MICROBIOLOGY AND MEDICINE

Medical Microbiology deals with: biology of infectious agents, relationships between the infectious agents and human organism, patogenicity of infectious agents, antiinfectious resistance, etiological diagnosis of infectious diseases, bases of antiinfectious therapy and prevention.

Applications of microbiology have transformed the diagnosis, prevention and cure of disease. Along with improved nutrition and living conditions, they have – at least in developed communities – revolutionized human health, doubled the average length of life and ensured the safe upbringing of most children born, where before only a minority survived.

Even in the developed world, infection is still extremely common: at least a quarter of all illnesses for which patients consult their doctors are infective, a substantial proportion of patients acquire infection while in hospital, sometimes with multiresistant organisms.

Intensive farming methods and a shift in eating habits to pre-prepared 'fast foods' have led to a sharp increase in food-related infection.

In hospitals, new approaches to therapy that deplete the competence of the patient's immune system to cope with infection, as well as the increasing use of shunts, intravenous cannulae and prosthetic devices, all provide the ever-resourceful microbes with new opportunities to invade the host. Previously unsuspected links between microbes and diseases such as cancer, peptic ulcer, inflammatory bowel disease and rheumatoid arthritis have been uncovered.

Surprisingly, 'new' agents of infectious disease continue to be recognized. The most notorious of these is undoubtedly the human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS).

1.1 DEVELOPMENT OF MICROBIOLOGY

Microbiology is the study of living organisms of microscopic size. The term was introduced by the French chemist **Louis Pasteur**, whose demonstration that fermentation was caused by the growth of bacteria and yeasts (1857-60) provided a main impetus for the development of the science.

Microorganisms were first seen about 1675 by the Dutchman **Antony van Leeuwenhoek**. His microscopes consisted of a single biconvex lens that magnified about x 200 and resolved bodies with diameters down to about 1 μm. He found many microorganisms in materials such as water, mud, saliva and the intestinal contents of healthy subjects, and he recognized them as living creatures ('animalcules') because they swam about actively.

Other techniques essential for the rapid progress of bacteriology were developed by the German bacteriologist **Robert Koch**, who in 1877 described methods for the easy microscopically examination of bacteria in dried, fixed films stained with aniline dyes, and in 1881 devised the simple method for isolating pure cultures of bacteria by plating out mixed material on a solid culture medium on which the progeny of single bacteria grow in separate colonies.

1.2 MICROORGANISMS AND DISEASE

The germ theory of disease was slow in gaining acceptance, though it was early recognized that epidemic diseases such as smallpox, measles, typhus and syphilis were probably spread from person to person. The Italian scholar **Girolamo Fracastoro**, in his book De Contagione (1546), distinguished three modes of transmission: (1) by direct contact, i.e. touching a patient's body; (2) by contact with clothing and household goods contaminated by a patient; and (3) at a distance through the air. He explained contagion as being due to the transmission of invisible seeds, or germs, different kinds of which were the specific causes of the different diseases. Fracastoro's views were largely forgotten by the time Leeuwenhoek

discovered microorganisms, but speculations then began that these organisms might be the cause of certain diseases.

Koch's postulates, which **Jacob Henle** had earlier stated would be needed to prove that the particular micro-organism was the cause of a particular disease, namely: that the microbe be found in the body in all cases of the disease; that it be isolated from a case and grown in a series of pure cultures in vitro; and that it reproduce the disease on the inoculation of a late pure culture into a susceptible animal.

The viruses were more difficult to demonstrate, for most were too small to be seen with the light microscope and none could be grown on an inanimate culture medium. At first they could be demonstrated only by observation of the disease they produced when infected tissue was inoculated into a susceptible animal. Thus, in studies of rabies, Pasteur and his colleagues in 1881 failed to isolate any microorganism capable of causing the disease. **Virology** did not progress rapidly, how-ever, until after the Second World War, when the availability of the electron microscope enabled viruses to be visualized and the use of living human and animal tissue cells for the in-vitro culture of viruses was developed by John Enders (1949) and others from the earlier work of pioneers such as Alexis Carrel.

1.3 IMMUNITY AND IMMUNIZATION

It was known from ancient times that persons who had suffered from a distinctive disease, such as small-pox or measles, resisted it on subsequent exposures and rarely contracted it a second time. Such an acquired immunity is generally effective only against the same type of infection as that previously suffered.

Artificial immunization against smallpox was practiced in various communities by the unsafe method of inoculating smallpox exudates through the skin (variolation), until, in 1796, the safer method of inoculating cowpox exudate (vaccination) was discovered by the Gloucestershire doctor **Edward Jenner.**

The natural existence of a safe immunization agent such as vaccine virus is exceptional, and we owe to **Pasteur** the development of immunization strains of pathogenic microbes which are artificially attenuated (reduced in virulence) by prolonged or repeated culture under laboratory conditions.

The first step in elucidating the mechanisms of acquired immunity was the discovery of antibodies by **Behring and Kitasato** in 1890. They found that the injection of sublethal doses of diphtheria or tetanus toxin into guinea-pigs rendered the animals immune to the later injection of large doses of the same toxin. The immunity was associated with the appearance in the animal's blood of a substance, antitoxin, that specifically neutralized the toxin.

1.4 SEROTHERAPY AND CHEMOTHERAPY

The work of Behring and Kitasato led to the successful use of antisera raised in animals for the treatment of patients with diphtheria, tetanus, pneumonia and other diseases. Because, however, the antisera contained animal proteins foreign to the human body, and were given by injection, serotherapy often caused unpleasant allergic responses, called serum sickness. For this reason, and also because **serotherapy** was unsuccessful against many kinds of infection, further progress depended on the development of drugs that exhibited selective toxicity - the ability to inhibit or kill the microbe without harming the patient.

Although agents active against bacteria now form the most abundant group of antimicrobial drugs, the earliest therapeutic successes were achieved with antiprotozoal and anthelminthic compounds. Indeed, effective treatment for malaria (cinchona bark), amoebic dysentery (ipecacuanha root), tapeworm (male fern) and roundworm (wormseed) have been known for centuries. In contrast, effective therapy for systemic bacterial disease was unknown before the use of hexamine (methenamine) at the turn of the 20th century and **Paul Ehrlich**'s development of the arsenical Salvarsan (arsphenamine) for spirochaetal disease in 1909. Even these discoveries were of limited value. The true beginning of the therapeutic revolution in infection dates from **Gerhard Domagk**'s description of Prontosil (the forerunner of sulphona-mides) in 1935, the development of **Alexander Fleming**'s penicillin by **Howard Florey** and his colleagues in 1940, and **Selman Waksman**'s exploitation of the potential for antibiotic production among soil microorganisms in the 1940s. Within 25 years of these discoveries, most of the major groups of antimicrobial agents had

been recognized and more recent developments have chiefly involved chemical alteration of existing molecules.

The discovery of the first miracle drugs - sulphonamides, penicilin and streptomycin - was declared by some the disappearence of bacterial infection- as a disease entity of any importance.

Many things have happened to modify this view of the capabilities of antimicrobial drugs, but four things stand out:

- **bacteria have displayed an amazing ability to avoid, or repel the antibiotic,**
- **the pattern of bacterial disease has altered considerably,**
- **the use of antibiotics often disturbs the delicate bacterial ecology of the body allowing the proliferation of resistant species and sometimes initiating new infections that are worse then the originally treated,**
- **it turns out that no antibacterial drug is entirely free from toxic side effects.**

Progress in the **development of antiviral, antifungal and antiparasitic compounds** has been much slower and therapeutic options in non-bacterial infection consequently remain severely limited. Meanwhile, the explosion of knowledge in immunology has renewed hopes that it may be possible to manipulate immunological processes triggered by infection to the benefit of the host.

1.5 *ROMANIAN MICROBIOLOGY*

Romanian microbiology was represented by many personalities.

Victor Babeş (1854-1926) was bacteriology professor in Bucharest. He studied in Budapesta, Viena, Berlin, Paris. In 1885 he wrote "Les bactéries et leur rôle dans l'étiologie, l'anatomie et l'histologie pathologique des maladies infectieuses". He also descovered metacromatique granulas in *Corynebacterium diphteriae* (Babeş-Ernst corpuscles), he had studies in antrax, lepra, tuberculosis, introduced antirabic vaccination, antirabic and antidiphteric seroterapy. He also organised the first institute of medical researches in Romania, "Victor Babeş Institute" and the first bacteriology and hygiene laboratories in our country.

Ion Cantacuzino (1863-1934) studied in Paris. In 1901 he become professor in experimental medicine in Bucharest. He had studies in holera, and antiholeric vaccination. In 1906 he introduced in Romania, soon after France, the BCG vaccination. He built the first Infection diseases hospital in Romania. In 1921 he organised in Bucharest "the Institut of serums and vaccines I. Cantacuzino", which becomes the main important Microbiology school in our country.

Constantin Levaditi (1874 - 1953), was student of Victor Babeş and professor in Pasteur Institut in Paris. He had important studies in virology, bacteriology and parasitology.

2. CLASSIFICATION OF ORGANISMS

Living organisms are grouped according to similar characteristics (classification), and each organism is assigned a unique scientific name.

Scientific Nomenclature

In a world inhabited by millions of living organisms, biologists must be sure they know exactly which organism is being discussed. We cannot use common names, because the same name is often used for many different organisms in different locales. Because common names are rarely specific and can often be misleading, a system of scientific names, referred to as scientific nomenclature, was developed in the eighteenth century.

Every organism is assigned two names, or a binomial. These names are **the genus name and specific epithet (species)**, and both names are printed underlined or **italicized**. The genus name is always capitalized and is always a noun. The species name is lowercase and is usually an adjective. Because this system gives two names to each organism, the system is called binomial nomenclature.

Binomials are used by scientists worldwide, regardless of their native language, which enables them to share knowledge efficiently and accurately. Rules for naming newly classified bacteria and for assigning bacteria, are established by the International Committee on Systematic Bacteriology and are published in the Bacteriological Code. Descriptions of bacteria and evidence for their classifications are published in the International Journal of Systematic Bacteriology before being incorporated into a reference called Bergey's Manual. According to the Bacteriological Code, scientific names are to be taken from Latin (a genus name can be taken from Greek) or latinized by the addition of the appropriate suffix. Suffixes for order and family are -ales and -aceae, respectively.

2.1 THE TAXONOMIC HIERARCHY

All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy. A eukaryotic species is a group of closely related organisms that breed among themselves. (Bacterial species will be discussed shortly.) A genus consists of species that differ; from each other in certain ways but are related by descent. Just as a number of species make up a genus, related genera make up a family. A group of similar families constitutes an order, and a group of similar orders makes up a class. Related classes, in turn, make up a division. Thus, a particular organism (or species) has a genus name and specific epithet and belongs to a family, order, class, and phylum or division. All phyla or divisions that are related to each other make up a kingdom, and related kingdoms are grouped into a domain (the Bacteriological Code uses the term imperial).

Classification of Bacteria

The taxonomic classification scheme for bacteria found in **Bergey's Manual of Systematic Bacteriology**. In Bergey's Manual, bacteria are divided into four divisions three divisions consist of eubacterial cells, and the fourth division consists of the archaea.

A bacterial species is defined somewhat differently than a eukaryotic species, which is a group of closely related organisms that can interbreed. Unlike reproduction in eukaryotic organisms, cell division in bacteria is not directly tied to sexual conjugation, which is infrequent and does not always need to be species-specific. A bacterial species, therefore, is defined simply as a population of cells with similar characteristics. The members of a bacterial species are essentially indistinguishable from each other but are distinguishable from members of other species, usually on the basis of several features. In some cases, pure cultures of the same species are not identical in all ways. Each such group is called a strain, which is a

collection of cells derived from a single cell. Strains are identified by numbers, letters, or names that follow the specific epithet.

Bergey's Manual provides a reference for identifying bacteria in the laboratory, as well as a classification scheme for bacteria. Suggested evolutionary relationships are given for most groups. Information used for building (and modifying) the phylogenetic models come from analyses of nucleotide sequences in DNA and RNA.

The algae (excluding the blue-green algae), the protozoa, slime moulds and fungi include the larger and more highly developed microorganisms.

The viruses are the smallest of the infective agents; they have a relatively simple structure that is not comparable with that of a cell, and their mode of reproduction is fundamentally different from cellular organisms. Even simpler are **viroids**, protein-free fragments of single-stranded circular RNA that cause disease in plants. Other types of infectious particles have been postulated: **prions** are described as infectious proteins devoid of nucleic acid, while virinos are said to consist of a small amount of nucleic acid complexed with protein derived from the host cell. There is considerable debate about the true status of prions and virinos and of the relationship, if any, between them.

2.2 TAXONOMY

Taxonomy consists of three components: classification, nomenclature and identification. Classification allows the orderly grouping of microorganisms while nomenclature concerns the naming of these organisms and requires agreement so that the same name is used unambiguously by everyone. Changes in nomenclature may give rise to confusion and are subject to internationally agreed rules. In clinical practice, microbiologists are generally concerned with identification - the correct naming of isolates according to agreed systems of classification.

Table 1: Comparison of medically important organisms

Characteristic	Viruses	Bacteria	Fungi	Protozoa and helmints
Cells	No	Yes	Yes	Yes
Diameter	0,02 – 0,2	1 – 5	3 – 10	15 – 25
Nucleic acid	Either DNA or RNA	Both DNA and RNA	Both DNA and RNA	Both DNA and RNA
Type of nucleus	None	Prokaryotic	Eukaryotic	Eukaryotic
Ribosomes	-	70S	80S	80S
Mitochondria	-	-	+	+
Outer surface	Protein capsid and lipoprotein envelope	Rigid wall (peptidoglycan)	Rigid wall (chitin)	Flexible membrane
Motility	None	Some	None	Most
Replication	Not binary fission	Binary fission	Budding or mitosis	Mitosis

2.3 CLASSIFICATION IN CLINICAL PRACTICE

The identification of microorganisms in routine practice requires a pragmatic approach to taxonomy.

Protozoa: These are non-photosynthetic unicellular organisms with protoplasm clearly differentiated into nucleus and cytoplasm. They are relatively large with transverse diameters mainly in the range 2-100 μm. Their surface membranes vary in complexity and rigidity from a thin, flexible membrane in amoebae, which allows major changes in cell shape and the protrusion of pseudopodia for the purposes of locomotion and ingestion, to a relatively stiff pellicle in ciliate protozoa, which preserves a characteristic cell shape.

Fungi: These are non-photosynthetic organisms possessing relatively rigid cell walls. They may be saprophytic or parasitic, and take in soluble nutrients by diffusion through their cell surfaces.
Moulds grow as branching filaments (hyphae), usually between 2 and 10 μm in width, which interlace to form a meshwork (mycelium). The hyphae are coenocytic (i.e. have a continuous multinucleate protoplasm), being either non-septate or else septate with a central pore in each cross-wall. Moulds reproduce by the formation of various kinds of sexual and asexual spores that develop from the vegetative (feeding) mycelium, or from an aerial mycelium that effects their air-borne dissemination.

Yeasts: are ovoid or spherical cells that reproduce asexually by budding and also, in many cases, sexually, with the formation of sexual spores. They do not form a mycelium, although the intermediate yeast-like fungi form a pseudomycelium consisting of chains of elongated cells. The dimorphic fungi produce a vegetative mycelium in artificial culture, but are yeast-like in infected lesions. The higher fungi of the class Basidiomycetes (mushrooms), which produce large fruiting structures for aerial dissemination of spores, are not infectious for humans or animals, although some species are poisonous.

Bacteria: The main groups of bacteria are distinguished by the microscopical observation of their morphology and staining reactions. The Gram staining procedure, which reflects fundamental differences in cell wall structure, separates most bacteria into two great divisions: Gram-positive bacteria and Gram-negative bacteria.

Details of structure provide a basis for a separate division into:

1. Filamentous bacteria (Actinomycetes), most of which are capable of true branching and which may produce a type of mycelium
2. 'True' bacteria, which multiply by simple binary fission
3. Spirochaetes, which divide by transverse binary fission
4. Mycoplasmas, which lack a rigid cell wall
5. *Rickettsia*e and *Chlamydia*e, which are strict intracellular parasites.

True bacteria: Most medically important bacteria fall into this group. They are classified on the basis of their shape*:*

1. Cocci - spherical, or nearly spherical cells
2. Bacilli - relatively straight, rod-shaped (cylindrical) cells
3. Vibrios and spirilla - curved or twisted rod-shaped cells.

The main groups of **cocci** are distinguished by their predominant mode of cell grouping and their reaction to the Gram stain. The different cocci are relatively uniform in size (usually about 1 μm in diameter). Some species are capsulate and a very few are motile.

1. *Streptococcus*. Gram-positive; cells mainly adherent in chains, due to successive cell divisions occurring in the same axis (e.g. *Streptococcus pyogenes*); sometimes predominantly diplococcal (e.g. *Streptococcus pneumoniae*).
2. *Staphylococcus* and *Micrococcus*. Gram-positive cells mainly adherent in irregular clusters, due to successive divisions occurring irregularly in different planes (e.g. *Staphylococcus aureus*).
3. Sarcina. Gram-positive cells mainly adherent in cubical arrays of eight, or multiples thereof, due to division occurring successively in three planes at right angles (e.g. *Sarcinia sp*).
4. *Neisseria*. Gram-negative; cells mainly adherent in pairs and slightly elongated at right angles to axis of pairs (e.g. *Neisseria meningitidis*).
5. Veillonella. Gram-negative; generally very small cocci arranged mainly in clusters and pairs; anaerobic (e.g. *Veillonella parvula*).

Bacilli: The primary subdivision of the rod-shaped bacteria is made according to their staining reaction by the Gram method and the presence or absence of endospores.

Gram-positive spore-forming bacilli. Apart from some rare saprophytic varieties, the only bacteria to form endospores are those of the genera *Bacillus* (aerobic) and *Clostridium* (anaerobic). They are Gram-positive, but liable to become Gram-negative in ageing cultures. The size, shape and position of the spore may assist recognition of the species, e.g. the bulging, spherical, terminal spore ('drumstick' form) of *Clostridium tetani*.

Gram-positive non-sporing bacilli. These include several genera. *Corynebacterium* is distinguished by a tendency to slight curving, a club-shaped or ovoid swelling of the bacilli, and their arrangement in parallel or angular clusters due to the snapping mode of cell division. *Erysipelothrix* and *LactoBacillus* are distinguished by a tendency to grow in chains and filaments, and *Listeria* by flagella that confer motility.

Gram-negative bacilli. This large grouping includes numerous genera such as the pseudomonads and the family *Enterobacter*iaceae ('coliform bacilli') as well as small, often pleomorphic bacilli represented by *Haemophilus, Brucella*, etc. ('parvobacteria'), and anaerobes such as *Bacteroides*.

Vibrios and spirilla: Vibrios and the related campylobacters are recognized as short, non-flexuous, comma-shaped bacilli (e.g. *Vibrio cholerae*) and spirilla as non-flexuous spiral filaments (e.g. *Spirillum minus*). They are Gram-negative and mostly motile, having polar flagella and showing very active 'darting' motility.

Spirochaetes. These organisms differ from the 'true' bacteria in being slender flexuous spiral filaments which, unlike the spirilla, are motile without possession of flagella. The staining reaction, when demonstrable, is Gram-negative. The different varieties are recognized by their size, shape, wave form and refractility, observed in the natural state in unstained wet films by dark-ground microscopy.

1. *Borrelia*. Larger and more refractile than the other pathogenic spirochaetes and more readily stained by ordinary methods; coils large and open, with a wavelength of 2-3 μm, by electron microscopy a leash of 8-12 fibrils, each about 0.02 μm thick, is seen twisted round the whole length of the protoplast (e.g. *Borrelia recurrentis*).
2. *Treponema*. Thinner filaments in coils of shorter wavelength (e.g. 1.0-1.5 μm), typically presenting a regular 'corkscrew' form; feebly refractile and difficult to stain except by silver impregnation methods; by electron microscopy, a leash of four fibrils is seen wound round the protoplast within the cell wall (e.g. *Treponema pallidum*).
3. *Leptospira*. The coils are so fine and close (wavelength about 0.5 μm) that they are barely discernible by dark-ground microscopy, though clearly seen winding round two axial filaments by electron microscopy. One or both extremities of the organism are hooked, or recurved, so that it may take the shape of a walking-stick, an 'S' or a 'C' (e.g. *Leptospira interrogans*).

Mycoplasmas: These are prokaryotes that differ from 'true' bacteria in their smaller size and their lack of a rigid cell wall, which leads to extreme pleomorphism and sensitivity to external osmotic pressure. The viable elements range from 0.15 to over 1 μm in diameter, the smallest being capable of passing through filters that retain conventional bacteria. Mycoplasmas can be cultivated on cell-free nutrient media, and are the smallest and simplest organisms capable of autonomous growth.

***Rickettsia*e and *Chlamydia*e:** The Rickettsiae are rod-shaped, spherical or pleomorphic Gram-negative organisms. They are generally smaller than 'true' bacteria, but are still resolvable in the light microscope. Most are strict parasites that can grow only in the living tissues of a suitable animal host, usually intracellularly (e.g. *Rickettsia prowazekii*). Chlamydiae are similar to Rickettsiae, but have a more complex intracellular cycle (e.g. *Chlamydia trachomatis*).

Viruses: Viruses usually consist of little more than a strand of DNA or RNA (never both) enclosed in a simple protein shell known as a capsid. Sometimes the complete nucleocapsid may be enclosed in a lipoprotein envelope largely derived from the host cell. Viruses are capable of growing only within the

living cells of an appropriate animal, plant or bacterial host; none can grow in an inanimate nutrient medium. The viruses that infect and parasitize bacteria are named bacteriophages or phages.

3. MORPHOLOGY AND NATURE OF MICROORGANISMS

Living material is organized in units known as *cells*. Each cell consists of a body of *protoplasm*, the protoplast, enclosed by a thin semipermeable membrane, the *cytoplasmic* or *plasma membrane*, and also, in some cases, by an outer, relatively rigid *cell wall.* The protoplast is differentiated into a major part, the *cytoplasm*, and an inner body, *the nucleus*, which contains the hereditary determinants of character, *the genes*, borne on thread-like *chromosomes.*

Microorganisms are generally regarded as living forms that are microscopic in size and relatively simple, usually unicellular, in structure. The diameter of the smallest body that can be resolved and seen clearly with the naked eye is about 100 um. Nearly all bacteria are smaller than this, and a microscope is therefore necessary to see them. **Viruses** are even smaller, and in most cases an electron microscope is needed to visualize them.

However, when bacteria or fungi are allowed to grow on a nutritive, solid supporting medium, their numerous progeny form *colonies* that are readily visible to the naked eye. Many viruses can be grown within cultures of living cells *(tissue cultures*) in the laboratory and many cause characteristic *cytopathic effects* visible in the light microscope.

It is useful to draw a clear **distinction between relatively primitive (*prokaryotic*) cells and more advanced (*eukaryotic*) cells.** The bacteria and related organisms (rickettsiae, chlamydiae and mycoplasmas) are **prokaryotic** cells whereas the cells of fungi, protozoa, plants and animals are **eukaryotic**. **Viruses** fall into neither category; they are not cells in the accepted sense and rely on the biochemical processes of the host cell for their replication and propagation.

The main distinguishing features of the prokaryotic cell are:

1. Its **nucleus** appears as a simple, homogeneous body not possessing a nuclear membrane separating it from the cytoplasm, nor a nucleolus, nor a spindle, nor a number of separate non-identical chromosomes.
2. It **lacks the internal membranes** isolating the respiratory and photosynthetic enzyme systems in specific organelles, comparable with the membrane-bound mitochondria and chloroplasts of eukaryotic cells. Thus, the respiratory enzymes in bacteria are located mainly in the peripheral cytoplasmic membrane, and their effective functioning is dependent upon the integrity of the cell protoplast as a whole.
3. Its **rigid cell wall** usually contains as its main strengthening element a specific peptidoglycan substance not found in eukaryotic organisms.

3.1 ANATOMY OF THE BACTERIAL CELL

The *protoplast*, i.e. the whole body of living material (*protoplasm)*, is bounded peripherally by a very thin, elastic and semipermeable ***cytoplasmic membrane***. Outside, and closely covering this, lies the rigid, supporting ***cell wall***, which is porous and relatively permeable. Cell division occurs by the development, from the periphery inwards, of a transverse cytoplasmic membrane and a transverse cell wall, or *cross-wall*.

The *cytoplasm*, or main part of the protoplasm, consists of a watery sap packed with large numbers of small granules called *ribosomes* and a few convoluted membranous bodies called mesosomes (see below). The nuclear material, nucleus or *chromatin*, is not normally seen with the light microscope. In addition to these essential structures, other intracellular and extra¬cellular structures may be present in some species of bacteria; their occurrence sometimes depends upon particular conditions of growth.

Inclusion granules of storage products such as volutin (polyphosphate), lipid (poly-β-hydroxybutyrate), glycogen or starch may occur in the cytoplasm. Outside the cell wall, there may be a protective gelatinous covering layer called ***a capsule*** or, when it is too thin to be resolved with the light microscope (<0.2 μm), a ***microcapsule***. Soluble large-molecular material may be dispersed by the bacterium into the environment as ***loose slime***. Some bacteria bear, protruding outwards from the cell wall, one or more kinds of filamentous appendages: ***flagella***, which are organs of locomotion; *fimbriae*, which appear to be organs of adhesion; ***and pili***, which are involved in the transfer of genetic material. Because

they are exposed to contact and interaction with the cells and humoral substances of the body of the host, the surface structures of bacteria are the structures most likely to have special roles in the processes of infection.

3.1.1 Bacterial nucleus (DNA)

The genetic information of a bacterial cell is contained in a single, long molecule of double-stranded deoxyribonucleic acid (DNA) which can be extracted in the form of a closed circular thread about 1 mm long. The cell solves the problem of packaging this enormous macromolecule by condensing and looping it into a *supercoiled* state. As well as the chromosome, the bacterium may contain one or more additional fragments of DNA, known as ***episomes or plasmids***.

Because the absence of a nuclear membrane in prokaryotes, the physical separation of the mRNA synthesis (transcription) and translation does not occur in prokaryotes, the two processes often occur simultaneously and are said to be coupled.

Some bacteria contain additional DNA molecules beyond the genomic DNA. These addidonal molecules are referred to as **PLASMIDS** - double stranded and normally circular - significantly smaller in size than the chromosomal DNA. Plasmids are most commonly found in gram-negative bacteria, and although not usually essential for cellular survival.

Many plasmids confer resistance to one or more antibiotics. Because they can be spread from cell to cell, they contribute to proliferation of multidrug-resistant bacterial strains, making treatment of infections caused by these organisms very difficult.

Bacteria, in contrast, with eukaryotes reproduce by a process known as **binary fission**: the bacterial cell grows to a genetically predetermined length at which point a cross-wall is formed -and two new cells are produced. coupled with this process is the replication of the chromosome and any plasmid DNA, so that the daughter cell contains a duplicate of the parental genome.

3.1.2 Cytoplasm of bacteria

The cytoplasm of the bacterial cell is a viscous watery solution, or soft gel, containing a variety of organic and inorganic solutes, and numerous *ribosomes*. The cytoplasm of bacteria differs from that of the higher eukaryotic organisms in not containing an endo-plasmic reticulum or membrane-bearing microsomes, in not containing mitochondria and in not showing signs of internal mobility such as cytoplasmic streaming, the formation, migration and disappearance of vacuoles, and amoeboid movement.

Ribosomes: Bacterial ribosomes are slightly smaller (10-20nm) than those of eukaryotic cells and they have a sedimentation constant of 70S, being composed of a 30S and a 50S subunit (cf. 40S and 60S in the 80S eukaryotic counterparts). They may be seen with the electron microscope, and number tens of thousands per cell.

Protein synthesis in both bacteria and eukaryodc cells takes place on ribosomes, which are a complex of protein and ribonucleic acid (rRNA). This difference in ribosome structure is the mechanism by which certain antibiotics selectively inhibit protein synthesis by prokaryotic but not eukaryotic ribosomes. Bacteria, do not possess mitochondria, but here are localized some enzymes to carry out these same functions.

Inclusion granules: In many species of bacteria, round granules are observed in the cytoplasm. These are not permanent or essential structures, and may be absent under certain conditions of growth.

Mesosomes: These are convoluted or multilaminated membranous bodies visible in the electron microscope. They develop by complex invagination of the cytoplasmic membrane into the cytoplasm, sometimes in relation to the nuclear body and often from the sites of cross-wall formation in Gram-positive bacteria. Mesosomes are thought to be involved in mechanisms responsible for the compartmenting of DNA

at cell division and at sporulation. They may also have a function analogous to the mitochondria of the eukaryotic cell - providing a membranous support for respiratory enzymes.

3.1.3 Cytoplasmic membrane

The classical model of a 'unit membrane' is: **lipid molecules are arrayed in a double layer with their hydrophilic polar regions externally aligned and in contact with a layer of protein at each surface.** The functions of membranes differ widely in nature and it is clear that this simplified model cannot account for all of the variations of function that are already known.

The cytoplasmic membrane constitutes an **osmotic barrier** that is impermeable to many small molecular solutes and is responsible for maintaining the differences in solute content between the cytoplasm and the external environment. **It permits the passive diffusion inwards and outwards** of water and certain other small molecular substances, especially lipid-soluble ones, and it actively effects the selective transport of specific nutrient solutes into the cell and that of waste products out of it. In addition to the enzymes, *or permeases*, responsible for the active uptake of nutrients, the cytoplasmic membrane contains many other kinds of enzymes, notably respiratory enzymes and pigments (cytochrome system), certain enzymes of the tricarboxylic acid cycle and, probably, polymerizing enzymes that manufacture the substances of the cell wall and extracellular structures. It has little mechanical strength and is supported on the outside by the cell wall.

3.1.4 Cell wall

The cell wall encases the protoplast and lies immediately external to the cytoplasmic membrane. It is 10-25 nm thick, strong and relatively rigid, though with some elasticity, and openly porous, being freely permeable to solute molecules smaller than 10 kDa in mass and 1 nm in diameter. It supports the weak cytoplasmic membrane against the high internal osmotic pressure of the protoplasm (usually between 5 and 25 atm) and maintains the characteristic shape of the bacterium in its coccal, bacillary, filamentous or spiral form.

The integrity of the cell wall is essential to the viability of the bacterium. If the wall is weakened or ruptured, the protoplasm may swell from osmotic inflow of water and burst the weak cytoplasmic membrane. This process of lethal disintegration and dissolution is termed *lysis*.

The *cell wall* plays an important part **in *cell division***.

The **chemical composition** of the cell wall differs considerably between different bacterial species, but in all species the main strengthening component is *peptidoglycan (syn. mucopeptide or murein*). **Peptidoglycan** is composed of **N-acetylglucosamine and N-acetylmuramic acid molecules linked alternately in a chain. The N-acetylmuramic acid units each carry a short peptide,** usually consisting of L-alanine, D-glutamic acid, either was **o-diaminopimelic acid** (in Gram-negative bacteria) or L-lysine (in Gram-positive bacteria) and D-alanyl-D-alanine. The wall is given its strength by cross-links that form between adjacent strands. Several antibiotics interfere with the construction of the cell wall peptidoglycan. **The β-lactam antibiotics (penicillins and cephalosporins)** inhibit peptidoglycan synthesis. Thus the interruption of the integrity of the peptidoglycan usually results in death of the bacterial cell.

Biologically, peptidoglycan **interferes with phagocytosis, is mitogenic** (stimulates mitosis of lymphocytes), and **has pyrogenic activity** (induces fever).

The peptidoglycan of some streptococcal species contributes to the **establishment of the chronic inflammatory response in a rheumatoid arthritis and rheumatic fever model system**.

The walls of Gram-positive bacteria contain more peptidoglycan and are thicker and stronger (more extensively cross-linked) than those of **Gram-negative bacteria**. This difference is associated with a higher internal osmotic pressure in Gram-positive cells.

The peptidoglycan provides rigidity and it also determines the shape of the particular bacterial cell. Chemically represents a regular heteropolymer of alternating molecules of N-acetylglucoseamine (GlcNAc) and N-acetylmuramic acid (MurNAc).

The bacterial cell wall also contains other components whose nature and amount vary with the species. Many **Gram-positive** bacteria contain relatively large amounts of *teichoic acid* (a polymer of ribitol or glycerol phosphate complexed with sugar residues) interspersed with the peptidoglycan; some of this material (*lipoteichoic acid*) is linked to lipids buried in the cell membrane. **Gram-negative** bacteria possess a complex membrane-like structure external to the peptidoglycan layer. In *E.coli* this comprises lipid, polysaccharide, lipoprotein and lipopolysaccharide, and makes up over 80% of the wall weight. This outer membrane confers several important properties on Gram-negative bacteria: it protects the peptidoglycan from the effects of lysozyme and it impedes the ingress of many antibiotics that are thus rendered impotent. Components of the lipo-polysaccharide, in particular the core structure, lipid A, *forms endotoxin*, which, when released in the bloodstream, may give rise to *endotoxin shock.*

Both gram-positive and gram-negative bacteria contain peptidoglycan in their cell walls (multiple layers are often cross-linked in three dimensions, providing a very strong, rigid cell wall); the two types of cell walls are very different.

First, the amount of peptidoglycan is quite different in that the peptidoglycan of gram-positive cell walls is usually 30 to 200 molecules thick, providing 40% to 80% of the total cell wall dry weight. In contrast, the peptidoglycan in gram-negative cell walls is usually only one molecule (layer) thick.

Gram-positive cell walls also contain so called teichoic acids, which are common surface antigens and function as structures that attach to other bacteria, as well as to specific receptors on mammalian cell surfaces. These teichoic acids are important factors in virulence.

Gram-negative cell wall is much more complex, both structurally and chemically. Structurally, the gram-negative cell wall contains two layers external to the cytoplasmic membrane.

Immediately external to the CM is a thin peptidoglycan layer, which accounts for only 5% to 10% of the gram-negative cell wall by weight. It is unusual for this layer to contain interpeptide bridges; thus it is much less rigid than the gram-positive peptidoglycan.

External to the peptidoglycan is **the outer membrane**. The area between the external surface of the CM and the internal face of the outer membrane is referred to as the **periplasmic space**. This space is containing:

- hydrolytic enzymes - breakdown of large macromolecules into smaller products that the cell uses for metabolism (proteases, phosphatases, lipases, nucleases, carbohydrate-degrading)
- many of the lytic virulence factors (collagenases, hyaluronidases, and proteases) - in the case of pathogenic gram-negative species
- binding proteins, (PBPs)which bind specific molecules (penicillin) for transport into the cytoplasm.

Outer Membrane is External to the peptidoglycan. It is unique to gram-negative prokaryotes. It functions by regulating the passage of molecules greater than 700 daltons (d) into the cell. This membrane is a typical lipid bilayer but differs from any other biological membrane in its lipid composition and number of proteins.

The inner lipid membrane contains phospholipids that are normally found in bacterial membranes. The outer one is composed by a so called **lipopolysaccharide (LPS).** Because of its toxic and other biological properties this molecule was originally named the endotoxin. This is the only location where LPS molecules are found. Importantly, many of the proteins of the outer membrane traverse the entire lipid bilayer and are trans-membrane proteins. A group of these proteins are known as porins because they form pores (channals) through the membrane that allow the diffusion of hydrophilic molecules through the membrane. The outer membrane also contains integral proteins, which, maintain the integrity of the outer membrane, as well as peripheral proteins or surface proteins, some of which are receptor molecules for bacteriophages. The association between the outer membrane and the peptidoglycan is maintained by a lipoprotein, which is an intrinsic protein of the outer membrane and is linked to the peptidoglycan.

The LPS molecule is composed of three main regions:

(1) the hydrophobic lipid A

consists of a glucosamine disaccharide whose hydroxyl groups are esterified with β-hydroxy fatty acids, the only β-hydroxy acids found in prokaryotes.

(2) the core polysaccharide - links the lipid A region to the 0-antigen polysaccharide

(3) **the hydrophilic O-antigen or O-specific polysaccharide-** consists of repeating units of a tetrasaccharide or pentasaccharide subunit. The specific composition of these repeating units varies, producing the 0-somatic antigen on which serotyping of many different gram-negative species is based (there are hundreds of different serotypes of Salmonella based on the 0-antigen composition.

All chemical forms of LPS that have very little or no O-antigen are called "rough" LPS molecules (R), as opposed "smooth" molecules(S). The presence of the O-antigen is important in resistance to certain antibiotics and may be important for survival in the host.

LPS is a heat stable toxin (even stable to autoclaving), which is liberated from gram-negative bacteria upon lysis and cell death. This endotoxic activity is associated with the lipid A component and produces a broad spectrum of pathophysiological effects.

In **lesser amounts, LPS** functions as an activator of many inflammatory mediators including activation of the complement cascade by the alternate pathway, and activation of tumor necrosis factor, interleukin 1, and prostaglandins.

When present **in sufficient quantities** in the blood, LPS will cause death within 1 or 2 hours because of irreversible shock and cardiovascular collapse. In addition, it is pyrogenic, causes platelet aggregation, increases resistance to certain antibiotics, causes resistance to phagocytosis, and plays a role in bone resorption.

LPS of GNB is among the most active components of prokaryotes.

Lysozyme is a natural body defence substance which lyses bacteria of many species. It cleaves the link between N-acetylglucosamine and N-acetylmuramic acid. Bacteriophages possess a lysozyme-like enzyme that allows their initial penetration into the bacterium and, after they have reproduced, causes lysis of the bacterium.

Biologically, peptidoglycan interferes with phagocytosis, is mitogenic (stimulates mitosis of lymphocytes), and has pyrogenic activity (induces fever). The peptidoglycan of some streptococcal species contributes to the establishment of the chronic inflammatory response in a rheumatoid arthritis and rheumatic fever model system.

3.1.5 Capsules, microcapsules and loose slime

Many bacteria are surrounded by a discrete covering layer of a relatively firm gelatinous material that lies outside and immediately in contact with the cell wall. When this layer, in the wet state, is wide enough (0.2 μm or more) to be resolved with the light microscope, it is called a capsule. When it is narrower, and detectable only by indirect, serological means, or by electron microscopy, it may be termed *a micro-capsule*. The capsular gel consists largely of water and has only a small content (e.g. 2%) of solids. In most species, the solid material is a complex polysaccharide, though in some species its main constituent is polypeptide or protein.

Loose slime, or free slime, is an amorphous, viscid colloidal material that is secreted extracellularly by some non-capsulate bacteria and also, outside their capsules, by many capsulate bacteria. In capsulate bacteria the slime is generally similar in chemical composition and antigenic character to the capsular substance. When slime-forming bacteria are grown on a solid culture medium, the slime remains around the bacteria as a matrix in which they are embedded and its presence confers on the growths a watery and sticky 'mucoid' character. The slime is freely soluble in water and, when the bacteria are grown or suspended in a liquid medium, it passes away from them and disperses through the medium.

Microcapsules may not be seen at all, and for this reason their presence has generally to be deduced from serological evidence that the cell wall antigen (e.g. the O, or somatic, antigen in Enterobacteriaceae) is masked by a covering layer (e.g. of K, or 'capsular', antigen).

Function: It is not known with certainty what the functions of capsules and microcapsules are, but it is probable that their principal action is in **protecting** the cell wall against attack by various kinds of antibacterial agents, e.g. bacteriophages, colicines, complement, lysozyme and other lytic enzymes, that otherwise would more readily damage or destroy it. In the case of certain capsulate pathogenic organisms

(e.g. pneumococcus, pyogenic streptococci, anthrax bacillus and plague bacillus) there is good evidence that the capsule protects the bacteria against ingestion by the phagocytes of the host. The capsule is thus an **important virulence determinant**, and non-capsulate mutants of these bacteria are non-virulent. In some organisms the capsule contains more than one functional component. Thus, *Str. pyogenes*, which under favourable conditions of growth may form an antiphagocytic capsule composed of hyaluronic acid, also possesses a second surface substance, M protein, which inhibits either the ingestion or the intracellular digestion of the cocci by the phagocytes; the M protein occurs in association with the hyaluronic acid capsule or, when the latter is absent, by itself in the form of a microcapsule

The capsular substance is usually **antigenic** and the capsular antigens play a very important part in determining the antigenic specificity of bacteria. When capsulate pneumococci are treated with type-specific antiserum, the sharpness of outline of the capsule is greatly enhanced. This is referred to as the *capsule-swelling reaction.*

3.1.6 Flagella and motility

Motile bacteria possess filamentous appendages known as *flagella*, which act as organs of locomotion. The flagellum is a long, thin filament, twisted spirally in an open, regular wave form. It is about 0.02 μm thick and is usually several times the length of the bacterial cell. It originates in the bacterial protoplasm and is extruded through the cell wall. According to the species, there may be one, or up to 20 flagella, per cell. In elongated bacteria the arrangement of the flagella may *be peritrichous,* or *lateral*, when they originate from the sides of the cell, or *polar*, when they originate from one or both ends. Where several occur on a cell, they may function coiled together as a single 'tail'. Flagella consist largely or entirely of a protein, ***flagellin***, belonging to the same chemical group as myosin, the contractile protein of muscle.

They are invisible in ordinary preparations by the light microscope, but may be shown by the use of special staining methods, and in special circumstances by dark-ground illumination.

Motility may be observed microscopically or by the occurrence of spreading growth in semi-solid agar medium. By microscopy of wet films, motile bacteria are seen swimming in different directions across the field, with a darting, wriggling or tumbling movement.

Function: It is not known with certainty what advantage a bacterium derives from its ability to move actively. **Motility** may be beneficial in increasing the rate of uptake of nutrient solutes by continuously changing the environmental fluid in contact with the bacterial cell surface. Random movement and dispersion through the environment may be beneficial ensuring that at least some cells of a strain reach every locality suitable for colonization.

3.1.7 Fimbriae

Certain Gram-negative bacilli, including intestinal commensal and pathogenic species, possess filamentous appendages of a different kind from the flagella. These are called fimbriae and they occur in non-motile, as well as in motile strains. They are far more numerous than flagella (e.g. 100-500 being borne peritrichously by each cell) and are much shorter and only about half as thick (e.g. varying from 0.1 to 1.5 μm in length and having a uniform width between 4 and 8 nm). They do not have the smoothly curved spiral form of flagella and are mostly more or less straight. They cannot be seen with the light microscope but are clearly seen with the electron microscope in preparations that have been metal-shadowed or negatively stained with phosphotungstic acid.

Fimbriate strains of bacteria often undergo a reversible variation between a fimbriate phase and a non-fimbriate phase that is affected by the conditions of growth. Most bacilli become fimbriate, as a result of prolonged culture or serial subculture, in static liquid medium incubated aerobically. The non-fimbriate phase predominates when subcultures are made serially on a solid culture medium.

Function: Fimbriae probably function as organs of **adhesion**. The possession of fimbriae confers on bacilli the power of adhering firmly to solid surfaces of various kinds, including those of the cells of animals, plants and fungi. Comparable non-fimbriate bacilli do not adhere when they collide with such surfaces.

The presence of fimbriae is a characteristic most common among pathogenic bacteria of the mucosal surfaces. As such, urinary tract pathogens such as *Escherichia coli* typically are fimbriated. The presence of specific fimbriae is a requirement for *E. coli* to colonize and infect the urinary tract.

One of the strongest cases for the direct relationship of fimbriation and virulence is *Neisseria gonorrhoeae*. Virulent strains of *N. gonorrhoeae* are fimbriated and are able to adhere to genital tract mucosal surfaces.

Pili (sex pili): These structures are similar to fimbriae, but are functionally different. They are longer than fimbriae and confer the ability to attach specifically to other bacteria that lack these appendages. Pili appear to be involved in the transfer of DNA during conjugation; they also act as receptor sites for certain bacteriophages described as being 'donor-specific'.

3.1.8 Bacterial spores

Some bacteria, notably those of the genera *Bacillus and Clostridium*, develop a highly resistant resting phase *or endospore*, whereby the organism can survive in a dormant state through a long period of starvation or other adverse environmental condition. The process does not involve multiplication: in *sporulation*, each vegetative cell forms only one spore, and in subsequent *germination* each spore gives rise to a single vegetative cell. Certain specific antigens develop in the spore that are not found in the vegetative cells.

Sporulation: Sporulation occurs as a response to starvation or, at least, the exhaustion of a limiting substance. It does not take place as long as conditions continue to favour maximal vegetative growth. In certain species, sporulation may be induced by depletion of nutrients necessary for vegetative growth, e.g. the carbon and energy source, the nitrogen source, sulphate, phosphate or iron salt; at the same time, the process requires a continued supply of other minerals (potassium, magnesium, manganese and calcium salts), and favourable conditions of moisture, temperature, pH, oxygen tension, etc. The spore is formed inside the parent vegetative cell (hence the name 'endospore'). It develops from a portion of protoplasm near one end of the cell (the 'forespore'), incorporates part of the nuclear material (equivalent to one genome) of the cell and acquires a thick covering layer, the 'cortex', and a thin, but tough, outer 'spore coat' consisting of several layers. Spores of some species have an additional, apparently rather loose covering known as *the exosporium*. Finally, the remainder of the parent cell disintegrates and the spore is freed.

Viability: Spores are much more resistant than the vegetative forms to exposure to disinfectants, drying and heating. Thus, application of moist heat at 100-120°C for a period of 10-20 min may be needed to kill spores, whereas heating at 60°C suffices to kill vegetative cells. In the dry state, or in moist conditions unfavourable to growth, spores may remain viable for many years. The marked resistance of spores has been attributed to several factors in which they differ from vegetative cells: the impermeability of their cortex and outer coat, their high content of calcium and dipicolinic acid, their low content of water and their very low metabolic and enzymic activity.

Germination: Germination of the spore occurs when the external conditions become favourable to growth by access to moisture and nutrients. It is irreversible and involves rapid degradative changes. The spore successively loses its heat resistance and its dipicolinic acid, it loses calcium, it becomes permeable to dyes and its refractility changes. Spores that have survived exposure to severe adverse influences such as heat are much more exacting than normal spores in their requirements for germination.

3.2 BACTERIAL REPRODUCTION

Among the 'lower' or true bacteria, multiplication takes place by *simple binary fission*. The cell grows in size, usually elongating to twice its original length, and the protoplasm becomes divided into two approximately equal parts by the ingrowth of a transverse septum from the plasma membrane and cell wall. In some species, the cell wall septum, or cross-wall, splits in two and the daughter cells separate almost immediately. In others, the cell walls of the daughter cells remain continuous for some time after cell division and the organisms grow, adhering in pairs, clusters, chains or filaments. If cross-wall splitting is thus delayed in an organism in which the cross-walls of successive cell divisions are all formed in parallel planes, the cells will be grouped in pairs, chains, rods or filaments.

4. BACTERIAL METABOLISM

All living cells require **water** as well as certain **nutrients** to grow and divide. Among the inorganic substances (trace elements) are ions such as Mg2+, Zn2+, Mn2+, Co2+, and Fe3+, which function as cofactors in a variety of enzyme systems. Similarly, Na+ is essential for certain nutrient transport systems. Phosphorous (as P043-) is an essential constituent of nucleotides and phospholipids, whereas sulfur is found in certain coenzymes (e.g., acetyl-CoA) as well as in two of the amino acids. Similarly, a source of nitrogen (either as NH3+ or organic nitrogen compounds) is essential for the synthesis of amino acids and nucleotides. Finally, oxygen is a component of a variety of essential compounds and, in addition, functions in its elemental form as the terminal electron acceptor in aerobic respiration, the process by which energy in the form of adenosine triphosphate (ATP) is produced.

Among the **organic compounds** necessary for growth are **carbohydrates**, which function in cellular structure and energy production, **amino acids and nucleotides**, which represent the building blocks for proteins and **nucleic acids**, respectively; and **lipids**, which are the major component of the cellular membrane. Finally, the vitamins, like the trace elements described above, are required in small quantities and function as cofactors for certain enzymatic reactions.

The vast majority of bacteria, however, fall in between and will grow in either the **presence or the absence of oxygen**. These bacteria are referred to as **facultative**. One may also classify bacteria on the basis of the predominant source of carbon. Those that utilize CO2 as the predominant source of carbon for the synthesis of organic compounds are referred to as **autotrophs (lithotrophs),** whereas cells, like animal cells, that utilize organic carbon sources are known as **heterotrophs (organotrophs).**

1. **Obligate aerobes:** grow in the presence of oxygen, with no fermentation and with oxidative phosphorylation
2. **Obligate anaerobes:** with no oxidative phosphorylation, they use fermentation, are killed by oxygen and lack certain enzymes (superoxide dismutase: $O_2^- + 2H^+$ to H_2O_2), catalase (H_2O_2 to $H_20 + O_2$), peroxidase (H_2O_2 to H_20 /NAD to NADH)
3. **Aerotolerant anaerobes:** respire anaerobically and not killed by oxygen
4. **Facultative anaerobes:** use fermentation, have aerobic respiration and survive in oxygen
5. **Microaerophilic bacteria:** grow in low oxygen concentration and are killed by the high oxygen concentration; they need a higher concentration of CO_2.

4.1 GROWTH - NUTRITIONAL REQUIREMENTS:

a. **Oxygen**. It is necessary to provide oxygen for a strict aerobe and to remove it completely from the environment of a strict anaerobe. The growth of an strict anaerobic bacteria may be inhibited by an oxygen tension as low as 10-5 atm.

b. **Carbon dioxide**. All bacteria require the presence of a small amount of carbon dioxide for growth, normally provided by the atmosphere or by oxidation and fermentation within the cell itself. Some bacteria, when first isolated from the body require a much higher concentration of carbon dioxide (5-10%), and this must provided in the environment of the culture medium.

c. **Inorganic ions**. Phosphate, potassium, magnesium, natrium, sulfur as well as numerous trace metals are essential for bacterial growth.

d. **Organic nutrients**: carbohydrates as energy source, amino acids, vitamins, purines and pyrimidines

4.2 GROWTH - PHYSICAL REQUIREMENTS:

a Influence of temperature.

i **On growth.** For each species there is a definite temperature range within growth takes place. In the laboratory, bacteria are grown at this optimum temperature in a thermostatically controlled incubator. The **optimum temperature** of bacteria that are parasitic on humans is about **37°C**. These are termed as mesophilic bacteria. A group of soil and water bacteria grow best below 20°C, quite well at 0°C, and are termed as phyhrophiles. The termophiles have a minimum growth temperature above 40°C. *B. stearothermophilus* is killed only after exposure to 121°C for 10-35 minutes.

ii **On viability**. Heat is an important agent in the artificial destruction of bacteria. The bacteria are most sensible to moist heat that to dry heat. (hot air oven and autoclave). At low temperatures some species die rapidly, but the majority survive well. Cultures may be preserved for a long time at between 3-5°C, in deep freeze cabinet or in liquid nitrogen.

b Influence of moisture and desiccation.

Drying in air is injurious to many microbes, and the different species vary widely in their ability to survive when died under natural conditions, as in infected exudates smeared on clothing or furniture, and in dust. Thus, the gonococcus, *Treponema pallidum* die quickly, whereas the *tubercule Bacillus, S. aureus* and the smallpox virus may survive for months. The spores of *B. anthracis* have survived for over 60 years. Organisms may survive drying for a long period of years in that are desiccated rapidly and completely, while frozen, and thereafter maintained in a high vacuum in a sealed glass ampoule stored at room temperature in the dark This is the basis of the lyophilization or freeze-drying process of preserving bacterial cultures in the laboratory.

c Influence of hydrogen concentration.

The majority of commensal and pathogenic bacteria grow best at a neutral or very slightly alkaline reaction (pH 7.2-7.4).

d Influence of light and other radiations.

Darkness provides a favorable condition for growth and viability. UV rays are rapidly bactericidal. Bacteria are also killed be ionizing radiation.

e Influence of osmotic pressure.

For most species the maximum concentration of sodium chloride permitting growth lies between 5 and 12%. Halophilic (osmophilic) species can grow at higher concentrations.

f Influence of mechanical and sonic stress.

A bacterial suspension may be largely disintegrated by very vigorous shaking with fine glass beads or to ultrasonic vibration. These measures are used in separating the large molecular components of the cell.

4.3 TYPES OF GROWTH

In the laboratory bacterial growth can be seen in three main forms:

1. By the development of **colonies**, the macroscopic product of 20-30 cell divisions of a single bacterium or clump of bacteria on solid media
2. By the transformation of a clear broth medium to a turbid suspension of 10^7 - 10^9 cells per millilitre
3. In **biofilm** formation, in which growth is spread thinly (300-400 μm thick) over an inert surface and nutrition obtained from a bathing fluid.

In natural systems only biofilms, such as those that develop on the surfaces of intravascular cannulae, appear to have the same appearance and properties as growth produced in the laboratory, while colonies, the other form of *sessile growth*, rarely reach macroscopic dimensions. Turbid liquid systems due to *planktonic* growth of a single organism are also a rarity in nature. However, in spite of these unrepresentative features,

growth in macroscopic colonies and growth to high cell densities in broth offer great practical advantages and remain central techniques.

4.3.1 Growth curve in liqiud medium

When an inoculum of bacterial cells from a pure culture of bacteria is introduced into a suitable nutrient medium and incubated under appropriate conditions, almost all of the cells have the potential to grow at a very high rate. Under optimal conditions, four main phases of growth are recognized:

a. **Lag phase**. In this period there is no appreciable multiplication of cells. The duration of this phase varies and represents the time taken for the organism to adapt itself to growth in the fresh medium.

b. **Logarithmic phase**. In this period, the cells divide at a constant rate, and as a result of growth by binary fission, there is a linear relationship between the time and the logarithm of the number of cells.

c. **Stationary phase**. The essential nutrients begin to disappear, and there is a balance between cell growth and division and cell death. At the onset of this phase, many species of bacteria produces secondary metabolites. These include many antibiotics and exotoxins. The sporulation of sporforming species starts at the end of this phase.

d. **Death or decline phase**. During this phase, bacterial lysis and cell destruction cause a reduction in cell number.

4.3.2 Multiplication of bacteria in the body

Conditions encountered by microorganisms in the body selects those that grow within certain limits of temperature, osmolarity and pH. After entering the body of a small number of these germs can multiply rapidly and cause: a serious infection with fatal acute (meningitis) or they will produce an evolving insidious infection, chronic (TB), in the case of bacteria with long generation time.

In the body, growth and multiplication of bacteria is stressed by: nutrition needs (by competing with normal flora) and anti-infectious defense mechanisms.

Higher nutritional demands are neded for bacteria with exclusively human habitat (gonorrhea) than in the other (E. coli). Extracellular bacteria are exposed to the action of antibodies, complement, phagocytosis. Intracellular bacteria are protected by the action of these factors and sometimes escapes immune surveillance. Bacteria develop adaptive mechanisms to bypass barriers opossing their multiplication.

4.4 METABOLISM AND THE CONVERSION OF ENERGY

All cells require a constant supply of energy to survive. This energy, typically in the form of ATP, is derived from the ordered breakdown of various organic substrates (carbohydrates, lipids, and proteins). This process of substrate breakdown and conversion into usable energy is known as catabolism. The energy produced may then be used in the synthesis of cellular constituents (cell walls, proteins, fatty acids, and nucleic acids), a process known as anabolism. Together these two processes, which are interrelated and rightly integrated, are referred to as **intermediary metabolism**.

The specific metabolic pathways used by bacteria for the breakdown and synthesis of organic substrates are, for the most part, shared by all living cells (prokaryotic and eukaryotic).