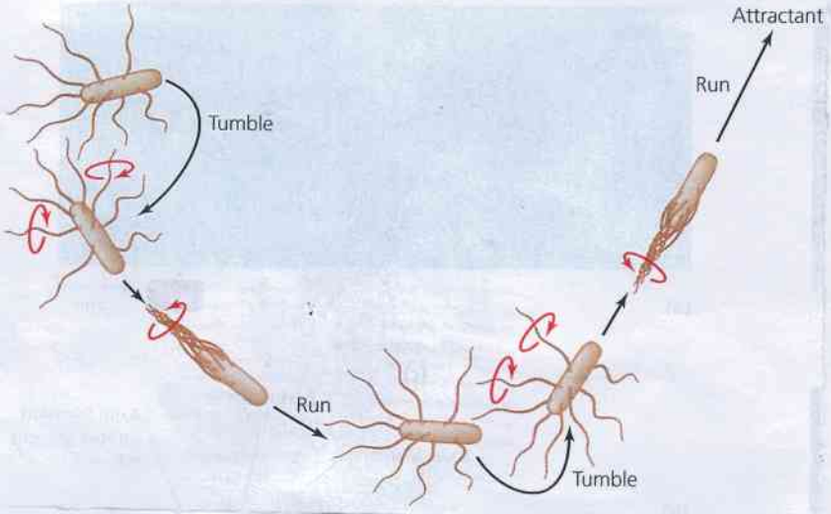
Figure **Axial filament.** **(a)** Scanning electron micrograph of a spirochete, *Treponema pallidum*. **(b)** Diagram of axial filament wrapped around a spirochete. **(c)** Cross section of the spirochete, which reveals that the axial filament is composed of endoflagella.







▲ **Figure** **Micrographs of basic arrangements of bacterial flagella.** **(a)** Peritrichous. **(b)** Single polar flagellum. **(c)** Tuft of polar flagella.



◀ **Figure** **Motion of a peritrichous bacterium.** In peritrichous bacteria, runs occur when all of the flagella rotate counterclockwise and become bundled. Tumbles occur when the flagella rotate clockwise, become unbundled, and the cell spins randomly. In positive chemotaxis (shown), runs last longer than tumbles, resulting in motion toward the chemical attractant. *What triggers a bacterial flagellum to rotate counterclockwise, producing a run?*

External structures

External structures which protrude from the cell into the environment are present in many bacteria . These structures are:

1 . Flagella : elongated filaments responsible for motility ; composed of a Protein ' flagellin' – chemically similar to myosin. Movement respone to stimulus is termed taxis, either Light (Phototaxis) or chemical (chemotaxis).

2. Pili : finer shorter filaments extruding from the cytoplasmic membrane ; Also protein (pilin), they are responsible for adhesion (common pili) And probably for conjugation when genes are transferred from one bacterium to another (sex pili).and can act as receptor sites for some bacteriophage.

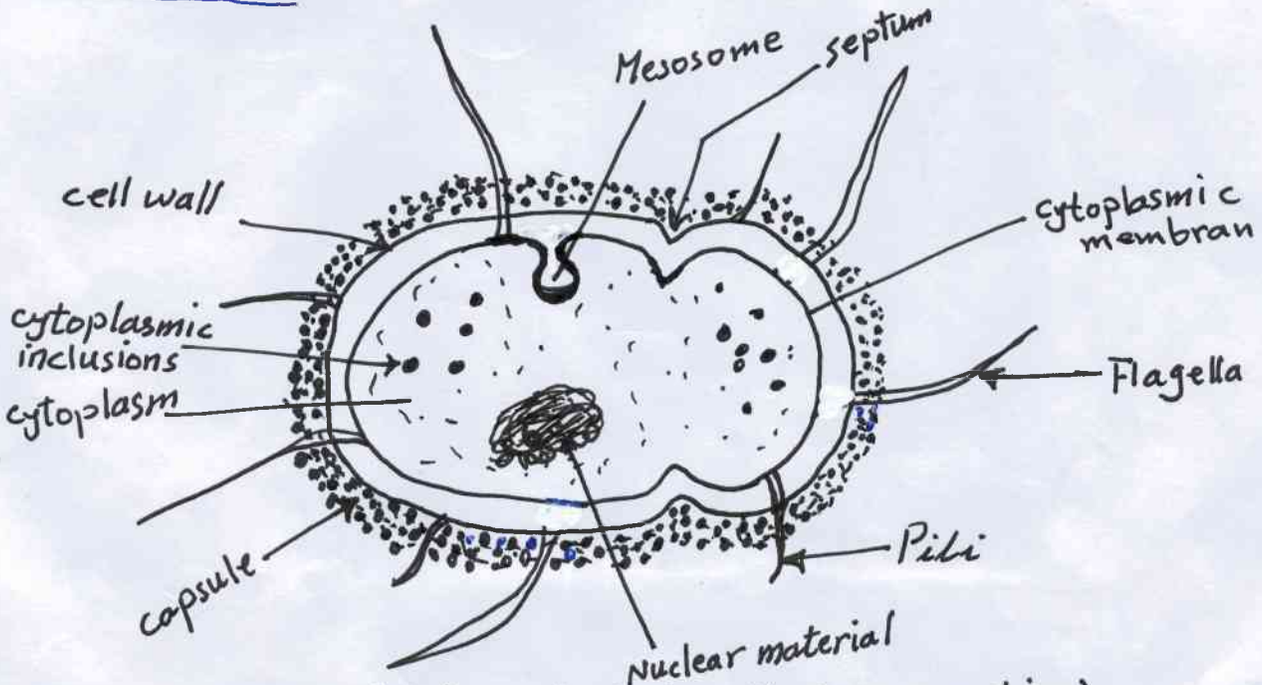


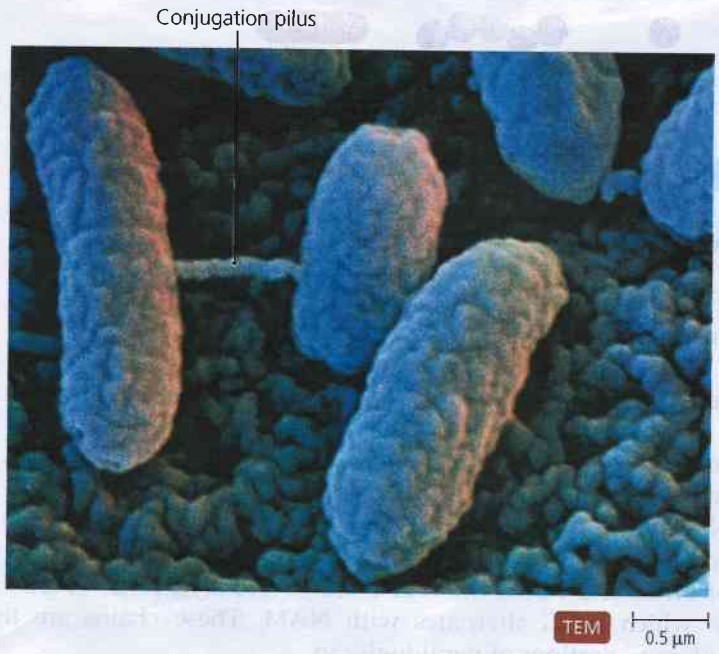
Fig. 2.1 : Typical bacterial cell (cross section).

3. Capsules : amorphous material , which surrounds many bacterial species As their outermost layer ; usually polysaccharide , occasionally protein ; often inhibit phagocytosis and so their presence correlates with virulence in certain bacteria .

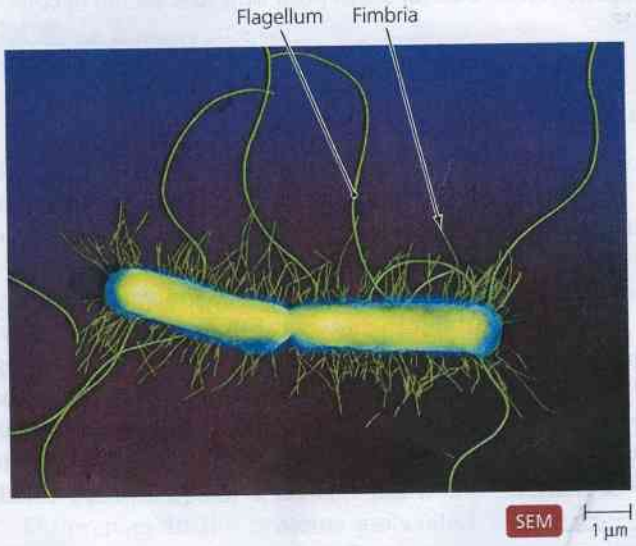
4.Sheaths: It is a filamentous structure enables bacteria to attach to solid surfaces .These sheaths afford protection against predators & parasites .

Cell wall :

In addition to conferring rigidity upon bacteria, the cell wall also protects against osmotic damage . It is porous and permeable to substances of low molecular weight .



▲ **Figure** **Pili.** Two *Salmonella* cells are connected by conjugation pili. *How are pili different from bacterial flagella?*



▲ **Figure** **Fimbriae.** *Proteus vulgaris* has flagella and fimbriae.

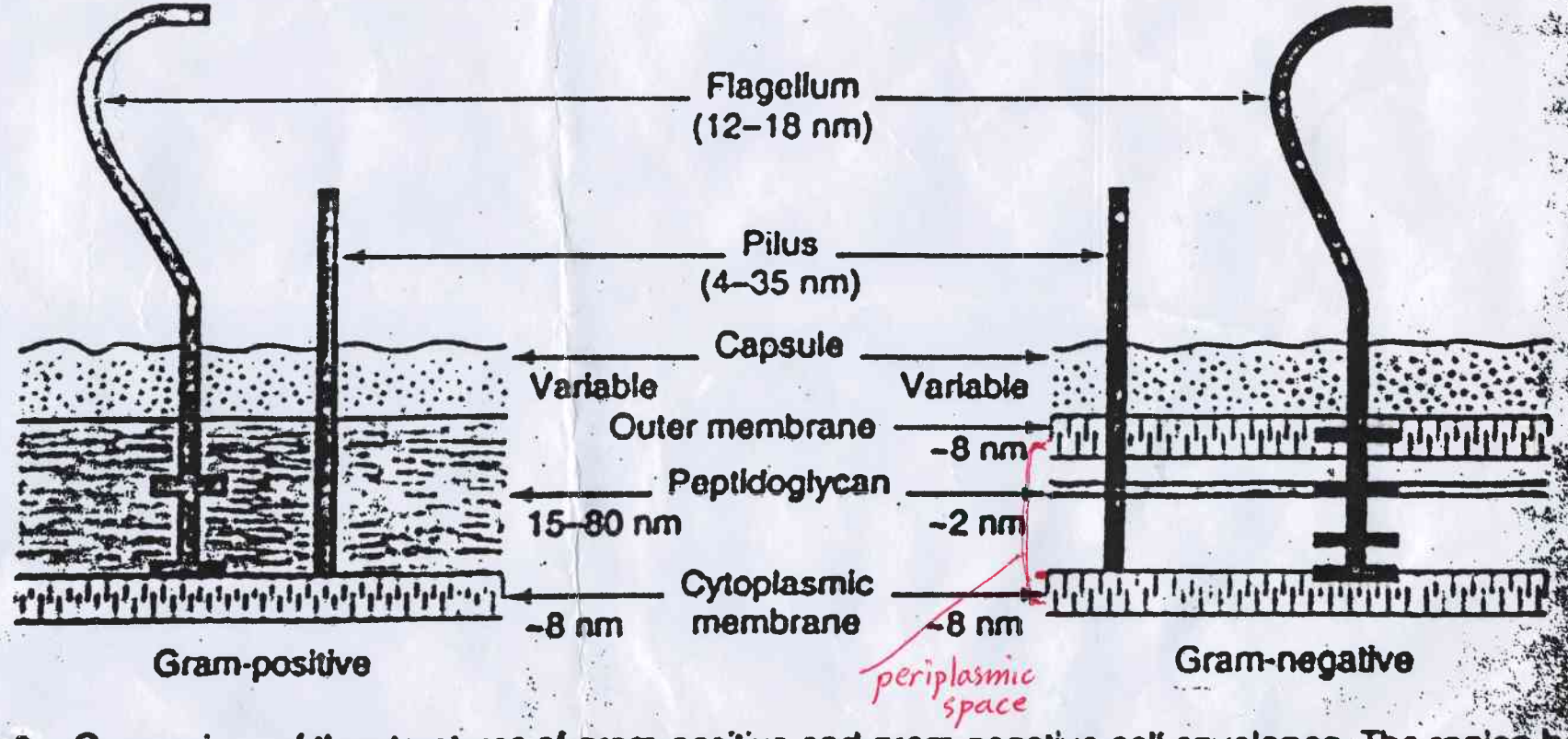
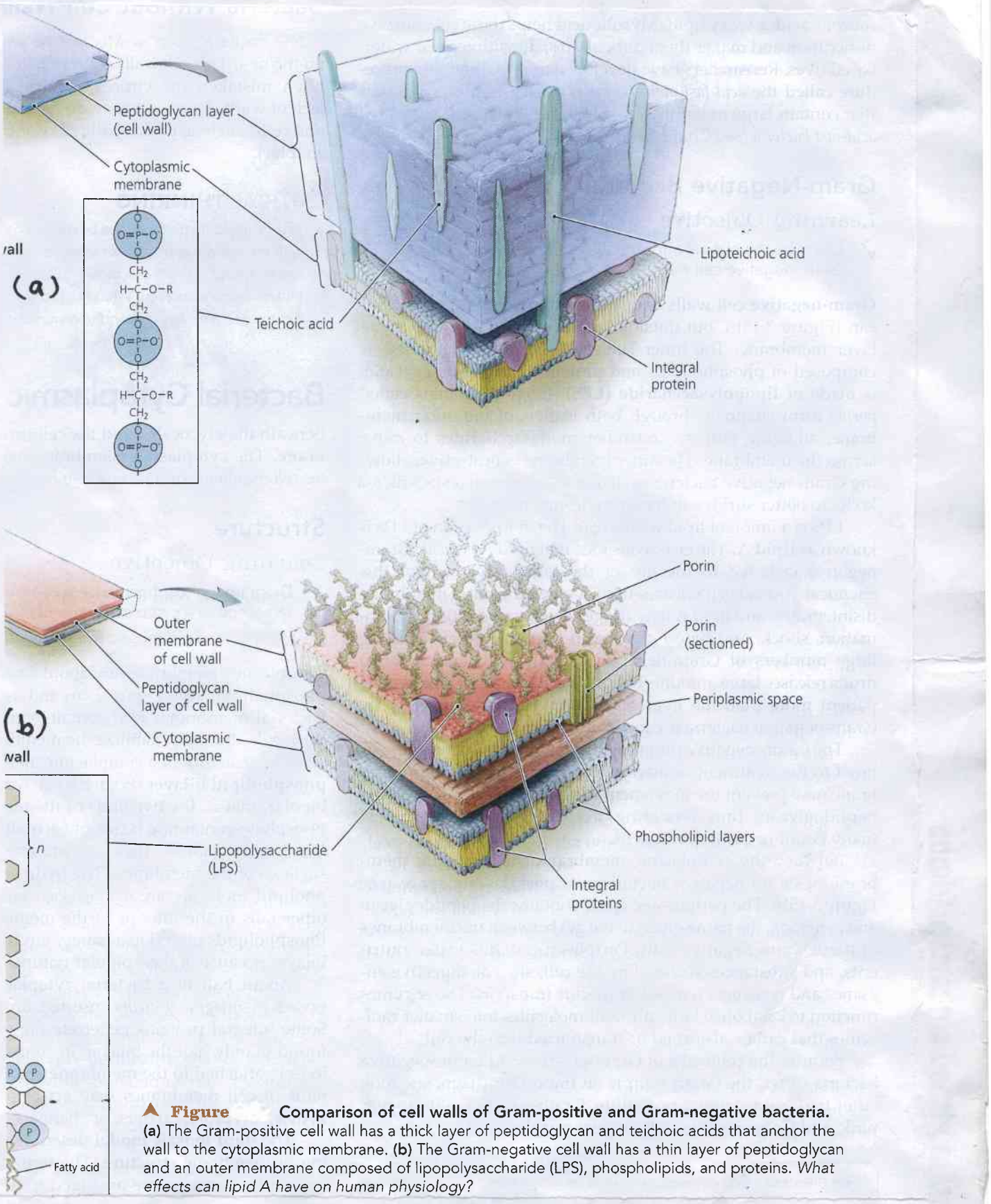


Figure 2–2.: Comparison of the structures of gram-positive and gram-negative cell envelopes. The region be cytoplasmic membrane and the outer membrane of the gram-negative envelope is called the periplasmic sp produced, with permission, from Ingraham JL, Maalee O, Neidhardt FC: *Growth of the Bacterial Cell.* Sinaue ates, 1983.)

enolpyruvate; the phosphorylated carrier pro



▲ **Figure** **Comparison of cell walls of Gram-positive and Gram-negative bacteria.** **(a)** The Gram-positive cell wall has a thick layer of peptidoglycan and teichoic acids that anchor the wall to the cytoplasmic membrane. **(b)** The Gram-negative cell wall has a thin layer of peptidoglycan and an outer membrane composed of lipopolysaccharide (LPS), phospholipids, and proteins. *What effects can lipid A have on human physiology?*

Structure of the cell wall differs in –Gram- positive and Gram- negative bacteria; this is illustrated in Figure 2.2.

Chemically the cell wall is peptidoglycan: this is a mucopeptide composed of alternating strands of N- acetyl muramic acid and N- acetyl glucosamine cross – linked with peptide subunits.

Teichoic or teichuronic acids are part of the cell wall of Gram- Positive bacteria: they maintain the level of divalent cations outside the cytoplasmic membrane.

Other components which may be present in the cell wall are antigens such as the polysaccharide (Lancefield)and protein (Griffith) antigens of streptococci and the lipopolysaccharide O antigens of Gram-negative bacilli.

Function of cell wall:

1- protects cells from osmotic pressure .

2 - Plays an essential role in cell division.

3- sites of major antigenic determination of the cell surface .

4- LPS responsible for non-specific endotoxin activity.

Bacteria with defective cell walls:

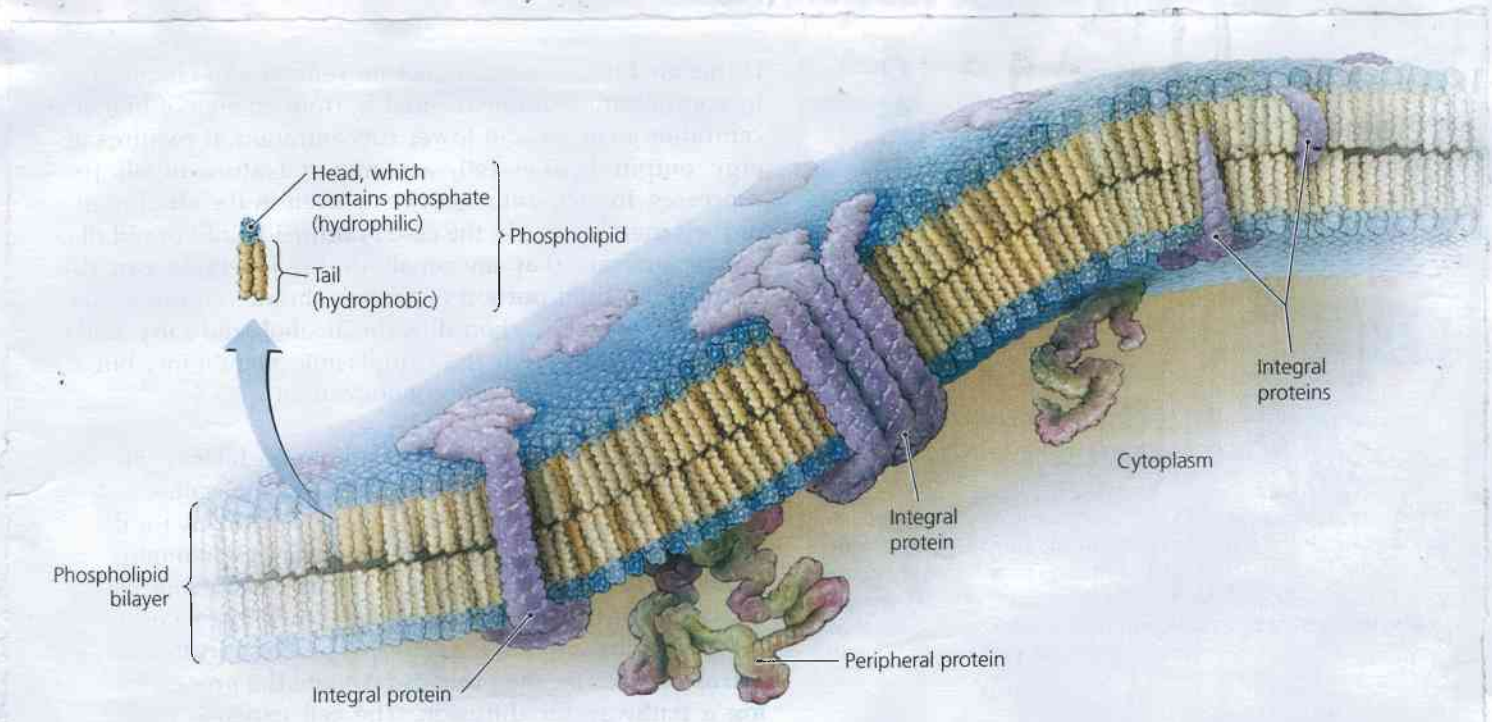
Bacteria develop and can survive with defective cell walls: these can be induced by growth in the presence of antibiotics and a hyperosmotic environment to prevent lysis. Bacteria without cell walls are of four types:

1.protoplasts: derived from Gram-positive bacteria and totally Lacking cell walls; unstable and osmotically fragile; produced artificially by lysozyme and hyperosmotic medium: require hyperosmotic conditions for maintenance .

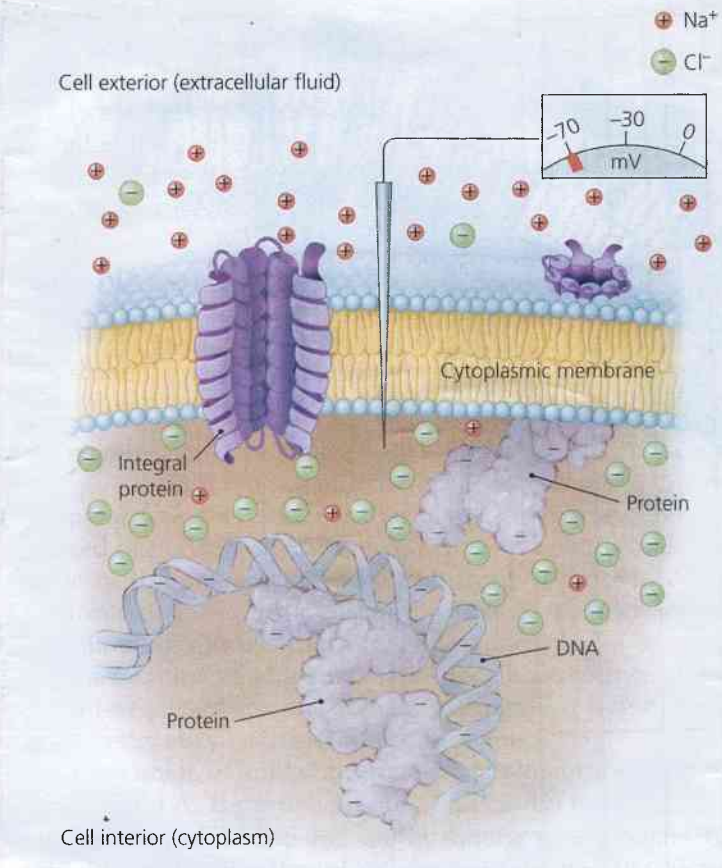
2.Spheroplasts: derived from Gram-negative bacteria; retain some residual but non-functional cell wall material; osmotically fragile; produced by growth with penicillin and must be maintained in hyperosmotic medium.

3.L-forms: cell wall –deficient forms of bacteria usually produced in the laboratory but sometimes spontaneously formed in the body of patients treated with penicillin; more stable than protoplasts or spheroplasts,they can replicate on ordinary media.

4. Mycoplasma: an independent bacterial genus of naturally occurring bacteria which lack cell walls; also stable and do not require hyperosmotic conditions for maintenance.



▲ **Figure**
The structure of a prokaryotic cytoplasmic membrane: a phospholipid bilayer.



▲ **Figure** **Electrical potential of a cytoplasmic membrane.**
The electrical potential exists across a membrane because there are more negative charges inside the cell than outside it.

peptidoglycan

Enzymes that attack c.w.

1- Lysozyme : attack the B1 – 4 linkage of the P.G backbone and hydrolyze it. This enzymes found in animal secretions (tears, saliva and nasal secretions).

2 – Autolysins enzymes : hydrolytic enz . that attack P.G, including glycosidases, amidases & peptidase.

These enzymes play an essential role in cell growth & division.

Cytoplasmic membrane

A' unit membrane' i.e. a double-layered structure composed of lipid and protein which acts as a semipermeable membrane through which there is uptake of nutrient by passive diffusion. It is also the site of numerous enzymes involved in the active transport of nutrient to the cell which important for metabolic processes .

Function of cell membrane :

1- selective permeability and transport of solutes .

2- Electron transport and oxidative phosphorylation in aerobic species.

3 – Extract hydrolytic enzyme .

4 – bearing enzymes and carrier molecules that function in the DNA synthesis .

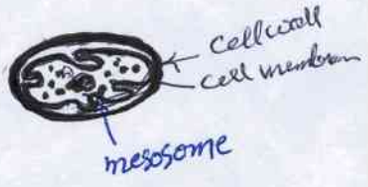
5 – Bearing receptors and other proteins .

Mesosomes

Convoluted invaginations of cytoplasmic membrane often at sites of septum formation : involved in DNA segregation during cell division and respiratory enzyme activity .mesosomes of two types:

1-Septal mesosomes.

2-lateral mesosomes .



Nuclear material

The single circular chromosome which is the bacterial genome or DNA undergoes semiconservative replication by directionally from a fixed point, the origin.

Plasmids:

In addition to the bacterial chromosome , bacteria may contain one or more small, circular macromolecules of DNA known as plasmids which Contain specific genetic information such as resistance to antibiotics , Production of toxins, and tolerance to toxic of metals .

Ribosomes

Ribosomes are distributed throughout the cytoplasm and are the sites of protein synthesis.

Inclusions

Sources of stored energy, e.g. polymetaphosphate (volutin),poly-B-hydroxybutyrate (lipid), polysaccharide (starch or glycogen).

Spores

Spores produced by bacteria in the genera Bacillus and Clostridium enable them to survive adverse environmental conditions: developed from and at the expense of the vegetative cell. Spores are dense, contain a high concentration of calcium dipicolinate and are resistant to heat , desiccation and disinfectants; they often remain associated with the cell wall of the bacillus from which they develop and are described as central, terminal , sub terminal , etc. When growth conditions become favorable they germinate to produce vegetative cells.

TAXONOMY تصنيف

Taxonomy is the classification or division of organisms into ordered groups.

Nomenclature تسمية is the labeling of the groups and of individual members within groups.

Organisms fall into three kingdoms:

1.Animals

2.Plants

3.Protista – contains all unicellular organisms including bacteria.

Higher organisms are classified phylogeneticaly (i.e. on the bases of evolution)but this classification is impossible in the case of bacteria as they lack sufficient morphological features. There for , different characters have been chosen arbitrarily اختيار اجباري so that the various characters can be distinguished between species .these characteristics include :

1-Morphology .

2- Staining .

3- Culture characteristics .

4- Antigenic structure .

5- Biochemical reactions .

6- Base composition (i.e. GC ratio) of bacterial DNA .

Pare

Classification of Microorganisms (prokaryote) :

Classification was started 200 years ago , several thousand species of bacteria have been identified , .named and cataloged .tracing the origins of and evolutionary relationships among bacteria has not been an easy task .one of the questions that has been plagued taxonomist is . what characteristics are the most indicative of closeness in classifying . Early bacteriologists found classifying according to shape ,variation in arrangement , growth characteristics , and habitat .

As a results of technique development , it became clear that similarities in cell shape , arrangements and staining reactions do not automatically indicate relatedness .

At the time being , classification schemes are turning to genetic and molecular level that cannot be visualized under microscope or in culture .

The methods that a microbiologist uses to identify bacteria to the level of genus and species fall into the main categories of morphology (1) (microscopic and macroscopic) ,bacterial physiology (2) or biochemistry (3) , serological (4) analysis and genetic (5) techniques.

Many of the identification system are automated and computerized to process data and provide a "(best fit) identification . However , not all methods are used on all bacteria . A few bacteria can be identified by placing them in a machine that analyzes only the kind of fatty acids they contain ,in contrast , some are identifiable by a Gram stain and a few physiological tests .others may require a diverse spectrum of morphological , biochemical , and genetic tests .

1a. Gram-positive cells..... Gram-positive bacteria
1b. Gram-negative cells..... 2

2a. Rod-shaped cells..... 3
2b. Non-rod-shaped cells..... Cocci and pleomorphic bacteria

3a. Can tolerate oxygen..... 4
3b. Cannot tolerate oxygen..... Obligate anaerobes

4a. Ferments lactose..... 5
4b. Cannot ferment lactose..... Non-lactose-fermenters

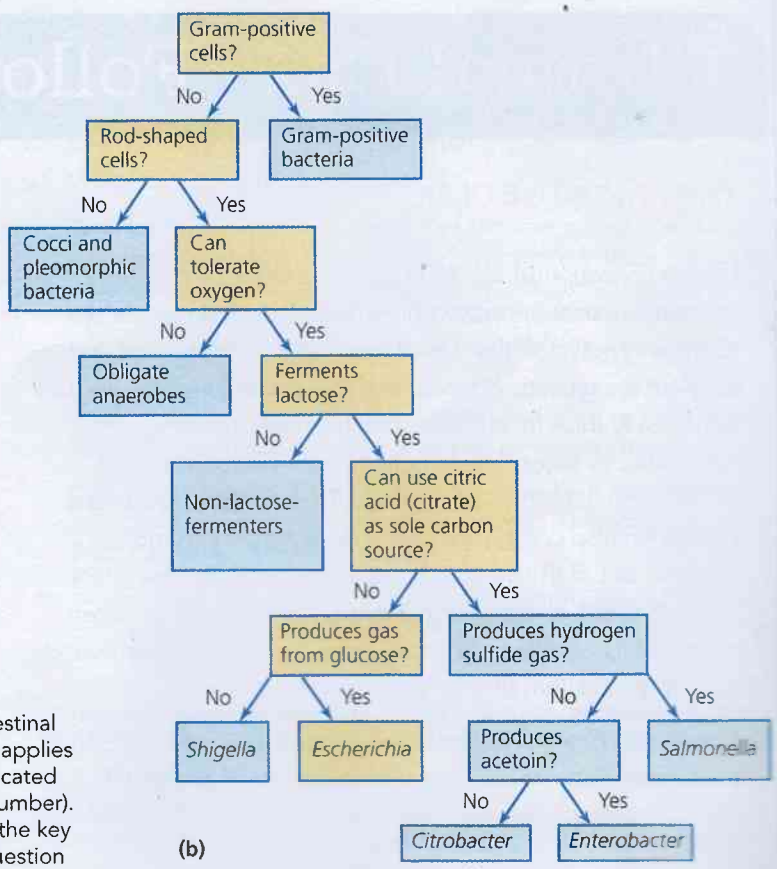
5a. Can use citric acid as a sole carbon source..... 6
5b. Cannot use citric acid alone..... 8

6a. Produces hydrogen sulfide gas..... *Salmonella*
6b. Does not produce hydrogen sulfide gas.... 7

7a. Produces acetoin..... *Enterobacter*
7b. Does not produce acetoin..... *Citrobacter*

8a. Produces gas from glucose..... *Escherichia*
8b. Does not produce gas from glucose..... *Shigella*

(a)



(b)

Figure 4.27 Use of a dichotomous taxonomic key. The example ented here involves identifying the genera of potentially pathogenic intestinal eria. **(a)** A sample key. To use it, choose the one statement in a pair that applies e organism to be identified, and then either refer to another key (as indicated ords), or go to the appropriate place within this key (as indicated by a number). flowchart that shows the various paths that might be followed in using the key ented in part (a). Highlighted is the path taken when the bacterium in question cherichia.

Species : The units of classification .

Species consists of an assemblage of individuals that share a high degree of phenotypic similarity . coupled with an appreciable dissimilarity from other assemblages of the same general kind .

Accordingly , the best working definition of a bacterial species is the following ; a group of strains that show a high degree of overall phenotypic similarity and that differ from related strain groups with respect to many independent characters .

The characterization of species :

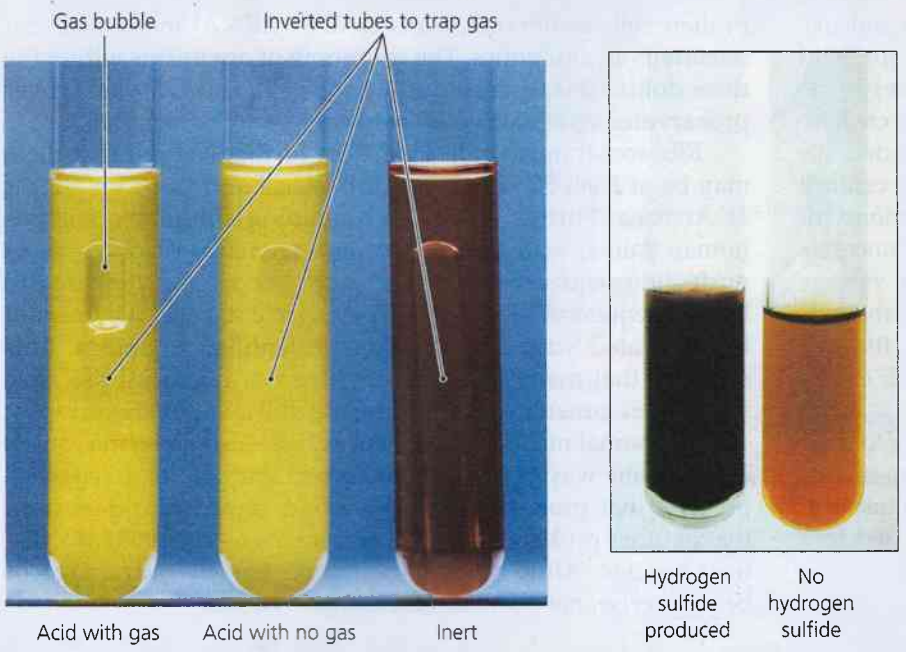
Species should be characterized by complete descriptions of their phenotype or – even – better – of their genotypes .

The bacterial taxonomist has always been forced to seek other kinds of characters other than structural or anatomical used for eukaryote, so he look for biochemical , physiological , ecological – with which to supplement structural data . Most bacteria can be identified only by finding out what they can do , not simply how they look .

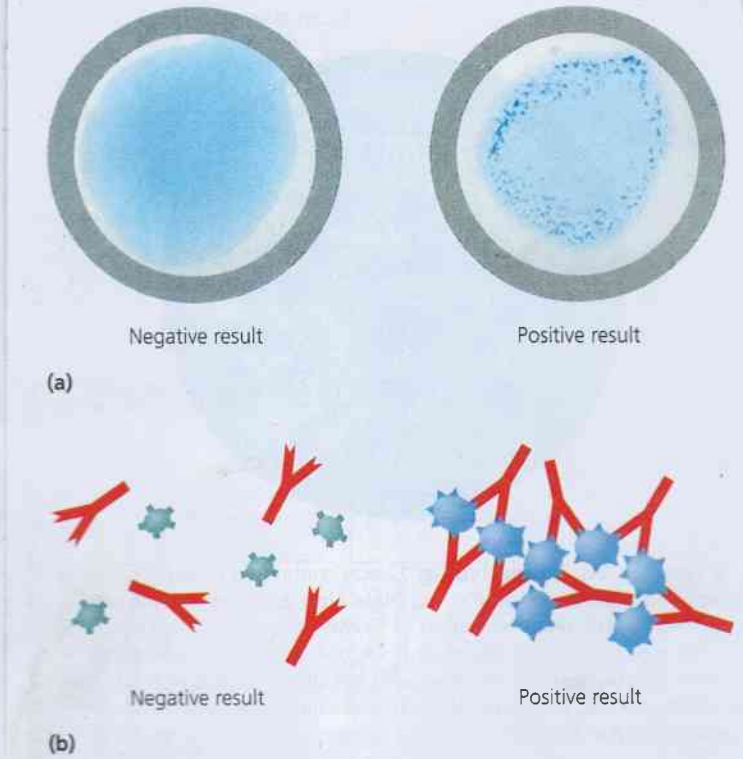
The naming of species :

According to a convention known as the binomial system of nomenclature, every biological species bears a Latinized name that consists of two words .the first word indicates the taxonomic group of immediately higher order, or genus to which the species belongs , and the second word identifies it as a particular species of that genus .

The first letter of the generic name is capitalized ,and specific name in small (Escherichia coli) .



◀ **Figure 4.23 Two biochemical test bacteria. (a)** A carbohydrate utilization tes tube in which the bacteria have metabolize carbohydrate to produce acid (which chan pH indicator, phenol red, to yellow) and ga by the bubble. At center is a tube with anc that metabolized the carbohydrate to proc gas. At right is a tube inoculated with bact "inert" with respect to this test. **(b)** A hydr test. Bacteria that produce H_2S are identifi precipitate formed by the reaction of the l present in the medium.



▲ **Figure 4.25 An agglutination test, one type of serological test. (a)** In a positive agglutination test, visible clumps are formed by the binding of antibodies to their target antigens present on cells. **(b)** The processes involved in agglutination tests. In a negative result, antibody binding cannot occur, because its specific target is not present; in a positive result, specific binding does occur. Note that agglutination occurs because each antibody molecule can bind simultaneously to two antigen molecules.

Binomial nomenclature is used for all biological groups except viruses . The virologists are currently divided over the best way to design at members of this groups ; some wish to extend the binomial system to the viruses , whereas others would prefer another system , which gives in coded form information about the properties of the organism .

In bacterial taxonomy , when a new species is named , a particular strain is designated as the type strain . Type strains are preserved in culture collections,

The type strain is important for nomenclature purposes .

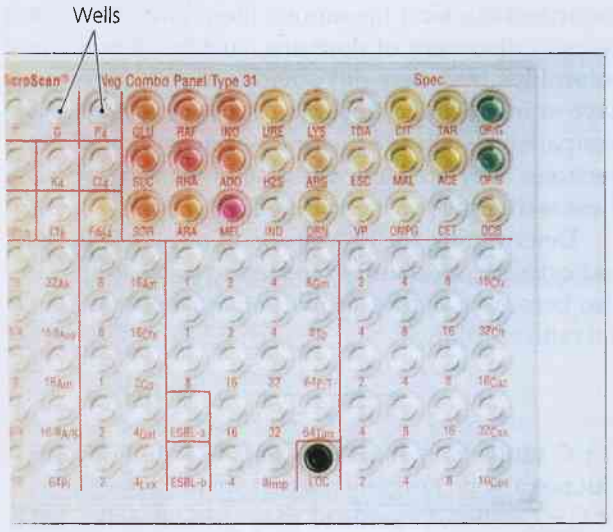
Species , Genus , family , order , class , division .

New approaches to bacterial taxonomy :

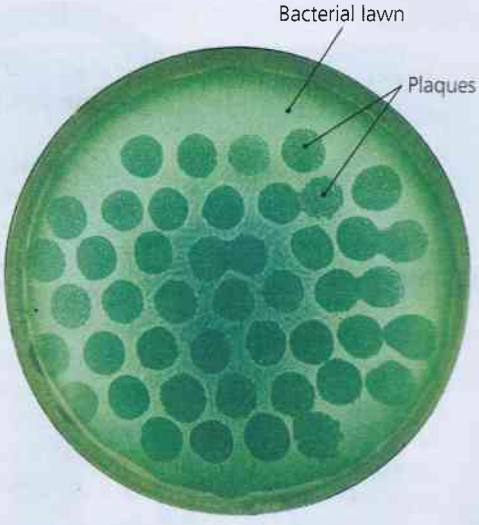
The growth of molecular biology has opened up a number of new approaches to the characterization of organisms , which have had a profound impact on the taxonomy of bacteria . the base – pairing rules require that A = T and G = C and molar ratio (G + C) : (A + T) , analysis showed that this ratio in fact varies over a rather wide range in DNA preparation from different organisms , and subsequent work has revealed that the base composition of DNA is a character of profound taxonomic importance , particularly among microorganisms .

The recently developed molecular approaches to the analysis of bacterial relationships have provide a very valuable supplement to earlier purely phenotypic characterizations . It is now possible to recognize among the bacteria a considerable number of subgroups that appear to be natural ones .

Many of these groups contain a large number of species , really distinguishable by both phenotypic and genotypic criteria .



4.24 One tool for the rapid identification of bacteria, ted MicroScan system. A MicroScan panel, a plate merous wells, each the site of a particular biochemical test. nt ascertains the identity of the organism by reading the lors in the wells after the biochemical tests have been



▲ **Figure 4.26 Phage typing.** Drops containing type A bacteriophages were added to this plate after its entire surface was inoculated with an unknown strain of *Salmonella*. After 12 hours of bacterial growth, clear zones, called plaques, developed where the phages killed bacteria. Given the great specificity of phage A for infecting and killing its host, the strain of bacterium can be identified as *Salmonella enterica* serotype Typhi.

- Prokaryotes –

Microorganisms that lack nuclear enveloped (nuclear membrane) and the genetic material is free in the cytoplasm . Individuals of this microorganisms [Bacteria and cyanobacteria] are characterized by :

1 – Unicellular organisms , sometime cells aggregated (0.2-10 µm).

2 – All cells are not differentiated ,

3 – Genetic material is free in cytoplasim .

4 – Cells are lack of organilles (mitochondria, Golgi bodies, endoplasmic reticulum .....) .

5 – No spindle formation through cell division .

6 – Ribosomes are distributed in cytoplasm .

7 – Nutrients absorbed in form of molecules or ionic .

8 – Cells are not motile , and if motile using flagella.

9 – Some time cells in form of mycelium or in colony form .

10 – The cytoplasim is not motile .

A certain degree of structural diversity exists among prokaryotes especially in metabolic terms , particularly with respect to the mechanisms of energy – yielding metabolism. it characterized of photosynthesis, aerobic respiration , and fermentation – all occur in prokaryotes . In addition ,many groups of prokaryotes can fix molecular nitrogen and use it as a nitrogen source. The largest unicellular bacteria are considerably larger than the smallest unicellular protists . Nevertheless , the average cell size of prokaryotes is considerably less than that of protist , and the smallest bacteria have cells far below the lower limit of cell size in eukaryotic groups .

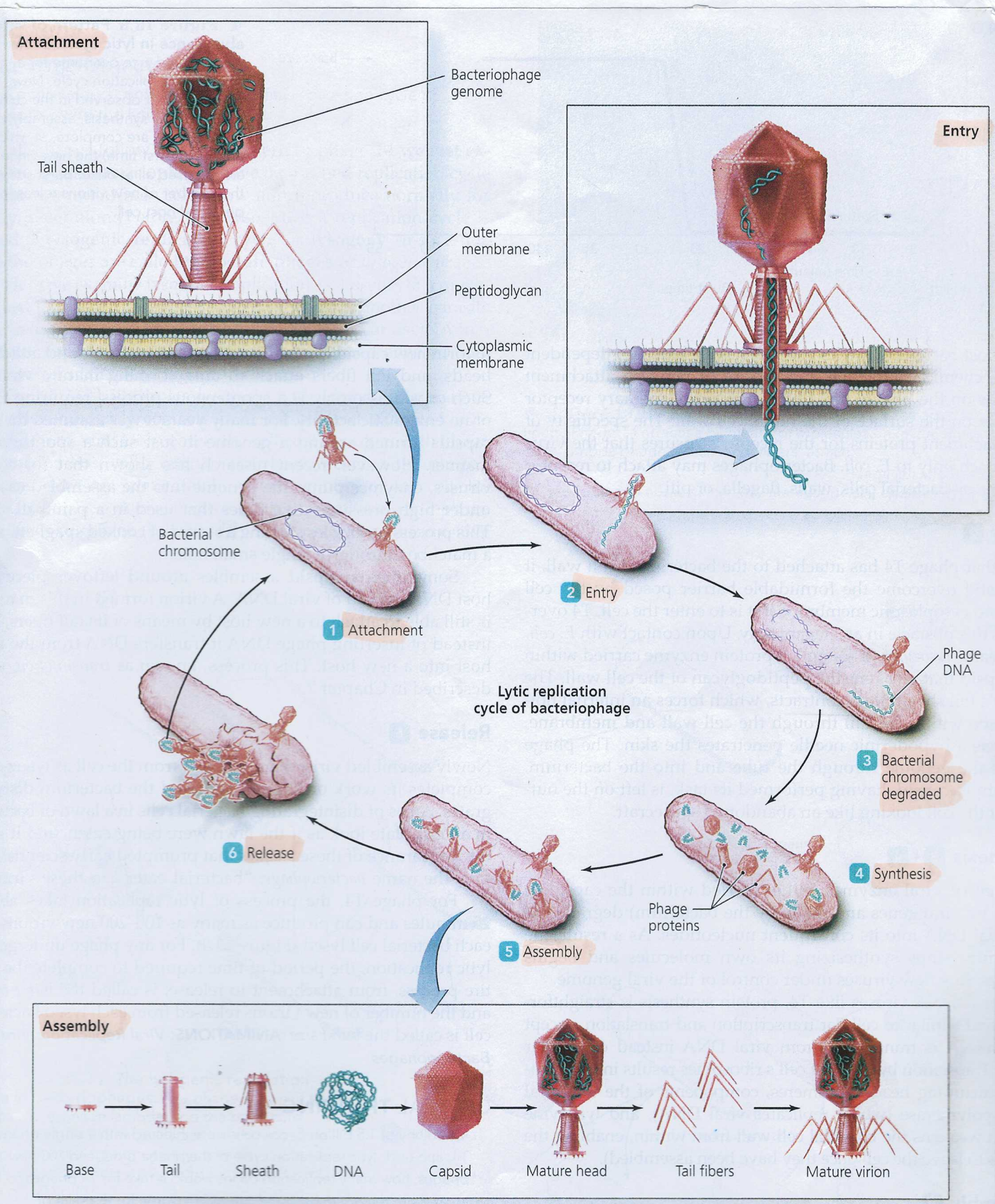
The Viruses :

The term virus was used to denote any agents capable of producing disease . the word is Latin in originally ment venom or poisonous fluid .

In 1892 D.J. Ivanowsky found that on infectious extract from tobacco plants with mosaic disease retained its infectivity after passage through a filter able to prevent the passage of bacteria . he assumed that the infectious agent was a small microorganisms. during the following two or three decades , it was shown that many major diseases of both plant and animals are caused by similar infectious agents , so small that they cannot be seen with light microscope . with the passage of time, the adjective "filterable" was gradually dropped, and the word "Virus" became a specific group designation for these ultramicroscopic ,filter passing infectious agents . Studies of their behavior in the laboratory led to the conclusion that they were obligate intracellular parasites able to multiply only within the host cells. scientist were concluded that the virus was not a living organism but rather a fluid infectious principle. The infectious principle of a virus is therefore not a protein molecule but a molecule complex ,built up from two different kinds of macromolecule :protein and nuclic acid . The nucleic acid is specific to the virus and may be either DNA or RNA .

Bacteria are susceptible to infection by ultramicroscopic ,filter – passing agents , designated as bacteriophages (eaters of bacteria) , This designation is often shortened to phages .

A virus alternates in its life cycle between two phases ,one extracellular and the other intracellular . In its extracellular Phase, it exists as an inert, infectious particle , or " virion " .In the intracellular phase ,a virus exists in the form of replicating nuclic acid , either DNA or RNA . during the intracellular phase, the genetic material of the virus is not only replicated by the host cell but also serves as a genetic determinant for the synthesis by the cell of specific viral proteins . These proteins include the subunits , or capsomers , from which the capsid of the mature particle is assembled .



▲ **Figure** **The lytic replication cycle in bacteriophages.** The phage shown in this illustration is T4, and the bacterium shown is *E. coli*. The circular bacterial chromosome is represented diagrammatically; in reality it would be much longer.

The reproduction of viruses :

The process of viral reproduction can be considered to take

Place in five stages :

1- Penetration of the host cell .
2- Synthesis of enzymes needed for viral nucleic acid replication.
3- Synthesis of viral constituents .
4- Assembly of the constituents to form mature virons .
5- Release of the natural viron from the host cell .

The Lytic cycle of infection: (Bacteriophages)

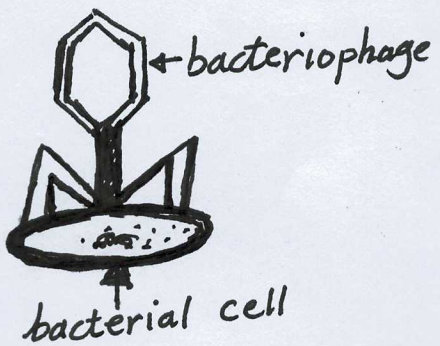
1 – Adsorption and pentration .

2 – The formation of " early " proteins .

3 – The replication of DNA .

4 – Maturation .

5 – The liberation of mature virons .



Virus detecting assays in laboratory :

1 – Radio immuno assay (RIA) . الدوز المناعي المرتبط بالمواد المشعه

2 – ELISA test. الدوز المناعي المرتبط بالحميره

3 – Neutralization. التعادل

4 – Hemoagglutination inhibition . تثبيط التوازن الدموي

5 – Gel diffusion . الانتشار

6 – Immunofluoresence . التألق المناعي

Microbial Nutrition and physiology

Every Microorganism must find in its environment both the structural units and the energy sources for building and maintaining its structure and organization .

These materials are termed nutrients .

The Essential Elements :

Water is a prime requirement of all living organisms and is an essential nutrient for almost all .

aw = available water , or water activity. The cell constituent of water (Microorganism) (MO) 80 – 90% of total weight . All chemical reaction happened inside cell need water in liquid form .

$aw = \frac{p}{po}$]

P = solute water vapor pressure

p^{o} = water vapor pressure .

$aw = \frac{vm\emptyset}{55.5}$

Maximum value of aw = 1 . 000 , this decrease as the solute concentration is increased .

All microorganisms can grow within the level of aw.between o.63 – 0.99. some yeast can grow in aw = o.73 but all bacteria and fungi needs aw = o.93 – 0.99, the fungus Aspergillus can grow at 0.62. Chemical forms in which elements serve as nutrients are carbon and to a lesser extent of nitrogen , sulfur and oxygen , inorganic salt . Each nutrient must serve either in the energy metabolism or as a suitable building block for living substance , or in both capacities .

- The main source of energy is the sun , the organisms that can utilized

Sun energy are Algae , photo bacteria and protozoa .

Organotroph = can utilize organic compound .

Lithotroph = M.O. can utilize inorganic compound .

There for , there are four groups of M.O. according to the energy source and exogenous electron donors :

1-Photolithotrophs ضوئية غير عضوية التغذية Depend on radiant energy.

2-photoorganotrophs ضوئية عضوية التغذية

3-Chemolithotrophs كيميائية لا عضوية التغذية

4-Chemoorganotrophs كيميائية عضوية التغذية

Inorganic electron donor : (Hydrogen gas , H_2s, sulfur, Iron...)

Organic electron donor : organic compounds (fermentation , glycolsis ,TCA,.....)

Main types of Nutrition

- autotrophic (plant)
- heterotrophic (animal)

on the base of the type of energy source utilize

- phototroplic (photosynthetic) radiant energy
- chemotroplic (chemosynthetic) dark oxidation reduction

on the baase of organic or inorganic nature

- Lithotrophic (inorganic electron doner) H_2S , S
- organotrophic (organic compounds) sugars, proteins

وإذا قمت على باب التداخل بين مصدر الطاقة ونوعية الإلكترونات الموجودة يكون التقسيم كالآتي:

NUTRITIONAL CLASSIFICATION OF ORGANISMS

Originally, biologists recognized two main types of nutrition: *autotrophic*, characteristic of plants, in which the organism depends entirely on inorganic compounds, and *heterotrophic*, characteristic of animals, in which organic compounds are required as nutrients. Today, the simple categories of autotrophs and heterotrophs are no longer sufficient to encompass the variety of nutritional patterns known to exist in the living world. A sharp separation between the two groups became difficult to make when it was discovered that specific growth factors may be required by some organisms that can use inorganic compounds as their principal nutrients. In recent years, therefore, attempts have been made to establish new categories for nutritional classification.

If the needs for growth factors are disregarded, it is occasionally useful to classify organisms on the basis of the type of energy source that they normally utilize. Those organisms that use radiant energy for growth are termed *phototrophic* (photosynthetic), and those that use dark oxidation-reduction reactions as a source of energy are said to be *chemotrophic* (or chemosynthetic).

A further differentiation of the nutritional types can be made on the basis of the organic or inorganic nature of the exogenous electron donors that are normally required for growth. Those organisms that can use inorganic electron donors (for example, hydrogen gas, ammonia, H_2S or sulfur) are said to be *lithotrophic*, and those that require organic compounds as electron donors are said to be *organotrophic*.

A combination of the types of energy sources and exogenous electron donors required for growth as criteria for the nutritional classification leads to the following nomenclature:

(1) *Photolithotrophic* organisms are dependent chiefly on radiant energy and use inorganic oxidizable substrates (electron donors) for growth. The most familiar examples of the photolithotrophs are the green plants which use water as the electron donor for the reduction of CO_2 to cell material. The blue-green algae and certain photosynthetic bacteria (the green and purple sulfur bacteria) are also photolithotrophic. With the exception of some organisms that require organic growth factors, the photolithotrophs are autotrophic in their nutrition.

(2) *Photoorganotrophic* organisms are dependent chiefly on radiant energy and use organic oxidizable substrates (electron donors) for growth. The category of photoorganotrophs comprises the heterotrophic photosynthetic "non-sulfur purple bacteria."

(3) *Chemolithotrophic* organisms are dependent on oxidation-reduction reactions as a source of energy for growth and are capable of using inorganic oxidizable substrates (electron donors) for such oxidations. A number of specialized groups of bacteria (for example, the hydrogen bacteria, the colorless sulfur bacteria, the nitrifying and the iron bacteria) belong in this category. Most of them are autotrophic, capable of using CO_2 as the sole source of carbon.

(4) *Chemoorganotrophic* organisms are dependent on oxidation-reduction reactions as a source of energy for growth and on organic compounds as oxidizable substrates (electron donors). The organisms belonging in this group are, by definition, heterotrophic and include the animals and the great majority of the microorganisms.

The combination between the types of energy sources and oxygenous electron donors leads to the following nomenclature of nutritional classification:

1- photolithotrophic organisms: (green plants)

2- photoorganotrophic " : (non-sulfur purple bacteria)

3- Chemolithotrophic " : (iron bacteria) hydrogen bacteria

4- Chemoorganotrophic " : (animals, majority of Microorganisms) MO.

AEROBIOSIS AND ANAEROBIOSIS

Living organisms differ strikingly from one another in their response to the presence of atmospheric oxygen. Most animals and many microorganisms are completely dependent on respiration as their ultimate source of energy. Such organisms are said to be *obligately (or strictly) aerobic,* for they cannot grow or even survive without molecular oxygen, which is consequently an essential nutrient for them.

At the other extreme, there are many microorganisms and a few animals that depend for their energy on reactions not involving oxygen and that are inhibited or killed by exposure to air. These are said to be *obligately (or strictly) anaerobic.* Some obligate anaerobes are so sensitive that the presence of minute traces of molecular oxygen in their environment causes a cessation of growth. The inhibitory effect of oxygen on obligate anaerobes is not completely understood. There is, however, good reason to believe that the sensitivity of such organisms reflects the fact nature of the cell membrane is undoubtedly due in part to its lipo-protein composition. No simple explanation, however, can be offered for the high degree of specificity that is exhibited by cells with regard to absorption, retention, and accumulation of certain specific nutrients.

PENETRATION OF NUTRIENTS INTO THE CELL

In order to be used for metabolism, nutrients must penetrate the cell boundaries, namely the cell wall and the cell membrane, and reach the sites of action of the appropriate enzymes. Bacterial cell walls are very porous and do not constitute a barrier to the penetration of materials other than those that are insoluble and particulate (for example, cellulose). It is therefore the cell membrane that is primarily concerned with the selective permeation of nutrients. It presents the principal barrier between the external environment and the enzymatic complement of the cell. Although certain enzymes appear to be located in the external surface of the membrane and are therefore accessible to substrates in the environment, other enzymes associated with the membrane are probably located on the inner surface. Such particulate enzymes, like the soluble cytoplasmic enzymes, do not come into contact with externally supplied chemical substances until these have penetrated the lipo-protein barrier.

The passage of some compounds into the cell is relatively nonspecific, being dependent on the general properties of the membrane. For example, lipid solvents like alcohol may enter by dissolving in the surface lipids. Other substances (for example, long-chain aldehydes) may enter through "pores" in the lipo-protein meshwork. In most cases, however, the penetration of nutrients is apparently dependent on the presence of specific "combining sites" in the membrane that somehow effect the selective transport of particular compounds and ions into the cell. In many cases such transport requires metabolic activity on the part of the cell.

The specific transport of a given substance across the membrane may be passive or active. *Passive transport* involves the diffusion of a substance from a region where it is more concentrated to one where it is less concentrated. Hence, it requires a relatively high external concentration of the compound in question. *Active transport,* in contrast, involves the accumulation of specific nutrients against a concentration gradient, so that their *internal concentration within the cell may become hundreds of times greater than their external concentration in the medium.* Such accumulation requires an expenditure of energy on the part of the organism.

The agents responsible for active transport, which have been called *permeases,* act as "pumps" for carrying their specific substrates across the cell membrane and possess properties that resemble those of enzymes.

Conditions of cultivation

The media described below can support the development of certain organisms only if all other requirements for the growth are statisfied . These

Common ingredients	
Water	1 Liter
NH_4CL	1 g
K_2HPO_4	1 g
$MgSO_4.7H_2$	200mg
$Feso_4.7H_2o$	10 mg
$Cacl_2$	10 mg
Trace elements	
As inorganic (Mn, MO,Cu_2,Co, Zn)salts	0.02-0.5mg of each.
Yeast extract	5g
Glucose	5g

requirements include suitable temperatures of incubation , favorable osmotic conditions , and a hydrogen ion concentration within the range tolerated by a particular organism . The effects of physical and chemical environmental factors on growth will be the following :

1- The control of PH:

The PH value for a given solution can calculated according the fallowing equations .

$$PH = Log\left[\frac{1}{(H+)}\right]$$

For example , a solution of acid , which is 0.1N with

respect to hydrogen ions ,has a pH value of 1.0

$$Log\left[\frac{1}{0.1}\right]$$

equals The logarithm of 10 which is 1.0 .

2- The control of oxygen concentration :

oxygen an essential nutrient for the obligately aerobic bacteria, but it is a metabolic poison for obligate anaerobes. Aerobic bacteria can be grown easily on the surface of agar plates and in shallow layers of liquid medium . To obtain Large population of aerobic bacteria in liquid cultures , it is therefore necessary to aerate the medium .

Culture Media: الأوساط الزرعية

The substrate that contain all nutritional requirements of essential elements supporting growth of microorganisms .

Media can classified according to the constituent to : المكونات

1-Natural media : contain all nutritional requirements of unknown concentration , ex . Milk , urin, yeast extracted, blood , fruit extract : vegetable extract .....etc.

2- Synthetic media : The media that (contain) made of known concentration of required nutritional elements .

3- Inorganic synthetic media : The simplest culture media used for cultivations of iron and sulfur bacteria .

4- Organic synthetic media : it is a complex organic media used for cultivation of virulence pathogenic bacteria ex. Corynobacterium diphtheria which need 8 amino acids and 3 vitamins , and inorganic salts , and carbohydrates for growth .

Selective Media :

It is clear that no single medium or set of conditions will support the growth of all different types of organisms that occur in nature. Therefore , any medium that is suitable for the growth of a specific organisms (to some extent) is selective for it,selective media are also valuable in the detection of undesirable contaminants in food , milk , and drinking water ,as well as for the identification of disease – producing organisms in feces .selective media can be used to abtain bacterial cultures from nature either by direct isolation or by enrichment .

Direct isolation is based on the ability of organisms to grow on a selective medium , solid media are employed when a mixed inoculum containing a variety of different organisms is spread directly on the surface . Direct isolation is the most effective technique to use when the goal is to isolate the maximum possible number of types of bacteria that are able to grow in a particular environment .

The enrichment culture technique is based on free competition among different organisms in liquid media .

A gram of soil generally serves as an excellent source of variety of microorganisms for the inoculation of enrichment media

There are four types of selective media :

1- Enrichment media . الوسط الزرعي الغني

2- Selective media : contain chemicals that can inhibit the growth of some groups of bacteria and can allow the other to grow . for ex . sodium azide can used to isolate lactic acid bacteria , and Krystal violate stain to isolate Brucilla,.....etc. الوسط الزرعي الانتخابي

3 – Differential media : الوسط الزرعي التفريقي

This media can differentiate between differ bacterial

colory after incubation period as a result of reaction of Some stains or chemical compounds or detectors . for ex . If sewage sample cultured on Eosine Methyl Blue (EMB) Medium, some colonies appear metallic green sheen (Pseudomonadus spp.) and other appear colonies with pink color of dark center (Fish eye) indicating fecal E.coli (Entero bacteria) or Acetobacter .

4 – Assay media : الوسط لتزكين الأختباري

used for quantitative determination (the amount) of antibiotics or vitamins or hormones produced by microorganisms .

ROUTINE MAINTENANCE OF LABORATORY CULTURES .

The maintenance of a culture collection is a tedious task, for the cultures must be transferred at regular intervals to prevent their dying . The media and the conditions of cultivation for this purpose are designed to insure the maximal survival of the organisms . Although good growth must be obtained in each culture , the massive accumulation of metabolic products in the medium must be avoided because these often accelerate the death of the organisms.

To prevent a rapid senescence of " stock cultures" they are maintained , whenever possible , at relatively low temperatures . Incubation at elevated temperatures , essential for the growth of some bacteria , is carried out only for a minimal period of time . The cultures are then kept at room temperature or in a refrigerator .

In the cultivation of aerobic bacteria on the surface of solid media , another means is sometimes used for decreasing the metabolic rate and thus increasing the longevity of cultures.

Bacterial growth

Growth : The increase in the numbers of individuals of unicellular organisms , while in the multicellular organisms lead to increase in the size of individuals .

The measurement of growth :

I - Direct Methods

Since growth represents an orderly increase of all the components of an organisms , its measurement must involve the determination of the total amount of living substance in an individual or population . Such measurement may be made by direct or indirect means .

1 – Dry weight :

The most commonly used methods for the direct measurement of the growth are based on determinations of the dry weight of cell materials or on the assay of one of their elementary constituents for example , Carbon or nitrogen . also, it can measured by catalytic power of living substances increase in particular enzyme , respiration Co_2 evolved .

The disadvantages of dry weight method :

1- It is difficult to determine accurately dry weight of less than a milligram (5×10^9 cells) .

2 – The increase in dry weight or in the carbon or nitrogen content does not necessarily reflect true growth for instance , the formation of extracellular capsular material or intracellular food reserves may Increase dry weight .

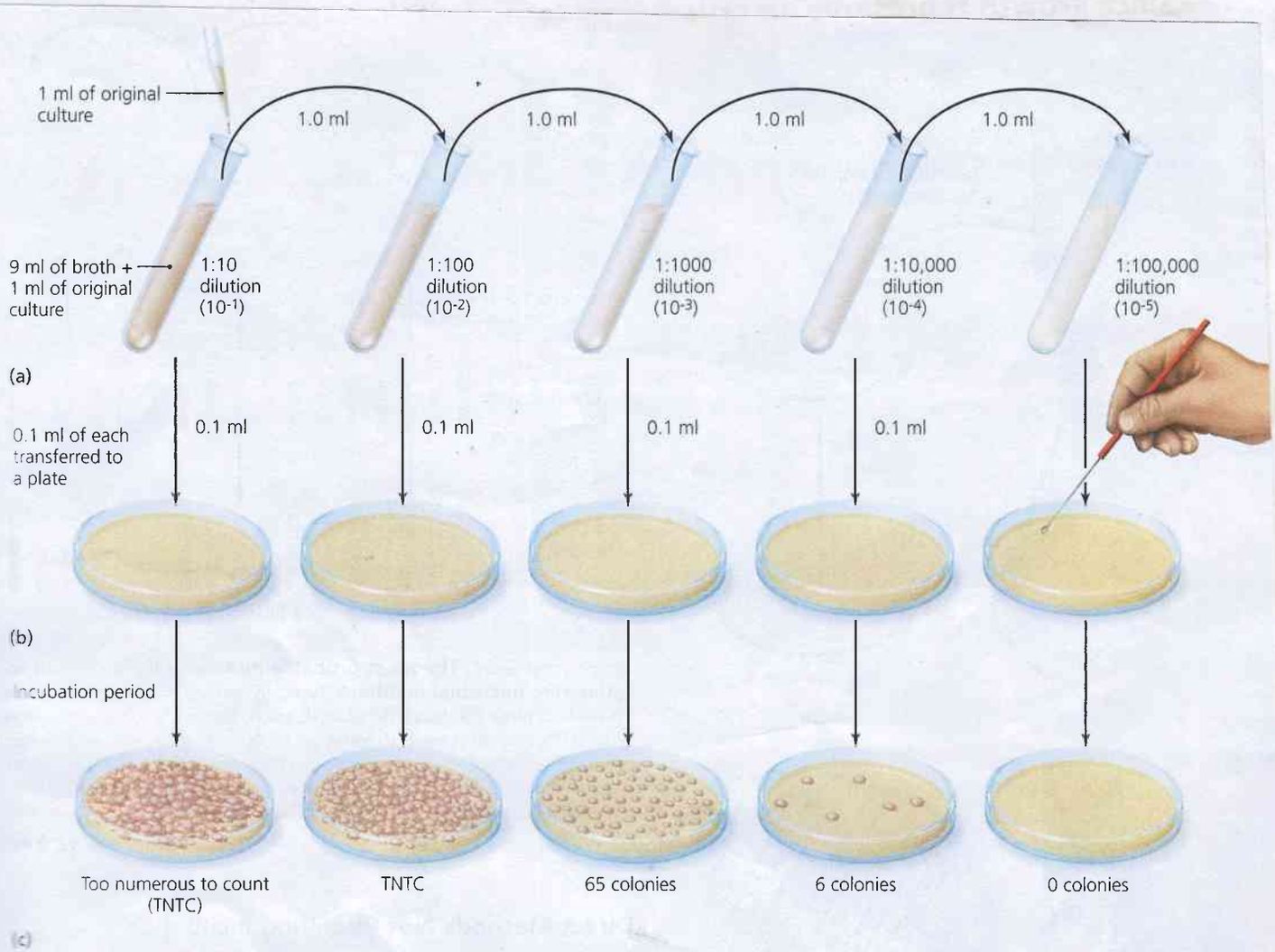


Figure 6.22 A serial dilution and viable plate count for estimating microbial population size. (a) Serial dilutions. A series of 10-fold dilutions is made. **(b)** Plating. A 0.1-ml sample from each dilution is poured onto a plate and spread with a sterile rod. Alternatively, 0.1 ml of each dilution can be mixed with melted agar medium and poured into plates. **(c)** Counting. Plates are examined after incubation. Some plates may contain so many colonies that they are too numerous to count (TNTC). The number of colonies is multiplied by 10 (because 0.1 ml was plated instead of 1 ml) and then by the reciprocal of the dilution to estimate the concentration of bacteria in original culture—in this case, 65 colonies × 10 × 1000 = 650,000 bacteria/ml.

2 – Total count :

The most direct of these is to count the number of cells in the population with the aid of microscope, this may be done by counting the number of bacterial cells in a very small volume of the culture using special slides known as counting chamber . This method counting both viable and nonviable cells which cannot be distinguished from one another by microscopic examination . only suspensions that contain ten million or more cells per milliliter ($\leq 10^7$ cells /ml) can be counted with any degree of accuracy .

3 – Viable count :

This method can be done by spreading known diluted suspension on agar medium , each cell will give rise to a macroscopically visible colony . one can determine the number of viable cells in the original culture by determining the number of colonies that developed after incubation multiplied by reverse dilution in one milliliter .

(No . colonies x reverse dilution x ml . fraction)= Viable numbers

This method sofar the most sensitive once for estimation of bacterial growth. Therefore , it is the only method that can be used for the study of the development of cultures of low cell density .

Ex: If we found 50 colonies in 0.1 ml of culture suspention of dilution 10^{-6}. caculate the viable cells in origin culture.

No. of Viable cells = $50 \times 10 \times 10^6$

$= 5 \times 10^8$ cells/ml.

II- Indirect methods :

1-Turbidimetry :

The determination of the degree of light scattering caused by a suspension of cells . The scattering of light is proportional (within limits) to the cells concentration.This scattering of light that makes bacterial culture appear turbid to the naked eye .

Turbid metric measurement can be made only with homogeneous suspension especially for unicellular organisms . Accurate measurements require suspensions containing ten million or more bacteria per milliliter ($\leq 10^7$ cells / ml) .

2-Colorimetric method :

The growth can be measured indirectly by the density of color Produced by nutritional elements like glucose, amino acids ,.....etc. Organisms when grow consumed nutrient leading to decrease the concentration which lead to decrease the color produced , as the grow continue, the concentration decreased which decrease the color density . Growth measurement follow the decrease in color density and comparison with standard calibration curve .

Generation time (G.T) :

The time need for multiplying the culture number .
(time need for completing cell cycle).

Growth rate :No . of divisions / hour ,

$$G.R. = \frac{nG.T.}{total\ time/h}$$

$$NO.G.T. = \frac{10\log n2 - 10\log n1}{10\log 2}$$

n_1=number of cells at initial incubation time
n_2= number of cells after period of incubation time .

Example: Determine the number of generation happened and growth rate (gr) in a culture have a 100 cells /ml in the first time and became 1000 cells /ml after 10 hours of incubation.

NO. G.T. = $\frac{10\ log\ 1000 - 10\ log\ 100}{10\ log\ 2}$.

= $\frac{3-2}{0.3}$ = $\frac{1}{0.3}$ = 3.3

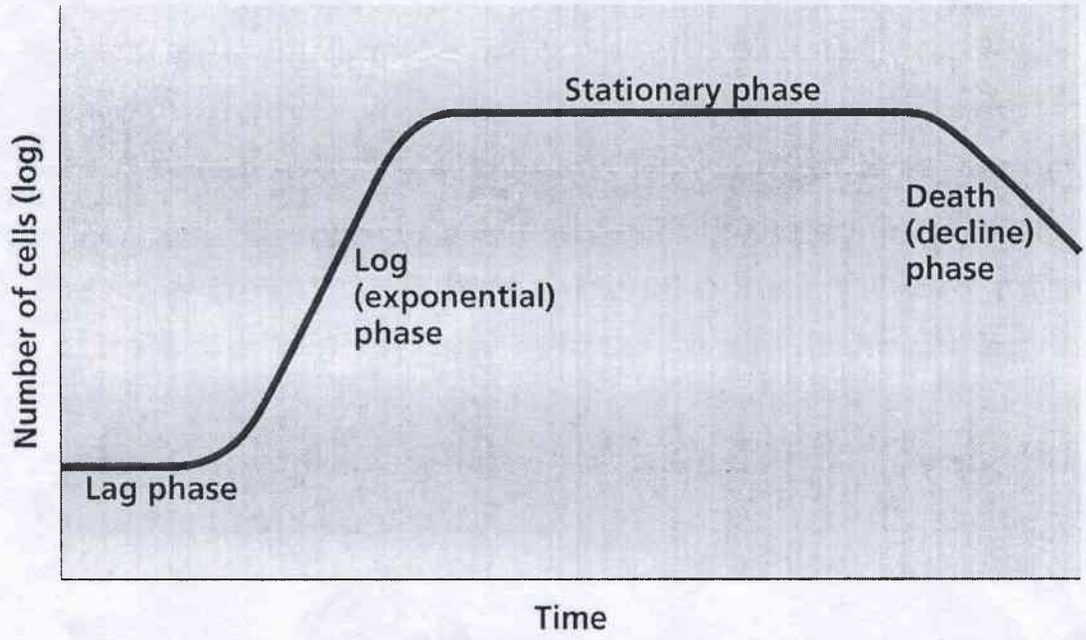
GR= $\frac{no.G.T.}{total\ time}$

G.R. = $\frac{3.3}{10}$ = 0.33 generation time/hr.

The Growth Curve :

Both in nature and in the laboratory , the growth of bacterial population becomes limited either by the exhaustion of available nutrients or by the accumulation of toxic substances . Since such changes in the environment result from the growth of the bacteria themselves . The development of bacterial populations is self – limited. Culture grow exponentially only for a short period ; eventually , growth ceases and death of the population ensues.

a typical growth curve for a bacterial culture is shown in the figure. at least four principal phases in the history of the culture may be recognized . These are the lag phase , the phase of exponential growth (Logarithmic phase) , the stationary phase and the decline (death) phase .



The Lag phase :

In this phase the cells are not increased in numbers but in Size that reach the critical size and become ready for dividing , in this phase the culture cells need time for adaptation to the new environment . this period is known as the lag phase . Such lags results from the fact that induced enzymes must be formed by the cells before growth in the new environment can take place.

The Exponential (logarithmic) phase :

A constant rate of growth is reached with a maximum reproductive potential of that organism in the specific environment .

The environmental factors that govern the rate of growth include the nature and concentration of the nutrients , PH, temperature and other physical and chemical variables . when the environment becomes less favorable , the growth rate decrease and the culture enter the stationary phase . The physiological characters of the cells in this phase are vegetative , producing primary metabolite (protein , amino acids , carbohydrates ,..etc.) . active in production energy (glycolsis , fermentation , TCA cycle) . [living cells greater than dead cells] .

The Stationary phase:

In this phase , the viable count remains constant at its maximum value .

The stationary phase is brief and is followed rapidly by the death of the

Cells , with a consequent decrease in viable count .

In most cases , however , the culture remains in the stationary phase For hours , or even days , before death of cells becomes noticeable . the total cell count of the culture remains constant . The physiological character of cells starting with secondary metabolites like antibiotics, hormones , vitamins , ...etc. .

Also , sporulation starting in this phase .

The Decline (death) phase:

In this phase , the viable count decreased (dead cells higher than viable cells) . The statistical death rate for the population , which is zero during stationary phase , begin to increase and eventually reach's a maximum constant value . After a constant death rate is established, the culture dies exponentially . The physiological status of cells in this phase is that almost all Living cells converted to spores or autolysis at the end releasing cell contents to the medium .

Synchronous growth :

When a single bacterial cell reproduces in a suitable medium , the first few divisions well synchronized , but the divisions become randomized as the population increases . Recently , the synchronization of cell divisions have been achieved in large populations of bacteria by use of special tricks :

1 –Manipulation of temperature :

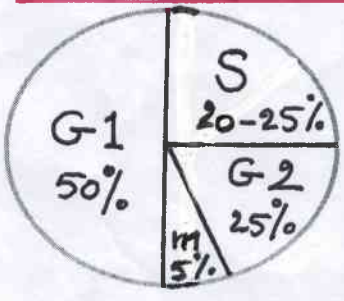
Cell divisions can be inhibited by keeping a bacterial culture at a temperature that is considerably below the optimal one for growth. The individual cells , however , may continue to grow slowly but only up to the point of dividing . if the temperature is then raised , the cells divide simultaneously, so that a sudden doubling of the entire population is observed .

2 –Differential filtration :

The inoculum is filtered through layers of filter paper . The smallest cells , which are collected in the first fraction are in approximately the same phase of the dividing cycle and divide simultaneously when placed in suitable media .

Cell Cycle :

The time required for completing the division of cells (G . T .) which varied



from prokaryotes (EX . E . Coli 20 – 25 minutes) and eukaryotes organisms (2hours - many days) .This time is environmental factors dependent, like nutrition element concentration , pH , and temperature .

Cell cycle go through four stages :

1- First stage(G 1) : It take 50% of generation time . during this stage , the cell reach the critical size and be ready for DNA replication .

2 – Second stage (S) : It take 20 – 25 % of generation time , in this stage , DNA is synthesis , it is constant and not affected by environment factors .

3 – Third stage (G2) : It take 25% of G.T. time , there is no dramatic changes in this stage , and stable against environmental factors .

4 – Fourth stage (m) : In this stage , the cell divide to two cells and take 5% of G . T . period , this stage end by Dividing nucleus and nucleolus .

Factors effecting growth :

The environment factors, chemical or physical , can affects the inhabitant and distribution of microorganisms in nature , these factors are :

1- Temperature :

Temperature is very important factor that affecting the enzyme activity . The temperature range for growth is wide for microorganisms (30 C^{o}) , but the optimum temperature of growth is so narrow (2 – 3C^{0}) .

According to temperature of growth microorganisms divide to :

a . Psychrophilic : Grow in low temperature , optimum temperature for growth zero – $20C^0$.

b . Thermophilic : It grow in high temperature , optimum temperature for growth are 40-80 C^0 .

C . Mesophilic : The majority of microorganisms in this group , which are pathogenic for humans and animals . The optimum temperature for growth in the range 25- 40 C^0 .

2 – Osmotic pressure :

Most of microorganisms live in the normal osmotic pressure , when the osmatic of the solute is higher in the cytoplasm , and even though the cells is not lyses because the raged cell wall .

Some of microorganisms live in high osmotic pressure and they call it osmophilic. If it in salt environment it called hallophilic or saccharophillic in high concentration of sugar .

3 - Hydro static pressure :

It is the pressure of water column , which effect the marine and fresh water living microorganisms . The pressure increased one atmosphere for each 10 meter depth .

4 – PH :

The range of PH that can suitable for growth of microorganisms is 3 – 4 units but the optimum PH within one unit .

Most organisms (prokaryote and eukaryote) can have metabolic activity within the range of 5 – 8 PH .

5 - Electromagnetic Rays :

The energy transport in the atmosphere in wave form and named electromagnetic rays , If it in form of atoms of high energy , then called atoms rays which coming from radioactive element , as the wave length decrease , the energy is increased . The microorganisms utilize the visible light , while the ultraviolet and high energy atoms rays (gamma , alpha , beta , x-ray) can killed the microorganisms , therefore It can used for sterilization of materials .