Lec. 1

Physical and Chemical agents

For Microbial control

Microbial control of microorganisms mean either destroy or remove contaminants. Contamination are microbes present at a given place and time that are undesirable or unwanted . Most decontamination methods employ either physical agents , such as heat or radiation ,or chemical agents, such as disinfectants and antiseptics . Some time , the two categories are used overlap in some cases . For instance , radiation can cause damaging chemicals form , or chemical can generate heat . A Flowchart summarize the major applications and aims in microbial control .

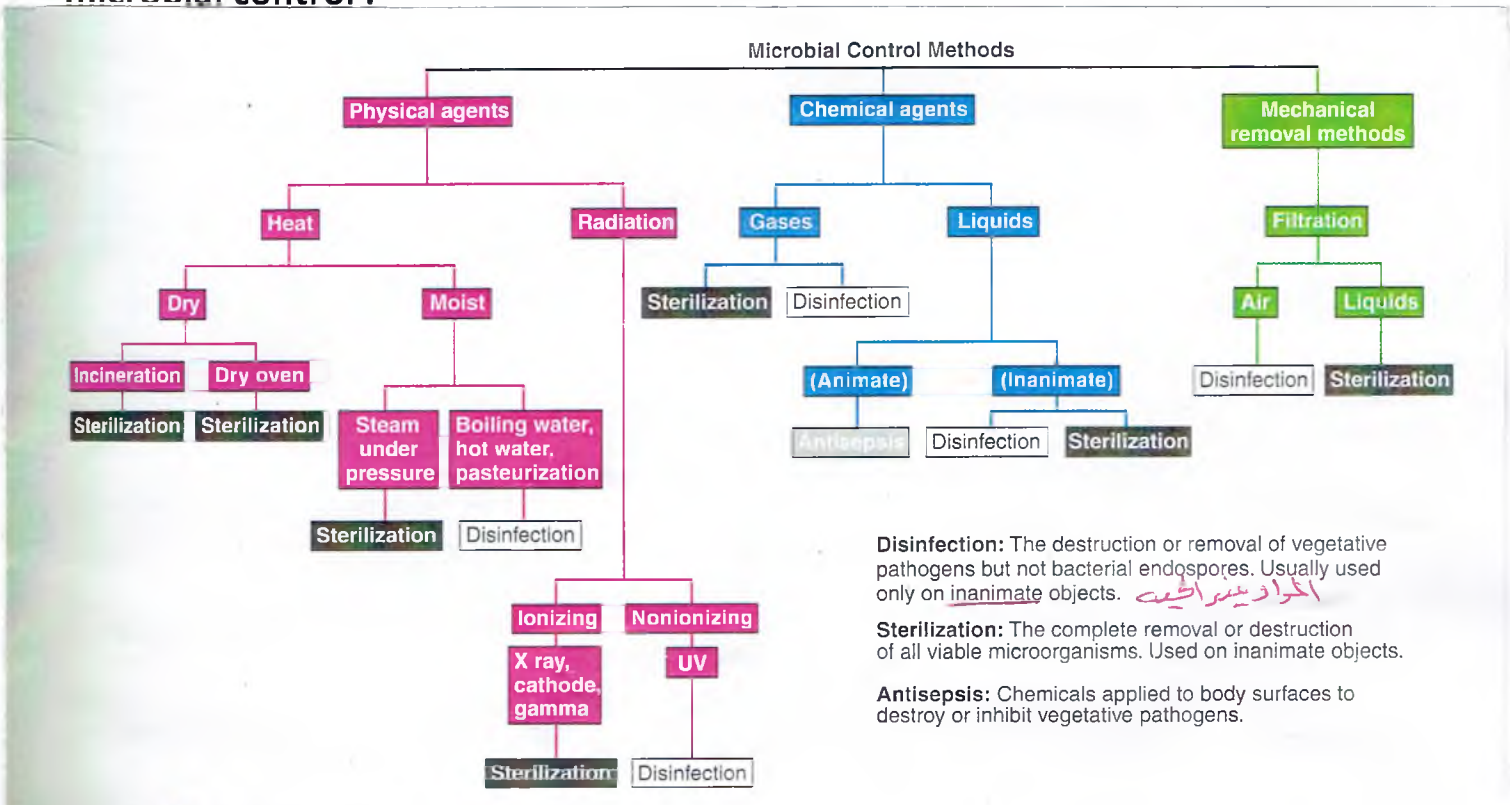


Figure 1: Microbial control methods.

Terminology of Microbial Control

Term	Definition	Examples	Comments
Antisepsis	Reduction in the number of microorganisms and viruses, particularly potential pathogens, on living tissue	Iodine; alcohol	Antiseptics are frequently disinfectants whose strength has been reduced to make them safe for living tissues.
Aseptic	Refers to an environment or procedure free of pathogenic contaminants	Preparation of surgical field; handwashing; flame sterilization of laboratory equipment	Scientists, laboratory technicians, and health care workers routinely follow standardized aseptic techniques.
-cide -cidal	Suffixes indicating destruction of a type of microbe	Bactericide; fungicide; germicide; virucide	Germicides include ethylene oxide, propylene oxide, and aldehydes.
Degerming	Removal of microbes by mechanical means	Handwashing; alcohol swabbing at site of injection	Chemicals play a secondary role to the mechanical removal of microbes.
Disinfection	Destruction of most microorganisms and viruses on nonliving tissue	Phenolics; alcohols; aldehydes; soaps	The term is used primarily in relation to pathogens.
Pasteurization	Use of heat to destroy pathogens and reduce the number of spoilage microorganisms in foods and beverages	Pasteurized milk and fruit juices	Heat treatment is brief to minimize alteration of taste and nutrients; microbes still remain and eventually cause spoilage.
Sanitization	Removal of pathogens from objects to meet public health standards	Washing tableware in scalding water in restaurants	Standards of sanitization vary among governmental jurisdictions.
-stasis -static	Suffixes indicating inhibition, but not complete destruction, of a type of microbe	Bacteriostatic; fungistatic; virustatic	Germistatic agents include some chemicals, refrigeration, and freezing.
Sterilization	Destruction of all microorganisms and viruses in or on an object	Preparation of microbiological culture media and canned food	Typically achieved by steam under pressure, incineration, or ethylene oxide gas.

Resistance of Microbial forms :

The target of microbial control processes are microorganisms capable of causing infection or spoilage that are constantly present in the external environment and on the human body. This targeted population is rarely simple or uniform : in fact, it often contains mixture of microbes with extreme differences in resistance and harmfulness . Contaminants that can have far-reaching effects if not adequately controlled include bacterial vegetative cells , endospores , fungalhyphe, spores , yeasts , protozoa,
trophozotes, cysts, worms , viruses and prione .

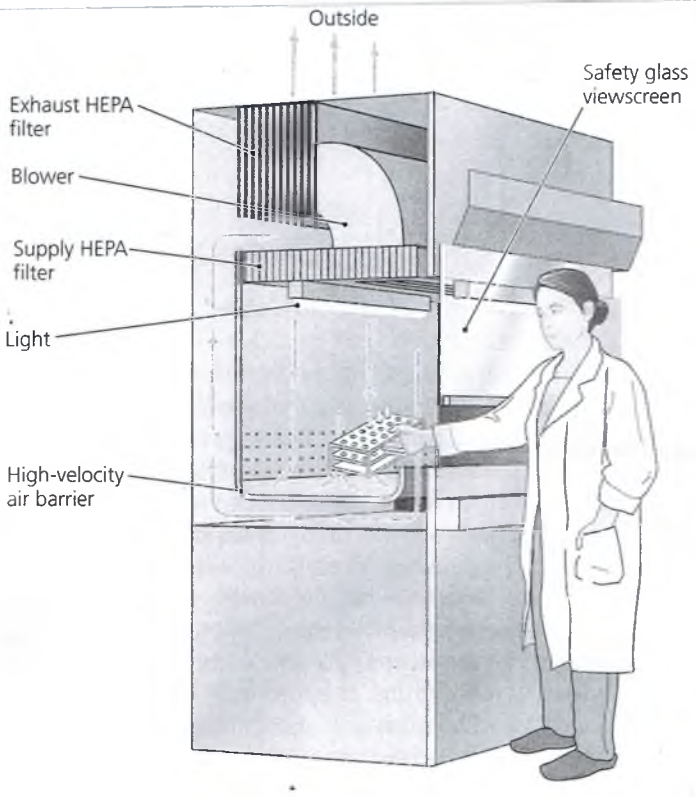
Highest resistance : Prions , bacterial endospores .

Moderate resistance : protozoan cysts , fungal spores (zygospores) , viruses , among the most resistant viruses are the hepatitis B, bacterial vegetative cells .

Least resistance : Most vegetative bacterial cells , fungal hyphae , yeast, enveloped viruses , and protozoan trophozoites .

Bacterial endospores have traditionally been considered the most resistant microbial entities , being as much as 18 times harder to destroy than vegetative cells : destruction of spore is the goal of sterilization . Other methods of control (disinfection , antiseptic) act primarily upon microbes that are less hardy than endospores .

Method	Endospores	Vegetative forms	Relative resistance
Heat(moist)	12OC^0	80 C^0	1.5 X
Radiation (x-ray) dosage	4000 grays	1000 grays	4 X
Sterilization gas (ethylene oxide)	1200 mg IL	700 mg IL	1.7 X
Sporicidal liquid (2% gluter aldehyde)	3 h	10 min	18 X



▲ **Figure 9.10 The roles of high-efficiency particulate air (HEPA) filters in biological safety cabinets.** HEPA filters protect workers from exposure to microbes (by maintaining a barrier of filtered air across the opening of the cabinet). Hospital also use HEPA filters in air ducts of operating rooms and of the rooms of highly contagious or immunocompromised patients.

TABLE 9.3 Membrane Filters

Pore Size (μm)	Smallest Microbes That Are Trapped
5	Multicellular algae, animals, and fungi
3	Yeasts and larger unicellular algae
1.2	Protozoa and small unicellular algae
0.45	Largest bacteria
0.22	Largest viruses and most bacteria
0.025	Larger viruses and pliable bacteria (mycoplasmas, rickettsias, chlamydias, and some spirochetes)
0.01	Smallest viruses

Sterilization:

Sterilization is a process that destroys or removes all viable microorganisms , including viruses . Any material that has been subjected to this process is said to be sterile.

Bactericide: chemical that destroyed bacteria except for those in the endospore stage .

Fungicide : Chemical that can kill fungal spores , hyphae and yeast .

Virucide : Any chemical known to inactivate viruses , especially on living tissue.

Sporcide : Agent capable of destroying bacterial endospores .

Germicides , Disinfection , Antisepsis :

Germicide , and also called a microbicide , is any chemical agent that kills Pathogenic microorganisms .A germicide can be used on nonliving materials or on living tissue , but it ordinarily cannot kill resistant microbial cells .

Disinfection : Refer to the use of a physical process or a chemical agent to destroy vegetative pathogens but not bacterial endospores . normally used only for nonliving objects because they can be toxic to human And other animal tissue .

EX . application of 5%bleach to an examining table, Immersing thermometers in on iodine solution between uses .

Antisepsis :Chemical agents are applied directly to exposed body surfaces (skin and mucous membranes), wounds , and surgical incisions to destroy or inhibit vegetative pathogens .Examples of antisepsis include preparing the skin before surgical operation with iodine compounds , swabbing a wound with hydrogen peroxide , and ordinary hand washing with a germicidal soap .

Practical concerns in microbial control agents :

1-Dose the application require sterilization or is disinfection adequate, for spore or vegetative ?

2- Is the item to be reused or permanently discarded ? if it will be discarded , then the quickest and least expensive method should be chosen ?

3- If it will be reused , can the item withstand heat , pressure, radiation or chemicals ?

4 – Is the control method suitable for a given application ? (for example material) ultraviolet radiation is good sporicidal agent , but it will not penetrate solid or, in the case of a chemical , will it leave on undesirable residue ?

5 – will the agent penetrate to the necessary extent ?

6 – Is the method cost and labor – efficient , and is if safe ?

Modes of Action of Antimicrobial agents :

The mechanism action of antimicrobial agents affect in one or more of the following targets (chemical and physical) :

1-The cell wall.⇐ Drugs (penicillin) , detergents , alcohol.

2 – The cell membrane . ⇐ Detergents(surfactants), important ions seep out.

3 – Cellular synthetic processes (DNA , RNA) . (chloramphenicol bind ribosome)

4 – Synthesis of proteins ⇐ chloramphenicol binds to the ribosomes of bacteria in a way that stops peptide bounds from forming .

I – Methods of physical control :

Many microorganisms have adapted to a tremendous diversity of habitat on earth, even severe conditions of temperature , moisture , pressure and light .

1 – Heat :

The elevated temperatures exceeding the maximum growth temperature are micrtistatic . The two physical states of heat used for microbial control are moist and dry . Moist heat occure in the form of hot water , boiling water , or steam . In practical , the temperature of moist heat usually ranges from 60 C^0 to 135 C^0 . The temperature of steam can be regulated by adjusting its pressure in a closed container, dry heat denote air with flame or electric heating coil . In practice, the temperature of dry heat ranges from 160 C^0 to several thousands degrees Celsius .

Mode of Action :

Moist heat and dry heat differ in their modes of actions as well as in their efficiency . Moist heat operates at lower temperature and shorter time of exposure to achieve the same effectiveness as dry heat . Moist heat effect are the

Physical Methods of Microbial Control

Method	Conditions	Action	Representative Use(s)
Moist heat			
Boiling	10 min at 100°C	Denatures proteins and destroys membranes	Disinfection of baby bottles and sanitization of restaurant cookware and tableware
Autoclaving (pressure cooking)	15 min at 121°C	Denatures proteins and destroys membranes	Autoclave: sterilization of medical and laboratory supplies that can tolerate heat and moisture; pressure cooker: sterilization of canned food
Pasteurization	15 sec at 72°C	Denatures proteins and destroys membranes	Destruction of all pathogens and most spoilage microbes in dairy products, fruit juices, beer, and wine
Ultrahigh-temperature sterilization	1–3 sec at 140°C	Denatures proteins and destroys membranes	Sterilization of dairy products
Dry heat			
Hot air	2 h at 160°C or 1 h at 171°C	Denatures proteins, destroys membranes, oxidizes metabolic compounds	Sterilization of water-sensitive materials such as powders, oils, and metals
Incineration	1 sec at more than 1000°C	Oxidizes everything completely	Sterilization of inoculating loops, flammable contaminated medical waste, and diseased carcasses
Refrigeration	0–7°C	Inhibits metabolism	Preservation of food
Freezing		Inhibits metabolism	Long-term preservation of foods, drugs, and cultures
Desiccation (drying)	Varies with amount of water to be removed	Inhibits metabolism	Preservation of food
Lyophilization (freeze drying)	−196°C for a few minutes while drying	Inhibits metabolism	Long-term storage of bacterial cultures
Filtration	Filter retains microbes	Physically separates microbes from air and liquids	Sterilization of air and heat-sensitive ophthalmic and enzymatic solutions, vaccines, and antibiotics
Osmotic pressure	Exposure to hypertonic solutions	Inhibits metabolism	Preservation of food
Ionizing radiation (electron beams, gamma rays, X rays)	Seconds to hours of exposure (depending on wavelength of radiation)	Destroys DNA	Sterilization of medical and laboratory equipment and preservation of food
Nonionizing radiation (ultraviolet light)	Irradiation with 260-nm-wavelength radiation	Formation of thymine dimers inhibits DNA transcription and replication	Disinfection and sterilization of surfaces and of transparent fluids and gases

coagulation and denaturation of proteins , which quickly and permanently halt cellular metabolism .

Dry heat of a moderate temperature dehydrate the cell , removing the water necessary for metabolic reactions and also alters protein structure . the use of higher temperature when dry heat is used lead to oxidizes cell , burning them to ashes .

The effects of cold and desiccation :

The principal benefit of cold temperature is to slow growth of cultures and microbes in food during processing and storage . Freezing temperature ranging from -70 C^0 to – 135 C^0 used for preserve culture of bacteria , viruses and fungi for long periods .

2 – Radiation:

Radiation is defined as energy emitted from atomic activities and dispersed at high velocity through matter or space . Also , radiation exists in many states and can be described and characterized in various way . we will consider only those types suitable for microbial control : gamma rays , x- rays and ultraviolet radiation .

Modes of action :

When the cell is bombarded by certain wave or particles , its molecules absorb some of the available energy , leading to one of two consequences .

(1) if the radiation ejects orbital electrons from an atom , it causes ions to form, this type of radiation is termed ionizing radiation , one of the most sensitive targets for ionizing radiation is DNA . which will undergo mutation on a broad scale . Secondary lethal effects appears to be chemical change in organelles and the production of toxic substances . Gamma rays . x-rays , and high – speed of electrons are all ionizing in their effects . (2) non – ionizing radiation , test examplified by U.V. excites atoms by raising them to higher energy state , but it does not ionize them . this atomic excitation , in turn , leads to the formation of abnormal bonds within molecules such as DNA and is thus a source of mutation .

Application of ionizing radiation :

It applied for foods since 50 years ago (meat ,flour , fruits, and vegetables) . radiation used to kill bacteria , insects , worms and inhibit the sprouting of white potatoes . sterilization of medical products , drugs ,vaccines , medical Instruments , syringes, surgical gloves , tissue such as bone and skin , heart valves. Its main advantages include speed , high penetrating power , and the absence of heat . Its main disadvantages are potential dangers from factory exposure to radiation and possible damage to some materials .

Application of UV. Radiation :

Ultraviolet radiation is usually characterized at disinfection rather than sterilization .Airborne microbes can cut down by 99% used for hospital rooms , food preparation area , dental offices , operating rooms .

M.O. Filtration :

Techniques for removing microbes . Filtration is an efficient method to remove microbes from air and liquids . In practice, a fluid is strained through a filter with openings large enough for the fluid to pass through but too small for microorganisms to pass though. Most filters are thin membranes of cellulose acetate, polycarbonate , Varity of plastic materials (Teflon, nylon) .

Applications :

It used to prepare liquids that cannot withstand heat, including serum and other blood products , vaccines , drugs , IV fluids , enzymes and media . it is an alternative methods for sterilizing milk and beer without altering their flavor . Also , for water purification . Other type of filter like , crystals , fibers , and so on , this type has disadvantages of not removing soluble molecules (toxins) that can cause disease , also used efficiently for removing air born contaminants that are a common of infection sources , especially widely used to provide a flow of sterile air to hospital rooms and sterile rooms .

II- Chemical Agents in Microbial Control :

Chemical control of microbes probably emerged as a serious science in the early 1800s , when physicians used chloride of lime and iodine solution to treat wounds

Chemical Methods of Microbial Control

Method	Action(s)	Level of Activity	Some Uses
Phenol (carbolic acid)	Denatures proteins and disrupts cell membranes	Intermediate to low	Original surgical antiseptic; now replaced by less odorous and injurious phenolics
Phenolics (chemically altered phenol; bisphenols are composed of a pair of linked phenolics)	Denature proteins and disrupt cell membranes	Intermediate to low	Disinfectants and antiseptics
Alcohols	Denature proteins and disrupt cell membranes	Intermediate	Disinfectants, antiseptics, and as a solvent in tinctures
Halogens (iodine, chlorine, bromine, and fluorine)	Presumably denature proteins	Intermediate	Disinfectants, antiseptics, and water purification
Oxidizing agents (peroxides, ozone, and peracetic acid)	Denature proteins by oxidation	High	Disinfectants, antiseptics for deep wounds, water purification, and sterilization of food-processing and medical equipment
Surfactants (soaps and detergents)	Decrease surface tension of water and disrupt cell membranes	Low	Soaps: degerming; detergents: antiseptic
Heavy metals (arsenic, zinc, mercury, silver, copper, etc.)	Denature proteins	Low	Fungistats in paints; silver nitrate cream: surgical dressings, burn creams, and catheters; copper: algicide in water reservoirs, swimming pools, and aquariums
Aldehydes (glutaraldehyde and formaldehyde)	Denature proteins	High	Disinfectant and embalming fluid
Gaseous agents (ethylene oxide, propylene oxide, and beta-propiolactone)	Denature proteins	High	Sterilization of heat- and water-sensitive objects
Enzymes	Denature proteins	High against target substrate	Removal of prions on medical instruments
Antimicrobials	Act against cell walls, cell membranes, protein synthesis, and DNA transcription and replication	Intermediate to low	Disinfectants and treatment of infectious diseases

and to a wash their hands before surgery . at the present time, approximately 10,000 different chemical agents are manufactured .antimicrobial chemicals occur in the liquid , gaseous or even solid state .they serve as disinfectants , antiseptics, sterilants . in most cases , solid or gaseous antimicrobial chemicals are dissolved in water , alcohol or a mixture of the two to produce a liquid solution .

Character of chemical used as antimicrobial agents :

Chemical chose as antimicrobial agents should have the following characters:

1- Rapid action in low concentration .

2-Solubiling in water or alcohol and long – term stability .

3-have broad spectrum against microbes without being toxic to human and animal tissue .

4- Penetrate of surfaces to sustain accumulative or persistent action .

5-Resistant to becoming inactivated by organic matter .

6-Non corrosive or non staining properties .

7 – Sanitizing and deodorizing properties .

8 - Affordability and ready availability .

As yet, no chemical can completely fulfill all of those requirements , but gluteraldehyde and hydrogen peroxide approach this ideal . the action of antimicrobial chemical classified to : high , intermediate and low groups .

Factors Effecting the Germicidal activity :

Factors that control the effect of a germicidal include the nature of the microorganisms being treated , the nature of the material , the degree of contamination , the time of exposure , and the strength and chemical action Of the germicidal.

Germicidal Categories :

1- The halogen antimicrobial chemicals : fluorine , bromine ,chlorine and iodine . These elements are microbcidal and not microbstatic .

- Halogen is the active ingredient .

2 – Phenol and its derivatives : phenol (carbolic acid) is an acid , Poisonous compound derived from the distillation of cool tar. Phenol now used only in certain limited cases . It destroy vegetative bacterial cells ,

fungi and most viruses . The toxicity of Phenolic compounds lead to limiting in use as antiseptics .

3 - Chlorhexidine :

The compound chlorhexidine is a complex organic has containing chlorine and two phenolic rings . Its mode of action targets both cell membranes by lowering surface tension and protein structure by causing denaturation. At high concentration it is bactericidal for both gram-positive and gram - negative but inactive against spores . low toxicity and rapid action and not absorbed into deeper tissue .

4 - Alcohols :

Alcohols are colorless hydrocarbons with one or more - OH functional groups. Several alcohols available , only ethyl and isopropyl are suitable for microbial control . Alcohols mechanism of action depends in its concentration . 50%and higher dissolve membrane lipids , disrupt cell surface tension . If enter cytoplasm denatures proteins through coagulation (50% - 95 %) . Greater activity at 70% .

5 -Hydrogen peroxide :

The germicidal effects of hydrogen peroxide are due to the direct and indirect actions of oxygen . oxygen forms hydrogen free radicals which are highly toxic and reactive to cells .Hydrogen peroxide is bactericidal , virucidal , fungicidal and in higher concentration sporicidal .

6 - Aldehyde sterilants and disinfectants :

Organic substances bearing a – (CHO) functional group (strong reducing group). The two aldehydes used most often in microbial control are gluteraldehyde and formaldehyde . The mechanism of activity involves cross-linking protein molecules on the cell surface . it is rapid and broad – spectrum .

Gaseous sterilants and Disinfectants:

The broadest applications currently are ethylene oxide (ETO) , propylene oxide , and chlorine dioxide .

ETO is a very strong alkylating agent , and it reacts vigorously with functional groups of DNA and proteins and it blocks both DNA replication and enzymatic actions . it toxic and can damage the lungs , eyes and mucous membrane .

Detergents and soaps :

Detergents are polar molecules that act as surfactants . Most anionic detergents have limited microbiological power. This includes most soaps .Much more effective are positively charged (cationic) detergents , Particularly the quaternary ammonium compounds . It effective against some gram – positive bacteria , viruses , fungi and algae . At low concentration , they exhibit only micro static effects . ,soaps functions Primarily as cleansing agents and sanitizers in industry , the home and the medical setting . Brushing the hands for 15 – second with germicidal soap is effective to remove some resident microbes , but is unable to sterilize the skin .

Lec 2

Enzyme and metabolism

The enzymes:

They are large biological molecules responsible for the thousands of metabolic Processes , every microbial cell must possess many enzymes . Thy are highly selective catalysts , greatly accelerating both the rate and specificity of metabolic Reactions, from the digestion of food to the synthesis of DNA . Most enzymes are Proteins , although some catalytic RNA molecules have been identified .

- The chemicals which an enzyme acts on is called its substrate .
- The enzyme combines with its substrate to form an enzyme – substrate complex .
- The complex than breaks up into product and enzyme .
- A metabolic pathway is a number of reactions catalyzed by sequence of enzymes .
- Substrate : The starting molecules for a chemical reaction are called the substrates .
- Enzyme substrate complex : The enzyme substrate complex is transitional Step when the substrates of a chemical reaction are bound to the enzyme .
- Active site : The area on the enzyme where the substrate or substrates attach to.
- Enzymes are usually very large proteins and the active site is just a small region of the enzyme molecule .

Factors influencing enzyme activity:

1. PH- the optimum (best)PH – in living cells is close to 7 , higher or lower optimum PH- usually slow the enzyme activity .
2. Temperature : strongly influences enzyme activity optimum (best) temperature for maximum enzyme function is usually About 35 – 40 C .
   - Reactions proceed slowly below optimal temperatures .

- Above 45 C . most enzymes are denatured (change in their shape so the enzyme active site no longer fits with the substrate and the enzyme can't function) .

3 • Substrate concentration, Enzyme concentration .

Isoenzymes:- Many enzymes occur in various forms these are called Isoenzymes , they carry out the same function but have different structural features .

Allosteric enzymes :- These enzymes have a regulatory role , it has an active site where the substrate bind and another site known as the regulatory site to which the regulatory molecule (effector) binds to regulate the biochemical reaction .

CLASSIFICATION OF ENZYME

- These are approximately 3000 enzymes which have been characterized .
- These are grouped into six main classes according to the type of reaction catalyzed .
- At present , only a limited number are used in enzyme electrodes or for other analytical purposes .

1- Oxidoreductases

- These enzymes catalyzed oxidation and reduction reactions involving the transfer of hydrogen atoms or electrons.
- The following are of particular importance in the design of enzyme electrodes .
- This group can be further divided into main classes .

Dehydrogenases

- catalyzed hydrogen transfer from the substrate to a nicotinamide adenine Dinucleotide cofactor (NAD +) . An example of this is lactate dehydrogenase which catalyzes the following reaction :
- Lactate + NAD + = Pyruvate + NADH + H +

Oxygenizes

- Catalyze substrate oxidation by molecular oxygen .

- The reduced product of the reaction in this case is water and not hydrogen peroxide .
- An example of this is the oxidation of lactate to acetate catalyzed by lactate – 2 –monooxygenase .
- Lactate + O2 = acetate + CO2 + H2,O

2 -Transferases

- These enzymes transfer C , N , P or S containing groups (alkyl , acyl , aldehyde , amino, phosphate or glucosyl) from one substrate to another .
- Transaminases, transketolases ,transaldolases and transmethylases belong to this group .

3 - Hydrolases

- These enzymes catalyse cleavage reactions or the reverse fragment condensations .
- According to the type of bond cleaved , a distinction is made between peptidases , esterases , lipases , glycosidases , phosphatases and so on .
- Examples of this class of enzyme include ; cholesterol esterase , alkaline phosphatase and glucoamylase .

4- Lyases

- These enzymes non – hydrolytically remove groups from their substrates with the concomitant formation of double bonds or alternatively add new groups across double bonds .

5 – Isomerases

- These enzymes catalyse intramolecular rearrangements and are subdivided into ;
  - racemases
  - epimerases
  - mutases
  - cis- trans –isomerases
- An example of this class of enzyme is glucose isomerase which catalyses the isomerization of glucose to fructose . 6 – ligases

Ligases split C-C C-O C-N C-S and C-halogen bonds with out hydrolysis or oxidation

The reaction is usually a companied by the consumption of a high energy compound such as ATP and other nucleoside triphosphates

An example of this type of enzyme is pyruvate carboxylase.

ENZYME INHHIBITORS

- Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner .
- There are a variety of types of inhibitors including : nonspecific , irreversible , reversible – competitive and moncompetitive .
- Poisons and drugs are examples of enzyme inhibitors .

NON SPECIFIC INHIBITORS

- A nonspecific inhibition effects all enzymes in the same way .
- Non – specific methods of inhibition include any physical or chemical changes which ultimately denatures the protein portion of the enzyme and are therefore irreversible .

EXAMPLE:

- Temperature : Usually , the reaction rate increases with temperature, but with enzyme reaction rate decreases with increasing temperature .
- At high temperatures the protein part of the enzyme begins to denature , thus inhibiting the reaction .

ENZYME COFACTORS

- A non protein component of enzymes is called the cofactor.
- If the cofactor is organic , then it is called a coenzyme
- Coenzymes are relatively small molecules compared to the protein part of the enzyme .
- Many of the coenzymes are derived from vitamins .

- The coenzymes make up a part of the active site , since without the coenzyme , the active site , since without the coenzyme , the enzyme will not function .

Metabolism : is the total of all chemical reactions that occur in the cells .

Metabolism is the activity by which an organism synthesizes its constituents and converts energy from outer sources to energy rich bonds .

Metabolism represents as :

1- Catabolism : It is the break down of large complex organic molecules into smaller , simpler molecules . The process is usually accompanied by release of energy in the form of ATP(adenosine triphosphate) , the cell store this energy until in need . This is an energy producing reaction .
2- Anabolism : Is the synthesis of complex organic molecules from simpler once . This process consumes energy .
3- Amphebolicpath ways :biosynthetic pathways are found to branch from intermediates form in the major energy – yielding pathways (glycolysis , pyruvate oxidation and krebs – cycle) , these are known as amphibiotic pathways .

Energy production in microorganisms

Microorganisms can be divided into two groups according to their nutritional requirements of carbon sources , autotrophs and heterotrophs .

1- Energy production by heterotrophs

Most heterotrophic microorganisms can generate ATP through many metabolic pathways depending on the electron acceptors . Several of these Energy – producing pathways will be mentioned : Fermentation ,respiration , and photosynthesis .

I-Fermentation : in fermentation organic substance is serve both as electron donor and acceptor and the yield of energy is lower than respiration is considered as an incomplete oxidation process of which its end products contain (low) considerable amounts of energy , the end products could be organic acids such as lactic a acid , acetic acid , prop ionic acid , or alcohols

and all are released to the surrounding environment . an important intermediate that serves as a terminal electron acceptor and which is of importance in energy production is pyruvate . there are three main pathways that produce pyruvate or pyruvic acid through fermentation (the rich energy form is phosphoenol pyruvate) :

1 - Glycolysis :L the best – known process by which energy is obtained from glucose an aerobically also known as Embden Meyerhof pathway . in this pathway one molecule of glucose is converted to two molecules of pyruvate and two NAD (reduced coenzyme molecules) and a net of two ATP molecules (4ATP molecules are produced two of which are consumed during the reaction leaving only two free ATP molecules) pyruvate is an intermediate compound that participates in many fermentation processes that produce energy .

2- Phosphogluconate pathway : (pentose phosphate pathway) : glucose metabolism proceeds by decarboxylation when glucose – 6 – phosphate (G- 6 P) is converted to ethanol , lactic acid , and CO2 (only one pyruvate and one ATP molecule are generated) .Through this process pentose – phosphate, (ribose 5 – phosphate)is generated with NADPH , which is required for reductive steps in cell biosynthesis , while ribose – 5 – phosphate is of importance in nucleic acids biosynthesis .

a . Lactic acid fermentation : this is simple one reaction fermentation where pyruvate reduced to lactate catalyzed by lactic dehydrogenase , no gas is forced and net yield of 2ATP molecules this pathway is characteristics in lactobacillus and streptococcus bacteria .

b – Alcoholic fermentation : pyruvate is converted to CO_2andacetaldehyde, which is reduced to ethanol . This fermentation is characteristic of yeasts and uncommon bacteria .

c – Mixed acid fermentation : is characteristic of most enterobacteriaceae,It generates a molecule of ATP, pyruvate by fermentation could be converted to formic acid or acetic acid or ethanol

d – Other types of fermentation are methane fermentation as by methanobacterium, and acetone fermentation by bacillus spp. and anterobacter it produces 2ATP per glucose molecule . Pyruvate could be completely oxidized through the krebs cycle or (Tricarboxylic acid (TCA) cycie) , which is considered to be of the most important pathways for producing ATP in aerobic bacteria .

II – Respiration : energy – yielding metabolism can make use of exogenous or externally derived electron acceptors . This metabolic process is called respiration and may be divided into two different types . in aerobic respiration , the final electron acceptor is oxygen , whereas the acceptor in anaerobic respiration is a different exogenous acceptor . Most often the acceptor in anaerobic respiration is inorganic

(e . g ., $NO3^-$, SO^{-2}, CO2 , Fe3 , $SeO4^{-2}$, and many others) , but organic acceptors such as fumarate may be used . Type of respiration :

1 – Aerobic respiration : the electron donor could be organic or inorganic compound and it could be similar to that in fermentation (pyruvate) and the electron acceptor is oxygen , this is known as Complete oxidation and the energy yield is obtained by complete conversion of the organic substance (the electron donor) to CO2 and H_2O.

Substance (oxidized pyruvate) $\longrightarrow 2H^+ + 2e^- + CO_2 + H_2O$

Pyruvate (generated from the glycolytic pathway) is oxidized Through the krebs or TCA cycle to acetyl COA and CO_2

2 - Anaerobic respiration : Electrons derived from sugars and other organic molecules are usually donated either to endogenous organic electron acceptors or to molecular O2 by way of an electron transport chain .

However , many bacteria have electron transport chains that con operate with exogenous electron acceptors other than O2. As noted earlier , this energy – yielding process is called anaerobic respiration .

The major electron acceptors are nitrate , sulfate , and CO2, but metals and a few organic molecules can also be reduced [redacted], some bacteria con use nitrate

as the electron acceptor at end of their electron transport chain and still produce ATP .

According to types of respiration bacteria fall into several groups :

a - Obligate or strict aerobes : such as Mycobacterium tuberculosis and some spore forming bacteria , they require O_2.

b – Facultative anaerobes : they can survive anaerobically but in the presence of air shift from fermentation to aerobic oxidation as in anterobacteria and yeast.

c - Obligate or strict anaerobes : grow only in the absence of O_2 the terminal electron acceptors are sulphate and carbonate as in clostridium .

d– Microaerophiles : bacteria grow in the presence of minute quantities of free O_2 as , well as CO_2 .

III – PHOTSYNTHESIS : energy from light is used to provide cellular energy such as ATP molecules . The light is absorbed by special pigment (chlorophyll) in which electrons are transferred through a chain of electron carriers similar to that in phosphorylation .

Antibiotics

Antibiotics: solid molecules produced by som fungi and bacteria, controle growth of pathogenic bacteria at very low concentration.

Antibiotics or **antibacterial** antimicrobial agents produced by microorganisms that kill or inhibit other microorganisms, and are often used in medical treatment of bacterial infections. They **destroy or slow down the growth of bacteria.** Several antibiotic agents are also effective against a number of fungi, protozoans and some are toxic to humans and animals, even when given in therapeutic dosage. Antibiotics are not effective against viruses such as the common cold or influenza, and may be harmful when taken inappropriately.

Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites.

- **Antibiotics that kill bacteria are called "bactericidal"**
- **Antibiotics that stop the growth of bacteria are called "bacteriostatic"**

Historical Perspective

The modern era of antimicrobial chemotherapy began in 1929 with Fleming's discovery of the powerful bactericidal substance penicillin, and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. In the early 1940's, penicillin was isolated, purified and injected into experimental animals, where it was found to not only cure infections, but also possess incredibly low toxicity for the animals.

- The most important property of a clinically-useful antimicrobial agent, especially from the patient's point of view, is its **selective toxicity**, i.e., that the agent acts in some way that inhibits or kills bacterial pathogens but has little or no toxic effect on the animal taking the drug This implies that the biochemical processes in the bacteria are in some way different from those in the animal cells, and that the advantage of this difference can be taken in chemotherapy. Antibiotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes. The range of bacteria or other microorganisms that are affected by a certain antibiotic are is expressed as its **spectrum of action**. Antibiotics effective against procaryotes which kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be **broad spectrum** . If effective mainly against Gram-positive or Gram-negative bacteria, they are **narrow spectrum** . If effective against a single organism or disease, they are referred to as **limited spectrum**.

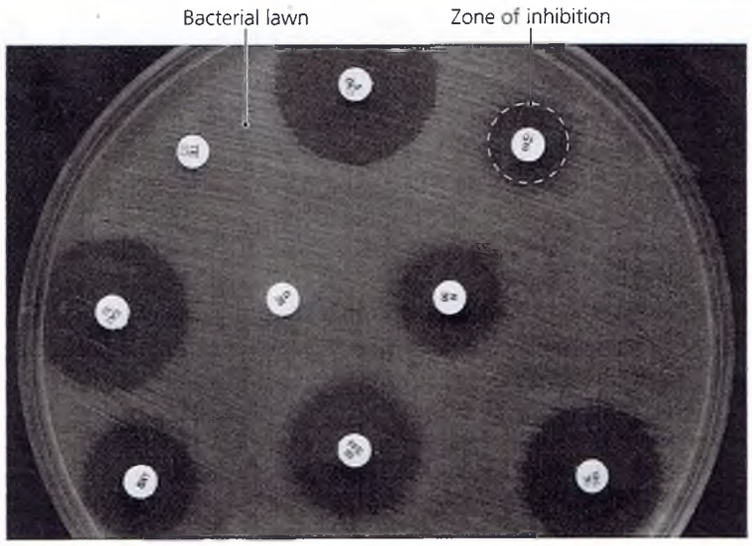
antimicrobial → active in height molecular weight

Antibiotics → active in low molecular

Sources of Some Common Antibiotics and Semisynthetics

Microorganism	Antimicrobial
Fungi	
Penicillium chrysogenum	Penicillin
Penicillium griseofulvum	Griseofulvin
Cephalosporium spp.[a]	Cephalothin
Bacteria	
Amycolatopsis orientalis	Vancomycin
Amycolatopsis rifamycinica	Rifampin
Bacillus licheniformis	Bacitracin
Bacillus polymyxa	Polymyxin
Micromonospora purpurea	Gentamicin
Pseudomonas fluorescens	Mupirocin
Saccharopolyspora erythraea	Erythromycin
Streptomyces griseus	Streptomycin
Streptomyces fradiae	Neomycin
Streptomyces aureofaciens	Tetracycline
Streptomyces venezuelae	Chloramphenicol
Streptomyces nodosus	Amphotericin B
Streptomyces avermitilis	Ivermectin

[a]spp. is the abbreviation for multiple species of a genus.



▲ **Figure 10.9 Zones of inhibition in a diffusion susceptibility (Kirby-Bauer) test.** In general, the larger the zone of inhibition around disks, which are impregnated with an antimicrobial agent, the more effective that antimicrobial is against the organism growing on the plate. The organism is classified as either susceptible, intermediate, or resistant to the antimicrobials tested, based on the sizes of the zones of inhibition. *If all of these antimicrobial agents diffuse at the same rate and are equally safe and easily administered, which one would be the drug of choice for killing this pathogen?*

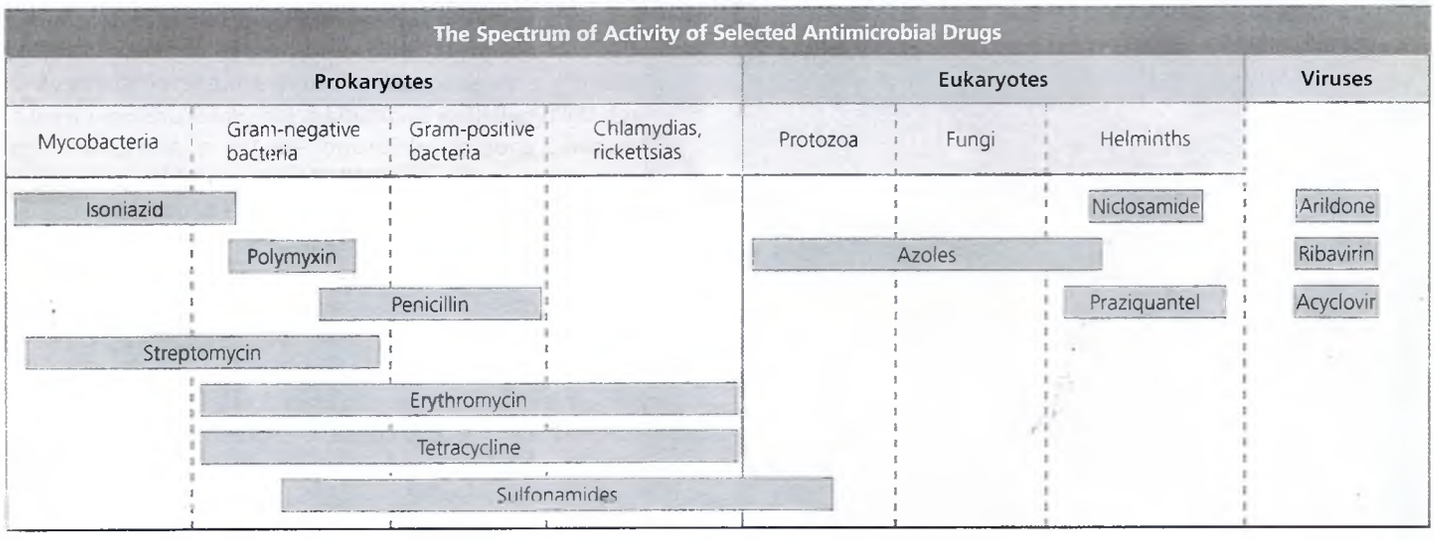


Figure 10.8 Spectrum of action for selected antimicrobial agents. The more kinds of ıogens a drug affects, the broader its spectrum of action.

Kinds of Antimicrobial Agents and their Primary Modes of Action

1. **Cell wall synthesis inhibitors** Cell wall synthesis inhibitors generally inhibit some step in the synthesis of bacterial peptidoglycan. Generally they exert their selective toxicity against eubacteria because human cells lack cell walls.

- **Beta lactam antibiotics** Chemically, these antibiotics contain a 4-membered beta lactam ring. They are the products of two groups of fungi, *Penicillium* and *Cephalosporium* molds, and are correspondingly represented by the penicillins and cephalosporins. The beta lactam antibiotics inhibit the last step in peptidoglycan synthesis. Beta lactam antibiotics are normally bactericidal and require that cells be actively growing in order to exert their toxicity.

- **Natural penicillins**, such as **Penicillin G** or **Penicillin V**, are produced by fermentation of *Penicillium chrysogenum*. They are effective against *streptococcus*, *gonococcus* and *staphylococcus*, except where resistance has developed. They are considered narrow spectrum since they are not effective against Gram-negative rods.
- **Semisynthetic penicillins** first appeared in 1959. A mold produces the main part the molecule (6-aminopenicillanic acid) which can be modified chemically by the addition of side chains. Many of these compounds have been developed to have distinct benefits or advantages over penicillin G, such as increased spectrum of activity (effectiveness against Gram-negative rods), resistance to penicillinase, effectiveness when administered orally, etc.
- **Amoxycillin** and **Ampicillin** have broadened spectra against Gram-negatives and are effective orally; **Methicillin** is penicillinase-resistant.
- **Clavulanic acid** is a chemical sometimes added to a semisynthetic penicillin preparation.

Although nontoxic, penicillins occasionally cause death when administered to persons who are allergic to them. In the U.S. there are 300 - 500 deaths annually due to penicillin allergy.
In allergic individuals the beta lactam molecule attaches to a serum protein which initiates an IgE-mediated inflammatory response.

- **Cephalolsporins** are beta lactam antibiotics with a similar mode of action to penicillins that are produced by species of *Cephalosporium*. They have a low toxicity and a somewhat broader spectrum than natural penicillins. They are often used as penicillin substitutes, against Gram-negative bacteria, and in surgical prophylaxis. They are subject to degradation by some bacterial beta-lactamases, but they tend to be resistant to beta-lactamases from *S aureus* .

2. Cell membrane inhibitors disorganize the structure or inhibit the function of bacterial membranes. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize the membranes rapidly kill the cells.

. The only antibacterial antibiotic of clinical importance that acts by this mechanism is **Polymyxin**, produced by *Bacillus polymyxis*. Polymyxin is effective mainly against Gram-negative bacteria and is usually limited to topical usage. Polymyxins bind to membrane phospholipids and thereby interfere with membrane function. Polymyxin is occasionally given for urinary tract infections caused by Pseudomonas that are gentamicin, carbenicillin and tobramycin resistant.

The balance between effectiveness and damage to the kidney and other organs is dangerously close, and the drug should only be given under close supervision in the hospital.

3. Protein synthesis inhibitors Many therapeutically useful antibiotics owe their action to inhibition of some step in the complex process of translation. Their attack is always at one of the events occurring on the ribosome and rather than the stage of amino acid activation or attachment to a particular tRNA. The most important antibiotics with this mode of action are the **tetracyclines**, **chloramphenicol**, the **macrolides** (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

- The **aminoglycosides** are products of *Streptomyces* species and are represented by streptomycin, kanamycin, tobramycin and gentamicin. These antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-negative bacteria. **Streptomycin** has been used extensively as a primary drug in the treatment of tuberculosis. **Gentamicin** is active against many strains of Gram-positive and Gram-negative bacteria, including some strains of *Pseudomonas aeruginosa*.

Tetracyclines consist of eight related antibiotics which are all natural products of *Streptomyces*, although some can now be produced semisynthetically. **Tetracycline**, **chlortetracycline** and **doxycycline** are the best known. The tetracyclines are broad spectrum antibiotics with a wide range of activity against both Gram-positive and Gram negative bacteria. The combination of their broad spectrum and low toxicity has led to their overuse and misuse by the medical community and the wide-spread development of resistance has reduced their effectiveness.

Chloramphenicol has a broad spectrum of activity but it exerts a bacteriostatic effect. It is effective against intracellular parasites such as the rickettsia. Chloramphenicol inhibits the bacterial enzyme peptidyl transferase thereby preventing the growth of the polypeptide chain during protein synthesis. Its unfortunate toxicity towards the small proportion of patients who receive it .

The **Macrolides** are a family of antibiotics whose structures contain large lactone rings linked through glycoside bonds with amino sugars. The most important members of the group are **erythromycin** and **oleandomycin**. Erythromycin is active against most Gram-positive bacteria, *Neisseria*, *Legionella* and *Haemophilus*, but not against the *Enterobacteriaceae*.
Macrolides are bacteriostatic for most bacteria but are cidal for a few Gram positive bacteria.

4. Effects on Nucleic Acids Some chemotherapeutic agents affect the synthesis of DNA or RNA, or can bind to DNA or RNA so that their messages cannot be read. The majority of these drugs are unselective, affect animal cells and bacterial cells alike and therefore have no therapeutic application.
Two nucleic acid synthesis inhibitors which have selective activity against procaryotes and some medical utility are nalidixic acid and rifamycins.
Nalidixic acid is a synthetic chemotherapeutic agent which has activity mainly against Gram negative bacteria. Nalidixic acid belongs to a group of compounds called **quinolones**.
Nalidixic acid is a bactericidal agent that binds to the DNA gyrase enzyme (topoisomerase) which is essential for DNA replication and allows supercoils to be relaxed and reformed.
However, the main use of nalidixic acid is in treatment of lower urinary tract infections (UTI). The compound is unusual in that it is effective against several types of Gram-negative bacteria such as *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae* and species which are common causes of UTI. It is not usually effective against *Pseudomonas aeruginosa*, and Gram-positive bacteria are resistant.

The **rifamycins** are also the products of *Streptomyces*. **Rifampicin** is a semisynthetic derivative of rifamycin that is active against Gram-positive bacteria (including *Mycobacterium tuberculosis*) and some Gram-negative bacteria.

5. Competitive Inhibitors The competitive inhibitors are mostly all synthetic chemotherapeutic agents. Most are "growth factor analogs" which are structurally similar to a bacterial growth factor but which do not fulfill its metabolic function in the cell. Some are bacteriostatic and some are bactericidal.

Sulfonamides were introduced as chemotherapeutic agents by Domagk in 1935, who showed that one of these compounds (prontosil) had the effect of curing mice with infections caused by beta-hemolytic streptococci. Chemical modifications of the compound sulfanilamide gave compounds with even higher and broader antibacterial activity. Bacteria which are almost always sensitive to the sulfonamides include *Streptococcus pneumoniae*, beta-hemolytic streptococci and *E. coli*. The sulfonamides have been extremely useful in the treatment of uncomplicated UTI caused by E. coli. The sulfonamides (e.g. **Gantrisin**) and **Trimethoprim** .

Chemical class	Examples	Biological source	Spectrum (effective against)	Mode of action
Beta-lactams (penicillins and cephalosporins)	Penicillin G, Cephalothin	Penicillium notatum and Cephalosporium species	Gram-positive bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Semisynthetic penicillin	Ampicillin, Amoxycillin		Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Clavulanic Acid	Clavamox is clavulanic acid plus amoxycillin	Streptomyces clavuligerus	Gram-positive and Gram-negative bacteria	Suicide inhibitor of beta-lactamases
Polypeptides	Polymyxin	Bacillus polymyxa	Gram-negative bacteria	Damages cytoplasmic membranes
Aminoglycosides	Streptomycin	Streptomyces griseus	Gram-positive and Gram-negative bacteria	Inhibit translation (protein synthesis)
	Gentamicin	Micromonospora species	Gram-positive and Gram-negative bacteria esp. Pseudomonas	Inhibit translation (protein synthesis)
Macrolides	Erythromycin	Streptomyces erythreus	Gram-positive bacteria, Gramnegative bacteria	Inhibits translation (protein
Rifamycins	Rifampicin	Streptomyces mediterranei	Gram-positive and Gram-negative bacteria, Mycobacterium tuberculosis	Inhibits transcription (eubacterial RNA polymerase)
Tetracyclines	Tetracycline	Streptomyces species	Gram-positive and Gram-negative bacteria, Rickettsia	Inhibit translation (protein synthesis)
Chloramphenicol	Chloramphenicol	Streptomyces venezuelae	Gram-positive and Gram-negative bacteria	Inhibits translation (protein synthesis)

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Pathogenic Microorganisms

Different types of microorganisms interact with human bodies on a regular basis. They can be harmless, harmful or beneficial. Harmful microorganisms are also called pathogenic.

The ability of a microorganism to cause disease is called **pathogenicity**. There are several pathogens that can cause serious harm or even immediate death.

Invasion and multiplication of pathogenic microorganisms in the body is called an **infection**. When we are infected by pathogens we become sick, which means that our bodies stop functioning properly. **Infectious agents**, such as bacteria, a virus, fungi or protozoa cause communicable diseases. **Communicable diseases** can be spread from one person to another.

Infection transmission :

All living organisms have a natural or acquired resistance mechanism called immunity. When we get sick, for example, we use different body cells and chemicals to fight bacteria. Bacteria in their turn use different chemicals to fight us. That is why infection is sometimes referred to as a race between pathogen and host organism. The infection can be transmitted by direct or indirect contact.

Direct contact transmission - involves any direct contact with an infected individual. Infection can be passed in water droplets through a sneeze, cough, laugh or exhalation and through bodily fluids.

Indirect contact transmission - is a method of spreading infection from person to person that involves contact with a contaminated object. Objects can become contaminated when touched by someone with an infection, by ingesting contaminated water, or animals and insects.

Types of pathogens

Bacteria

E.coli causes food poisoning and urinary tract infections.
Mycobacterium tuberculosis causes tuberculosis.

Viruses

Influenza virus causes 'flu'.
Herpes simplex virus causes herpes.

Protozoa

Plasmodium causes malaria.

Fungi

Tinea causes ringworm.

Toxins is a poisonous substance produced within living cells or organisms ,toxins can be small molecules, peptides, or proteins that are capable of causing disease on contact with or absorption by body tissues interacting with biological macromolecules such as enzymes or cellular receptors. Toxins vary greatly in their severity, ranging from usually minor (such as a bee sting) to almost immediately deadly (such as botulinum toxin).

Exotoxin :- excreted by living cell secreted by gram positive bacteria, and can cause damage to the host by destroying cells or disrupting normal cellular metabolism. They may exert their effect locally or produce systemic effects. Well-known exotoxins include: botulinum toxin produced by *Clostridium botulinum*; *Corynebacterium diphtheriae* . The toxic properties of most exotoxins can be inactivated by heat or chemical treatment to produce a toxoid. These retain their antigenic specificity and can be used to produce antitoxins and, in the case of diphtheria and tetanus toxoids, are used as vaccines.

Endotoxins: - is the integral part of the cell walls of Gram-negative bacteria, and are liberated when bacteria are disintegrated (lysed). Cell wall of Gram negative bacteria contain lipopolysaccharides (LPS, endotoxin)

1- Gram positive- spore forming anaerobic bacteria

A- Tetanospasmin (toxin of *Clostridium tetani*)

Clostridium tetani is an anaerobic gram-positive rod that is widespread in the environment.

Clostridium tetani contaminates wounds, and the spores germinate in the anaerobic environment of the dead tissue.

B- Botulotoxin (toxin of *Clostridium botulinum*)

Clostridium botulinum is found in soil or water and may grow in foods if the environment is appropriately anaerobic.

C- Toxins of *Clostridium perfringens*

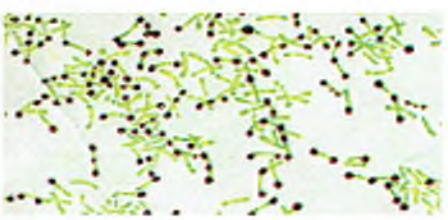
Spores of *Clostridium perfringens* are introduced into the wounds by contamination with soil or faeces. In the presence of necrotic tissue (an anaerobic environment),Many of these are necrotizing and hemolytic and favour the spread of gangrene

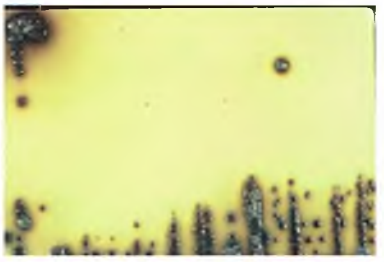
Diphtheria toxin (toxin of *Corynebacterium diphtheriae*)

Aerobic/Facultative Gram Positive Bacilli

Diphtheria bacilli colonize and grow on mucous membranes, and start to produce toxin, which is then absorbed into the mucous membranes, and even spread by the bloodstream.

Gram's stain: beaded rods in typical arrangement (unreliable)





2- Gram positive non spor forming bacteria

A- Staphylococcus aureus

- Normal flora of the skin, nose, throat, and mucous membranes; cause suppuration, abscess, pyogenic infections, fatal septicemia.
- Hemolysis blood, coagulate plasma and produce a variety of extracellular enzymes and toxins
- At least 30 species: *S aureus, S epidermidis, S saprophyticus*-UTI in young women
- Staphylococci are non-motile; aerobic or microaerophilic and relatively resistant to drying and heat
- *S aureus* is catalase +, coagulase +; form grey to golden yellow colonies, ferment mannitol.

B- The Streptococci

- Heterogenous group of bacteria, characterised by colony growth characteristics, haemolysis patterns on blood agar, antigenic composition of group-specific cell wall.
- Spherical cocci in chains, Gram +.
- Haemolysin: streptolysin O (SLO)-inactivated in O_2, anti-SLO level increases following infection (ASO serum titer of 160-200 units)
- *S pyogenes* – Group A; β-haemolytic; human pathogen only; diseases such as local tonsillitis.
- *S pneumoniae*

3- Gram-negative bacilli

A- Pseudomonads

- Motile; aerobic; occur widely in soil, water, plants and animals.
- *P aeruginosa* is an obligate aerobe, sometimes producing sweet or grape-like odour; forms smooth round colonies with fluorescent greenish pyoverdin in agar; bluish pigment pyocyanin.

B- *Vibrio cholera*

- Vibrios are comma-shaped aerobic rods, motile and possess a polar flagellum; **oxidase +.**
- *V cholerae* produces enterotoxin that causes cholera.
- The culture presented as convex, smooth, opaque round colonies and granular; grow well at 37°C on thiosulfate-citrate-bile-sucrose agar with yellow colonies. Colonies are rapidly killed by acid

C- *Helicobacter pylori*

- A spiral-shaped G-; multiple flagella at one pole and highly motile; grow at 37°C pH 6-7 and killed in acidic; oxidase + and catalase +.
- Cause gastritis, duodenal ulcer/peptic disease and gastric carcinoma. Urease producer.
- Produces: protease – makes mucous impermeable to acid; urease which yields ammonia production and buffers the acidic pH.

D- **Neisseria species** •

Are all gram negative cocci • Oxidase, catalase positive • Multiply intracellularly • Neisseria meningitidis/ Neisseria gonorrhea

A- Neisseria meningitidis

Gram negative diplococci • Infection from aerosol transmission in close contacts • Polysacharide capsule is antiphagocytic • LPS, • Meningitis, fever, pneumonia, meningococcemia with hemorrhagic lesions are major clinical manifestations .

B- Neisseria gonorrhea

- Gonorrhoea , in ancient times it was thought that the pus discharge associated with the disease contained semen.

Enterobacteriaceae

gram negative facultative anaerobic rods

All ferment glucose (dextrose)

All reduce nitrates to nitrites

All are oxidase negative

All except Klebsiella, Shigella and Yersinia are motile

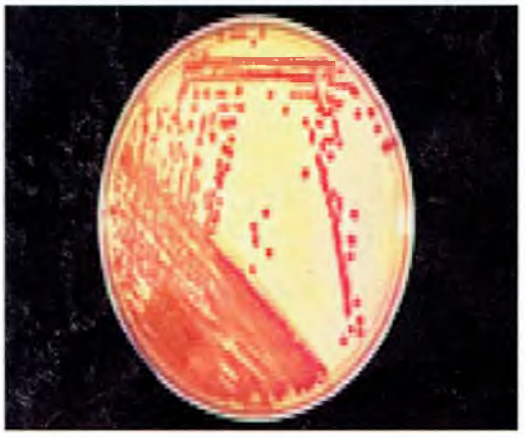
Non-Spore forming

A- Escherichia coli

- Common isolate from colon flora

Characteristics

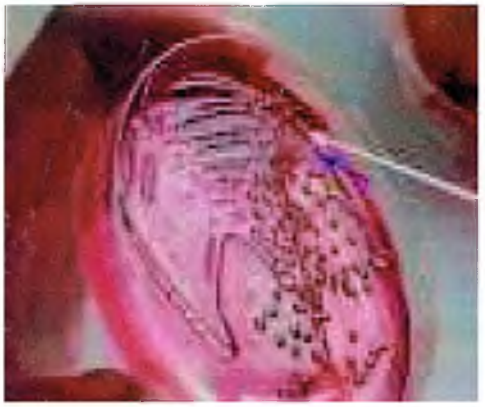
Dry, pink (lactose positive) colony with surrounding pink area on MacConkey, Usually motile •

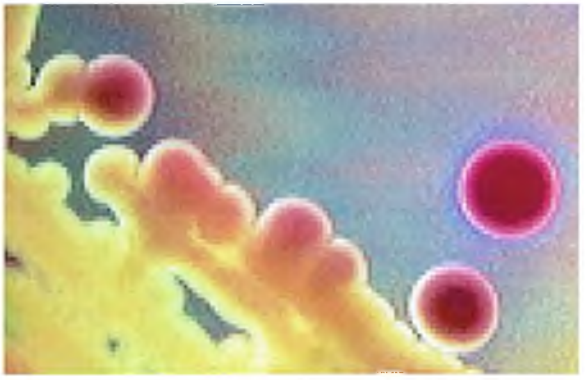


B- Klebsiella,

- Usually found in GI tract
- *K. pneumoniae* is mostly commonly isolated species
  - Possesses a polysaccharide capsule, which protects against phagocytosis and antibiotics AND makes the colonies moist and mucoid

- **Significant biochemical reactions**
  - Lactose positive
  - Most are urease positive
  - Non-motile





C- Proteus species

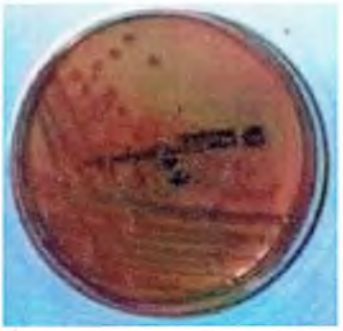
- *P. mirabilis* and *P. vulgaris* are widely recognized human pathogens
- Isolated from urine, wounds, and ear and bacteremic infections
- Both produce swarming colonies on non-selective media and have a distinctive "burned chocolate" odor
- Both are strongly urease positive

- A exhibits characteristic "swarming"
- B shows urease positive on right

D- Salmonella

- Produce significant infections in humans and certain animals
- On differential selective agar, produces clear, colorless, non-lactose fermenting colonies with black centers (if media contains indicator for hydrogen sulfide)

Salmonella on MacConkey



E- Shigella

- does not ferment lactose ,facultative anaerobe , non-motile
- All species cause bacillary dysentery

GRAM-POSITIVE BACTERIA:

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Staphylococcus				
Staphylococcus aureus (nose, skin esp. hospital staff and pts; vagina)	1. gram stain: a. gram (+), clustered cocci 2. culture: a. β-hemolytic **b. golden w/ sheep blood** 3. Metabolic: a. catalase (+) **b. coagulase (+)** c. facultative anaerobe	1. Protective a. microcapsule b. Protein A: binds IgG c. Coagulase: fibrin formation around organism d. hemolysins e. leukocidins f. penicillinase 2. Tissue-Destroying a. hyaluronidase b. staphylokinase (lysis of clots) c. lipase	1. Exotoxin Dependent a. enterotoxin→ **gastroenteritis** (rapid onset and recovery) b. TSST-1 → **toxic shock syndrome** (fever, GI sx w/diarrhea, rash, hypotension, desquamation of palms and soles) c. exfoliatin→ **scalded skin syndrome** (children) 2. Direct Invasion of Organs a. pneumonia b. meningitis c. osteomyelitis (children) **d. acute bacterial endocarditis** e. septic arthritis **f. skin infection** **g. bacteremia/sepsis** h. UTI	1. penicillinase-resistant penicillins (eg. methicillin, naficillan) 2. vancomycin 3. clindamycin * if methicillin resistant, treat w/ IV vancomycin
Staphylococcus epidermidis (skin, mucous membranes)	1. gram stain: a. gram (+), clustered cocci 2. Metabolic: a. catalase (+) b. coagulase (-) c. facultative anaerobe	1. Protective a. polysaccharide capsule (adherence to prosthetic devices) * high antibiotic resistance	1. Nosocomial Infection **a. prosthetic joints, valves** **b. sepsis from intravenous lines** c. UTI 2. skin contamination in blood cultures	1. vancomycin
Staphylococcus saprophyticus	1. gram stain: a. gram (+), clustered cocci 2. culture: a. γ-hemolytic 3. Metabolic: a. catalase (+) b. coagulase (-) c. facultative anaerobe		1. **UTIs** in sexually active women	1. penicillin

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Streptococcus				
Streptococcus pneumoniae (oral colonization)	1. gram stain: a. gram (+), diplococci 2. culture: a. does not grow in presence of optochin and bile b. α-hemolytic 3. Metabolic: a. catalase (-) b. facultative anaerobe 4. (+) quellung test (encapsulated bacteria)	1. capsule (83 serotypes)	1. **pneumonia** 2. **meningitis** 3. sepsis 4. **otitis media** (children) (secretes pneumolysins that bind cholesterol of host-cell membranes, actual effect is unknown)	1. penicillin G (IM) 2. erythromycin 3. ceftriaxone 4. vaccine: against the 23 most common capsular Ag's
Streptococcus pyrogenes (group A)	1. gram stain: a. gram (+), chains 2. culture: a. inhibited by bacitracin b. β-hemolytic (streptolysin O→oxygen labile, antigenic; S→oxygen stable, non-antigenic) 3. Metabolic: a. catalase (-) a. microaerophilic	1. M-protein (adherence factor, antiphagocytic, antigenic) 2. lipoteichoic acid (adherence factor) 3. steptokinase 4. hyaluronidase 5. DNAase 6. Anti-C5a peptidase	1. Direct Invasion/toxin a. **pharyngitis** (purulent exudates on tonsils, fever, swollen lymph nodes) **b. sepsis** c. skin infections **d. scarlet fever** **e. toxic shock syndrome** 2. Antibody-mediated a. **rheumatic fever** (fever, myocarditis, arthritis, chorea, rash, subcutaneous nodules) b. acute post-streptococcal **glomerulonephritis**	1. Penicillin G 2. Penicillin V 3. Erythromycin 4. Penicillinase-resistant penillicin (skin infections b/c might be staph) * après RF, cont. prophylaxis for repeat infection, if heart valve complications, prophylaxis avant certain procedures (eg. dental work)→ endocarditis ** invasive→ clindamycin
Steptococcus agalactiae (vaginal colonization)	1. gram stain: a. gram (+), chains (urine or CSF) 2. culture: (urine, CSF, blood) a. β-hemolytic 3. Metabolic: a. catalase (-) b. facultative anaerobe		1. **neonatal meningitis** 2. neonatal pneumonia 3. **neonatal sepsis**	1. penicillin G
Enterococci (group D) (normal colon flora)	1. gram stain: a. gram (+), chains 2. culture: a. bile, sodium chloride b. α,β,γ-hemolytic 3. Metabolic: a. catalase (-) b. facultative anaerobe	1. extracellular dextran helps bind to heart valves (high intrinsic resistance)	1. subacute bacterial **endocarditis** 2. biliary tract infections 3. **UTI**	1. ampicillin (combined w/ aminoglycosides in endocarditis) *resistance to penicillin G and emerging resistance to vancomycin
Streptococcus viridans (normal orophry nx flora & GI)	1. gram stain: a. gram (+), chains 2. culture: a. resistant to optochin b. α-hemolytic (green) 3. Metabolic: a. catalase (-) b. facultative anaerobe		1. subacute bacterial **endocarditis** 2. dental cavities 3. brain or liver abcesses	1. penicillin G

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Clostridium				
Clostridium tetani (soil; entry via wounds)	1. gram stain: a. gram (+), spore-forming rods (drumstick appearance) 2. metabolism: a. anaerobe	1. flagella (H-Ag (+))	1. tetanospasmin: inhibits release of GABA and glycine from nerve cells→ **sustained muscle contraction** a. muscle spasm b. lockjaw (trismus) c. risus sardonica (grin) d. opisthotones (pronounced back arch) e. respiratory muscle paralysis	1. tetanus toxoid: vaccine w/ formalin-inactivated toxin (DPT) 2. antitoxin: human tetanus immune globulin (for those never immunized) 3. clean the wound 4. penicillin or metronidazole 5. ventilatory assistance
Clostridium botulinum (soil, smoked fish, canned food, honey)	1. gram stain: a. gram (+), spore-forming rods 2. metabolism: a. anaerobe	1. flagella (H-Ag (+))	1. neurotoxin: inhibits release of ACh from peripheral nn (not secreted; released upon death of organism) a. cranial nerve palsies b. muscle weakness c. respiratory paralysis * infants: constipation and **flaccid paralysis**	1. antitoxin 2. penicillin 3. hyperbaric oxygen 4. ventilatory assistance and intubation
Clostridium perfringes (soil, food)	1. gram stain: a. gram (+), spore-forming rods 2. metabolism: a. anaerobe	1. non-motile	1. alpha toxin: **lecithinase** (splits lecithin into phosphocholine & diglyceride) **a. gaseous gangrene** • cellulites/wound infection • clostridial myonecrosis: fatal if no tx 2. superantigen (spores in food) a. food poisoning	1. radical surgery (amputation) 2. penicillin & clindamycin 3. hyperbaric oxygen
Clostridium difficile (GI, hospitals and nursing homes)	1. gram stain: a. gram (+), spore-forming rods 2. metabolism: a. anaerobe 3. immunoassay for *C. difficile* toxin 4. colonoscopy	1. flagella (H-Ag (+))	1. toxin A a. diarrhea 2. toxin B a. cytotoxic to colonic epithelial cells → **pseudomembranous enterocolitis** (antibiotic-associated diarrhea)	1. metronidazole 2. oral vancomycin 3. terminate use of responsible antibiotic

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Bacillus				
Bacillus anthracis (herbivores; cutaneous, inhaled, ingested endospores)	1. gram stain: b. gram (+), spore-forming rods 2. metabolism: b. aerobe (can be facultative) 3. serology	1. unique protein capsule (polymer of **γ-D-glutamic acid: antiphagocytic** 2. non-motile 3. virulence depends on acquiring 2 plasmids; one carries gene for protein capsule, other carries gene for exotoxin	1. **anthrax toxin** (exotoxin): 3 proteins (protective Ag (PA), edema factor (EF), lethal factor (LF)) a. **anthrax**: painless black vesicles; can be fatal if untreated, woolsorter's pulmonary disease, abdominal pain, vomiting and bloody diarrhea (infection results in permanent immunity if pt survives)	1. **penicillin G** 2. erythromycin 3. vaccine: for high-risk individuals a. composed of protective Ag b. animal vaccine composed of live strain, attenuated by loss of its protein capsule

GRAM-NEGATIVE BACTERIA:

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Enterobacteriaceae				
Salmonella species (zoonotic: turtles, chicken, uncooked eggs)	1. gram stain: a. gram(-) rods 2. culture: (EMB/MacConkey) **a. H_2S production** 3. metabolism: a. catalase (+) b. oxidase (-) c. glucose fermenter d. **does not ferment lactose** e. facultative anaerobe	1. flagella (H antigen) 2. **capsule** (Vi antigen): protects from intracellular killing 3. siderophore * **lives in Mϕ** in lymph nodes **__asplenic or non-fxn splenic__ pts are at increased risk	1. paratyphoid fever (similar to typhoid fever) 2. **gastroenteritis** 3. **sepsis** 4. **osteromyelitis** (esp SS pts)	1. ciprofloxacin 2. ceftriaxone 3. trimethoprim & sulfamethoxazole 4. azithromycin 5. **diarrhea: only fluid and electrolyte replacement**
Salmonella typhi (fecal-oral transmission)	1. gram stain: a. gram(-) rods 2. culture: (urine, blood, CSF; EMB/MacConkey agar) **a. H_2S production** 3. metabolism: a. catalase (+) b. oxidase (-) c. glucose fermenter d. **does not ferment lactose** e. facultative anaerobe	1. flagella (H antigen) 2. **capsule** (Vi antigen): protects from intracellular killing 3. siderophore * **lives in Mϕ** in lymph nodes **can live in **gall bladder** for years *** asplenic or non-fxn splenic pts are at increased risk	1. **typhoid fever** a. fever b. abdominal pain c. hepatosplenomegaly d. rose spots on abdomen (light skinned pts) 2. **chronic carrier state**	1. **ciprofloxacin** 2. **ceftriaxone** 3. trimethoprim & sulfamethoxazole 4. azithromycin
Shigella dysenteriae (humans; fecal-oral transmission)	1. gram stain: a. gram(-) rods 2. culture: (stool; EMB/MacConkey agar) **a. no H_2S production** 3. metabolism: a. catalase (+) b. oxidase (-) c. glucose fermenter d. **does not ferment lactose** e. facultative anaerobe	1. **non-motile** (no H antigen) 2. invades submucosa not lamina propria *IgA best defense	1. **Shiga toxin**: inactivates the 60S ribosome, inhibiting protein synthesis and killing intestinal cells a. **bloody diarrhea** with mucus and pus	1. fluoroquinolones 2. trimethoprim & sulfamethoxazole
Klebsiella pneumoniae	1. gram stain: a. gram(-) rods 2. culture: (EMB/MacConkey) 3. metabolism: a. indole, oxidase (-) b. glucose , lactose fermenter c. facultative anaerobe	1. capsule 2. non-motile	1. **pneumonia**, with significant lung necrosis and bloody sputum, commonly in alcoholics, or those with underlying lung disease 2. **hospital acquired UTI and sepsis**	1. 3rd generation cephalosporins 2. ciprofloxacin

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Enterobacteriaceae				
Escherichia Coli (human GI and UT; transmitted fecal-oral, urethral migration, colonization of catheters, aspiration)	1. gram stain: a. gram(-) rods 2. culture: (urine, blood, CSF on EMB or MacConkey agar) a. grow at 45.5°C b. indole (+) c. β-hemolytic 3. metabolism: a. catalase (+) b. oxidase (-) c. glucose , **lactose fermenter** d. facultative anaerobe	1. fimbriae (pili): colonization factor 2. siderophore 3. adhesins 4. capsule (K antigen) 5. flagella (H antigen)	1. enterotoxins a. LT (heat labile) ↑cAMP (similar to cholera toxin) b. ST (heat stabile): ↑cGMP c. Shiga-like toxin (verotoxin): inhibits protein synthesis by inactivating the 60S ribosomal subunit → diarrhea a. *enterotoxigenic (ETEC)*: non-invasive; LT and ST toxins, causing traveler's diarrhea b. *enterohemorrhagic (EHEC)*: bloody diarrhea, no fever, no stool pus; secretes Shiga-like toxin→ hemorrhagic colitis and hemolytic uremic syndrome (E.coli strain O157:H7) c. *enteroinvasive(EIEC)*: bloody diarrhea w/ stool pus and fever; secretes small amounts of shiga-like toxin 2. LPS a. hospital acquired sepsis 3. newborn meningitis 4. UTI 5. hospital acquired pneumonia	1. cephalosporins 2. aminoglycosides 3. trimethoprim & sulfamethoxazole 4. fluoroquinolones

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Vibrionaceae				
Vibrio cholera (fecal-oral transmission)	1. gram stain: a. **short, comma shaped, gram(-) rods** w/ single polar flagellum 2. culture: (TCBS agar) a. flat yellow colonies 3. **dark field microscopy** of stool a. motile organisms immobilized with antiserum 4. metabolism: a. ferments sugar (except lactose)	1. flagellum (H antigen) 2. mucinase: digests mucous layer to attach to cells 3. fimbriae: helps with attachment to cells 4. **noninvasive**	1. **cholergen** (enterotoxin): like LT, ↑ cAMP → secretion of electrolytes from the intestinal epithelium (secretion of fluid into intestinal tract) a. **cholera**: severe diarrhea with **rice water stools**, no pus (death by dehydration) * epidemics 1991 Latin America 1993 Bangladesh and India	1. replace fluids 2. doxycycline 3. fluoroquinolone
Campylobacter jejuni (zoonotic: wild and domestic animals and poultry; transmitted by uncooked meat and fecal-oral)	1. gram stain: a. **curved** gram(-) rods w/ **singular polar flagellum** 2. culture: (stool; EMB/MacConkey agar) a. optimum temp is 42°C 3. metabolism: a. oxidase (+) b. **does not ferment lactose** c. microphilic aerobe	1. flagella (H antigen) 2. invasiveness	1. enterotoxin: similar to cholera toxin and LT 2. cytotoxin: destroys mucosal cells → secretory or **bloody diarrhea** (associated with Guillain-Barre syndrome—acute neuromuscular paralysis; autoimmune) * one of the three most common causes of diarrhea in the world	1. fluoroquinolone 2. erythromycin 3. ciprofloxacin
Helicobacter pylori	1. gram stain: a. **curved** gram(-) rods w/ **tuft of flagella** 2. metabolism: a. urease (+) b. microaerobe		1. **duodenal ulcers** 2. **chronic gastritis**	1. bismuth, ampicillin, metronidazole & tetracycline 2. clarithromycin & omeprazole * both reduce duodenal ulcer relapse

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Pseudomonadaceae				
Pseudomonas aeruginosa (opportunistic)	1. gram stain: a. gram(-) rods 2. culture: (blood agar) a. greenish-metallic appearance w/ fruity smell 3. metabolism: a. oxidase (+) b. non-lactose fermenter c. obligate aerobe	1. polar flagellum (H antigen) 2. hemolysin 3. collagenase 4. elastase 5. fibrinolysin 6. phospholipase C 7. DNAase 8. some strains possess an antiphagocytic capsule	1. exotoxin A (similar to diptheria toxin): inhibits protein synthesis by blocking EF2 a. pneumonia (cystic fibrosis and immunosuppressed pts) b. osteomyelitis (diabetics, IV drug users, children) c. burn wound infections d. sepsis e. UTI f. endocarditis (IV drug users) g. malignant external otitis h. corneal infections in contact lens wearers	1. ticarcillin 2. timentin 3. carbenicillin 4. piperacillin 5. mezlocillin 6. ciprofloxacin 7. imipenem 8. tobramycin 9. aztreonam

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Bacteroidaceae				
Bacteroides fragilis (normal GI flora)	1. gram stain: a. gram(-) rods b. non-spore forming c. polysaccharide capsule 2. metabolism: a. anaerobe	* infection when organism enters peritoneal cavity	1. does not contain Lipid A a. abcesses in GI tract, pelvis, lungs	1. metronidazole 2. clindamycin 3. chloramphenicol 4. surgically drain abcess
Actinomyces				

GRAM-NEGATIVE COCCI:

Organism	Diagnostics	Virulence Factors	Clinical Manifestations	Treatment
Neisseria				
Neisseria Meningitidis (neonates & army recruits)	1. gram (-) **diplococci** 2. culture (**Thayer-martin VCN**) a. high CO_2 environment 3. metabolism a. ferments maltose, glucose b. facultative anaerobe	1. capsule: a. A, B, C serotypes associated w/ meningitis 2. IgA_1 protease 3. can extract Fe from transferring 4. pili (adherence)	1. endotoxin: LPS a. **meningitis** (fever, **stiff neck**, vomiting, lethargy, altered mental state, **petechial rash**) b. **septocemia** (fever, petechial rash, hypotension, **waterhouse-friderichsen** syndrome: bilateral hemorrhage of adrenal glands along w/ hypotension & petechial rash) 2. asymptomatic carriage in nasopharynx * complement deficiency (MAC) renders susceptibility	1. vaccine against capsular Ag's: A, C, Y and W-135. not B 2. Antibiotics a. penicillin G b. ceftriaxone (3rd gen. c'sporins) c. rifampin used prophylactically for close contact of infected people
Neisseria gonorrhoeae (humans; sexually transmitted)	1. gram (-) **diplococci** 2. culture (urethral pus; **Thayer-martin VCN**) a. WBCs b. high CO_2 environment 3. metabolism a. ferments glucose b. facultative anaerobe	1. pili a. adherence b. antigenic variation c. antiphagocytic: binds bacteria tightly to host cell 2. IgA_1 protease 3. outer membrane proteins: a. Protein I: porin b. Protein II (opacity protein): presence associated w/dark, opaque colonies for adherence	1. endotoxin: LPS **a.** men: **urethritis** b. women: **cervical gonorrhea→ PID** (ascending) cx: sterility, ectopic pregnancies, abcesses, peritonitis, perihepatitis, salpingitis c. men and women: gonococcal bacteremia, septic arthritis d. neonates: opthalia neonatorum conjunctivitis (usu erupts in first 5 days) * complement deficiency (MAC) renders susceptibility	1. 1st line a. 3rd generation C'sporins (ceftriaxone) + doxycycline for chlamydia and syphilis 2. 2nd line (not effective for syphilis) a. fluoroquinolones b. spectinomycin 3. ophthalmia neonatorum a. erythromycin eye drops prophylaxis b. w/disease: ceftriaxone + erythromycin serum for chlamydia

Microbiology- 2nd stage

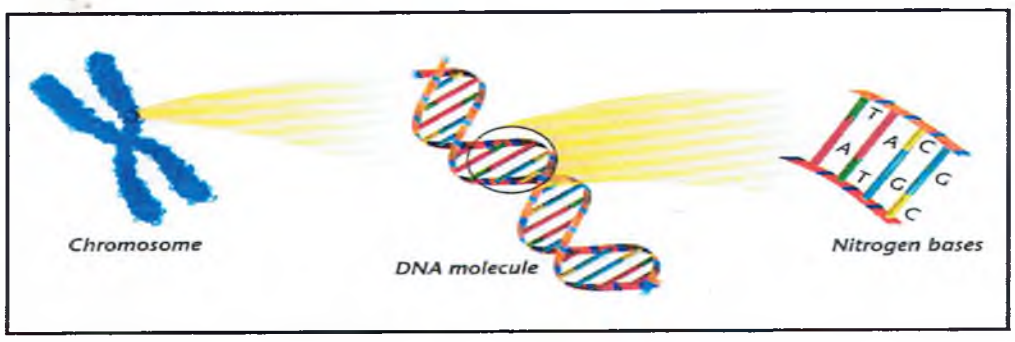
Lec -5-

Microbial Genetic

DNA stands for Deoxyribose Nucleic Acid. This chemical substance is present in the nucleus of all cells in all living organisms. DNA controls all the chemical changes which take place in cells.

The structure of nucleic acids and their replication

- Genetic material of living organisms is either DNA or RNA.
- DNA – Deoxyribonucleic acid
- RNA – Ribonucleic acid



Quick Review

- **DNA is organized into chromosomes, which are found within the nuclei of cells.**
- **A gene is a segment of DNA on a chromosome that codes for a specific protein and thus determines a trait.**
- **The genetic code is determined by the order of bases in the gene, which specifies what type of protein will be produced.**

DNA is a very large molecule made up of a long chain of sub-units

The sub-units are called **Nucleotides**

Structure of a nucleotide

A nucleotide is made of 3 components:

1- **Pentose sugar**

This is a 5 carbon sugar, The sugar in DNA is deoxyribose

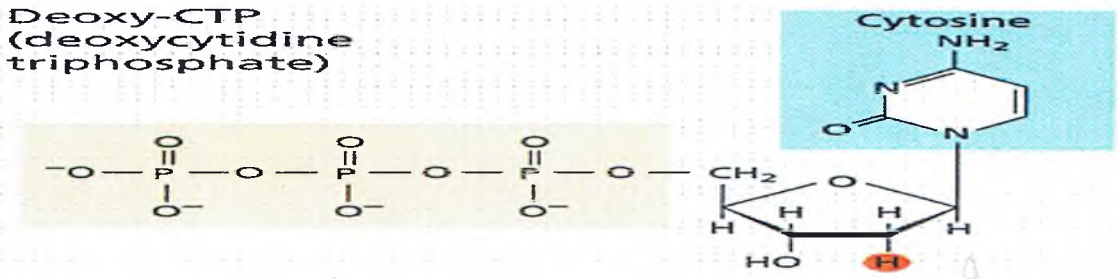
The sugar in RNA is ribose.

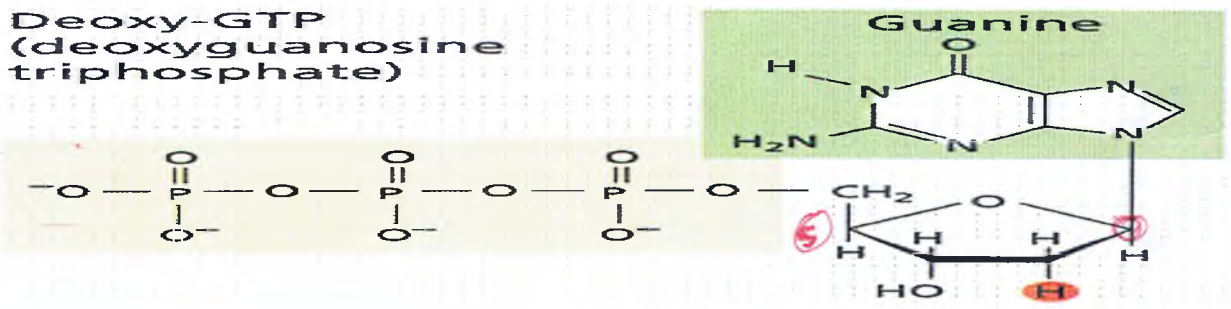
2- **Phosphate group** :- Phosphate groups are important because they link the sugar on one nucleotide onto the phosphate of the next nucleotide to make a polynucleotide

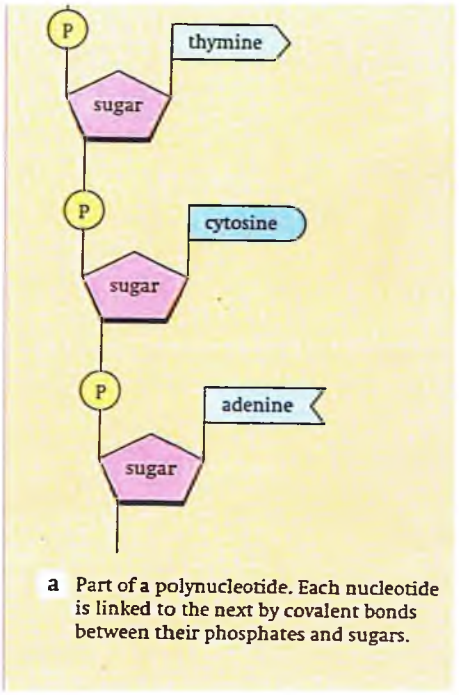
3- **Nitrogenous bases – Two types**

Pyramidines	Purines
Thymine – T	Adenine – A
Cytosine - C	Guanine - G

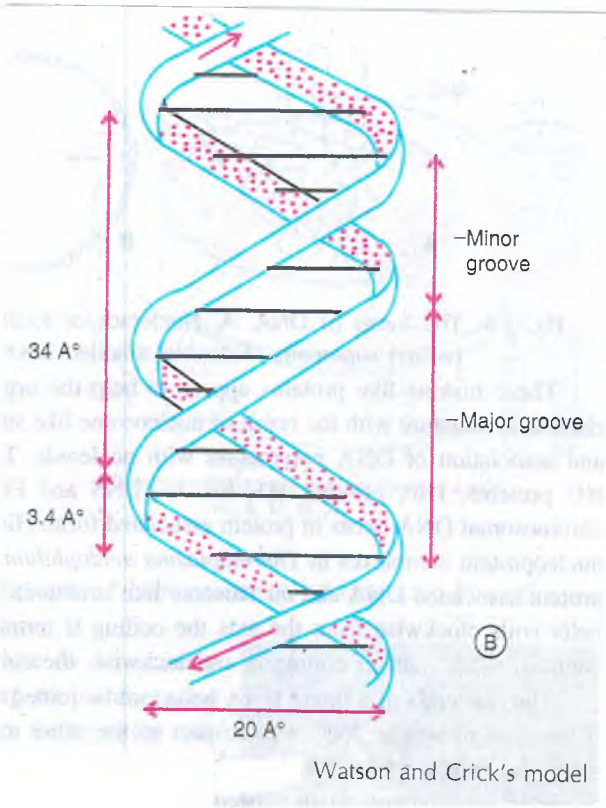
- In RNA the four bases are:
  - Uracil
  - Adenine
  - Cytosine
  - Guanine







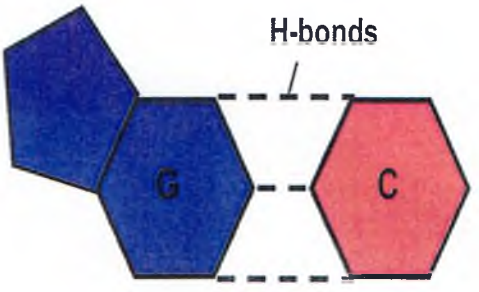
a Part of a polynucleotide. Each nucleotide is linked to the next by covalent bonds between their phosphates and sugars.



Watson and Crick's model

DNA Double Helix -- <u>Base pairing</u>

- **The Nitrogenous Bases pair up with other bases. For example the bases of one strand of DNA base pair with the bases on the opposite strand of the DNA.**
- **Adenine always base pairs with Thymine (or Uracil if RNA)**
- **Cytosine always base pairs with Guanine.**



- DNA has a regular structure. It's orientation, width, width between nucleotides, length and number of nucleotides per helical turn is constant. All of these features were described by Watson and Crick. Adenine is always opposite thymine, and cytosine is always oppostie guanine. The two strands are held together by hydrogen bonds: two bonds between adeninine and thymine and three bonds between guanine and cytosine

- The basic building block is the ***deoxyribose sugar***. This sugar is distinguished because it contains a hydrogen (H) atom at the number 2' carbon. Normal ribose has a hydorxyl (-OH) group at this position.

Attached to the 5' carbon is a triphosphate group. This group is important because in a DNA chain it undergoes a reaction with the 3' OH group to produce polydeoxynucleotide

A Single Strand Molecule of DNA

Each strand of the double-stranded DNA molecule has the same basic structure.
It is a series of series of deoxyribonucleotides linked together by phosphodiester bonds.
DNA is a polynucleotide. It consists of a series of deoxyribonucleotides that are joined by phosphodiester bonds. This bond joins the a phosphate group to the 3' carbon of the deoxyribose sugar.
Each strand is complementary to the opposite strand. If one strand has an adenine at a position, its anti-parallele strand would have a thymine at the corresponding position. Likewise, guanine and cytosine would be complementary.

Making a Phosphodiester Bond/Growing the DNA Chain

Phosphodiester bonds are formed when a news dideoxynucleotide is added to a growing DNA molecule.
During the reaction, a condensation reaction occurs between the a phosphate of the nucleotide and the hyroxyl group attached to the 3' carbon. This reaction is performed by the enzyme DNA polymerase.
This is also an energy requiring reaction. The energy is provided by the breaking of the high-energy phophate bond in the nucleotide. This results in the release of a pyrophosphate molecule.

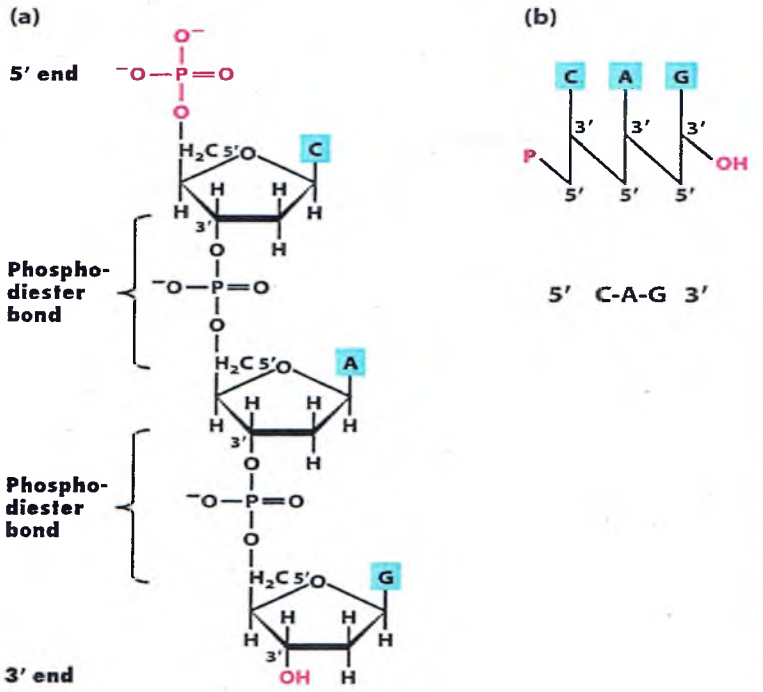


Figure 4-2
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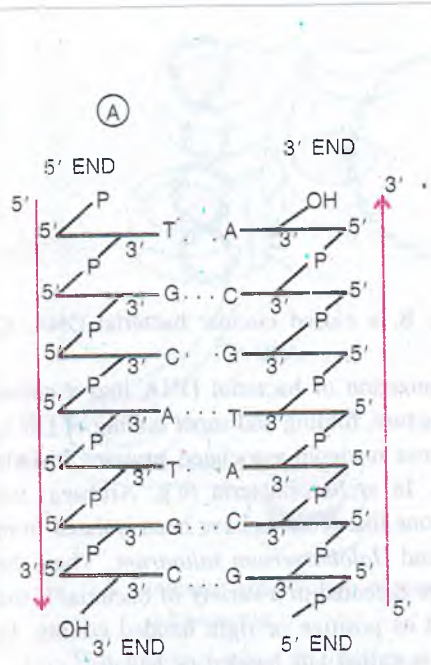


Antiparallel orientation of the complementary chains

DNA replication

DNA replication is essential biological process. Its primary function is to produce new DNA for cell division. The process has several distinct steps that are important to understand. The factors that are absolute requirements for DNA replication to begin are a free 3'-OH group and a DNA template. A RNA primer provides the free 3'-OH group. The DNA to be replicated serves as the template. It is important to remember that all DNA replication proceeds in the 5'-3' direction

1- **Initiation stage:** Unwinding of the double helix when each strand serves as a template.
The point where DNA stars unwinding is known as the (origin of replication)
2- **Elongation stage:** - process is different for the 5'-3' and 3'-5' template. a)5'-3' Template: The 3'-5' proceeding daughter strand -that uses a 5'-3' template- is called leading strand because DNA Polymerase ä can "read" the template and continuously adds nucleotides (complementary to the nucleotides of the template, for example Adenine opposite to Thymine etc).

3- **Termination**. This process happens when the DNA Polymerase reaches to an end of the strands.

RNA

Ribonucleic acid (RNA) is one of the three major bio molecules (along with DNA and proteins) that are essential for all known forms of life. The chemical structure of RNA is very similar to that of DNA, with two differences, (a) RNA contains the sugar ribose instead of sugar deoxyribose and (b) RNA has the nucleotide uracil in place of thymine .

Types of RNA

1- **Messenger RNA (mRNA)** :- It is formed in the nucleus eukaryotes and nuclear region of prokaryotes. It carries the information transcribed from the DNA to the Ribosome in the cytoplasm where protein is synthesized.

2- **Ribosomal RNA (rRNA) :- It is** the major component of the ribosome. **rRNA play** an important role in protein synthesis because it forms an essential part of the ribosome which is responsible for sequencing the amino acidsin the proper order according to the codon sequence of the DNA .

3- **Transfer RNA (tRNA) :-** It has two recognition sites .. one binds to an activated amino acid, the second is known as the ainticodon that recognizes the codon on the mRNA.

Codon:- (The genetic code)
A triplet of adjacent nucleotides in the messenger RNA chain that codesfor a specific amino acid in the synthesis of a molecule.
For each amino acid there is one or more **tRNA that** carries the specific activated amino acid to the ribosome.

Lec. 6-7

Immunity

Overview

A wide variety of organisms and their associated pose a constant threat to the human body. The human immune system – the defensive mechanism that identify and neutralize these threats is able to distinguish "non-self" organisms and molecules from "self" that which belongs within the body. Threats may enter the body from the outside (e.g., infectious organisms or toxic agents) or may arise from potentially harmful changes occurring within the body (e.g., the malignant transformation of a previously normal cell into a cancer cell).

Immunity: - is a specific defensive of a host when a foreign substance or organism invades it.

Antigens:- defined as an organism, a molecule, or part of molecule that is recognized by the immune system. Antigens may be simple or complex, protein, generally **high molecular weight** carbohydrate , and induce a **specific immune response** in organisms, foreign molecules , 4000 dalton -

Hapten:- Some foreign substances with low molecular weight must attach themselves to a long carrier molecules in order to become antigenic.

The immune system recognizes a body or substance within the organism as self or non-self.

Self:- is any substances that belongs to the organism.

Non-self:- is any substances or molecule that does not belong to the organism. An antigen is recognized as non-self by the immune system.

Immune response :- The immune response is how your body recognizes and defends itself against bacteria, viruses, and substances that appear foreign and harmful. An immune system response due to the presence of a particular foreign antigen, such as bacteria, fungi or virus.

Defensive mechanisms include :

1) Innate immunity (Natural or Non specific)

2) Acquired immunity (Adaptive or Specific)

1) Innate immunity

Component of Innate Immune system:-

First line	Second line
1) Mechanical barriers	A- cells
2) Chemical & biochemical inhibitors	1- Natural killer
3) Normal flora	2- Phagocytes
	B- Soluble factors
	C- Inflammatory barriers

First line

1) Mechanical barriers

- Intact skin
- Coughing and sneezing reflex
- Mucous secretion
- Lysozyme in tears
- Blinking reflex and tears
- The hair at the nares

2) Chemical & biochemical inhibitors

- Sweet and sebaceous secretion
- Hydrolytic enzymes in saliva
- HCl of the stomach
- Proteolytic enzyme in small intestine
- Lysozyme in tears

3) Normal bacterial flora

- Competition for essential nutrients
- Production of inhibitory substances

Second line

A) cells

1- Natural killer (NK): Large granular lymphocytes, Innate cytotoxic lymphocytes

2- Phagocytes:- Specialized cells for capture, Ingestion and destruction of invading microorganisms

* Polymorphoniclear leucocytes, mainly neutrophils: (granulocytes circulate in blood)

B- Soluble factors

1- Acute phase protein (Plasma protein, CRP=C reactive protein, Fibrin.)

2- Complement (proteins in serum, body fluids)

2- Interferons (Proteins against viral

C) Inflammatory Barriers

* Tissue damage by a wound or by invading pathogen

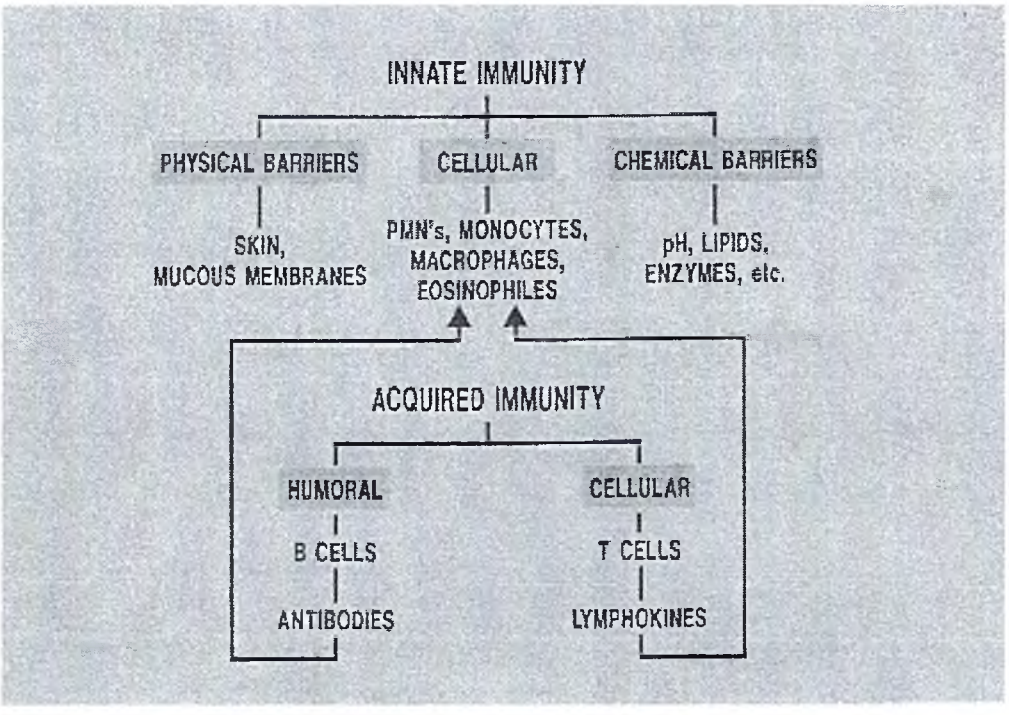
* **Inflammatory response:** Release of chemical mediators (Histamine, fibrin, cytokines)

From: 1-Tissue damage

2- Leukocytes

3- Invading microbe (these interaction lead to vasodilatation of capillaries (Redness of tissue).

NONSPECIFIC DEFENSE MECHANISMS		SPECIFIC DEFENSE MECHANISMS (IMMUNE SYSTEM)
First line of defense	Second line of defense	Third line of defense
• Skin • Mucous membranes • Secretions of skin and mucous membranes	• Phagocytic white blood cells • Antimicrobial proteins • The inflammatory response	• Lymphocytes • Antibodies



2) Acquired immunity

Is the protective defense mechanism an organism develops against foreign substances and microorganisms. This type of immunity is established throughout an individual's life.

There are two types of acquired immunity :-

1- Natural acquired immunity

A- Naturally acquired active immunity:-

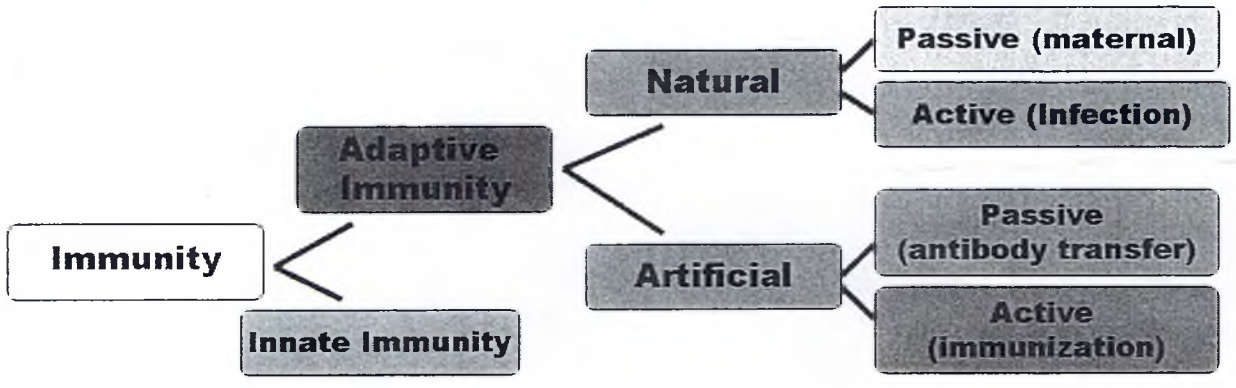
Occurs when an individual is exposed to an infectious disease . The individual's immune system respond's by making it's own lymphocytes and antibodies.

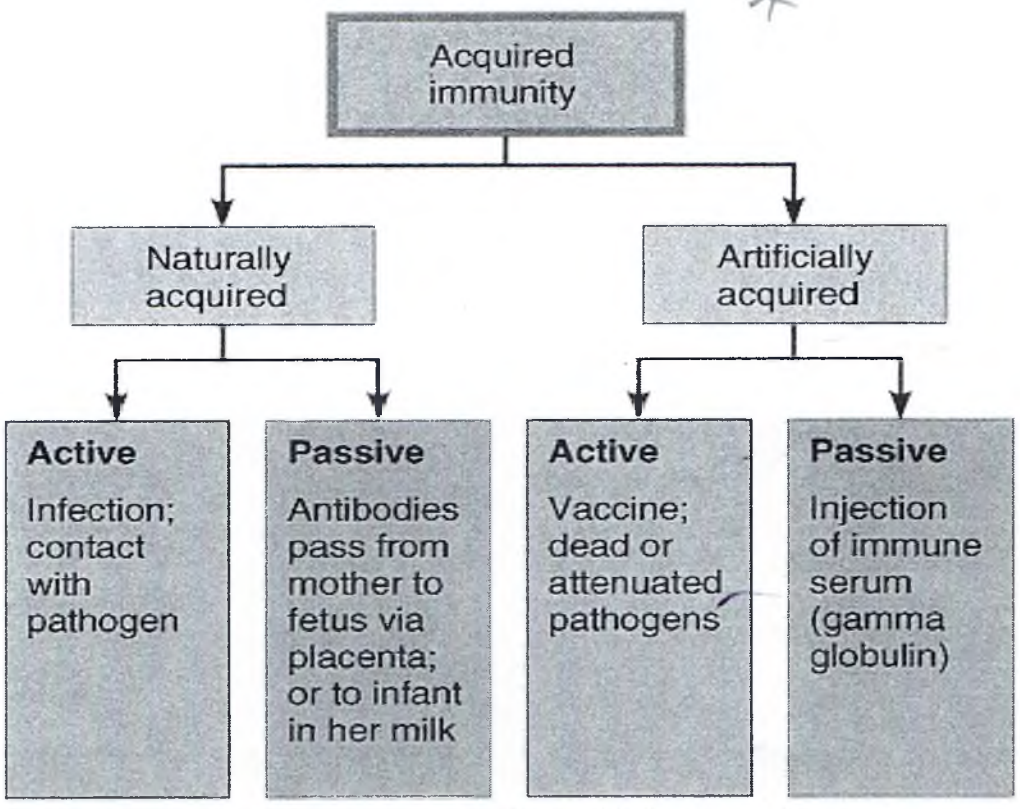
B- **Naturally acquired passive immunity:-** Occurs when an antibodies(IgG) are made by mother and passed on to the fetus through the placenta . IgA antibodies are also passed to the baby in the first secretion of breast milk, called colostrums during brest feeding.

2- Artificially acquired immunity

A- Artificially acquired active immunity:- Occurs when an individual is given a vaccine.(a vaccine is a substances that contains the weakened or dead organisms, these antigens stimulate the immune response, but do not cause major sickness. The body remembers the Ag with memory cells the next time if there is exposure to the same Ag.

B- Artificially acquired passive immunity:- Occurs when antibodies are developed outside the individual body, and intravenously injected in to the body.





Ag = antigene
Ab = antibody

The component of immune system

1-**Cellular immunity:-** Is involved a specialized lymphocyte called T cells.

2-**Humoral immunity (Antibody- mediated immunity):-** Uses antibodies in extracellular fluids, such as mucus secretion, blood plasma, and lymph to destroy Ag, these Ab produced by B-cells .

1- Cellular immunity:-

*T-cells :

T cells develop from stem cells in the bone marrow and migrate to the thymus gland where they mature. Then they migrate to the lymphatic system to begin their

fight against Ag. Some T-cells attack the Ag in a primary immune response, while others become memory cells and become a secondary immune response when the antigen is encountered later on.

Types of T-cells

Each identify by characteristics of their surface molecules. These are :-

- **Helper T (Th) cells :-** These cause the formation of cytotoxic Tcells, activated macrophage, produce cytokines, and are essential to the formation of antibodies by B-cells.
- **Cytotoxic T (Tc) cells:- These** destroy cells that have been infected by microbes.
- **Delayed hypersensitivity T(T_D) :-** These are associated with allergic reaction.
- **Suppressor T (T_S) cells :-** These turn off the immune response where there are no Ag.

  T cells are also identified by their surface receptors, called clusters of differentiation (CD)
  - **CD4- Helper T cells**
  - **CD8- Cytotoxic T cells and suppressor T cells**

*Maerophages :-

Macrophages are phagocytic cells that ingested Ag , they destroy virus, infected cells, and bacteria in intracellular location. They also eliminate some cancerous cells.

*Natural killer cells(NK) :-

NK cells are lymphocytes that destroy other cells such as tumor cells. These cells are always active and searching for an infected cells, different from other cells in the immune system, which become activated only when stimulated by an Ag.

2- Humoral immunity (Antibody- mediated immunity):-

*B- cells:-

B-cells are cells that develop from stem cells in the bon marrow, and the liver of fetuses. They are transported to the lymph node and spleen where they attach antigen receptor (antigen binding site) on the cell's surface to destroy antigens.
Once an antigen detected, the B cell with T cells activated a special group of lymphocytes antibodies used in the antibody- mediated immunity response.
T cell do not make antibodies, when B cells come in contact with extracellular antigen , the B cells transforms into plasma cells, that produced antibodies at about 2000 antibodies/ sec.

- **B cells react to one kind of antigen , because antigen receptors bind to one specific antibody.**
- **An antibody attaches to an antigen at an antigen binding site to form an antigen- antibody complex.**

Immunoglobulins :-

Immunoglobulins are synthesized by **B- lymphocytes (B-cell),** and are both synthesized and secreted by **plasma cell**.

Antibodies:- are proteins that made by the body in response to an antigen and can combined **specifically** with that antigen.

Microbes are antigenic and they contain and produce many antigens, antigens have specific sites that bind to antibodies called **"epitopes" also called "antigenic determinant". The epitope must be the right size, shape, and chemical structure for the antibody to bind to the epitop and then destroy the antigen.

The term antibody is applied to an immunoglobulin molecule with specificity for an epitope (binding site on the surface of antigen) , By this binding, antibodies can remove and destruct the antigen.

Antibodies facilitate the ability of other cells and molecules in the immune system to identify and interact with antigens.

ANTIBODY STRUCTURE

An antibody molecule is composed of two identical Ig heavy chains (H) and two identical light chains (L), each with a variable region (V) & constant region (C).

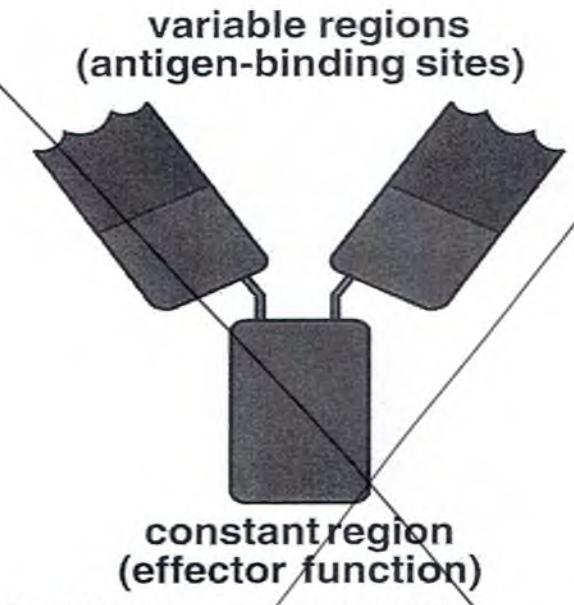


Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

Variable (V) and Constant (C) Regions

Each H-chain and each L-chain has V-region and C-region

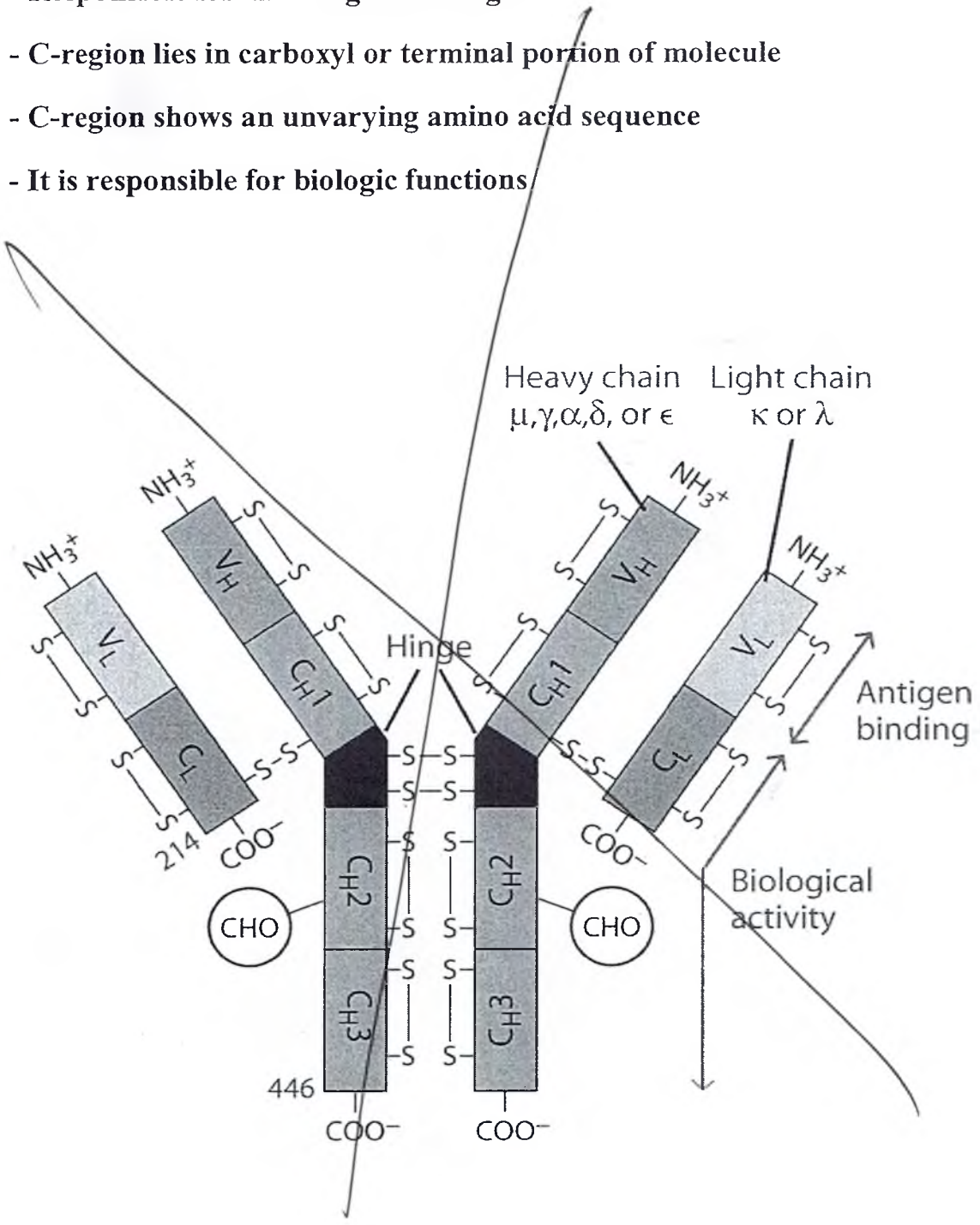
- **V-region lies in terminal portion of molecule**
- **V-region shows wide variation in amino a. sequences**

- **Responsible for the antigen binding.**

- **C-region lies in carboxyl or terminal portion of molecule**

- **C-region shows an unvarying amino acid sequence**

- **It is responsible for biologic functions**



Immunoglobulin Classes

IgG:-

Structure: Monomer

Percentage serum antibodies: 80%

Location: Blood, lymph, intestine

Half-life in serum: 23 days

Known Functions: Enhances phagocytosis, neutralizes toxins and viruses, protects fetus and newborn. IgG can cross blood vessel wall and placenta then enter tissue fluid.

IgM:-

Structure: Pentamer

Percentage serum antibodies: 5-10%

Location: Blood, lymph, B cell surface (monomer)

Half-life in serum: 5 days

Known Functions: First antibodies produced during an infection. Effective against microbes and agglutinating antigens.

IgA:-

Structure: Dimer

Percentage serum antibodies: 10-15%

Location: Secretions (tears, saliva, intestine, milk), blood and lymph.

Half-life in serum: 6 days

Known Functions: Localized protection of *mucosal* surfaces. Provides immunity to infant digestive tract, and fight antigens that affect the respiratory tract.

IgD:-

Structure: Monomer

Percentage serum antibodies: 0.2%

Location: B-cell surface, blood, and lymph

Half-life in serum: 3 days

Known Functions: on B cell surface, initiate immune response, also provides protection against parasitic worms.

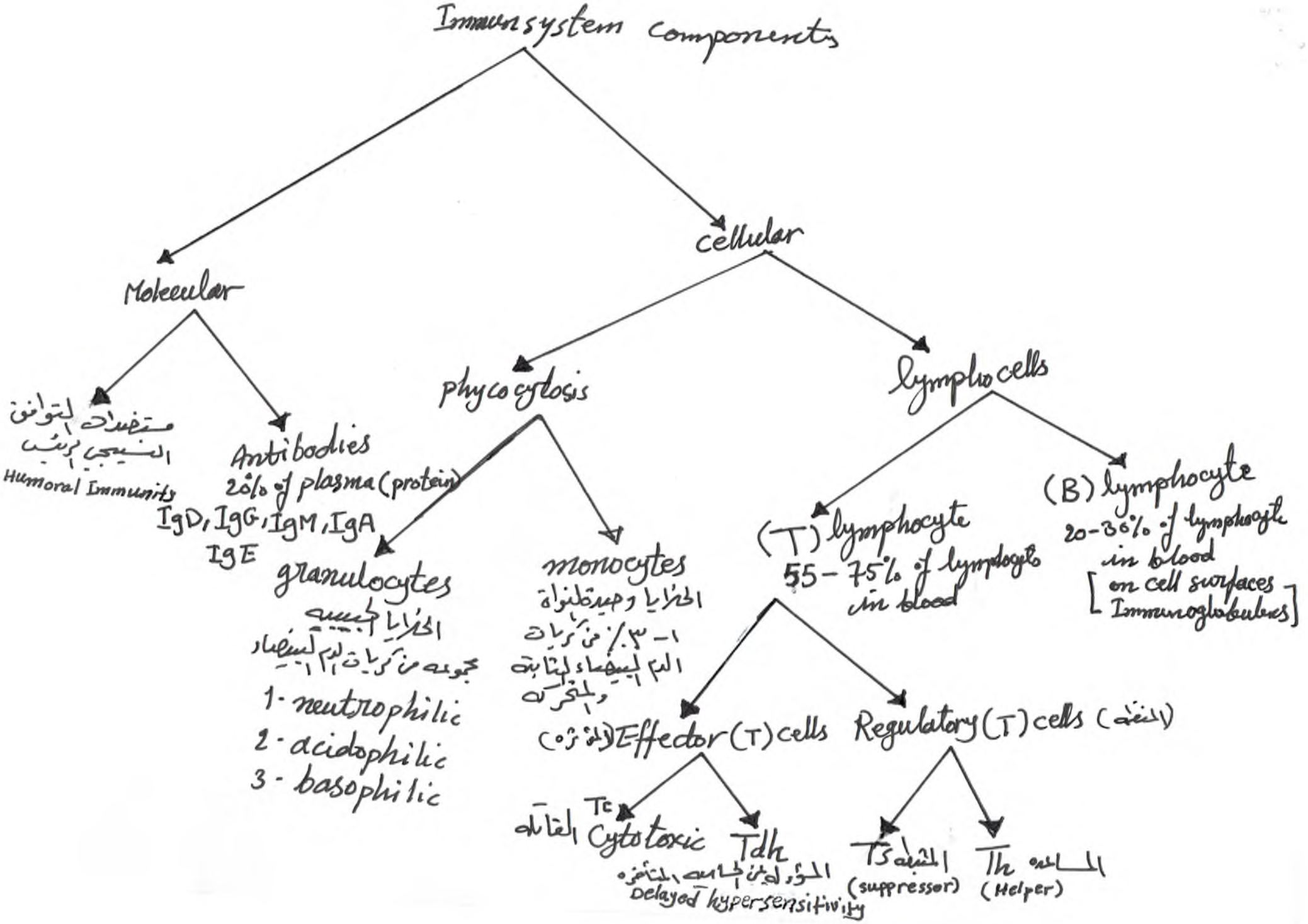
IgE:-

Structure: Monomer

Percentage serum antibodies: 0.002%

Half-life in serum: 2 days

Known Functions: IgE binds to basophil cells and mast cells surface by antigen triggers leads to release chemical mediators such as histamine, that cause an allergic reaction (responses). IgE is less than 1% of serum antibodies.


Immunsystem components
Molecular
cellular
مضادات التوافق
الشبيجي يرشب
Humoral Immunity
Antibodies
20% of plasma (protein)
IgD, IgG, IgM, IgA
IgE
phycocytosis
lymphocells
granulocytes
الخلايا الحبيبية
مجموعة من كريات الدم البيضاء
1- neutrophilic
2- acidophilic
3- basophilic
monocytes
الخلايا وحيدة النواة
١- ٣٪ من كريات
الدم البيضاء لها نواة
واحدة متحركة
(T) lymphocyte
55 - 75% of lymphocyte
in blood
(B) lymphocyte
20-30% of lymphocyte
in blood
[on cell surfaces
Immunoglobulines]
Effector (T) cells (المؤثر)
Regulatory (T) cells (المثبط)
Tc
Cytotoxic القاتلة
Tdh
المؤخر لفرط الحساسية
Delayed hypersensitivity
Ts المثبطة
(suppressor)
Th المساعدة
(Helper)