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History and Scope

Medical microbiology is the study of causative agents of infectious disease of human being and his reactions to such infections. In other words it deals with aetiology, pathogenesis, laboratory diagnosis, treatment, epidemiology and control of infection.

Historical Event

Varo and Columella (first century B.C.) suggested that diseases were caused by invisible organisms (Animalia minuta) inhaled or ingested.

Fracastorius of Verona (1546) felt that contagium vivum may be the cause of infectious disease.

Kircher (1659) could find minute worms in the blood of a plague patient. It is more likely that he observed perhaps only blood cells with the apparatus which he had at his disposal.

Antony van Leeuwenhoek (1693) could give description of various types of bacteria. He also invented microscope.

Von Plenciz (1762) proposed that each disease was caused by a separate agent

Augustion Bassi (1835) proposed that muscardine disease of silk worm was caused by fungus.

Oliver Wendell Holmes (1843) and Ignaz Semmdweis in Vienna (1846) independently put forward the view that puerperal sepsis was transmitted by the contaminated hands of obstetricians and medical students. Washing of hands in antiseptic solution was suggested for its prevention

Pasteur (1857) established that fermentation was the result of microbiol activity. Different types of fermantation was associated with different kind of micro-organism. He introduced techniques of sterilization and developed steam sterilizer, hot air oven anp autoclave. He started his work on pebrine, anthrax, chicken cholera and hydrophobia. He attenuated culture of anthrax

bacilli by incubation at high temperature (42°C—43°C) and proved that inoculation of such culture in animal induced specific protection against anthrax. Pasteur's development of vaccine for hydrophobia was the greatest breakthrough in medicine. He is remembered as a man who laid the foundation of microbiology. He is also known as father of microbiology.

Robert Koch (Father of Bacteriology) perfected bacteriological techniques during his studies on the culture of anthrax bacillus (1876). He also introduced staining techniques and also methods of obtaining bacteria in pure culture using solid media. He discovered bacillus tuberculosis (1832) and cholera vibrio (1883).

Lord Lister (1854) used carbolic acid spray on wound during operation. He is also called father of antiseptic surgery.

Hansen (1874) described leprosy bacillus.

Neisser (.879) described gonococcus.

Ogston (1881) discovered staphylococcus.

Loeffler (1834) isolated diphtheria bacillus.

Nicolaier (1884) observed tetanus bacilli in soil.

Fraenkel (1886) discribed pneu mo coccus.

Schaudin and Hoffman discovered the spirochaete of syphilis.

Roux and Yersin (1888) deicibed mechanism of pathogenesis when they discovered diphtheria toxin.

Loeffler and Frosch (1898) observed that foot and mouth disease of cattle was caused by a microbe i *e.* filter passing virus.

Walter Reed (1902) observed that yellow fever was caused by filterable virus and that it was transmitted through the dite of mosquitoes.

Landsteiner and Popper (1909) showed poliomyelitis was caused by filterable virus.

Towert (1951) andHerellc (1917) discovered lytic phenomenon in

bacterial culture. The agent responsible was termed as bacterio-phage (viruses that attack bacteria),

Ruska (1934) introduced electron microscope and hence detailed study of morphology of virus was possible

Fleming (1925) made the accidental discovery that the fungus pencillium produces a substance which destory staphylococci.

Jerne (1955) proposed natural selection theory of antiobody synthesis

Burnet (1957) putforward clonal selection theory.

Burnet (1967) introduced concept of immunological surveillance.

MICROBIOLOGY

In short this is the science dealing with the study of microorganisms.

Branches of Microbiology

1. Medical microbiology
2. Industrial microbiology
3. Food microbiology
4. Soil microbiology.

Here we are concerned with medical microbiology. It is studied under following headings:

a. Parasitology deals with the study of parasites causing diseases in human being.
b. Mycology deals with the study of fungus causing diseases in human being.
c. Immunology is concerned with mechanism involved in the development of resistance by body to infectious diseases.
d. Bacteriology deals with the study of bacteria,
e. Genetics is the study of heredity and variations. {/)
f. Virology is the study of viruses.

Scope of Microbiology

1. Diagnostic *e g.* isolation and identification of causative organism from the pathological lesions. We can also diagnose typhoid fever by doing Widal's test.
2. Prognosis of disease *e..g.* in Widal's test rising titre signifies active disease and ineffective treatment. Falling titre means effective treatment and curing of disease.
3. Guidance in treatment *e g.* by culturing the organism in pure from and then performing drugs sensitivity test we can suggest the effective drug for the treatment of that particular infection.
4. Source of infection *e.g.* in sudden outbreak of infectious disease we can find out the source of infection.

Morphology of Bacteria

Bacteria are unicellular free living organisms without chlor-phyll having both DNA and RNA. They are capable of performing all essentia processes of life *e.g.* growth, metabolism and. reproduction. They have rigid cell wall containing muramic acid. They were orginally classified under plan and animal kingdoms. This being unsatisfactory a third kingdom "PROTISTA" was formed for them. Protista is again divided into 2 groups (a) Prokaryotes (&) Eukaryotes. Bacteria and green algae are prokarytes while fungi, algae, slime moulds and protozoa arc eukaryotes. It is worth mentioning the prokaryotic and eukaryotic cells

Morphology of Bacteria

Size of Bacteria. Most of bacteria are so small that their siz\is measured in terms of micron. .

1 micron (μ) or micrometer (μm)=One thousand of a millimeter.

1 millimicron (mμ) or nanometer (nm) = 0ne thousandth of micron.

1 Angstrom units (A°)=One tenth of nanometer.

Generally cocci are about I/ μ in diameter and bacilli are 2—10 / μ in length and 0.2 to 0.5/ μ in width. The limit of resolution with unaided eye ii about 200 μ. Obviously bacteria can be visualized only under magnification.

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

Cocci (from kokkos, meaning berry):

They are spherical. On the basis of arrangementlTof individual organisms, they are described as staphylococci (clusters like bunches of grapes), streptococci (arranged in chains), diplp-cocci (forming pairs) tetrads and sarcina are cocci arranged in groups of four and cubical packet of eight cell respectively.

Fig. 1.

(a) Cocci in cluster=staphylococci

(b) Cocci in chain – streptococci

(c) Cocci in pair – diplococci

(d) Cocci in groups of four—tetrad

(e) Cocci in groups of eight—sarcina.

(1) Bacilli (from baculus, meaningods)

The cylendrical or rod shaped organism* are called bacilli.

In some of the organisms length may approximate the width of the organisms. These are called coccobocilli & g, Brucella.

(2) Chinese letter arrangement is seen in corynebacteria.

(3) Vibrio : They are comma shaped, curved rods and derive the name from their characteristic vibratory motility.

(4) Spirochaetes : (from speria meaning coil, chaite meaning hair). They are relatively longer, thin, flexible organisms having several coils.

(5) Actinomycets : (On actis meaning ray, mykes, meaning fungers) are branching filamentous bacteria, so called because of resemblance to radiating sun rays, tv

(6) Mycloplasma are organisms which lack cell wall, and so do'nt possess a stable morphology. They are round or oval bodies with interlacing filaments.

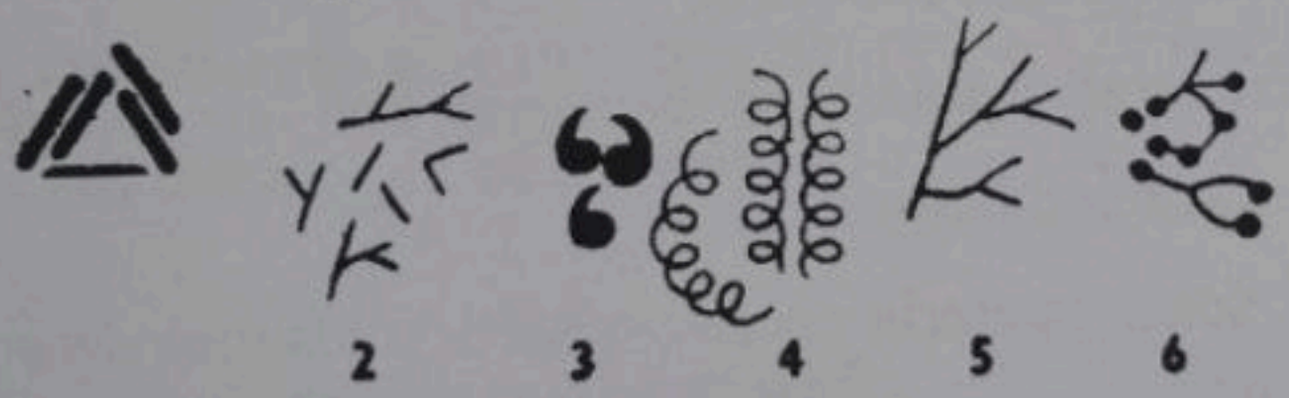


Fig. 2.

Many a time cell wall synthesis become defective either spontaneously or as a result of drugs *e.g.* in presence of pencillin bacteria lose their distinctive shape. Such organism are called protoplast, sphere plast or L forms.

Bacterial Anatomy

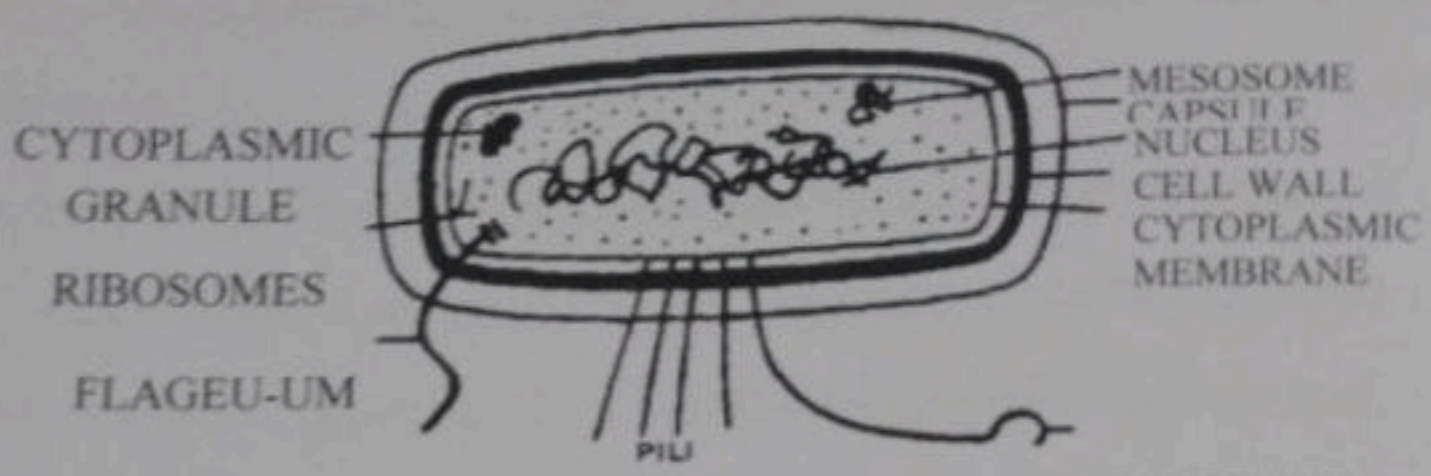


Fig. 3

Structure of bacteria.

The outermost layer consists of two components—(o) a rigid «11 wall (b) cytoplasmic membrane a plasma membrane. Inside this there is protoplasm comprising of the cytoplasm, cytoplasms inclusions such as ribosomes, mesosomes, granules, vacuoles and nuclear body. The cell may be enclosed in a viscid layer which may be loose slime layer or organised as a capsule Apart from this some bacteria carry filamentous appendages protruding from cell surface : the flagclla, organ of locomotion and the fimbriae which neem 1o be organ of adhesion.

The important structural features of bacterial cell as found tinder electron microscope are 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. Its presence can be shown only on immunolagical ground. Bacteria secreting large amount of slime produce tnucoid growth on agar with stringy consistency when touched with loop. Slime has little affinity far basic dye and so not visible in Gram stained smear.

CAPSULE. It is gelatinous secretion of bacteria which gets organised as a thick coat around cell wall & is known as capsule. It may be composed of complex polysaccharide *e.g.* pneumococi and klebsdla or polypeptide *e.g.*

bacillus anthrax or hyaluronic acid *e.g.* streptococcus pyogenes. Capsules have no affinity for dyes and so they are not seen in stained preparations.

Demonstration of capsule

(a) Negative staining with India ink ; in this procedure, bacterial bodies and spaces in between are filled with India ink and captule is seen as hollo around cell.

(b) Special capsule staining technique using copper salt as moderant.

(c) Serological methods : If suspension of capsulated bacterium is mixed with its specific anti-capsular serum and •examine under microscope, capsule becomes prominent and appears swollen due to increase in refractivty. (Quelling reaction)

Function :

(1) Protection against deleterious agents *e.g.* lytic enzymes

(2) Contribute to the virulence of pathogenic bacteria by inhibiting phagocytosis.

CELL WALL. The cell wall is the outermost supporting layer which protects the internal structure It is about 10—25 nm. in thickness and shares 20—30y_o of dry weight of the cells.

Chemical structure of cell wall :

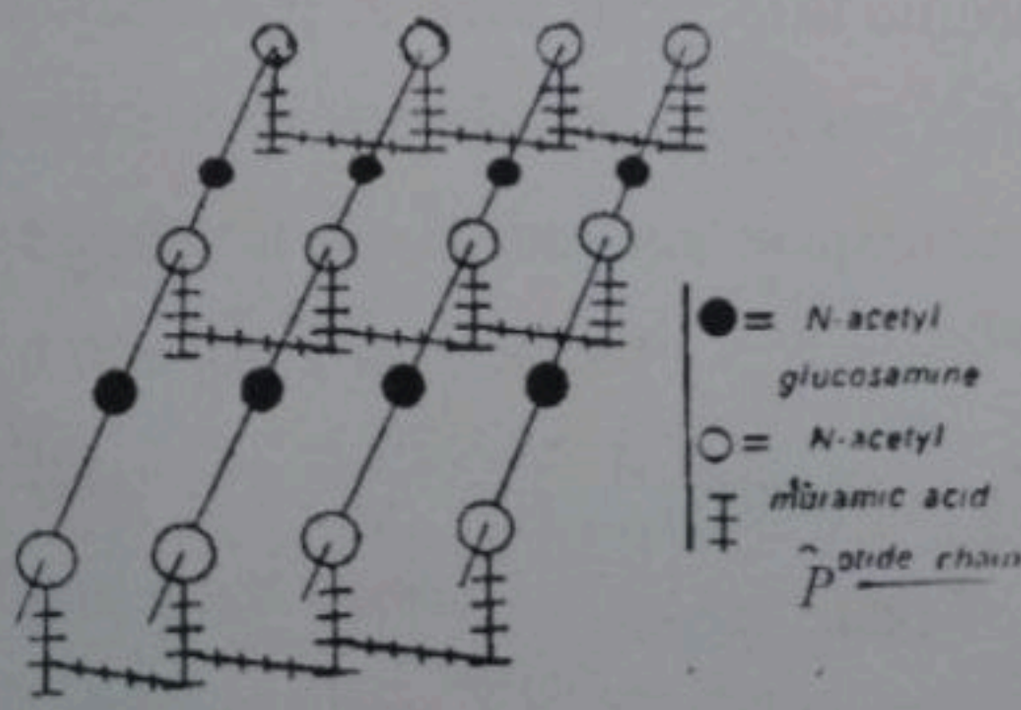


Fig. 4

Cell wall is composed of mucopeptide (muerin), scaffolding formed by N acetylc glucosaminc and N. acetyle muramic acid molecules alternating in chain cross linked by peptide chain. Cell wall antigens 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*
Thickness Variety of acid	15-23 mf* Few	10-15 mj* Several
Aromatic and sulfur containing amino acid	Absent	Present
Lipids. Techoic acids	Low 2—4% Present	High 15-20% 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 is formed consisting of cytoplasmic membrane and contents. With Gram negative bacteria the results is SPHEROPLAST. It differs from protoplast in that some cell wall material in retained. Protoplast and spheroplast are spherical regardless of orignal shape of the bacterium. Such organisms might have a role in persistent of certain chronic infections such as pyelonephritis.

Demonstration

(1) Plasmolysis.

(2) Microdissection,

(3) Reaction with specfic antibody.

(4) Mechanical rupture of cell.

(5) Differential staining procedure.

(6) Electron microscpy.

Function

(!) 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 semipermeable 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 mycoplaama.

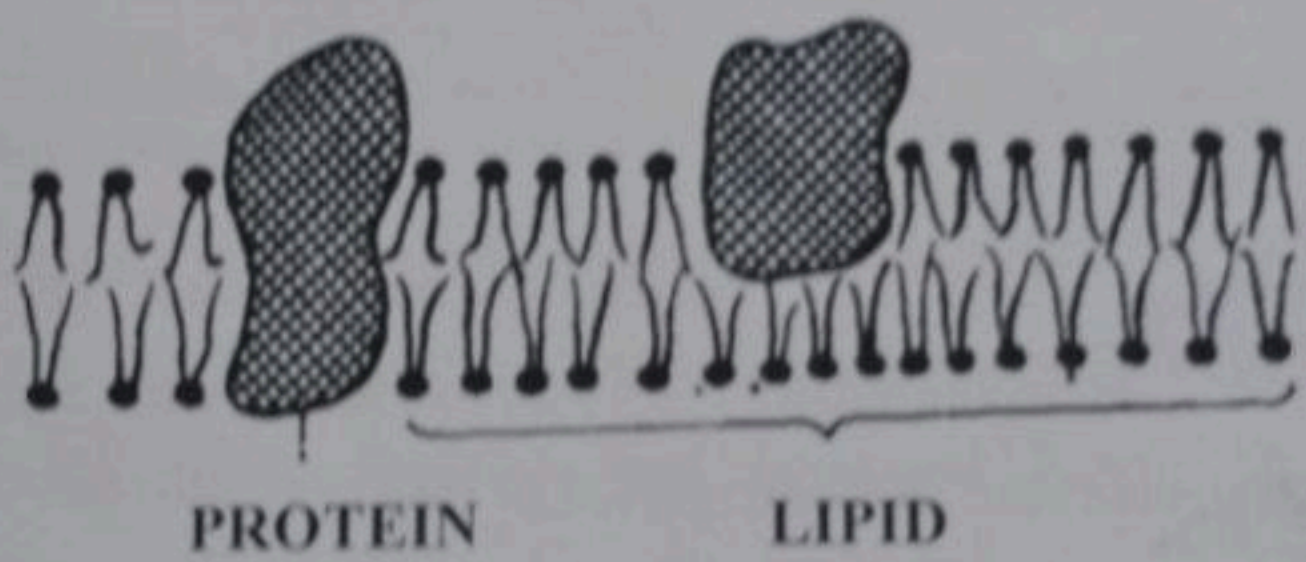


Fig. 5

Demonstration

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

pi asmo lysis.

(2) Electron microscope.

Function:

(1) It controls inflow and outflow of metabolites to and front protoplast.

(2) Presence ID the membrane of specific enzyme (permease) plays important role in passage through membrane.

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

It does'nt exhibit protoplasmic streaming (Internal mobility,) and it lacks endoplasmic rcticulum or mitochondria. It contains ribosomes, mesosomes, inclusions and vacuoles.

Ribosomes. These are ribonucleoprotein granules measuring 100-200 A° units in diameter and their sedimentation coefficient is 70 Svedberg units. The 70 s rebosome is composed of two smaller units of 50 s and 30 s.

Function. 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 multilami-nated structures formed as invaginations of plasma membrane into the cytoplasm. They are more prominent in Gram positive bacteria,

Functions

(I) They are the sites of respiratory enzymes in bacterea.

( ) Coordinate nuclear and cytoplasmic division during binary fission.

Intracytoplasmic inclusions

(a) VoJutin granules (Metachromatic or Babes-Eronst granules) are highly refractive, basophilic bodies consisting of polymeta phosphate.

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

Enaction. They are considered to represent a reserve of energy and phosphate for cell metabolism.

(6) 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) **Vacnoles.** 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. Jt mav open under certain, conditions to form long chain about 1000/* in length. Bacterial chromosomes in haploid and rclpicate by simple fission. Genes are arranged along the length of chromosome in fixed order and bear hereditary characters.

Bacteria may some times have extranuclear genetic material. These are called plasmid or episomes. They may be transmitted to daughter cell:

(a) during binary fission.

(6) by conjugation or

(e) through agency of bacterial phagcs.

Plastnids are not essential for the life of cell. They may-confer, certain properties like toxigenecity and drug resistance.

Flagella. These are long, sinnous 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;* or less), helical shaped, structure of uniform diameter throughout their length. Each flagellum originate in a spherical body (basal grunule) located just inside cell wall. They are antigenic and are composed of protein called 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.

Fig. 6, Arrangement of flagella in bacteria.

Monotricbate. One flagellum at one end of the org< nism *e g.* vibrio, pseudomonas, spirillum etc.

Amphitrichate. One flagellum at both the poles *e.g.* alcaligenes faecales.

Lophot rich ate. A tuft of flagella at the end *e.g.* Pscudo-*nonas.

Peritrichate. Several flagella present all over the surface of bacteria *e.g.* E. Coli, Salmonella.

Function. Bacterial motility.

Demonstratio

(1) Dark ground microscope.

(2) Special staining techniques in which their thickness is increased by mordanting.

(3) Electron microscope.

FIMBRIAE. They are flamentous short, thin, straight, hair like appendage (0 5P long and lets than 10 nm thick). They are also called pili

They project from cell surface, as straight flaments. They are best developed in freshly isolated strains and in liquid culture. They tend to disappear following subcultures on solid media.

Type* of pill (Fimbriae). Three types of pili.

(1) Common pili.

(2) F (fertility) pili.

(3) Col I (colicin) pili.

Common pili. They are numerous, short in size (1,5 f) and peritdchous in distribution. They are considered organs of adhering to the surfaces of other cells *e g* red cell of various animal species. This form the basis of haemagglatination.

F pili. They are associated with fertility (F+) and help in bacterial conjugation processes. They are longer (20f* length) than common and col I pili.

Col I pili They are about 2/* in length and associated with colicin factor I.

Demonstration, (1) Electron microscope

(2) Haemagglutination

(3) Fimbriated bacterial form pellicle in liquid media.

Function

(1) Organ of adhesion

(2) Conjugation tube through which genetic material is transferred from donor to recipient cell.

(3) They arc antigenic.

SPORES

They arc highly resistant dormant state of bacteria found in certain genera *e g.* bacillis and clostridium. They are not destroyed by ordinary methods of boiling for several hour. They are killed when autoclaved at 15 Ib

pressure at 121°C for 20 mts." The sporea 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.

Function, They make survival unfavourable codition like dry state, drying, freezing and toxic chemicals.

Formation of spore

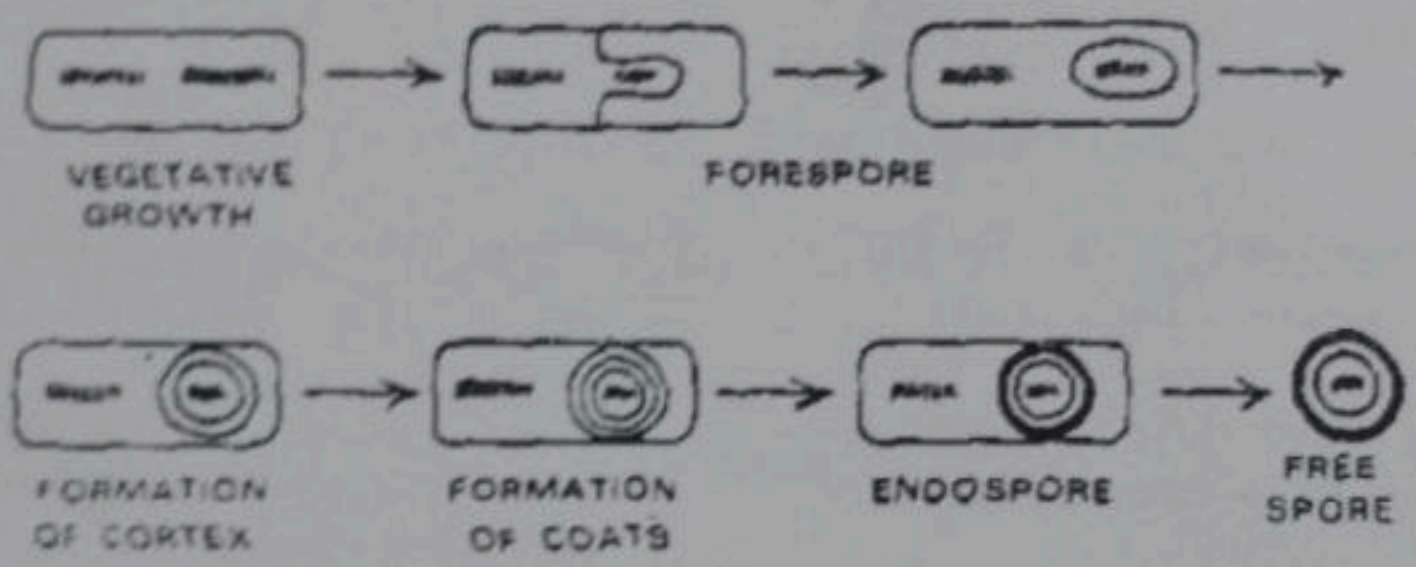
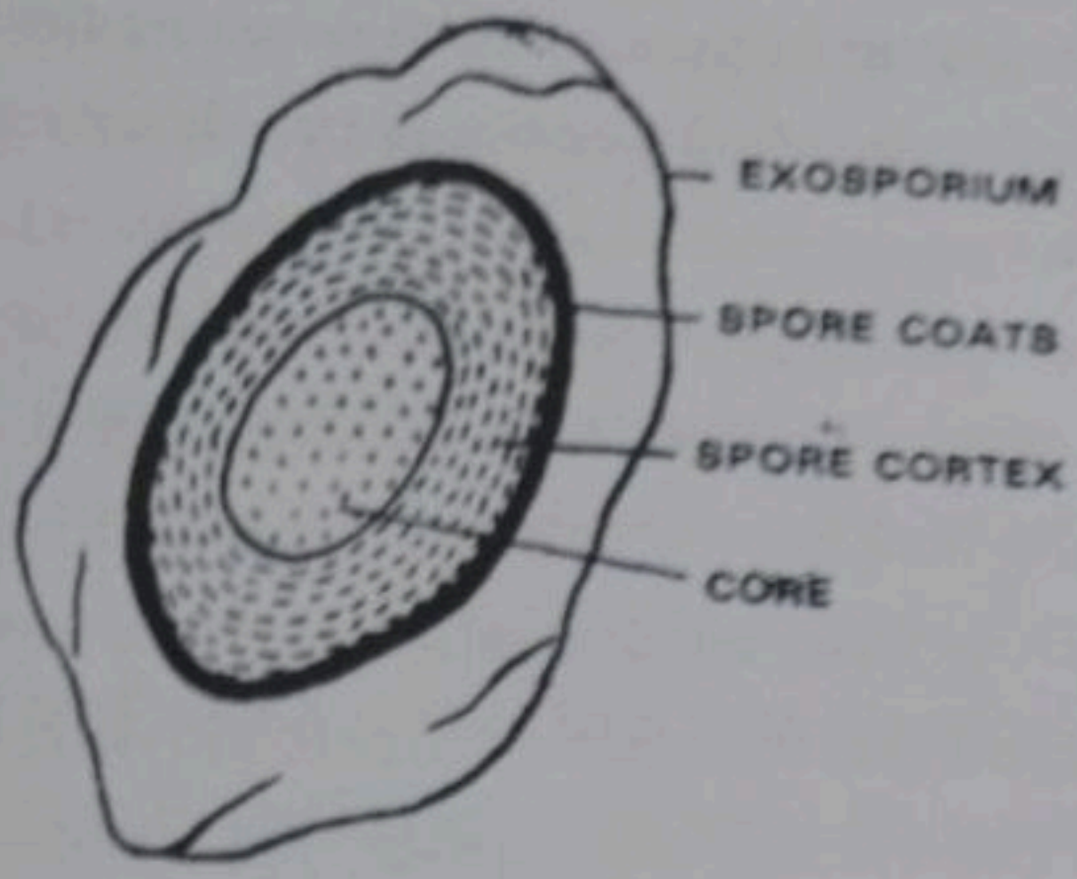


Fig. 7

Exact stimulus for sporulation is not known. Perhaps it is related to depletions of exogenous nutrient. Sporolation 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 multilayercd spore coat.

Some spores have an additional outer covering called exospb* rium having ridgea and grooves.



Fig, 8. Structure of spore. Spores may be :

(1) Central bulging

(2) Central not bulging

(3) Sub terminal bulging

(4) Sub terminal not bulging

(5) Terminal spherical

(6) Terminal oval.

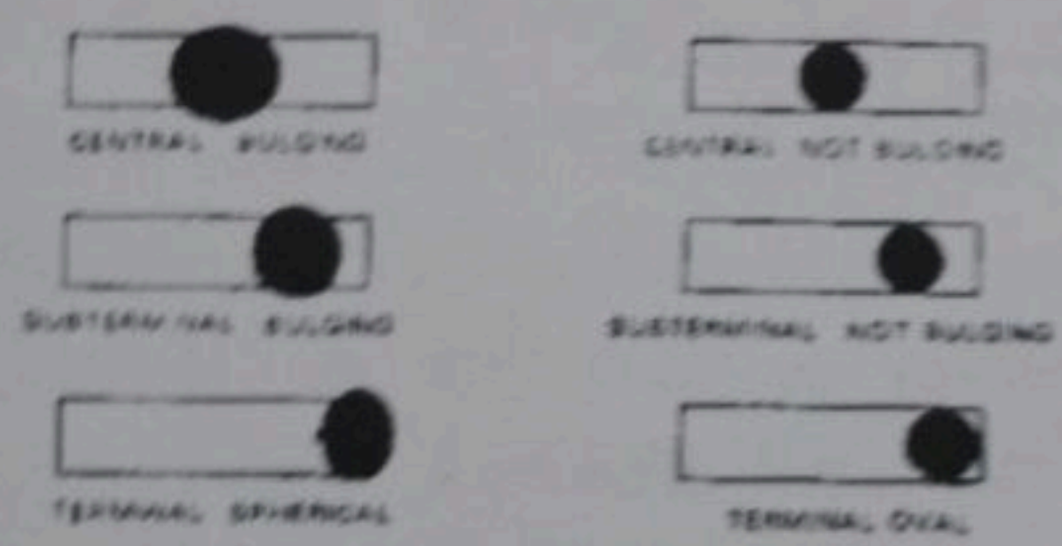


Fig. 9. Types of bacterial spore.

When transferred to favourable, rendition of growth, spores germinate. The spore loses its refractih'ty and swells. The **spore** wall is shed and germ cell appears by rupturing the spore coat. The germ cell elongates to form vegetative bacterium

Pleomorphism. Some species of bacteria show great variation in shape and «ze of undivided cell. This is called pleomor-phism. It may be due to defective cell wall synthesis.

Involution. Certain species *(e.g.* plaque bacillis, 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 moniliformcs was studied. They are ;« e in several species of bacteria developing either spontaneously or 3 i presence of pencillin like agent that interfere with cell w ^I synthesis.

L form resembles mycoplasma in several ways including morphology, type of growth on agar and filtrability. Possibly mycoplasma represent stable L forms of as yet unidentified parent bacteria. L form of bacteria have been isolated from patients of chronic urinary and suppurative infection. Yet their role is not clarified

Study of Morphology of Bacteria

It is of considerable importance that identification of bacteria be made. For this purpose following methods are employed. **Microscopy**

(a) **Light microscopy** is useful for the motility, size, shape arrangement of bacteria. Due to lack of contrast, details can't be appreciated.

(b) **Phase contrast microscopy** makes evident structure within cells that differ in thickness and refractive index. In the phase contrast microscope "phase" differences are converted into differences in intensity of light producing light and dark contrast in the image.

(c) **Dark ground microscopy.** Here reflected light is used instead direct transmitted light used in ordinary microscope. With its help extremely thin slender organism like spirochaetes can clearly be seen.

(d) **Oil immersion microscopy.** The magnification pro. duced by oil immersign objective of light microscope makes visible majority of bacteria.

(e) **Electron microscope** In electron microscope, beams of electron is used instead of beams of light used in optical microscope The electron beam is focmsed by circular electromagnets, analogous to the lenses in light microscope. The object which is kept on path of beam scatters the electrons and produce an image which is focussed on fluorescent viewing screen Resolving power of electron microscope is O.I nm.

(f) Interference microscope. It reveal cell organelles and also enable quantitative measurement of chemical constituent of cells *e.g.* lip d°. protein, nucleic acid etc.

(g) **Polarization microscope** It enables the study of intracellular structure using differences in birefrigenre.

UNSTAINED PREPARATIONS

The wet preparations of bacterial suspensions are mainly used for (a) bacterial motility, (6) for drmo stration of spirochaetes. 4-8 hours growth in fluid media are examined in hanging drop preparation or cover slip preparation. For the study of spirochaele *e.g.* Tre-ponema pallidum dark ground microscopic examination is done.

STAINING OF BACTERIA

The bacterial nucleic acid contains negatively charged phosphate group which combine with positively charged basic dye. Acidic dye do'nt stain bacterial cells and are used to stain background material. Most commonly used stains to study bacterial morphology are as under :

GRAM'S STATN

First described by Gram in 1884. It is used to study morphologic appearance of bacteria. Gram's itain differentiate all bacteria into two distinct groups.

(a) Gram's positive organisms (6) Gram's negative organisms.

Principle. Some organisms are not decolorized and retain colour of basic staining gentian violet (Gram pjsiiive organisms) while the other lose all gentian violet when treated with decolouring agent and take up the counter stain *t.g* dilute carbol fuschin or sufranin (Gram negative organisms).

Procedure. Bacterial suspensions is sprcaded in the form of thin film on the suiface of clean glass jlide and allowed to dry in air. It is called smear. Smear is fited by passing the slide over flame two or three times. Now proceed as follows:

- Cover the slide with gentian violet for 1-2 mts.
- Wash the smear with Gram's iodine and keep Gram's iodine on the slide for 1-2 mts.
- Decolourise the slide with acetone or alcohol carefully and immediately wash the smear with water.
- Counter stain with 0-5% aquous sufranin solution or dilute (1: 20) carbol fuschin for 1-2 mts.
- Finally wash the smear allow it to dry in air and put drop of oil and see under oil immersion lins.
- **Mechanism.** There are two major theories to explain thereaction.

I. Chemical Theory. In Gram's positive organism the iodine combines with chemical substance in cell (or cell wall) and helps in holding firmly the gentian violet the substance is thought to be magnesium ribonucleate or unsMurated fatty acid. Gentian violet iodine complex is attached to the protoplast of Gram stained bacterium The integrity of cell wall is a must for a positive stain.

II. **Physical Theory.** Gentian violet and iodine enter inside the cell and combine to form large molecule. In Grams positive bacteria cell wall acts as barrier so that iodine and gentian violet complex is retained in the cell even in the presence of acetone (or alcohol) This compex is soluble in acetone or alcohol,

ALBERT STA

eg. coryncbacteiiuai d
blue or a mixtures of t

Procedure. Hu
it is replaced by i >
washed with water a
look green while gra

Z'EHL NEELS

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Acid fast org

(1) Mycob
(2) Bacter
(3) Ascos
(4) Exosk
(5) Inclus
(6) Geroi

SPORE ST

Gram stain spore
Methods of sporo

ALBERT STAIN Some bacteria may have metachromatic black granules *eg.* coryncbactciiuai diphtheriae which stain dark bluish or black with mrthylene blue or a mixtures of taludine blue and malachite green.

Procedure. Htat fixed smear is covered with Albert stain. After 5 minutes it is replaced by i >dine solution and is kept there for 5 minutes. Smear is washed with water and studied under oil immersion lens. The body of bacilli look green while granules take dark bluish black colour.

Z'EHL NEELSEN STAIN. This is also called acid fast stain.

Principle. Som: organism retain carbol fuschin even when declorised with acid. Such organism are called acid fast organism. However, mycolic acid is thought to be responsible for acid fastness.

Procedure. Take heat 6xcd sraear and add concentrated carbol fuschin Now gently heat it tills steam romes out. Don't allow the stain to boil or dry. Keep it up for 8—10 mts. Now dectorise the sme3r with 3% solution of hydrochloric acid in 95% ethyl alcohol or 20% aquous sulfuric acid. Now wash the slide with water aiid counter stain it with methylene blue or melachitc green. Acid fast o'gar isms take red stain.

Acid fast organisms are :

(1) Mycobacteria.
(2) Bacterial spores.
(3) Ascospore of certain yeast.
(4) Exoskeleton of insects.
(5) Inclusion in lungs from cases of lipid pneumonia.
(6) Geroids in liver of rat.

SPORE STAIN. Spores are resistant to ordinary method of staining. In Gram stain spore appear as clear areas in deeply stained body of bacilli. Methods of sporq.staining are:

(1) **Modified acid fast stains.** Treat the heat fixed smear with steaming carbol fuschin for 3—6 mts. Decolorise with 0'5% sulfuric acid or 2% nitric acid in absolute alcohol. Wash and counter stain with 1% aq. methylene blue. Wash with water study it under oil immersion lens. Spores are stained bright red and vegetative part of bacilli blue.

(2) **Moller methods.** Here heat fixed smear is kept over beaker with boilii g water. As soon a? steam start condensing over under surface of slide add malachite green. Keep it up for 1 — 2 mts. wash it and counterstain it with dilute corbol fuschin.

CAPSULAR STAIN : They are not stained with ordinary aniline dyes. In *-ram, stain* they are shown as areas of haloes around bacteiia.

India ink method

Emulsify small amount of culture in a loopful of India ink on a slide and cover it with coverslip. Capsule is seen as clear halo between refractile outline of cell wall and greyish background of India ink. It i? best rapid method of capsule demonstration.

Hiss's Method

Treat thin freshly prepared smear with hot crystal violet for one minute. Now wash with 20% solution of copper sulfatc and blot.

The capsule is stained **blue** and the **body of the bacteria stains** deep purple.0.

3

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.

I. On the basis of energy sources.

(a) Phototropics which get energy from photochemical reactions.

(b) Chemotrophic gets energy from chemical reactions.

II. 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, phosphate! etc. to form new protoplasm of the cell.

(b) *Bet&Totrophic.* Here some of the essential metabolites are not synthesized. Organic compounds e g. proteinj 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 oxtdase system.

Aerobes The aerobe organisms grow only in the presence of oxygen e g. pscudomodaceae, 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-acrophilic 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 organism like neisseria gonorrhoea?, brucella abortus are greatly enhanced by the presence of extra amount of carbondioxide in atmospheric air.

3. **Moisture.** Bacteria require water for their growth. Dessication may kill most of bacteria.

4. **Accessary nutritional requirement.** Most often the accessary growth factors are vitamins. The requirement of growth factors differ widely in various bacteria *e.g.*

organism	*Growth factors*
N. gonorrhoae	Glutathionc
C. diphtheriae	β-alanine
S. aureus	Nicotinic acid, thiamine
H. influenza	Haematin (Co-enzyme I)

They are not syntheiized by bacteria and so supplied in media.

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 (Lag phase). This is followed by multiplication and increase in number of bacteria to the extent that media look turbid to the naked eye (Log phise).

After some time growth rate becomes stationary and later on declines. Counting of bacteria at different period after inoculation and then events of sequences are represented on & graph which is called growth curve,

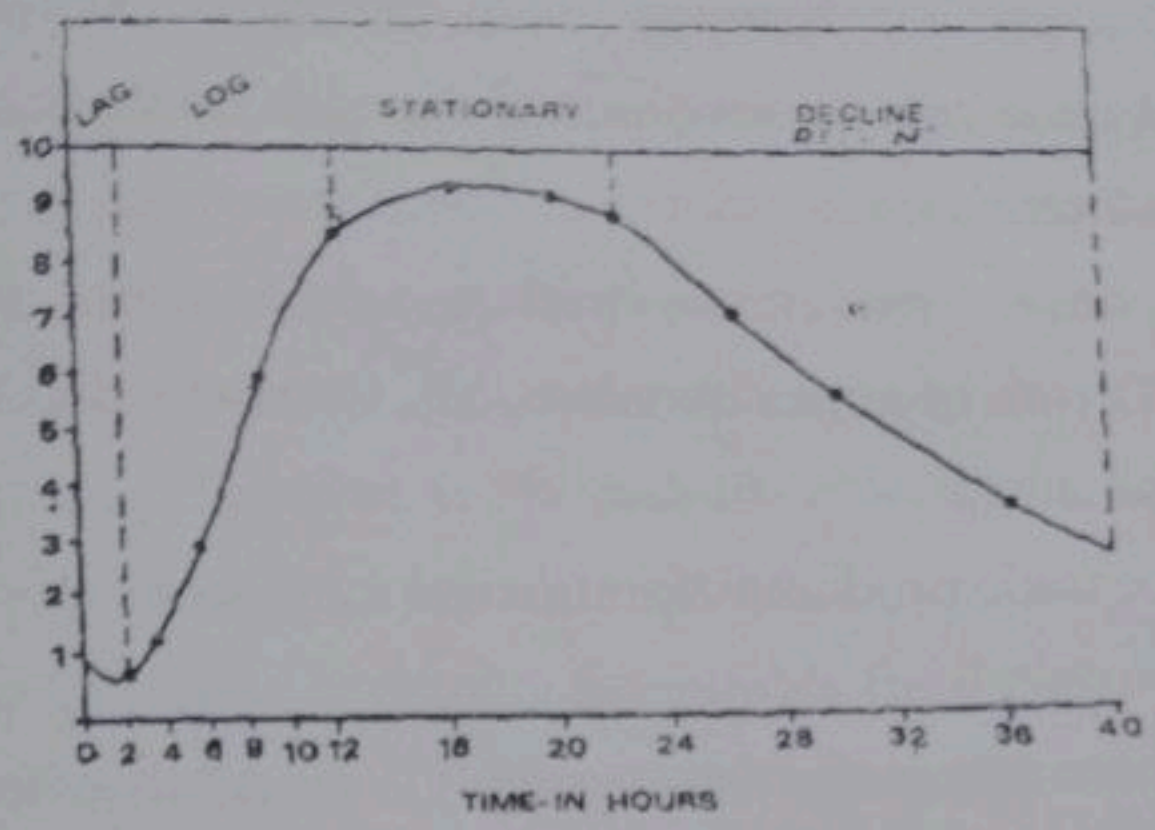

Fig. 10. Growth curve

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 enzymes 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 gives straight line] During thia periods.

(f) Bateria have high rate of metabolism.

(g) 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 some time 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 this phase are :

(i) Nutritional exhaustion

(ii) Toxic accumulation

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

Survival Phase

When most organism 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 LactobacilH grow at acidic pH while cholera vibrio grow at alkaline pH,

(3) **Moisture.** Water in quite essential for the growth of bac'eria. Organism like n eisaeria gonorrhoe and treponema pall id um 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 is usually favourable for the growth and viability of all the organisms. Direct light exposure shorten the survival of bacteria. Photochromogenic mycobacteria form pigment on exposure to light. Organism are scnsttivitive to ultraviolet and other radiations.

(6) **Mechanical and sonic** stress. Bacteria have tough cell walls, Vigrous shaking with glass beads, grinding and exposure to ultrasonic vibration may cause rupture or disintegration of sell 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. Generai gap of:

(a) Coliforms bacteria is 20 minutes.

(b) Mycobacterium tuberculous is 20 hours.

(c) Lepra bacilli is 20 days.

BACTERIAL COUNT

(A) Total bacterial count includes living as well as dead bacteria. It can be obtained by following methods.

1. **Direct** counting chamber.
2. Counting in an electronic device as in Coulter counter.
3. Direct counting using stained smears prepared by spreading known volume of culture over measured area of a slide.
4. Comparing relative number in smear of the culture mixed with known number of other cells.
5. By opacity measuring using nephalometer or absorptio-meter.
6. Measuring wet and dry weight of cells after ceutrifYfgation or filteration.
7. Chemical assay of nitrogen.

(B) The viable count measures number of living organism. It can be obtained as under.

(1) Dilution method.

Several tubes are inoculated with varying dilution and viable count calculated statistically from number of tube showing growth. This method doca'nt give accurate values. This method IB used for presumptive coliform count in drinking water.

(2) Plating method.

Here appropriate dilutions are inoculated on solid media either on the surface of plate or ai pour plates. The number of colonies that develop after incubation gives an estimate of viable count.

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On the basis of main characters of each order, further, families and genera arc classified.

(2) **Morphological classification.** All the organisms are classified into two groups,

(A) **Higher bacteria.** They are filamentous and grow by branching to form mycelium *e.g.* Actinomycetcs. Organism? producing true mycelium among Actinomycetales arc further classified into :

(a) Vegetative mycelium fragments into bacillary or coccoid element. Of course they are Gram positive.

(1) Anaerobic, acid fast *e.g.* Nocardia

(2) Anaerobic non acid fast. Actinomyces israeli, Actionomyces bovis.

(*b*) Vegetative mycelium does not fragment into bacillary or coccoid form. Conida are formed in chain from aerial hyphae *e.g.* Streptomyces,

(B) **Lower or true bacteria.** They are unicellular and never form mycelium. They are grouped an the bases of their shape.

(a) Cocci—spherical

(6) Bacilli—rod shaped

(c) Vibrio—Comma shaped

(d} Spirilla —Spiral twisted non flexous rods.

(e) Spirochaetes—Thin spirally twisted, fleious rods.

Cocci. Following types of arrangement is seen.

Diplococcus. Binary fission occurs in one plane *e.g.* Pncumo-cocci.

Streptococcus. Cocci are arranged in chain *e.g.* stiepto-coccus haemolyticus, streptococcus veridans.

Staphylococcus. Cocci are arranged in cluster *e.g.* staph pyogenes, staph albus.

Tetracoccus. Arrangement of cocci in group of four *e g.* Micrococcus tetragena.

The cocci are further classified (into Gram positive and Gram negative). Gram positive are again divided on the basei of arrangement of cells.

Cocci

Gram negative
Neisacria gonococcus
Neisteria meningococciu
—Pneumococcus
—Micrococcus
—Sarcinococcus

Gram positive
—Staphylococcus
—Streptococcui

Bacilli. They may be Gram positive or Gram negative. Gram positive, may be acid fast on the basis of staining reaction to-Ziehl Neelsen itain. Gram negative organism are further identified on the bails of biochemical reaction and antigenic analysis.

Bacilli

Gram positive

Spores not fann

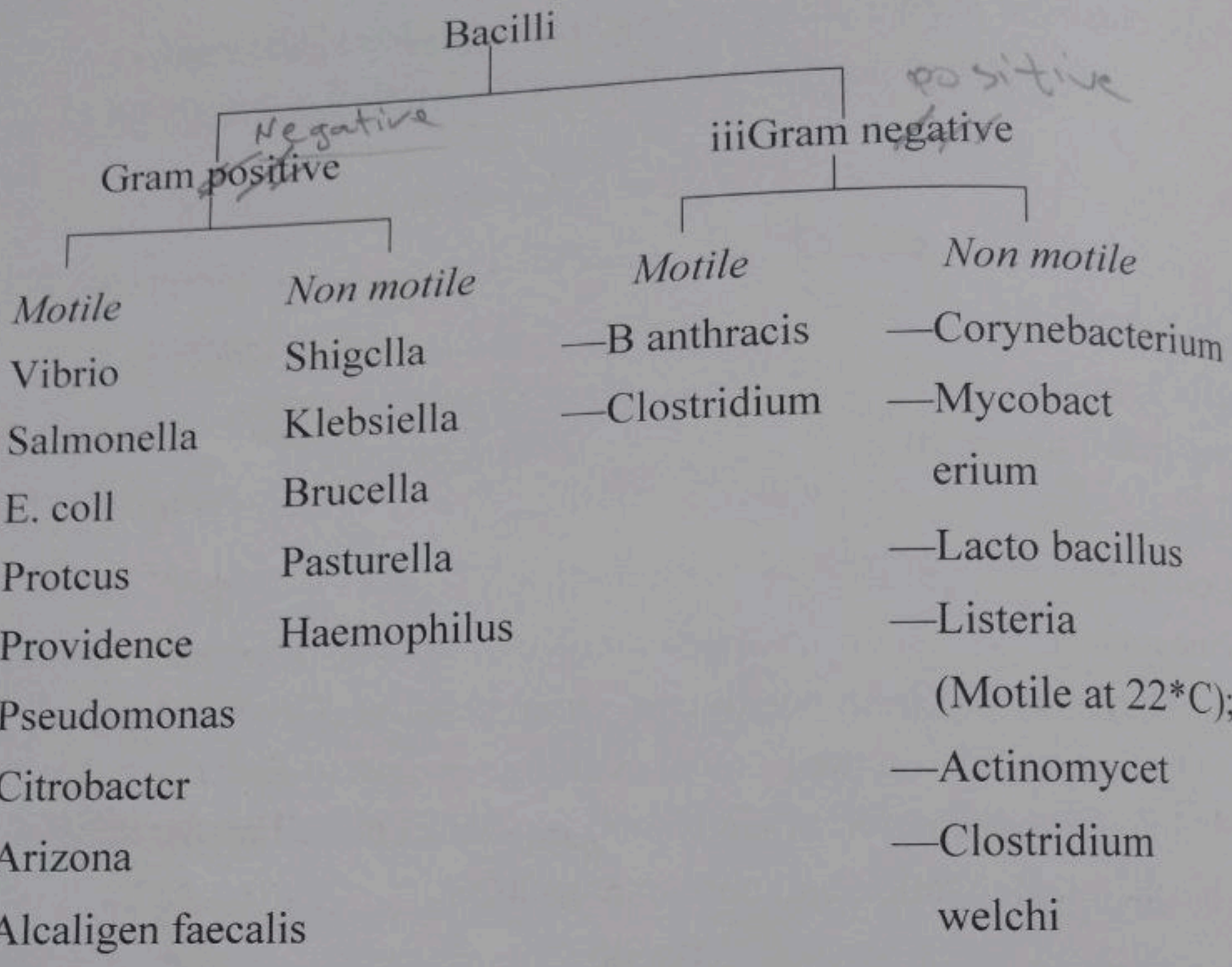
a) *Ziehl Neelsen stain (positive)*
Mycobacterium, Nocardia
(b) Ziehl Neelsen stain (negative)
—Lactobacillus
—Corynebactcrium
—Listeria
—Actinomycis

Spore formed

Aerobic
e.g.
—Bacillus anthracis
—Bacillus subtilis

Anaerobic
e.g.
clostridium

Gram negative
—Psendomonadaceae
—Entrobacteriaceae
—Brucellaceae
—Acromobacteriaceae
—bacteriodaceae



Vibrio. They are curved, non flexible, C.am negative, highly tactile' *e g.* Vibrio cholera,

Sprilla. Consists of coiled, non flexous motile celli *e.g.* spirilum minus.

Spirochaetis. They are slender, refractile and spiral filament. The pathological species are classified into 3 genera.

(a). Treponema *e.g.* Treponema pallidum.

(6) Leptospira *e.g.* L, icterohaemorrhagiae.

(c) Borrelia *e.g.* Boirelia recurrentis.

Identification of bacteria

For the identification of organism we proceed as under.

(1) Microscopic examination. It helps to find out whether the bacteria is cocci, bacilli, vibrio, spirillum or spirochaete. On Gram staining we can have two groups of organism ; Gram positive and Gram negative organisms.

(2) **Motility.** Pathogenic cocci are non motile. Among Gram negative bacilli, Salmonella, E. coli, proteus, Pseudomonas, Alcaligens faecalis, Vibrio cholera are motile. Among Gram positive bacilli clostridia and Bacillus are motile.

In Gram stained smears we note shape, size, arrangement, motility, spores and so on. Hanging drop preparation, dark ground microscopy, phase contrast, electron microscope help in their study.

Staining reaction

Simple stain bring out morphology best. Differential and special stain are necessary to bring out characteristics like flagella, capsule, spores and metachromatic granules.

Gram stain divide bacteria into Gram positive and Gram negative bacteria. Ziehl Neelsen stain into acid fast and non acid fast bacilli. Albert stain for the demonstration of metachromaic and fluorescent dye to bring out special character.

Study of morphology and staining characteristics helps in preliminary identification.

Culture character. Growth requirement and colonial characteristics in culture are useful for the identification of organism *e.g.* staphy pyogenes shows beta type haemolysis with pin head colonies where

as staph albus is without any haemolyses. Strepto haemolyticus are pin point colonies with beta haemolysis where as strepto viridans show alpha type haemolysis.

Resistance. Resistance to heat, concentration of disinfectant, antibiotic, chemotherapeutic agent and bacitracin help in differentiating and identification *e.g.* resistance of streptofaecalis to heat at 60°C for 30 mts and clostridial spores to boiling for various period.

Metabolism. Requirement of oxygen, need of carbondioxide, capacity to form pigment and power of haemolysis is helpful for classification of bacteria and to differentiate species.

Biochemical reaction

The more important and widely used tests are as under:

1. **Sugar fermentation.** This is tested in sugar media having indicator Andrade. Acid production changes the colour of medium into pink. Gas produced collects in Durham tube.

2. **Indole production.** This test demonstrate production of indole from tryptophane. This tryptophane is present in peptone water. In 48 hours peptone water culture 0'5 ml Kovac's reagent is added. Red colour ring indicates positive test.

3. **Methyle red test.** It is to detect the production of acid during fermentation of glucose and maintenance of pH below 4.5 Glucose phosphate culture is taken and few drops of 0.04% methyle red are added. Red color is positive while yellow colour means negative test.

4. **Voges-Proskauer test.** It depends on production of acetyl methyl carbinol from pyruvic acid. 48 hours growth of glucose phosphate culture is taken. To it we add 40% KOH (1vol.) and 3 volume of a naphthal. Deep pink color in 2-5 mts which deepens into magenta or crimson colour means positive test.

5. Cittrate utilization. Some organisms use carbon as sole source of carbon. Koser citrate medium (liquid) is taken for this test. Turbidity in this medium mean citrate has been used up. In Simmon', medium (solid) after overnight incubation colour of medium changes from green to blue if citrate is used up by the organism.

6. Nitrate reduction. Organism is grown in broth containing 1% KNO_3 for 5 days To it is added 1—2 drops of mixture of sulfanilic acid and a naphthalamine (mixed in equal proportion). Red colour appears within few minute if test is positive.

7. Urease test. It is done in christensen's, urease medium. Inoculate heavily the slope and incubate at 37°C. Urease producing organism produce pink colour. Urease producing bacteria reduce urea to ammonia and hence pink colour.

8. Hydrogen sulphide production. Some of the organism decompose sulphur containing amino acid producing H_2S among the product. It turns lead acetate paper strip into black. Instead of lead acetate we may use ferrous acetate or ferric ammonium citrate.

9. Catalase production. Pour a drop of 10 vol H_8O_{a2} on glass slide. Now touch straight wire charged with bacterial colony. In positive reaction gas bubbles are produced.

10. **Oxidase reactions.** The reaction is due to .cytochrome oxidase. 1% solution of tetra methyl-p-phenylene diamine hydrochlo-ride is made. The colony to be tested is smeared (5 mm line) over-paper soaked in above mentioned solution. Smeared area turns dark purple in 5-10 seconds in positive cases.

11. **Growth in KCN.** 1/13000 dilution of KCN is used to identify Gram negative bacilli.

Antigenic analysis

By using specific sera we can identify organism by agglutination reactions *e.g.* b, haemolytic streptococci is differentiated into 18 serological groups (A-T except I andj) on the bases of polysac-charides component. Like wise pneumococous is divisable into 77 capsular types by capsular swelling reactions with anticapsular sera.

Bacteriophage typing. Viruses that parasitize bacteria are called bacteriophage or phage. Phage brings about lysis of susceptible bacterial cells. Phage typing is useful in distinguishing strains among salmonella and staphylococcus. There is correlation between bacteriophage type and epidemic source.

Pathogenecity. For pathogenecity test commonly used laboratory animal models are, guinea pb, rabbit, rat and mouse. The route used may be subcutaneous, intramuscular, intraperitonial, intracerebral, intravenous, oral or nasal spray.

Resistance to antibiotic and other agents. Information about sensitivity pattern of strain is useful for selecting choice of drug. This may be useful as an epidemiological marker in tracing hospital infection *e.g.* staphy pyogenous sensitivity to mercury salt.

In other cases sensitivity of bacteria to agents help in identification of organism *e g.* Strepto haemolycus are sensitive to bacitracin and Pneumococcus to optochin.

6

Sterilization and Disinfection

The process of sterilization finds application in microbiology for prevention of contamination by extraneous organisms, in surgery for maintenance of asepsis, in food and drug manufacture for ensuring safety from contaminating organism and many other situation.

Sterilization, It is a process by which articles are freed of all microorganisms either in vegetative or spore state.

Disinfection. It is a process of destruction of pathogenic organisms capable of giving rise to infection.

Antiseptic. It means prevention of infection by inhibiting growth of bacteria.

Bacteriocidal agents. They are those which are able to kill bacteria.

Bacteriostatic agents. Only prevent multiplication of bacteria and they may remain alive.

Various agents used in sterilization are :

(A) **Physical**

(1) Sunlight.

(2) Drying.

(3) Dry heat.

(4) Moist heat.

(5) Filteration.

(6) Radiation.

(7) Ultrasonic vibrations.

(B) **Chemical**

(1) Acid.

(2) Alkalies.

(3) Salts.

(4) Halogens.

(5) Oxidising agents.

(6) Reducing agents.

(7) Formaldehyde.

(8) Phenol.

(9) Soap.

(10) Dyes.

(11) Aerosal etc.

Physical Methods

1. *Sunlight.* It possesses appreciable bacteriacidal activity. The action is due to ultraviolet rays. This is one of the natural methods of sterilization in cases of water in tanks, river and lakes.

2. *Drying.* Drying in air has deleterious effect on many bacteria. Spores are unaffected by drying. Hence it is very unreliable method.

3. *Heat.* The factors influencing sterilization by heat are:

(1) Nature of heat

(a) dry

(b) moist.

(2) Temperature and time.

(3) Number of organism present.

(4) Whether organism has sporing capacity.

(5) Type of material from which organism is to be eradicated.

(A) Dry Heat

Killing by dry heat is due to :

(1) Protein denaturation.

(2) Oxidative damage.

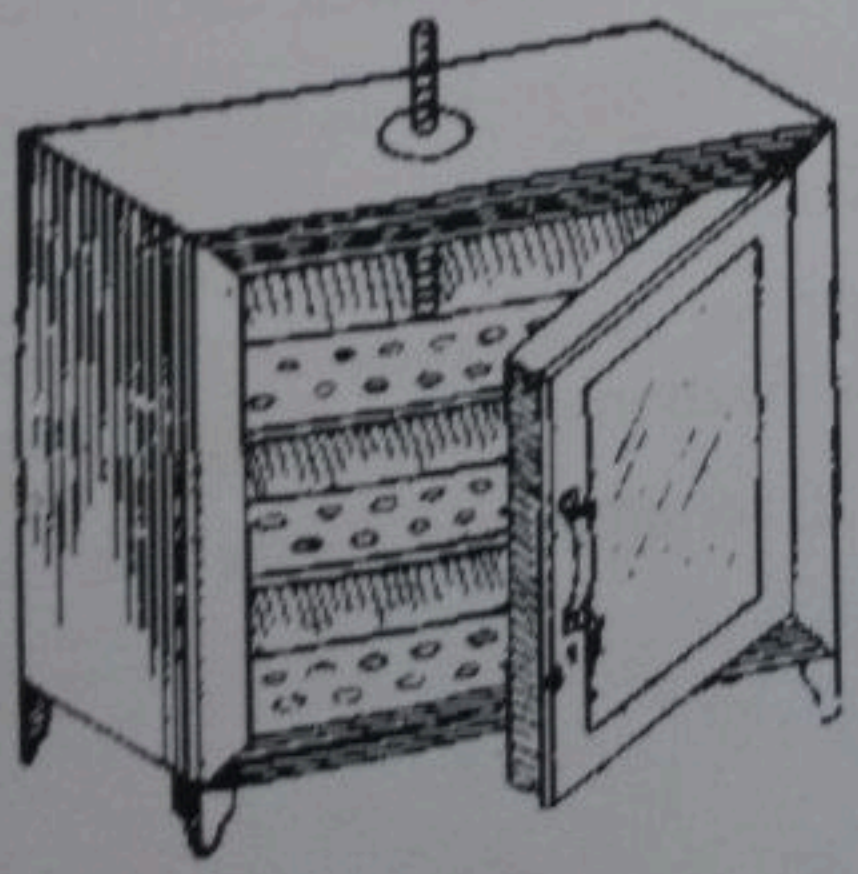
(3) Toxic effect of elevated levels of electrolytes.

(a) *Red heat.* It is used to sterilize metallic objects by holding them in flame till they are red hot *e.g.* inoculating wires, needles, scalpels, forceps etc.

(b) *Flaming.* The article *is* passed over flame without allowing it to become red hot *e.g.* mouth of culture tubes, cotton wool plugs, and glass slides.

(c) *Incineration.* This is excellent method for rapidly destroying material *e.g.* soiled dressing, animals carcasses, bedding and pathological material etc.

(d) *Hot air oven.* Sterilization by hot air oven requires temperature of 160°C for one hour. We can sterilize all glass syringes, petri dishes, test tubes, flask pipettes, cotton swabs, scalpel, scissors, liquid paraffin, dusting powder, etc.



Precautions

(1) It must be fitted with fans to ensure distribution of hot air.

(2) It should not be overloaded.

(3) Oven must be allowed to cool for about 2 hours before opening the doors otherwise glass wares are likely to get cracked.

Sterilization control

(1) The spores of non-toxigeous strain ofclost. tetani are used to test dry heat efficiency.

(2) Browne's tube (green spot) is available for dry heat. A green colour is produced after 60 minutes at ;60°G.

(3) Thermocouples may be used.

(B) **Moist Heat**

The lethal effect of moist heat is by denaturation and coagulation of protein.

(a) *Temperature below 100°G*

(i) *Paustvization of milk.* Temperature employed is either 6S°Cfor 30 minutes (Holder method] or 72°C for 15-20 seconds (flash method). Organism like Mycobacterium, Salmonellae and Biucellae are killed. Coxiella burnetti is relatively heat resistant and hence, may survive the holder method.

(ii) *Inspissation.* The slow soldifieation of serum or egg is carried out at 80°C in an inspissator *e.g.* serum slopes, Lowenstein Jensen's medium etc.

(b) Temperature at 100°C

(i) Tyndallization. This is the process by which medium is placed at 100°C in flowing steam for 30 minutes each on 3 successive days. The

mechanism under lying this method is vegetative cells are destroyed at 100°C and remaining spore which germinate during storage interval are killed on subsequent heating *e.g.* sterilization of egg or serum containing media.

(ii) *Boiling.* Most of vegetation form of bacteria, fungi and viruses are killed 50-70°C in short time. For needles and instruments boiling in water for 10 — 30 mts, is sufficient to sterilize them. Addition of little acid, alkaly, or washing soda, markedly increases the sterilizing power of boiling water. Spores and hepatitis virus are not readily destroyed by such procedure.

(iii) Steam at atmosphere' pressure (100°C). Here free steam is used to sterilize culture media which may decompose if subjected to higher temperature. A Koch or Arnold steamer is used. This is a cheap method of sterilization.

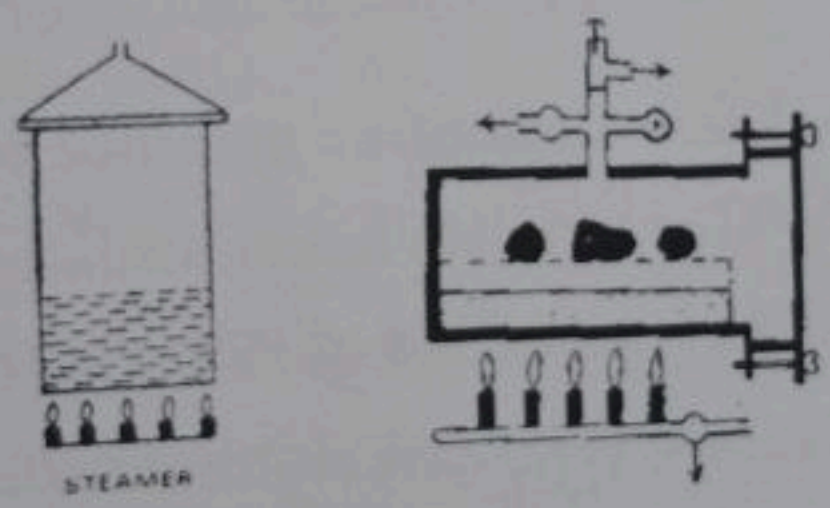


Fig. 16

(iv) Steam under pressure. For bacteriological and surgical work boiling is not sufficient because spores survive boiling. Hence high pressure sterilizer or autoclave Ked

AUTOCLVE

In this apparatus material for sterilization are exposed to 121° C for ,5 – 20 mts. at 15 lb pressure per square inch. Saturated steam heats the article to be sterilized rapidly by release of latent heat. On condensation 1600 ml of steam at 100° C and liberates 518 calories of heat. The condensed water ensures moist conditions for killing bacteria.

The air is a poor conductor of heat and must be removed from chamber. The contents must be so packed that free circulation of steam occurs.

Autoclave is used for culture media, rubber goods, syringes, gowns and dressing etc.

Sterilization control.

(1) Bacillus stearothermophilus

(2) Bro-wne, tube

(3) Autoclave tapes

(4) Thermocouples.

(4) *&teri'izationbyfilteration.* This is a method of sterlization useful for antibiotic solutions, sera, carbohydrate solution etc. We may also get bacteria free filterates of toxin and bacterophages. It is also useful when we want to separate microorganism" which are scanty in fluid.

Draw back. Viruses and mycoplasma may pass through filter. Hence serum filtered is not safe for clinical use as it may be containing viruses or mycoplasma.

Various types of filters are :

(i) Earthen-ware candles : Berkefeld, chamberland.

(ii) Asbestos disc filter *e g.* Seitz.

(iii) Sintered glass filters.

(iv) Collodion or Membranous filter.

(5) **Radiations**

(a) *Ultraviolet radiations.* It is chief bacteriocidal factor present in sunlight. It causes following changes in cell :

(1) Denaturation of protein

(2) Damage to DNA

(3) Inhibition of DNA replication

(4) Formation of H_2O_8 and organic peroxide in culture media

(5) Induction of colicin production in colicinogenic bacteria by destruction of cytoplasmic represser.

Ultraviolet lamps are used in :

(a) Killing of organism

(b) Making bacterial and viral vaccines

(c) Prevention of air borne infection in operation theatre, public places and bacteriological labora'ories.

X-rays and other ionizing radiations. Ionizing radiation have greater capacity to induce lethal changes in DNA of cell. They arc useful for the sterilization of disposable material like, cat gut, disposable syringes, adhesive dressing etc.

(6) **Ultrasnic and sonic vibrations.** They are bacteriocidal causing mechanical agitation and rupture of bacteria.

Chemical methods: Following methods are of common use:

(1) *Acids and alkalies.* They are inhibitory to the growth of bacteria. Mycobacteria are more resistant to acid than alkalies. Boric acid is weak antiseptic.

(2) Distilled water causes loss of viability. This4 action may be due to tracers of metal in distilled water.

(3) *Metallic ions,* $HgCl_2$ and $AgNO_8$ prevent the growth of many bacteria

in concentration less than *1* part per million. This action is due to affinity of certain protein for metallic,jons.

(4) *Inorganic anion.* They are much less toxic to bacteria. Potassium tellurite is inhibitory to Gram negative bacteria. Fluoride inhibits many enzymes of bacteria

(5) *Halogens.* Iodine is used chiefly for skin. Chlorine combines with water to form hypochloric acid which is bacteriocidal.

(6) *Oxidising agents.* They are weak antiseptic *e.g.* H_2O_8 potassium permanganate.

(7) *Formaldehyde.* It is useful in sterilizing bacterial vaccine and in inactivating bacterial toxin without affecting their antigeni-city. 5—10% solution in water kills many bacteria. It is bacterio-cidals, sporicidal and lethal to viruses also

(8) *Phenol.* It is used for sterilizing surgical instruments, and for killing culture accidentally split in the laboratory. It is generally used in 3% solution.

(9) *Soap and detergents.* They are bacteriocidal and bacterio-static for Gram positive and some acid fast organisms. Detergent acts by concentrating at cell membrane and thus disrupting its normal function or it may denaturate protein and enzyme.

(10) *Alcohol.,* Ethyl alcohol is most effective in 70% solution It does'ut kill spores.

(11) *Dyes.* Gentian violet and malachite green etc. are active against Gram positive bacteria. They have poor penetration and hence action is bacteriostatic. Acriflavin is bacteriostatic for staph in 1 : 3000,000 concentration.

(12) *Aerosale and gaseui disinfectant.* SO_2, chlorine and formalin vapour have been used as gaseous disinfectant. Propylcne glycol is powerful disinfectant.

Parasites. Are organism that can establish themselves and multiply on hosts. They may be pathogens or commensal 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.

Scarce of infection in man

(1) *Man.* Man is himself a common source of infection from a patient or carrier. Healtay carrier is a person harbouring pathogenic organism without causing any disease to him. A convalescent carrier is one who has recovered from disease but continues to harbour the pathogen in his body.

(2) *Animals.* Infectious diseases transmitted from animals to man are called zoonosis. Zoonosis may be bacterial (e.g. plague from rat), rickettseal *(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 (zoophilrc dermatophytes from cats and dogs.)

(3) *Insects.* The disease caused by insects are called arthropod borne disease. Insects like mosquitoes, flees, lice that transmit infection are called vector. Transmission may be mechanical (transmission of dysentry 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 *(e.g.* ticks in relapsing fever and spotted fever).

(5) *Soil.* Soil may serve as some of parasiting infection like roundworm and hookwarm. Spores of tetanus bacilli remain viable in soil for a long time, fungi like Histoplasma capsulatum and Nocardia

asteroides also survive in soil and cause human infection.

(6) *Water.* Cholera vibrio, infective hepatitis virus, guinea worm 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, tuberculousis small pox, measle, mumps etc.

(3) *Infection.* Cholera (water) food poisoning (food) dysentry (hand borne).

(4) *Inoculation.* Tetanus, (infection) rabies (dog), arbovirus (insects) serum hepatitis, (injection).

(5) *Insects,* Act as mechanical vector (dysentry and typhoid by house fly) or biological vector (malaria) of infectious disease.

(6) *Congenital.* Congenital syphilus, rubella, Listeria mono-cytogenes, toxoplasma, cytomegalic inclusion disease.

(7) *Laboratory infection.* Infection may be transmitted during procedure like, injection, L,P,, catheterization etc., if proper care is not taken.

Factors predisposing to microbial pathogenecity

Before discussing factor it is worth while to make fine distinction between the terms pathogenecity and virulence,

Pafnogenecity. Is referred to the ability of microbial species to produce disease.

Virulence. Is referred to the ability of microbial strains to produce disease, e g polio virus contains strain of varying degree of virulence.

Virulence is the sum of the following factors.

(A) **Invasiveness.** It is the ability of organism to spread in a host tissue after est iblishing infection. Less invasive organisms cause localized lesion *e.g.* staphylococcal abscess. Highly invasive organisms cause generalized infection *e.g.* streptococcal septicaemia.

(B) **Toxigenecity.** 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. 3* kg. 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 spacific tissue affinity.

(9) It is highly antigenic.

(10) Specific pharmacological effects for each exotoxin.

(11) Can be tozoided.

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 toxoided.

(11) Produce in Gram negative bacteria.

(C) **Communicability.** This is ability of parasite to spread from one host to another. It determines 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 (staph pyogenes) which prevents phagocytosis by forming fibrin barrier around bacteria.

(2) Fibrinolysin promotes the spread of infection by breaking down the fibrin barier in tissues.

(3) Hyaluronidase split hyaluronic acid (component of connective tissue) thus facilitating spread of infection along tissue spaces.

(4) Leucocidins damage polymorphonuclear leucocytes.

(5) Haemolysin is produced by some organism capable of destroying erythrocytes.

(E) **Bacterial appendages**

Capsulated bacteria like Pneumococcus, Klebsella pneumoniae and H. influenza will stand phagocytosis. Surface antigen *e.g.* Vi antigen of S. typhi and K. Antigen of £. 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 prodn<-«" clinical evidence of infection or death of susceptible animal.

(G) **Route of Infection**

Cholera vibrio is ineffective orally. No effect when it is introduced subcutanously. Streptococci can initite infection whatever be the mode of entry. They also differ in ability to produce damage to different organs in different species *e.g.* tubercle bacilli injected into rabbit cause lesion mainly in kidney and infrequently in liver and spleen. In guinea pig, main lesion is in liver, and spleen, kidney is spared.

8

Antimicrobial Therapy

Chemotherapeutic Agents

These are the agents which have lethal or inhibitory effect on the microbes responsible, but in 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, isoniazid, PAS, trimethoprim etc.

(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.* chlora-phenicol, the sulfonamidcs, tetracyclines etc.

(2) Bacteriocidal drugs are the drugs which kill the bacteria by virtue of their rapid lethal action *e g.* pencillins, cephalosporins, aminoglycosides, fucidin, nalidixic acid etc. Bacteriocidal drugs are more effective therapeutic agents than bacteriostatic drugs.

Mode of action. The problem can be considered from two aspects;

(A) Indentification of site of action of drug.

(B) Its precise mechanism of action.

(A) Site of action.

There are four major loci of action.

(1) Inhibition of synthesis of cell wall peptidoglycon *e.g.* pencillins, cephalosporin, cycloserine, vancomycin, ristocetin and bacitracin.

(2) Damage to the permeability of the cytoplasmic membrane *e.g.* tryocidin, gramicidin, polymyxin and antifungal polyene antibiotics.

(3) Inhibition of protein synthesis *e g.* aminoglycosides and tetracyclines. They bind to and inhibit the function of 30 S.

(4) Inhibition of nucleic acid synthesis Rifampicin inhibits the synthesis of messenger RNA by its action on the RNA poly-merase. Nalidixic acid inhibits DNA replication.

(E) Mechanism of action. There are three general mechanism of action.

(1) Competition with a natural substrate for the active site of enzyme *e.g.*

(a) Action of sulfonamides to interfere competitively with the utilization of para-amino benzoic acid (PABA).

(b) Action of para-amino benzoic acid with para-amino salicylic acid (PAS).

(2) Combination with an enzyme at a site sufficiently close to the active site as to interfere with its enzymatic function *e.g.* vanco-mycin, ristocetin and bacitracin.

(3) Combination with non enzymatic structural components *e.g.* drugs which inhibits protein synthesis and drugs which act by damaging cytoplasmic membrane,

Laboratory Uses of Antibiotics

(1) They may be incorporated as selective agents in culture media *e.g.* pencillin may be used for isolation of H. influenzae from material taken from upper respiratory tract (Pencillin inhibits the growth of Gram positive bacteria and Neisserias). Neomycin is used in Wi'lis and Hobb's medium for the isolation of clostridia.

(2) They are used for the central of bacterial contamination in tissue cultures used for virus isolation *e.g.* pencillin, streptomycin, nystatin etc.

(3) The pattern of sensitivity of an organism to a battery of antibiotics constitute a simple method of typing which is of considerable epidemiological value.

Some Antibiotics in Current Use

(1) Antibiotic mainly or exclusively active against Gram positive bacteria *e.g.* pencillin (G and V), methicillin, cloxacillin, ery-thromycin, novobiocin, vancomyein, bacitracin and fucidin.

(2) Antibiotics active against Gram negative bacteria *e g.* polymyxin, aminoglycoside.

(3) Antibiotics active against both Gram positive and Gram negative bacteria *e.g.* tetracycline, chloraphenicol, ampicillin, cephalosporins.

(4) Antibiotics active against fungi *e.g.* greseofulvin, iodides nystatin, amp otericin B.

Antibiotic Sensitivity Teats

These are used to determine the susceptibility of pathogenic bacteria to antibiotics to be used in treatment. Antibiotic sensitivity tests are very useful for clinician and hence constitute important routine procedure in diagnostic bacteriology. Mainly they are of two types:

(1) *Diffusion tests.* The principle of it is to allow the drug to diffuse through a solid medium. Concentration o'f drug being highest near the site of application of drug and decreasing with distance.

There are many methods for implementation of this diffusion tests. Most common, simple and easy method is to use filter paper discs impregnated with antibiotics (Disc diffusion method). Here filter paper discs 6 mm in diameter are charged with required concentration of drugs and are stored dry in the cold. Inoculation of pure bacterial growth in liquid medium, may be done by spreading with swabs on solid medium. After drying the plate at 37°C for 1/2 hour antibiotic discs are applied with sterilized forceps. After overnight incubation at 37*C, zone of inhibition of growth around each antibiotic disc is noted. Inhibition zone shows degree of sensitivity of antibiotic for that particular bacteria. The results are reported as sensitive or resistant.

Disc diffusion test is done only after the pathogenic bacteria are isolated from clinical specimen in pure form. Sensitivity tests should be done only with pathogenic bacteria and not with commensals. Further, Nitrofurantin need to be tested only against urinary pathogens. Sensitivity tests on methanamine mandelate are not a relevant as the drug is active only in vivo.

in case we require of drug sensitivity test still soon clinical material is directly inoculated uniformly on the surface of solid media plate and discs are applied. This is done only in emergency and results are subsequently verified by testing the pure isolates.

(2) *Dilution tests* These are quite laborious for routine use. However these are useful where therapeutic dose is to be regulated accurately *e.g.* in treatment bacterial endocarditis and to find out small degree of resistance

in slow growing bacteria like tubercle bacilli. In dilution test, serial dilution of drug are prepared and are inoculated with test bacterium. It may be done by tube dilution or agar dilution methods.

Antibiotic Assays in Body Fluids

In is done by making serial dilution of specimen and inoculating standard suspensions of bacteria of known minimum

inhibitory concentration (M.I.C). Lowest concentration of drug that inhibits bacterial growth is called minimum inhibitory concentration.

It is useful in verifying adequate drug concentration in blood and other body Suids. It also guards us against excessive blood., levels of toxic drugs.

Drag Resistance

During treatment with drugs, bacteria may acquire resistance to these drugs. Following are the various mechanisms of drug resistance :

(a) *Mutation.* All bacteria contain drug resistant mutant* arising spontoneously once in 10^7 to 10^{10} cell divisions. It is again of two types.

(b) Step wise mutation in which series of small step mutations result in high levels of resistance *e g.* pencellin, chloraphenicol, tetracycline, sulfonamides etc. However this type of resistance can be prevented by using adequate dosage of drugs.

(1) One step mutation in which case resistance develops suddenly even with first exposure of drug. This type of resistance is seen in tubercle bacilli developing resistance to streptomycin and isoniazid.

(2) Resistance transfer by transformation may be demonstrated experimentally but its role in nature is not known.

(3) Drug resistance by transduction is very commonly found in staphylococci. Pencillinase plasmid carrying determinant for resistance