Lec.1

Microbiology

Microbiology (Greek: *mīkros* small; *bios* life), so called because it primarily deals with organisms too small for the naked eye to see, encompasses the study of organisms that cause disease, the host response to infection and ways in which such infection may be prevented. For our purposes the subject can be broadly classified into **general**, **medical** and **oral microbiology**.

Dental students need both a basic understanding of general and medical microbiology, and a detailed knowledge of clinical oral microbiology in order to diagnose oral microbial infections, which are intimately related to the overall treatment plan for their patients. Moreover, the two major oral disorders—**caries** and **periodontal disease**—that the dental practitioner is frequently called upon to treat are due to changes in the oral bacterial ecosystem and the constituent oral microbiome, and a grasp of these disease processes is essential for their appropriate management

Eukaryotes and prokaryotes

Based on the organization of their cellular structures, all living cells can be divided into two categories:

- Cell that have membrane-bound organelles, called **Eukaryotic Cells**
  - ❖ Eukaryotic cell types - Animals, plants, fungi, protozoa, and algae
- Cells that **do not** have membrane-bound organelles, called **prokaryotic cells**
  - ❖ Prokaryotic cell types - **Unicellular** organisms such as **bacteria** and **blue green algae**

A major difference between prokaryotic and eukaryotic cells is the location of chromosomes. In prokaryotes, the bacterial genome, or chromosome, is a single, circular molecule of double-stranded DNA, lacking a nuclear membrane (smaller, single or multiple circular DNA molecules called plasmids may also be present in bacteria), whereas the eukaryotic cell has a true nucleus with multiple chromosomes surrounded by a nuclear membrane.

Morphology of bacteria

Shape and size

- The shape of a bacterium is determined by its rigid cell wall. Bacteria are classified by shape into three basic groups :
  1. cocci (spherical)
  2. bacilli (rod shaped)
  3. spirochaetes (helical).

Some bacteria with variable shapes, appearing as both coccal and bacillary forms, are called **pleomorphic** in appearance.

- The size of bacteria ranges from about 0.2 to 5 μm. The smallest bacteria approximate the size of the largest viruses (poxviruses), whereas the longest bacilli attain the same length as some yeasts and human red blood cells (7 μm)

Arrangement

Bacteria, whichever shape they may be, arrange themselves (usually according to the plane of successive cell division) as pairs (diplococci), chains (streptococci), grape-like clusters (staphylococci) or as angled pairs or palisades (corynebacteria).

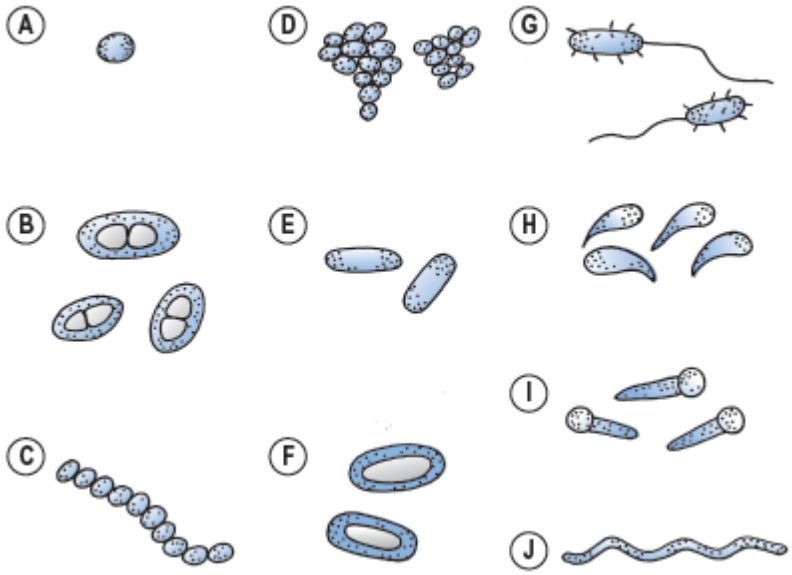


Fig. Common bacterial forms. (A) Coccus; (B) capsulated diplococci; (C, D) cocci in chains (e.g., streptococcus) and clusters (e.g., staphylococcus); (E) bacillus; (F, G) capsulated and flagellated bacillus (e.g., *Escherichia coli*); (H) curved bacilli (e.g., *Vibrio* spp.); (I) spore-bearing bacilli (e.g., *Clostridium tetani*); (J) spirochaete

Structure of bacteria

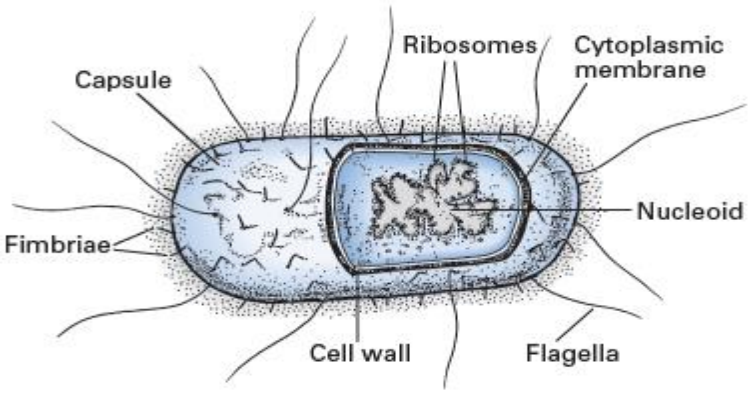


Fig. A bacterial cell.

1- Structures external to the cell wall

- **Flagella :** Flagella are whip-like filaments that act as propellers and guide the bacteria towards nutritional and other sources. The filaments are composed of many subunits of a single protein, **flagellin**
- **Fimbriae and pili:** Thin, hair like appendages on the surface of many Gram negative bacteria. Acts as organs of adhesion (attachment) - allowing bacteria to colonize environmental surfaces or cells and resist flushing. Made up of proteins called pilins. Pili can be of two types.
    a) Common pili - short and abundant
    b) Sex pili - very long pili and small number (one to six), helps in conjugation (process of transfer of DNA)
- **Glycocalyx (slime layer):** The glycocalyx is a polysaccharide coating that covers the outer surfaces of many bacteria and allows the bacteria to adhere firmly to various structures, for example, oral mucosa, teeth, heart valves and catheters, and contribute to the formation of biofilms. This is especially true in the case of *Streptococcus mutans*, a major cariogenic organism.
- **Capsule:** An amorphous, gelatinous layer surrounds the entire bacterium; it is composed of polysaccharide, and sometimes protein (e.g., anthrax bacillus). The capsule is important because:
    1. it mediates the **adhesion** of bacteria to human tissues or prosthesis such as dentures or implants, a prerequisite for colonization and infection.
    2. it hinders or inhibits **phagocytosis**; hence the presence of a capsule correlates with virulence.
    3. it helps in laboratory **identification** of organisms (in the presence of antiserum against the capsular polysaccharide the capsule will swell greatly, a phenomenon called the **quellung reaction**).
    4. its polysaccharides are used as antigens in certain vaccines because they elicit protective antibodies (e.g., polysaccharide vaccine of *S. pneumoniae*).

2- Cell wall

The cell wall confers rigidity upon the bacterial cell. It is a multilayered structure outside the cytoplasmic membrane. It is porous and permeable to substances of low molecular weight.

The inner layer of the cell wall is made of peptidoglycan and is covered by an outer membrane that varies in thickness and chemical composition, depending upon the Gram-staining property of the bacteria.

The cell walls of Gram-positive and Gram-negative bacteria have important structural and chemical differences

- The peptidoglycan layer is common to both Gram-positive and Gram-negative bacteria but is much thicker in the Gram-positive bacteria.
- The Gram-negative organisms have a complex outer membrane composed of lipopolysaccharide (LPS), lipoprotein and phospholipid. These form **porins**, through which hydrophilic molecules are transported in and out of the organism.

- The LPS of Gram-negative bacteria, which is extremely toxic, has been called the **endotoxin**. LPS is bound to the cell surface and is only released when it is lysed. It is responsible for many of the features of disease, such as fever and shock.
- The cell walls of some bacteria (e.g., *Mycobacterium tuberculosis*) contain lipids called mycolic acids, which cannot be Gram stained, and hence are called acid-fast organisms

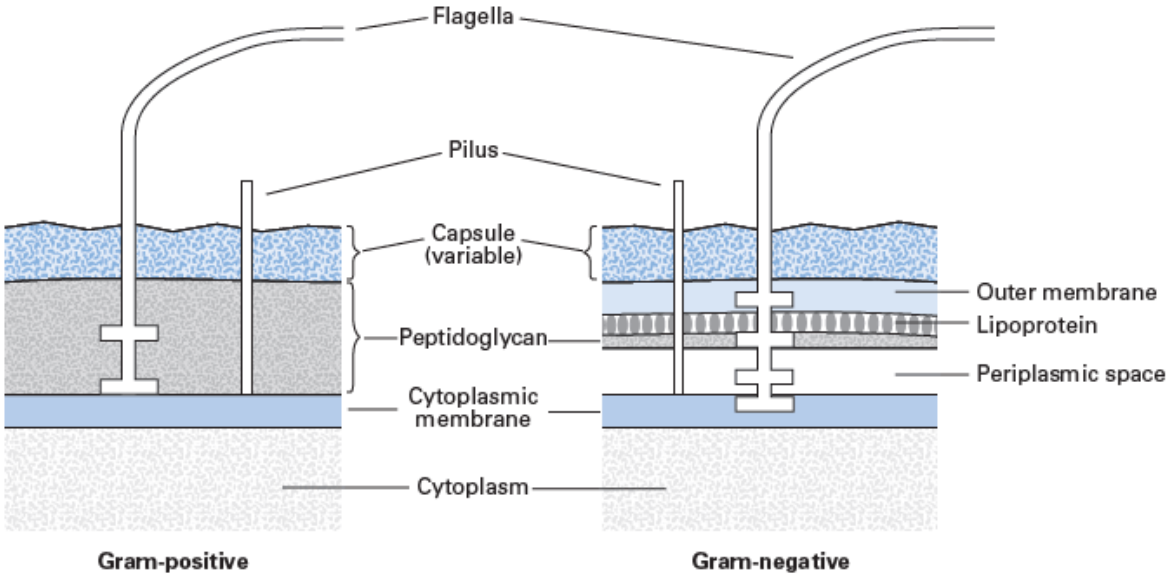


Fig. Structural features of Gram-positive and Gram-negative cell walls.

3- Cytoplasmic membrane

- Thin layer 5-10 nm, separates cell wall from cytoplasm.
- Acts as a semi-permeable membrane: controls the inflow and outflow of metabolites.
- Composed of lipoproteins with small amounts of carbohydrates.

4- Cytoplasm

The cytoplasm comprises an inner, nucleoid region (composed of DNA), which is surrounded by an amorphous matrix that contains ribosomes, nutrient granules, metabolites and various ions.

5- Nucleus: No nucleolus. No nuclear membrane. Genome – single, circular double stranded DNA (one chromosome). Divides by binary fission

6- Bacterial spores

- Spores are formed in response to adverse conditions by the medically important bacteria that belong to the genus Bacillus and the genus Clostridium.
- These bacteria sporulate (form spores) when nutrients, such as sources of carbon and nitrogen, are scarce.
- Once formed, the spore is metabolically inert and can remain dormant for many years. Spores are called either terminal or subterminal, depending on their position in relation to the cell wall of the bacillus from which they developed.
- When appropriate conditions supervene (i.e., water, nutrients), there is enzymatic degradation of the coat, and the spore transforms itself into a metabolizing, reproducing bacterial cell once again

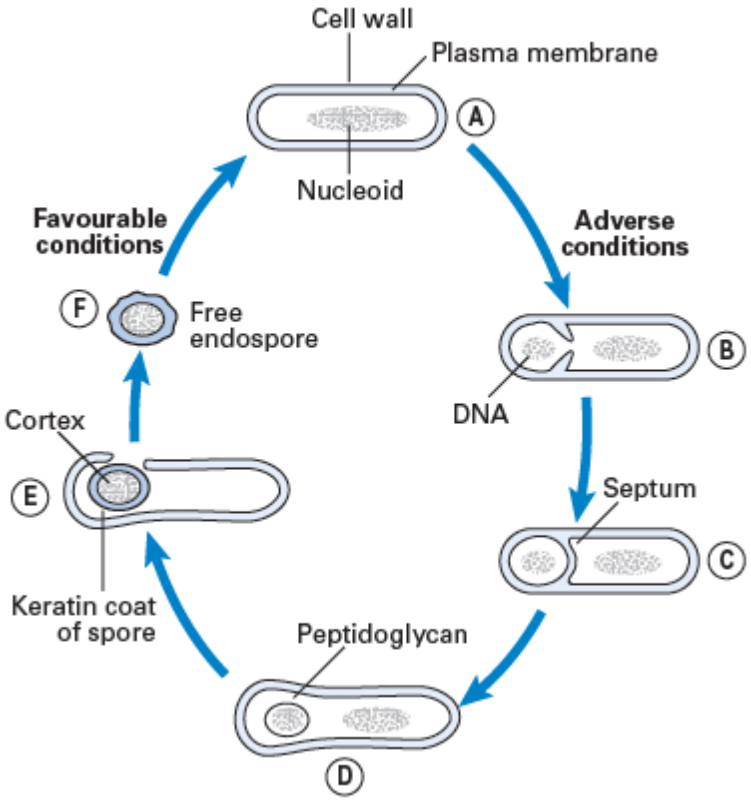


Fig. The cycle of sporulation. (A) Vegetative cell; (B) ingrowth of cytoplasmic membrane; (C) developing forespore; (D) forespore completely cut off from the cell cytoplasm; (E) development of cortex and keratin spore coat; (F) liberation of spore and conversion to vegetative state under favourable conditions.

Lec.2

Bacterial physiology

Growth

Bacteria, like all living organisms, require nutrients for metabolic purposes and for cell division, and grow best in an environment that satisfies these requirements. Chemically, bacteria are made up of polysaccharide, protein, lipid, nucleic acid and peptidoglycan, all of which must be manufactured for successful growth**.**

Nutritional requirements

Oxygen and hydrogen

Both oxygen and hydrogen are obtained from water; hence water is essential for bacterial growth. In addition, the correct oxygen tension is necessary for balanced growth. While the growth of aerobic bacteria is limited by availability of oxygen, anaerobic bacteria may be inhibited by low oxygen tension.

Carbon

Carbon is obtained by bacteria in two main ways:

1. Autotrophs, which are free-living, non-parasitic bacteria, use carbon dioxide as the carbon source.
2. Heterotrophs, which are parasitic bacteria, utilize complex organic substances such as sugars as their source of carbon dioxide and energy.

Inorganic ions

Nitrogen, sulphur, phosphate, magnesium, potassium and a number of trace elements are required for bacterial growth.

Organic nutrients

Organic nutrients are essential in different amounts, depending on the bacterial species:

1. Carbohydrates are used as an energy source and as an initial substrate for biosynthesis of many substances**.**
2. Amino acids are crucial for growth of some bacteria.
3. Vitamins, purines and pyrimidines in trace amounts are needed for growth.

Reproduction

Bacteria reproduce by a process called binary fission, in which a parent cell divides to form a progeny of two cells. This results in a logarithmic growth rate: one bacterium will produce 16 bacteria after four generations. The doubling or mean generation time of bacteria may vary (e.g., 20 min for Escherichia coli, 24 h for Mycobacterium tuberculosis); the shorter the doubling time, the

faster the multiplication rate. Other factors that affect the doubling time include the amount of nutrients, the temperature and the pH of the environment.

Bacterial growth cycle

The growth cycle of a bacterium has four main phases :

1. **Lag phase**: may last for a few minutes or for many hours as bacteria do not divide immediately but undergo a period of adaptation with vigorous metabolic activity.
2. **Log** (logarithmic, exponential) **phase**: rapid cell division occurs, determined by the environmental conditions.
3. **Stationary phase**: this is reached when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die. The bacteria have now achieved their **maximal cell density** or **yield**.
4. **Decline** or **death phase**: this is marked by a decline in the number of live bacteria

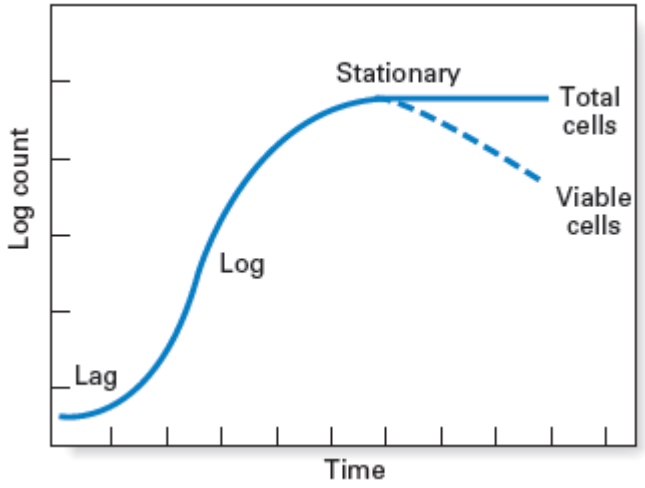


Fig. Bacterial growth curve. Lag, lag phase of growth; Log, logarithmic phase of growth.

Growth regulation

Bacterial growth is essentially regulated by the nutritional environment. However, both intracellular and extracellular regulatory events can modify the growth rate. **Intracellular factors include:**

1. **end product inhibition**: the first enzyme in a metabolic pathway is inhibited by the end product of that pathway
2. **catabolite repression**: enzyme synthesis is inhibited by catabolites.

Extracellular factors that modify bacterial growth are:

1- **temperature**: the optimum is required for efficient activity of many bacterial enzymes, although bacteria can grow in a wide range of temperatures. Accordingly, bacteria can be classified as:

- **mesophiles**, which grow well between 25° and 40°C, comprising most medically important bacteria (that grow best at body temperature)

- **thermophiles**, which grow between 55° and 80°C (*Thermus aquaticus*, for instance, grows in hot springs and its enzymes such as *Taq* polymerase are therefore heat resistant, a fact exploited by molecular biologists in the polymerase chain reaction (PCR) (see later text))
- **psychrophiles**, which grow at temperatures below 20°C.

2- **pH**: the hydrogen ion concentration of the environment should be around pH 7.2–7.4 (i.e., physiological pH) for optimal bacterial growth. However, some bacteria (e.g., lactobacilli) have evolved to exploit ecological niches, such as carious cavities where the pH may be as low as 5.0.

3- Oxygen

- **Obligate aerobes** – require O2
- **Obligate anaerobes** – die in the presence of O2
- **Facultative anaerobes** – can use O2 but also grow without it
- **Microaerophilic** -requires lower oxygen to survive.
- **Aerotolerant anaerobe:** tolerate the presence of oxygen but does not require it for its growth

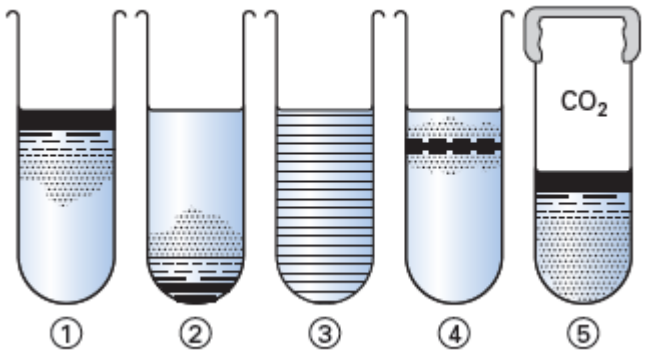


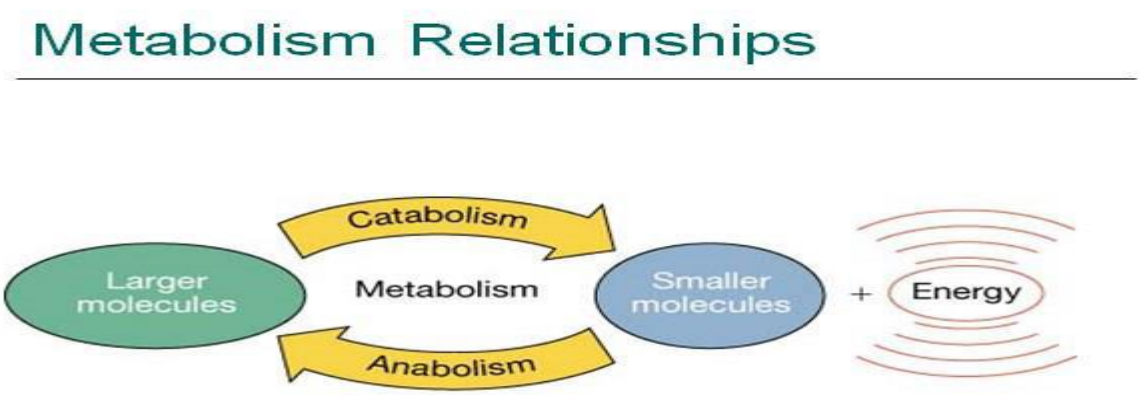
Fig. Atmospheric requirements of bacteria, as demonstrated in agar shake cultures. (1) Obligate aerobe; (2) obligate anaerobe; (3) facultative anaerobe; (4) microaerophile; (5) capnophilic organism (growing in carbon dioxide-enriched atmosphere)

Metabolism

Sum up all the chemical processes that occur within a cell

- **Anabolism**: Synthesis of more complex compounds and use of energy
- **Catabolism**: Break down a substrate and capture energy for growth and maintenance.

All cells require the energy supply to survive. The common energy form => ATP (Adenosine Tri-Phosphate)



Microbial metabolism -Is the means by which a microbe obtains the energy and nutrients, it needs to living and reproduce. Microbes use many different types of metabolic strategies, and microbes species can often be differentiated from each other based on metabolic characteristics.

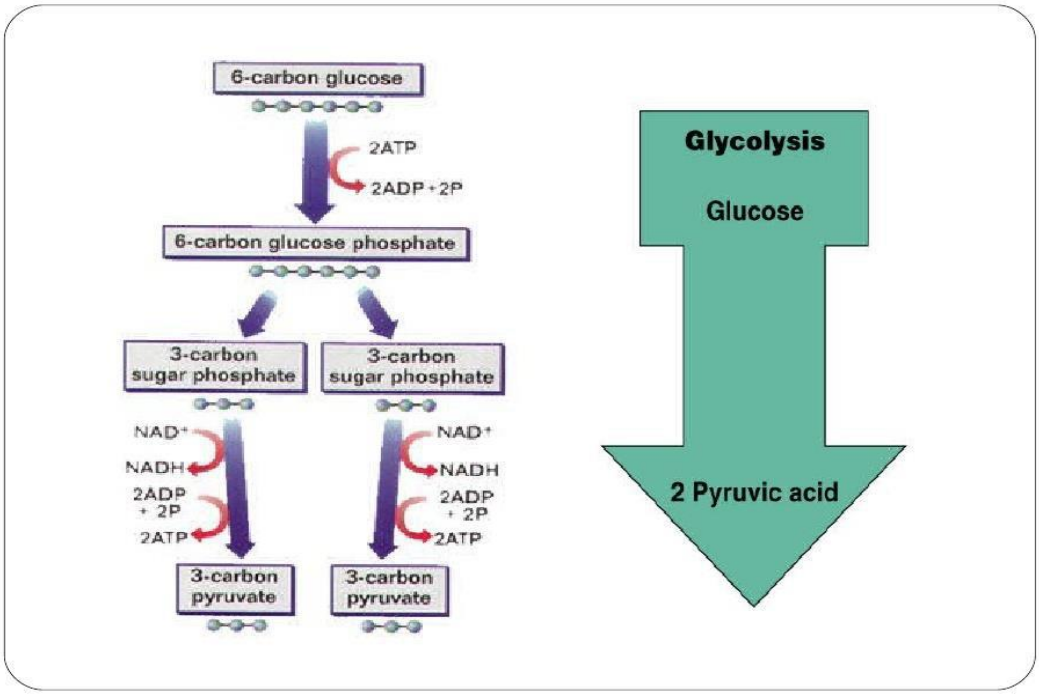
Metabolism of Glucose

- Bacteria can metabolism of glucose, proteins or lipids.
- Bacteria can produce energy from glucose. Glucose breakdown (Glycolysis) can be aerobic (using oxygen) or anaerobic (without oxygen).
- Anaerobic metabolism of glucose is also known as anaerobic glycolysis or fermentation.
- Aerobic metabolism of glucose is known as aerobic glycolysis and respiration.

Catabolism/Aerobic Respiration of Glucose : The breakdown of carbohydrates to release energy

- Glycolysis
- Krebs cycle
- Electron transport chain

Glycolysis : A nine-step biochemical reactions, each of which requires specific enzymes. Six-carbon molecule of glucose is broken down into three-carbon molecules of pyruvic acid - Can take place with or without oxygen - Produces very little energy–only 2 ATP. Takes place in the Cytoplasm of both prokaryptic and eukaryotic cells.



Krebs Cycle - The pyruvic acid produced during glycolysis are converted into acetyl-CoA. The Krebs Cycle is consists of eight reactions. -Acetyl-CoA combine with oxalate to produce citric acid (tricarboxylic acid). -Only 2 ATP produced, but a number of products like NADH, FADH 2 and H ions Mitochondria (eukaryotes); cell membrane (prokaryotes).

Electron Transport Chain - Certain of the products produced during the Krebs cycle enter the **electron transport chain** - Consist of a series of **oxidation-reduction reactions**, whereby energy is released as electrons are transferred from one compound to another. **- Oxygen** is the end of the chain; referred to as the final or terminal electron acceptor.

Cytochrome oxidase enzyme responsible for transferring electrons to oxygen.

Number of ATP Produced From One Molecule of Glucose by Aerobic Respiration

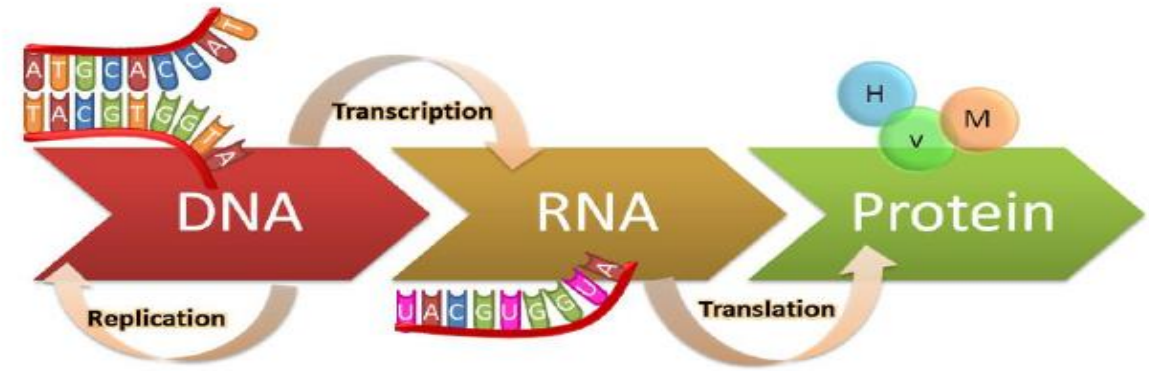
Biochemical pathways	Prokaryotic	Eukaryotic
Glycolysis	2	2
Kreps cycle	2	2
ETC	32	34
Total ATP	36	38

Lec.3

Molecular Biology

The study of the formation, structure, and function of macromolecules essential to life, such as nucleic acids and proteins, and their role in cell replication and the transmission of genetic information

Central Dogma of Molecular Biology

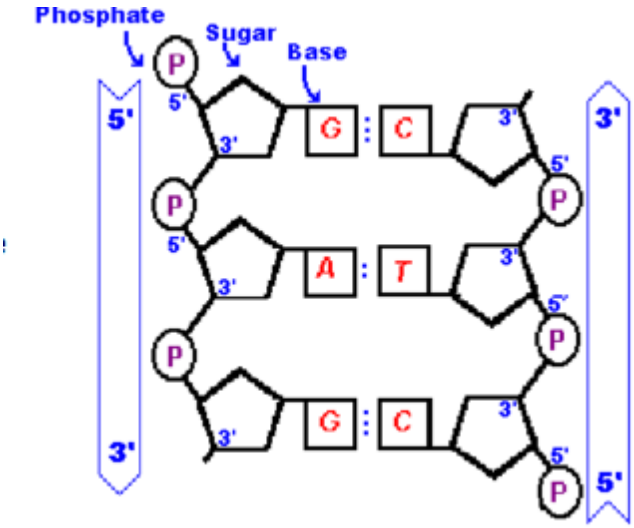


Structure and Function of Genetic Material

- Genetic Material = DNA or RNA. In the vast majority of organisms, genetic material is DNA (deoxyribonucleic acid)
- In some viruses, genetic material is RNA (ribonucleic acid)
- Genetic Material Basic Building Units: Nucleotides, Phosphate group, Pentose sugar, Nitrogenous base

Deoxyribonucleic Acid (DNA)

- Polynucleotide chains consisting of 4 bases
  - **A**denine, **T**hymine, **G**uanine, **C**ytosine
  - There are approximately 4 million base pairs (bps) in *M. tuberculosis* genome (~4,000 genes)
- Double-stranded
  - Chains are held together by complementary base pairing
- A-T
- C-G
- Complimentary and anti-parallel
  - Strands of nucleic acids have their **sugar-phosphate backbone** arranged in **opposite directions**, creating a stable **double helix**
- Replication driven by DNA Polymerase, in the 5’ to 3’ direction



Ribonucleic Acid (RNA)

- Polynucleotide formed by 4 bases
  _ Adenine, Cytosine, Guanine, Uracil
- Single-stranded
- Transcribed from DNA by RNA polymerase
- Can form hydrogen bonds with DNA, other RNAs, and itself
- Less stable than DNA
  _ hydroxyl group on the 2' carbon of the ribose

Gene

Segment of DNA that encodes a functional product, usually a protein

Gene Vs. Genomes

- Genome = All of the genetic material (DNA) in a cell
- Prokaryotic cell has only **one genome** located in the nuclear area
- Eukaryotic cell has **two genomes**
  - ❖ **Nuclear genome**
  - ❖ **Mitochondrial genome**
- If not specified, "genome" usually refers to the nuclear genome
- In human beings genes constitute only 1-3 % of the human genome
- The remaining 99% of the genome – have yet no known functions! These regions are called **non-coding regions**
- Genome = **Coding regions (genes) + non-coding regions.**

Bacterial genetics

Genetics is the study of inheritance and variation. All inherited characteristics are encoded in DNA, except in RNA viruses

The bacterial chromosome

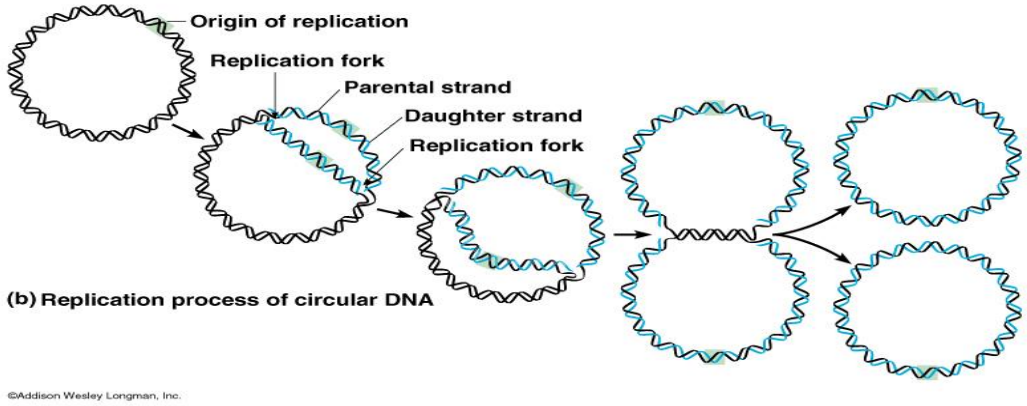
The bacterial chromosome contains the genetic information that defines all the characteristics of the organism. It is a single, continuous strand of DNA with a closed, circular structure attached to the cell membrane of the organism.

Genes

The genetic code of bacteria is contained in a series of units called **genes**. As the normal bacterial chromosome has only one copy of each gene, bacteria are called **haploid** organisms (as opposed to higher organisms, which contain two copies of the gene and hence are **diploid**).

The genetic material of a typical bacterium (e.g., *E. coli*) comprises a single circular DNA with a molecular weight of about 2 × 109 and composed of approximately 5 × 106 base pairs, which in turn can code for about 2000 proteins.

Replication of Bacterial DNA from a Single Origin



It is accurate process that insures that the progeny cells receive identical copies from the mother cell

Genetic variation in bacteria

Genetic variation can occur as a result of mutation or gene transfer.

Mutation

A mutation is a change in the base sequence of DNA, as a consequence of which different amino acids are incorporated into a protein, resulting in an altered phenotype. Mutations result from three types of molecular change, as follows.

1- Base substitution

This occurs during DNA replication when one base is inserted in place of another. When the base substitution results in a codon that instructs a different amino acid to be inserted, the mutation is called a missense mutation; when the base substitution generates a termination codon that stops protein synthesis prematurely, the mutation is called a nonsense mutation.

2- Frame shift mutation

A frame shift mutation occurs when one or more base pairs are added or deleted, which shifts the reading frame on the ribosome and results in the incorporation of the wrong amino acids 'downstream' from the mutation and in the production of an inactive protein.

3- Insertion

The insertion of additional pieces of DNA (e.g., transposons) or an additional base can cause profound changes in the reading frames of the DNA and in adjacent genes (Fig. 3.5).

Mutations can be induced by chemicals, radiation or viruses.

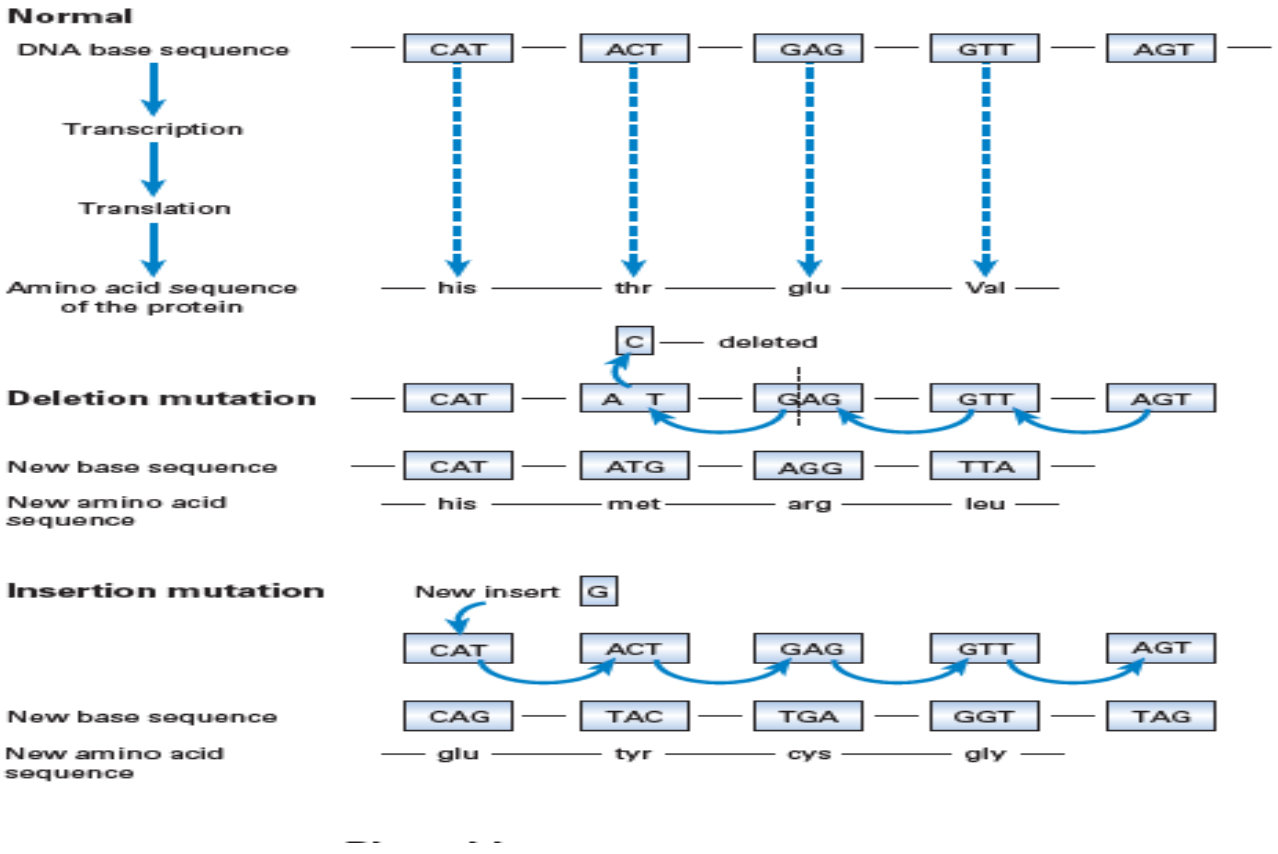


Fig. Events that entail mutation: the effect of the deletion and insertion of a single base on the amino acid sequence (and the quality of the protein thus produced) is shown

Gene transfer

Clinically, the most important consequence of DNA transfer is that antibiotic-resistant genes are spread from one bacterium to another. The transfer of genetic information can occur by:

1- Conjugation

This is the mating of two bacteria, during which DNA is transferred from the donor to the recipient cell. The mating process is controlled by an **F (fertility) plasmid**, which carries the genes for the proteins required for mating, including the protein pilin, which forms the sex pilus (conjugation tube). During mating, the pilus of the donor (male) bacterium carrying the F factor (F+) attaches to a receptor on the surface of the recipient (female) bacterium. The latter is devoid of an F plasmid (F−). The cells are then brought into direct contact with each other by 'reeling in' of the sex pilus. Then the F factor DNA is cleaved enzymatically, and one strand is transferred across the bridge into the female cell. The process is completed by synthesis of the complementary strand to form a double-stranded F plasmid in both the donor and recipient cells. The recipient now becomes an F+ male cell that has the ability to transmit the plasmid further. The new DNA can integrate into the recipient's DNA and become a stable component of its genetic material. Complete transfer of the bacterial DNA takes about 100 min.

2- Transduction

Transduction is a process of DNA transfer by means of a bacterial virus: **a bacteriophage (phage)**. During the replication of the phage, a piece of bacterial DNA is incorporated, accidentally, into the phage particle and is carried into the recipient cell at the time of infection.

3- Transformation

This is the transfer of exogenous bacterial DNA from one cell to another. It occurs in nature when

ng bacteria release their DNA, which is then taken up by recipient cells and recombined with the recipient cell DNA. This process appears to play an insignificant role in disease .

4- Transposition

This occurs when transposable elements (transposons) move from one DNA site to another within the genome of the same organism (e.g., *E. coli*). The simplest transposable elements, called 'insertion sequences', are less than 2 kilobases in length and encode enzymes (*transposase*) required for 'jumping' from one site to another.

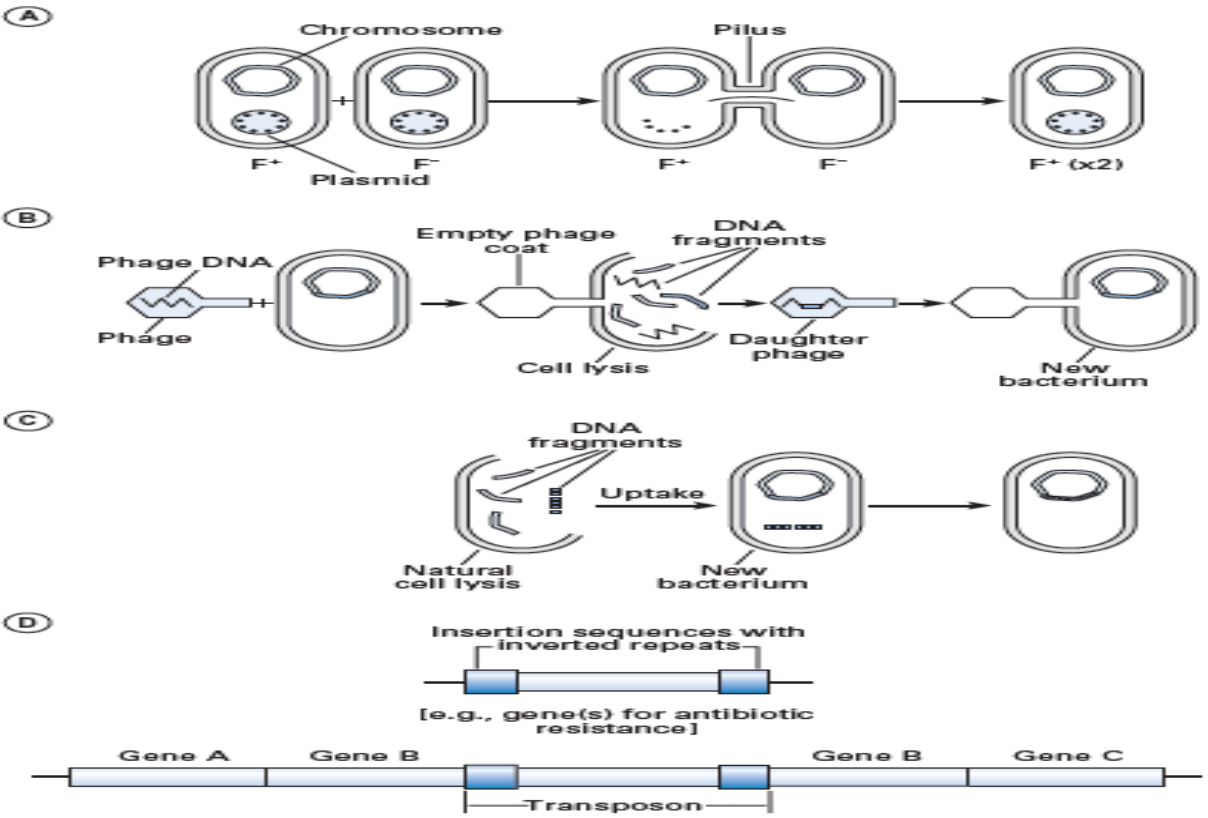


Fig. Gene transfer. (A) Conjugation: transfer of a plasmid gene by conjugation (see text); (B) transduction: phage-mediated gene transfer from one bacterium to another; (C) transformation: gene transfer by uptake of exogenous bacterial DNA by another bacterium in the vicinity (not mediated by plasmid or phage); (D) transposition: transposons (jumping genes) can move from one DNA site to another, thereby inactivating the recipient gene and conferring new traits such as drug resistance

Recombination

When the DNA is transferred from the donor to the recipient cell by one of the aforementioned mechanisms, it is integrated into the host genome by a process called recombination. There are two types of recombination:

1- **Homologous recombination**, in which two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage and reunion.
2- **Non-homologous recombination**, in which little homology is necessary for recombination to occur. A number of different enzymes (e.g., endonucleases, ligases) are involved in the recombination process.

Plasmids

Plasmids are extrachromosomal, double-stranded circular DNA molecules. They are capable of replicating independently of the bacterial chromosome. Plasmids occur in both Gram-positive and Gram-negative bacteria, and several different plasmids can often coexist in one cell.

Transmissible plasmids can be transferred from cell to cell by conjugation. They contain about 10–12 genes responsible for synthesis of the sex pilus and for the enzymes required for transfer; because of their large size, they are usually present in a few (one to three) copies per cell.

Non-transmissible plasmids are small and do not contain the transfer genes. However, they can be mobilized by co-resident plasmids that do contain the transfer gene. Many copies (up to 60 per cell) of these small plasmids may be present.

Clinical relevance of plasmids

A number of medically important functions of bacteria are attributable to plasmids (i.e., are plasmid coded). The plasmid-coded bacterial attributes include:

- antibiotic resistance (carried by R plasmids)
- the production of colicins (toxins that are produced by many species of enterobacteria and are lethal for other bacteria)
- resistance to heavy metals such as mercury (the active component of some antiseptics) and silver, mediated by a reductase enzyme
- pili (fimbriae), which mediate the adherence of bacteria to epithelial cells
- exotoxins, including several enterotoxins.

Lec.4

ANTIBIOTICS AND CHEMOTHERAPEUTIC AGENTS

The term antibiotic strictly refers to substances that are of biological origin whereas the term chemotherapeutic agent refers to a synthetic chemical. The distinction between these terms has been blurred because many of our newer "antibiotics" are actually chemically modified biological products or even chemically synthesized biological products. The generic terms to refer to either antibiotics or chemotherapeutic agents are antimicrobic or antimicrobial agent. However, the term antibiotic is often used to refer to all types of antimicrobial agents.

The current era of antimicrobial therapy began in 1935 with the discovery of sulfonamides. in1940 was found that penicillin which was discovered in 1929 has an effective therapeutic activity. During the next v 25 years researches on chemotherapy was concentrated on substances of microbial origin called antibiotics (penicillin, streptomycin, tetracycline, chloramphenicol & many other agents). Synthetic modification of antibiotics has been prominent in the development of new antimicrobial agents.

MAJOR PRINCIPLES AND DEFINITIONS

- **Selectivity:** Clinically effective antimicrobial agents all exhibit selective toxicity toward the bacterium rather than the host. It is this characteristic that distinguishes antibiotics from disinfectants. The basis for selectivity will vary depending on the particular antibiotic. When selectivity is high the antibiotics are normally not toxic. However, even highly selective antibiotics can have side effects.
- **Therapeutic index:** The therapeutic index is defined as the ratio of the dose toxic to the host to the effective therapeutic dose. The higher the therapeutic index the better the antibiotic.

CLASSIFICATION

Antimicrobial agents are classified in various ways:

1. According to microorganisms against which they are used- antibacterial, antifungal, antiparasitic, antiviral agents, etc.
2. According to their ability to kill (ends with suffix cidal) or inhibit (ends with suffix static) die microorganism, e.g. bactericidal and bacteriostatic.
3. According to the source:
   - Antibiotics: these are natural substances, produced by certain groups of microorganisms.
   - Chemotherapeutic agents: these agents are chemically synthesized.
4. According to their site of action and usage: Disinfectants destroy a wide range of microbes on non-living surfaces to prevent their spread. Antiseptics (which are applied to die living tissues and help to reduce infection during surgery), and Antibiotics (which destroy microorganisms within the body).

MECHANISMS OF ACTION OF ANTIMICROBIAL DRUGS

The mechanism of action of antimicrobial drugs one of the followings:

1. Inhibition of cell wall synthesis.
2. Inhibition of cell membrane functions.
3. Inhibition of protein synthesis (transcription & translation).
4. Inhibition of nucleic acid synthesis.

Inhibiting Cell Wall Synthesis.

- The integrity of the cell wall of a bacterium is essential to maintain its shape and provide structural support for the cell. Animal cells are enclosed only by a membrane, so the bacterial cell wall is a very suitable target for antimicrobial action. Peptidoglycan, a major component of the bacterial wall, is unique to bacteria and thus provides an ideal target for selective attack. A number of antibacterial drugs have been found that exert their action by interfering with the synthesis of peptidoglycan. One of the most important of these is **penicillin**, which belongs to a group of antibacterials known collectively as **beta-lactams**.
- β-Lactam antibiotics are bactericidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The final step in the synthesis of the peptidoglycan is facilitated by penicillin-binding proteins (PBPs). PBPs vary in their affinity for binding penicillin or other β-lactam antibiotics.
- Resistance to these compounds is frequently associated with the production of an enzyme, **beta-lactamase**, which breaks open this ring and destroys the antibacterial compound. Other members of the beta-lactam group are the cephalosporins, carbapenems and monobactams
- Beta-lactam drugs are particularly useful because they affect the synthesis of a structure unique to bacteria and are thus of low toxicity to humans. A small number of people develop an allergy to them; this is thought to be due to the formation of a conjugate of the beta-lactam ring with serum proteins, which elicits an inflammatory immune response, or to impurities in the product. People who are truly allergic to penicillin are often also allergic to most other beta-lactam antibacterial
- The second class of antimicrobial drugs that interfere with cell wall synthesis are the glycopeptide antibiotics, decaplanin, **vancomycin** and **teicoplanin**, are large molecules that interfere with cell wall synthesis by binding to the growing peptide chains that are part of the peptidoglycan molecule, thus preventing further synthesis of the cell wall. Because of their size, these compounds cannot penetrate the Gram-negative outer membrane and so they are active mainly against Gram-positive organisms.

Inhibition of cell membrane function

- Detergents which contain lipophilic hydrophilic groups, disrupt cytoplasmic membrane kill the cell. Polymyxins consist of detergent like cyclic peptide that selectively damage membrane
- There are several types of antimicrobial drugs that function by disrupting or injuring the plasma membrane. One example is daptomycin, a lipopeptide which has a distinct mechanism of action, disrupting multiple aspects of bacterial cell membrane function
- It appears to bind to the membrane causes rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis, which results in bacterial cell death.
- Another example is polymyxins antibiotics which have a general structure consisting of a cyclic peptide with a long hydrophobic tail. They disrupt the structure of the bacterial cell membrane by interacting with its phospholipids.

Inhibition of protein synthesis

- The mechanism of protein synthesis is essentially the same in eukaryotic and prokaryotic organisms. However, there are slight deference in the relative sizes and binding properties of the ribosomes in the two types of cell that permit some degree of selective toxicity. Antibacterial that inhibit protein synthesis act by interfering either with the translation of the messenger RNA into protein, or with the binding of the mRNA to the ribosomes.
- The 70S ribosomes in bacterial cells (made up of a 50S and 30S subunit) are smaller and less dense than the 80S eukaryotic host cell ribosomes. Therefore, drugs that target the 70S ribosomes are able to affect the bacterial cells adversely, while not binding significantly to the host ribosomes. However, eukaryotic mitochondria also contain 70S ribosomes, so drugs that inhibit protein synthesis in bacteria can also affect the mitochondria of the host cells.
- A number of different groups of antimicrobials affect protein synthesis in bacterial cells. They include the aminoglycosides, the tetracycline and the macrolides, as well as lincosamides, chloramphenicol and fusidic acid. Two newer classes of compounds are the oxazolidones and streptogramins. Compounds that inhibit the synthesis of protein in the cell may be bactericidal or bacteriostatic.

Inhibition of nucleic acid synthesis

- Examples of drugs acting by inhibition of DNA synthesis are quinolones, pyrimethrine, rifampicin, sulfonamides, trimethoprim Rifadin inhibit bacterial growth by binding to DNA dependent RNA polymerase enzyme of bacteria.
- All quinolones, fluroquinolones inhibit bacterial DNA synthesis by blocking DNA gyrase
- P-aminobenzoic acid is involved in the synthesis of folic acid, an important precursor to the synthesis of DNA.

- Sulfonamides are structural analogue of PABA inhibit its synthesis.
- Trimethoprim inhibits dihydrofolic acid reductase a stage in the synthesis of purines then DNA

ANTIMICROBIAL SUSCEPTIBILITY TESTING

- Bacteria exhibit great strain variations in susceptibility to amirnicrobial agents. it is, therefore, essential to determine the susceptibility of pathogenic bacteria isolated from the clinical specimens to antibiotics that are likely to be used in the treatment.
- Antimicrobial susceptibility test (AST) is performed only for pathogenic bacteria isolated from the specimen, and not for the commensal bacteria. For example, *E. coli* isolated from urine specimen should be subjected *to* AST, whereas *E. coli* isolated from stool is a commensal; hence, AST is not performed.
- The basic quantitative measures of the in vitro activity of antibiotics are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth (i.e. colonies on a plate or turbidity in broth culture) under standard conditions. The MBC is the lowest concentration of the antibiotic that kills 99.9% of the original inoculum in a given time
- For an antibiotic to be effective the MIC or MBC must be able to be achieved at the site of the infection. The pharmacological absorption and distribution of the antibiotic will influence the dose, route and frequency of administration of the antibiotic in order to achieve an effective dose at the site of infection.
- In clinical laboratories, a more common test for antibiotic susceptibility is a disk diffusion test. In this test the bacterial isolate is inoculated uniformly onto the surface of an agar plate. A filter disk impregnated with a standard amount of an antibiotic is applied to the surface of the plate and the antibiotic is allowed to diffuse into the adjacent medium.. The result is a gradient of antibiotic surrounding the disk. Following incubation, a bacterial lawn appears on the plate. Zones of inhibition of bacterial growth may be present around the antibiotic disk. The size of the zone of inhibition is dependent on the diffusion rate of the antibiotic, the degree of sensitivity of the microorganism, and the growth rate of the bacterium. The zone of inhibition in the disk diffusion test is inversely related to the MIC.
- The test is performed under standardized conditions and standard zones of inhibition have been established for each antibiotic. If the zone of inhibition is equal to or greater than the standard, the organism is considered to be sensitive to the antibiotic. If the zone of inhibition is less than the standard, the organism is considered to be resistant.

COMBINATION THERAPY

Combination therapy with two or more antibiotics is used in special cases:

- To prevent the emergence of resistant strains
- To treat emergency cases during the period when an etiological diagnosis is still in progress

- To take advantage of antibiotic synergism.

Antibiotic synergism occurs when the effects of a combination of antibiotics is greater than the sum of the effects of the individual antibiotics. Antibiotic antagonism occurs when one antibiotic, usually the one with the least effect, interferes with the effects of another antibiotic.

RESISTANCE TO ANTIMICROBIAL AGENTS

The ability of bacteria to become resistant to antibacterial agents is an important factor in their control Bacterial genes for resistance are either chromosomal or plasmid bearing

There are many different mechanisms by which microorganisms develop resistance to antimicrobial drugs

1. Microorganisms produce enzymes that destroy the active drug e g *S. aureus* resist penicillin by producing B-lactamase
2. Microorganisms change their permeability to drug e g tetracycline accumulate in susceptible bacteria but not in resistant bacteria
3. Microorganisms develop an altered structural target for the drug e. g. erythromycin resistant microorganisms have an altered receptor
4. Microorganisms develop an altered metabolic pathway that bypass the reaction inhibited by the drug e. g. sulfonamide
5. Microorganisms develop an altered enzyme that can still perform its metabolic function but it is much less affected by the drug e. g. trimethoprim

Origin of drug resistance

1- Non genetic origin of drug resistance

a. Microorganisms that are metabolically inactive (non multiplying) e.g. *M. tuberculosis*, Brucellae
b. Microorganisms may loss the specific target structure for a drug for several generations and thus be resistant e.g. Penicillin susceptible bacteria may change to cell wall deficient L form during penicillin administration
c. Microorganisms may infect the host at sites where antimicrobials are excluded or not active e.g. Gentamicin is not effective in treating salmonella enteric fever because the salmonella are intracellular the gentamicin do not enter the cell

2- Genetic origin of drug resistance

Most drug resistant microbes emerge as a result of genetic changes

a. **Chromosomal resistance:** This develop as a result of spontaneous mutation in the locus that control susceptibility to a given antimicrobial drug E g chromosomal resistant mutants to rifampicin occur with high frequency (about 10^7 to 10^5)

b. **Extrachromosomal resistance:** Bacteria often contain extra chromosomal genetic elements called plasmids Some plasmid carry genes for resistance to one or more than one antimicrobial drugs

Prophylaxis

Prophylaxis involves the administration of an antimicrobial drug before there is any evidence of infection, under conditions where the risks of an infection developing are considered to be very high. the risk of a patient acquiring an infection has to be weighed against factors such as cost, the toxicity of the drug and the risk of super infection (i.e. an overgrowth by opportunistic pathogens). Prophylaxis may be considered necessary for some surgical procedures, especially where prosthetic devices are involved. A single dose is usually given just before the procedure. It is recommended for patients who are undergoing abdominal surgery or who are susceptible to infection for other reasons. For example, patients who suffer from rheumatic heart disease involving damage to the heart valves are usually prescribed penicillin following certain dental procedures. this is to reduce the risk of infection by oral (viridians) streptococci, which may enter the bloodstream and infect the damaged valves, causing endocarditis. Pregnant women colonized with group B streptococci are given intra-partem antibiotics to reduce the risk of neonatal infection especially when premature rupture of the membranes occurs or there is prolonged labour.

Lec.5

The immune system and the oral cavity

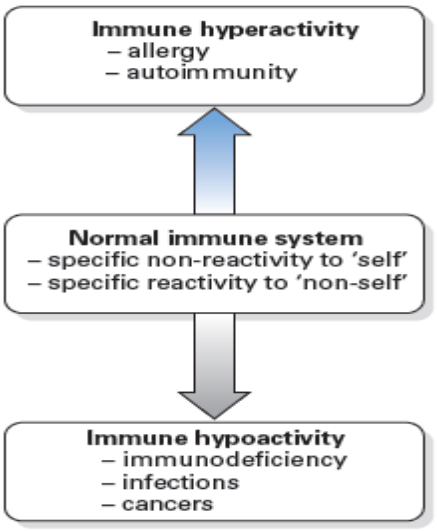
The immune system: general considerations

Immunology is the branch of biology concerned with the body's defense reactions. The word 'immunity' is derived from the Latin word *immunis*, meaning 'free of burden'. In essence, the immune system exists to maintain the integrity of the body by excluding or removing the myriad of potentially burdensome or threatening microorganisms, which could invade from the environment. Internally derived threats, mutant cells with malignant potential, may also be attacked by the immune system.

There are two kinds of immunological defense:

1. **Natural or Innate immunity**, comprising mainly pre-existing antigen-non-specific defenses
2. **Adaptive or Acquired immunity**, during which the immune system responds in an antigen-specific manner to neutralize the threat efficiently, and retains a memory of the threat so that any future encounter with the same threat will result in an accelerated and heightened protective response.

During its development, the immune system must be educated specifically to avoid reacting against all normal components of the body (**tolerance**). Immunology can be considered 'the science of self-non-self-discrimination'.



The innate immune system

These intrinsic defense mechanisms are present at birth prior to exposure to pathogens or other foreign macromolecules. They are not enhanced by such exposures and are not specific to a particular pathogen.

1- Mechanical and chemical barriers

- Intact skin is usually impenetrable to microorganisms.
- Membranous linings of the body tracts are protected by mucus, acid secretions and enzymes such as lysozyme, which breaks down bacterial cell wall proteoglycan.
- In the lower respiratory tract, the mucous membrane is covered by hair-like protrusions of the epithelial cell membrane called cilia.
- Although most pathogens enter the body by binding to and penetrating mucous membranes, several defense mechanisms, including saliva, tears and mucous secretions, are involved in preventing this entry.
- Apart from acting to wash away potential invaders, these secretions also contain antibacterial or antiviral substances.

2- Defensins and cathelicidins

- Defensins and cathelicidins are two major families of mammalian antimicrobial proteins. They contribute to host innate antimicrobial defenses by disrupting the integrity of the bacterial cell membrane.
- Further, several members of defensins and cathelicidins have been shown recently to have chemotactic effects on host cells.
- Their capacity to mobilize various types of phagocytic leukocytes, immature dendritic cells and lymphocytes, together with their other effects, such as stimulating interleukin-8 production and mast cell degranulation, provides evidence for their participation in alerting, mobilizing and amplifying innate and adaptive antimicrobial immunity of the host.

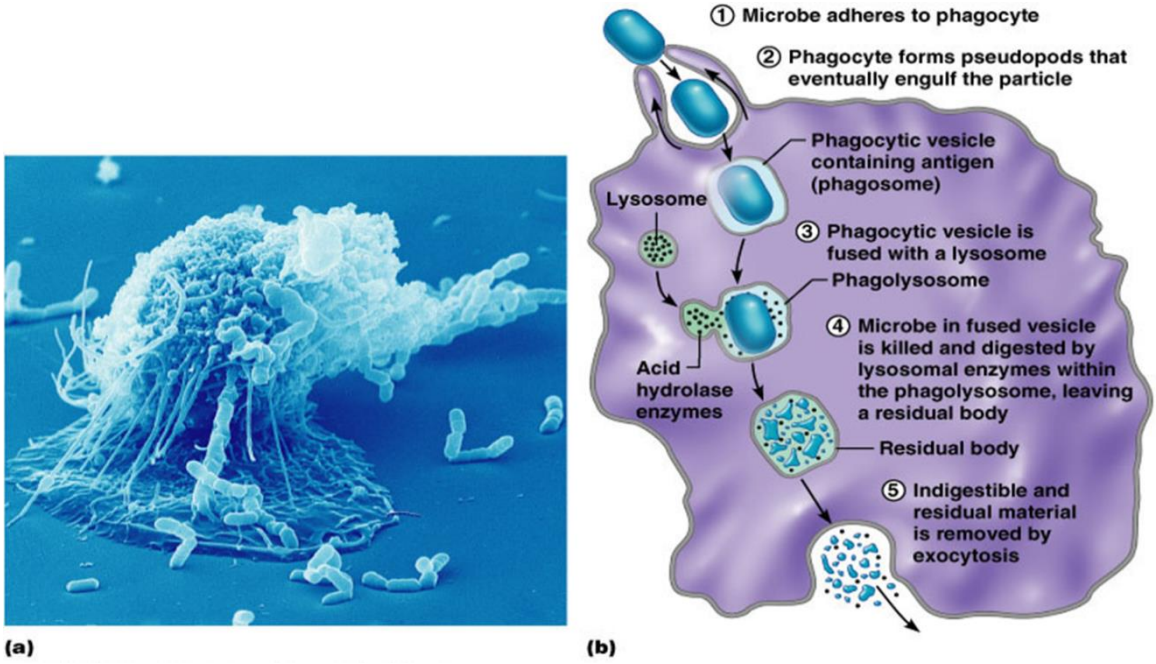
Table 8.1 Antigen-non-specific defence chemicals in oral secretions

Chemical	Antimicrobial function(s)	Major cell source(s)
Calprotectin	Divalent cation chelator, restricts microbe nutrition	Oral epithelial cells and neutrophils
Defensins (α and β types)	Membrane pore-forming peptides, cause osmotic lysis	Leukocytes and epithelial cells
Cathelicidins	Lysosomal antimicrobial polypeptides	Macrophages and neutrophils
Saliva	Ig, lysozyme, lactoferrin, peroxidases and GCF	Salivary acinar cells
Lysozyme	Muramidase activity, aggregates microbes and amphipathic sequences	Macrophages, epithelial cells and neutrophils
Peroxidase	Oxidizes bacterial enzymes in glycolytic pathways	Salivary acinar cells, neutrophils, eosinophils
Histatins, Cistatins	Various effects	Salivary acinar cells
SLPI, PRP	Antiviral activities	Various cell types
GCF	Provides blood components	Various cell types
Mucins	Aggregates bacteria, various effects, homotypic and heterotypic complexes	Salivary acinar cells

GCF, Gingival crevicular fluid; Ig, immunoglobulin; PRP, proline-rich proteins; SLPI, secretory leukocyte protease inhibitor.

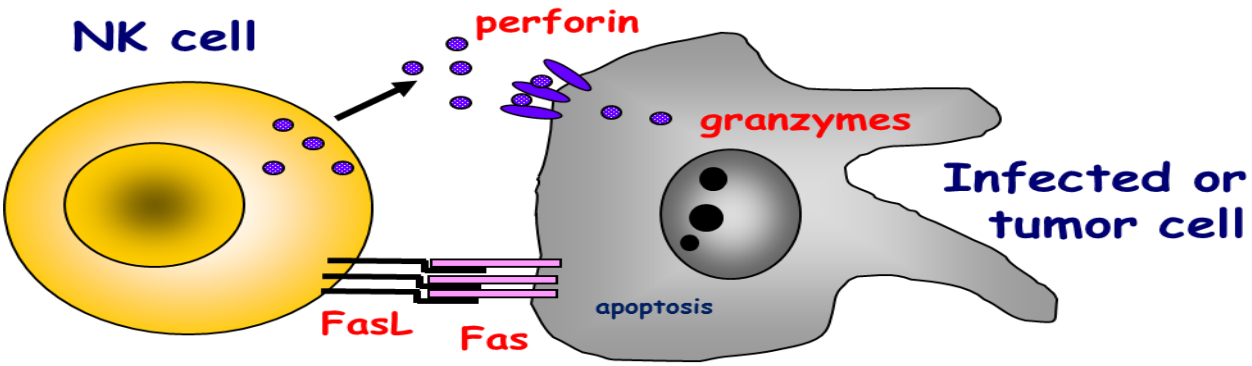
3- Phagocytosis

- Phagocytosis is a process by which phagocytic cells ingest extracellular particulate material, including whole pathogenic microorganisms. If the mechanical defenses are breached, the phagocytic cells become the next barrier. These include polymorphonuclear leukocytes (polymorphs) and macrophages.
- Macrophages are found in areas of blood filtration where they are most likely to encounter foreign particles, e.g., liver sinusoids, kidney mesangium, alveoli, lymph nodes and spleen.
- Phagocytes attach to microorganisms by non-specific cell membrane 'threat' receptors, after which pseudopodia extend around the particle and internalize it into a phagosome. Lysosomal vesicles containing proteolytic enzymes fuse with the phagosome, and oxygen and nitrogen radicals are generated, which kill the microbe. The phagocytes have several ways of dealing with the phagocytosed material. For example, macrophages reduce molecular oxygen to form microbicidal-reactive oxygen intermediates that are secreted into the phagosome.



Natural killer cells

- Large granular lymphocytes (not B-cell or T-cell)
- Kills tumor cells & viral inf.cells (intracellular pathogens)
- NK cells do not require prior immunization or activation
- They attach to 'target' cells, then cytotoxic granules are released onto surface of cell and effectors proteins penetrate cell membrane and induce death



Pathogen-associated molecular patterns, pattern-recognition receptors

The cells involved in innate immune responses such as phagocytes (neutrophils, monocytes, macrophages) and cells that release inflammatory mediators (basophils, mast cells and eosinophils) are designed to recognize only a few highly conserved structures present in many different microorganisms. These cells recognize microbial structures called **pathogen-associated molecular patterns** (PAMPs) in order to activate the innate immune response. PAMPs are molecular components common to a variety of microorganisms but not found as a part of eukaryotic cells and include:

- lipopolysaccharide (LPS) from the Gram-negative cell wall
- peptidoglycan, lipoteichoic acids from the Gram-positive cell wall
- mannose (common in microbial glycolipids and glycoproteins but rare in humans)
- bacterial DNA
- *N*-formylmethionine found in bacterial proteins
- double-stranded RNA from viruses
- glucans from fungal cell walls.

This promotes the attachment of microbes to phagocytes and their subsequent engulfment and destruction. Most defense cells (macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, lymphocytes) have on their surface a variety of receptors called pattern-recognition receptors (PRRs) capable of binding specifically to conserved portions of PAMPs so there is an immediate response against invading microbes

Acute-phase proteins

Acute-phase proteins are serum proteins produced by the liver in response to tissue-damaging infections and other inflammatory stimuli such as cytokines (e.g., interleukin-1 and interleukin-6). Although the physiological role of the acute-phase proteins is not fully understood, it has been recognized to enhance the efficiency of innate immunity.

The important role of Acute-phase proteins :-

1. It enhance the efficiency of innate immunity.
2. opsonization, coagulation, antiprotease activity and/or complement activation

The Compenant of Acute -phase proteins

1. C-reactive protein
2. α1-Antitrypsin
3. Mannose-binding protein

Interferon

Interferon, produced by virus-infected cells, comprises a group of cytokines that mediate innate immunity and includes those that protect against viral infection and those that initiate inflammatory reactions that protect against bacterial pathogens.

Inflammation: Triggered by tissue damage due to infection, heat, wound, etc.

Major Symptoms of Inflammation

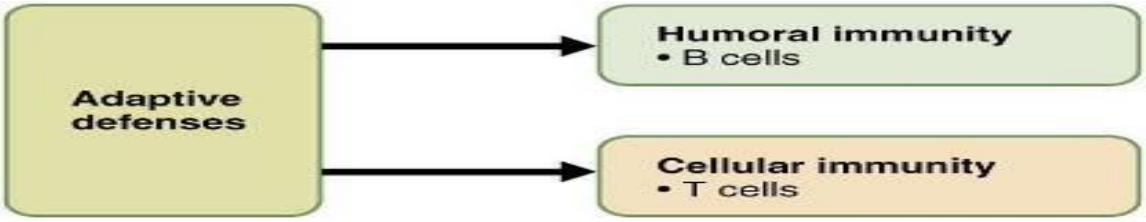
1. Redness
2. Pain
3. Heat
4. Swelling
5. Loss of function

Functions of Inflammation

1. Destroy and remove pathogens
2. If destruction is not possible, to limit effects by confining the pathogen and its products.
3. Repair and replace tissue damaged by pathogen and its products**.**

The adaptive immune system

- Is a type of resistance that is characterized by specificity for each individual pathogen, or microbial agent, and the ability to remember a prior exposure, which results in an increased response upon repeated exposure
- Adaptive immunity is often sub-divided into two major types depending on how the immunity was introduced:
  1. Naturally acquired immunity: is occurs through contact with a disease.
  2. Artificially acquired immunity: is develops only through deliberate actions such as vaccination.
- Both naturally and artificially acquired immunity can be induced in further subdivided depending on whether immunity is induced in the host or passively transferred from an immune host.



t **(b)**

Types of Acquired Immunity

1- Passive acquired immunity includes

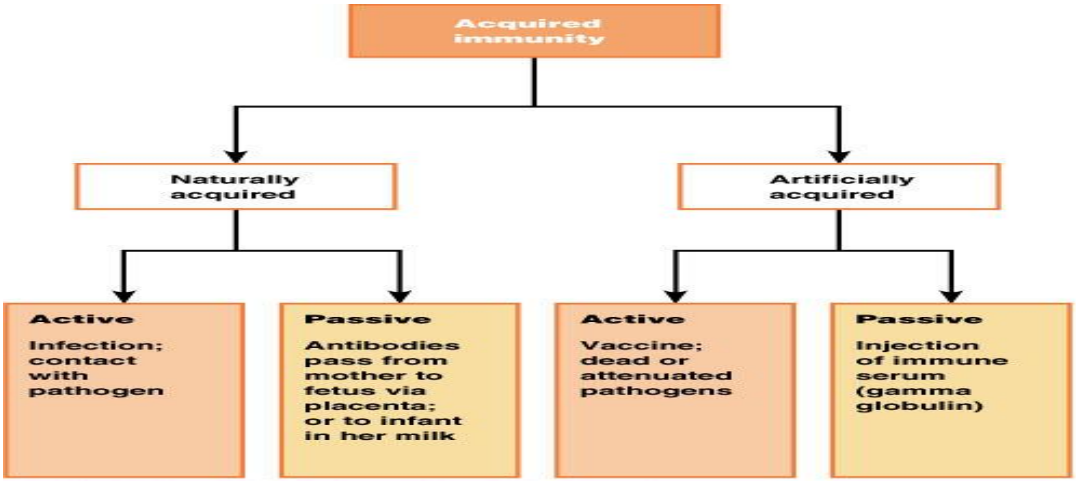
a) Naturally passive acquired immunity: antibodies are pass through placenta of fetus

b) Artificially passive acquired immunity: The injection of already prepared antibodies such as gamma globulin

2- Active immunity

a) Natural active acquired immunity: Following clinical or subclinical infections

b) Artificial active acquired immunity:_Following vaccination with live or killed infectious agents or their products



Adaptive immunity is mediated by B or T lymphocytes and stimulated by exposure to infectious agents.

1- Humoral Immunity (Antibody Immunity):

- Type of immunity that is mediated by secreted antibodies produced by the B-lymphocyte cells. Secreted antibodies bind to antigens on the surfaces of invading microbes (such as viruses or bacteria), which exposure them for destruction.
- Humoral immunity is called as such, because it involves substances found in the body fluids**.**

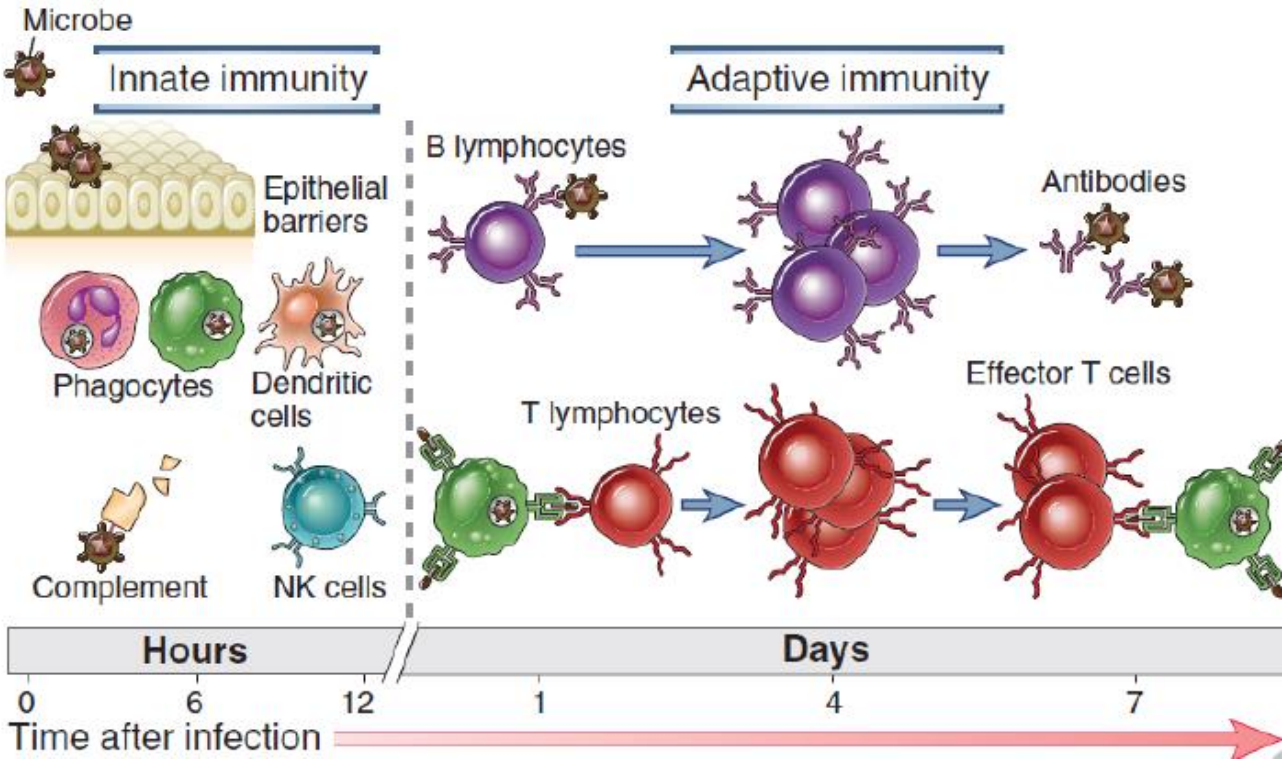
Cell-mediated immunity (Cellular Immunity):

- Since antibodies are useless against intracellular antigens, cell-mediated immunity is needed.

- Two major populations of T cells mediate cellular immunity:
  1. CD4 cells are helper T cells (TH).
  2. CD8 cells are cytotoxic T cells (TC) that destroy cells harboring foreign antigens.
- Regulatory T cells that release cytokines, which suppress the activity of both T cells and B

The innate and adaptive immune response

Characteristics	Cells	Molecules
Innate immunity		
Responds rapidly No memory No specificity No prior exposure is required	Physical barriers Phagocytes (PMNs and macrophages) Natural killer cells	Humoral factors Complement Acute phase Proteins Cytokines
Adaptive immunity		
Responds Slowly Memory Highly specific Present after exposure to an Ag	T cells B cells Dendritic cells	Antibodies Cytokines Granzymes



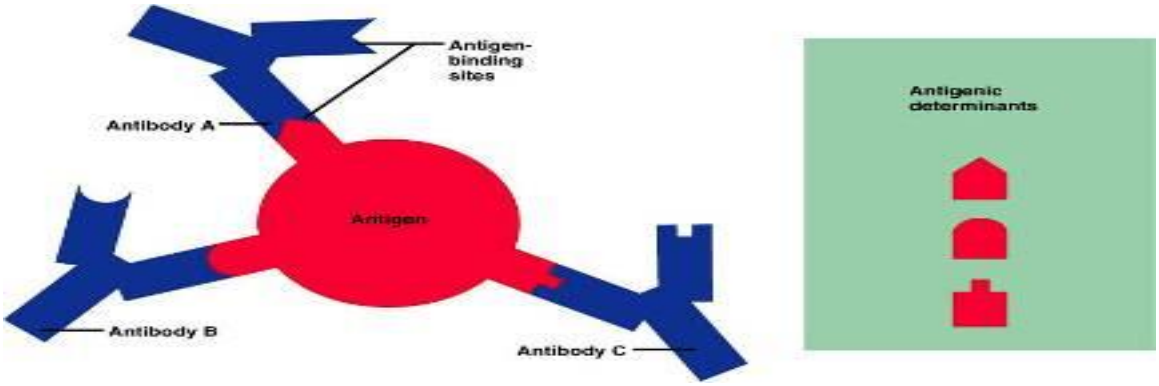
Lec.6

Antigen

The term *antigen* was coined from the words *antibody* and *generator*. Thus, an antigen was originally considered to be a substance that induced production of antibody. However, with greater insight into mechanisms of immune response following definition is considered appropriate for antigen. "An antigen when introduced into a host induces the formation of specific antibodies and T lymphocytes that are reactive against the antigen."

Antigenic Determinant Sites (Epitopes)

The whole antigen does not induce an immune response. Only a limited part of an antigen molecule is inducer of B and T cell responses. It is also that part of antigen with which the antibody or T cell reacts. This is called an antigenic determinant site or *epitope*



Classification of Ag

Based on Immunogenicity

1. **Complete antigen** : Substances which can induce Ab formation by themselves and can react specifically with these antibodies

2. **Incomplete antigen (haptens):** substances unable to induce Ab formation on its own but can become immunogenic when linked to proteins, called carrier proteins. They are of two types: **Complex & Simple**

Based on origin:

1. **Exogenous antigens** are antigens that have entered the body from the outside, for example by inhalation , ingestion , or injection . The immune system's response to exogenous antigens is often subclinical.
2. **Endogenous antigens** are antigens that have been generated within previously normal cells as a result of normal cell metabolism , or because of viral or intracellular bacterial infection .

Autoantigens

is usually a normal protein or complex of proteins that is recognized by the immune system of patients suffering from a specific autoimmune disease . These Ags should, under normal conditions, not be the target of the immune system, but, due to genetic and environmental factors, the normal immunological tolerance for such an Ag has been lost in these patients. Iso antigens & Heterophile

Super antigens are a class of antigens that cause nonspecific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release. Produced by » Bacteria » Virus » Mycoplasma. Ex.

1. Staphylococcal enterotoxins 2. Staphylococcal toxic shock toxin (TSST-1) 3. Streptococcal pyrogenic
2. Retrovirus in mice, is also known to produce superantigen antigen

Determinants of Antigenicity

There are several important determinants of any antigenicity.

- Macromolecular size: Proteins of molecular weight exceeding 10,000 daltons are good antigens. Polysaccharides are poor antigens.
- Molecular complexity: The antigenic potency of macromolecule increases with the complexity of structure and accordingly quaternary structures are antigenically most potent
- Biodegradibility: If a substance is insoluble in body fluids and cannot be converted to soluble forms by tissue enzymes, it may not act as an antigen. All cellular antigens, bacteria, viruses and red blood cells are quickly engulfed by phagocytic macrophages and digested to their soluble constituents.
- Foreignness: To be antigenic, the macromolecule must be foreign to the animal being immunized. Foreignness here denotes being of different antigenicity as the host. The more foreign the antigen source better it will be.
- Specificity: Antigen attaches specifically to an antibody because of the "fit" between the antigenic determinant on its surface as well as the receptor on antigen binding site on antibody. The effect of antibody is activated only after the "fit" has taken place.

Antibodies

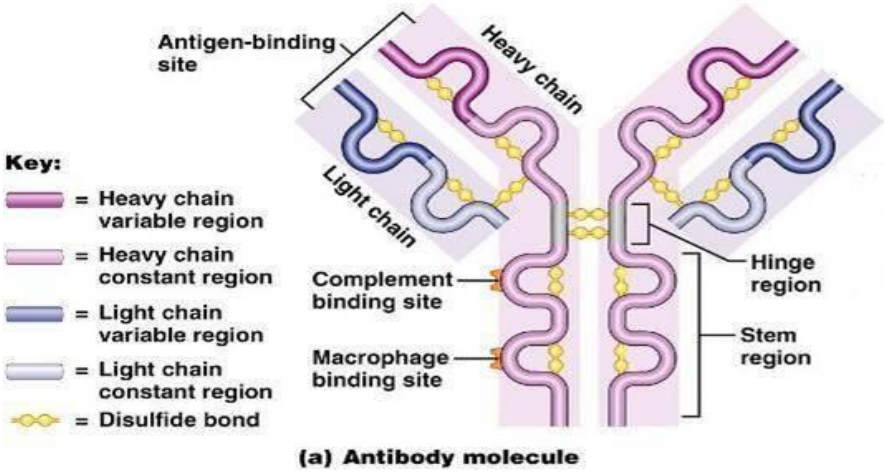
Antibody – “a Y-shaped protein, found on the surface of B-Cells or free in the blood, that neutralize antigen by binding specifically to it”. Also known as an Immunoglobulin.

They are synthesized by B lymphocytes and secreted by plasma cells. Depending on the electrophoretic migration, 3 types of globulins are present in the blood, namely α, β and γ. So antibodies are gamma (γ) globulin

Structure of Antibody

All antibodies have a common basic structure.

- Each antibody molecule has **4 polypeptide chains**.
- Two small identical light chain(L) and two longer identical heavy chain(H).
- L & H chains are further divided into variable & constant region.
- The central region of heavy chain at which the arms of the antibody molecule forms a Y is **called hinge region**.
- Fab: Fragment antigen binding - these fragments were called the Fab fragments because they contained the antigen binding sites of the antibody. It is also called paratope, is a part of an antibody which recognizes and binds to an antigen.The combining site of the antibody is created by both VH and VL.
- Fc : Fragment crystallization- this fragment was called Fc because it was easily crystallize



GENERAL FUNCTIONS Of IMMUNOGLOBULINS

A. Primary function: Antigen binding

Immunoglobulins bind specifically to one or a few closely related antigens.

B. Secondary function: Effector Functions

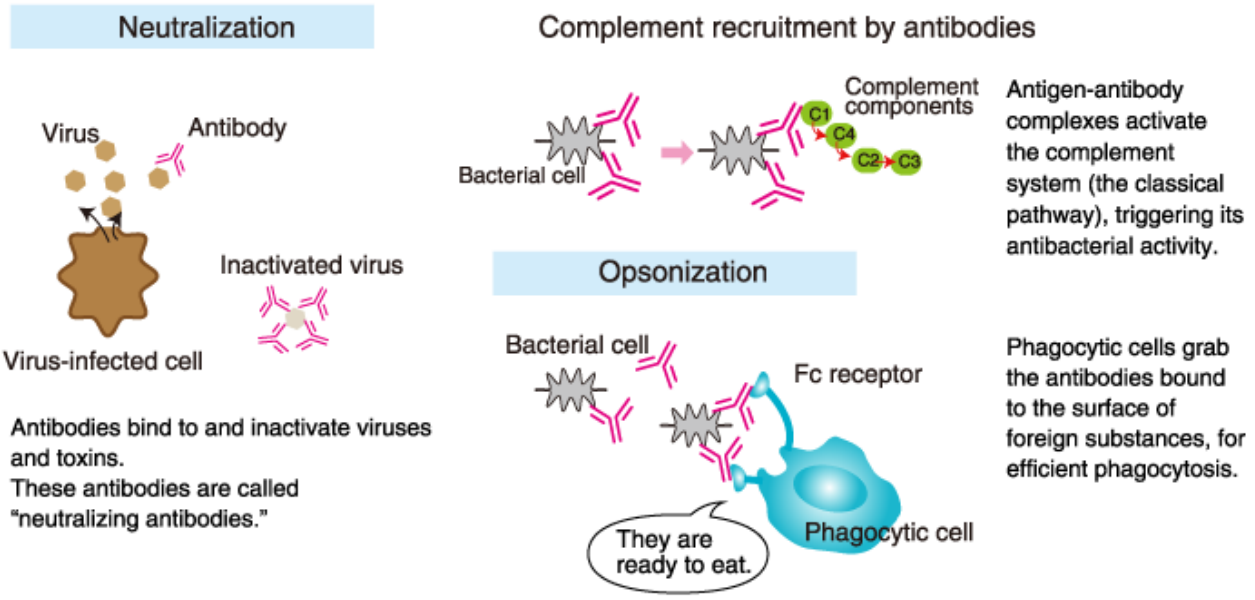
The binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are secondary "effector functions" of antibodies. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

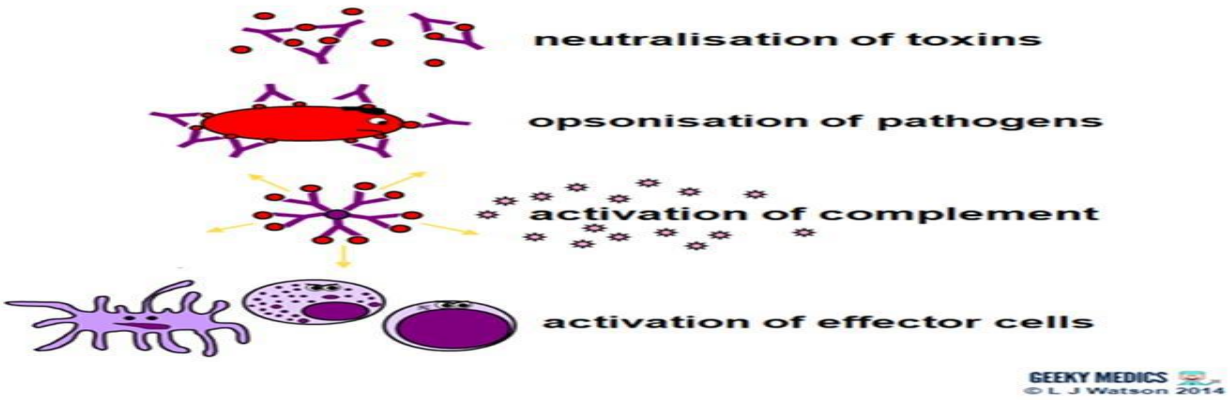
1- **Agglutination:** Agglutination of particulate antigen, including bacteria and viruses. IgM is particularly suitable for this function.

2- **Opsonization:** Opsonization i.e. coating of bacteria with antibody's Fab region (IgG). This facilitates phagocytosis by cells possessing Fc receptor, e.g. neutrophil, polymorphonuclear leucocytes".

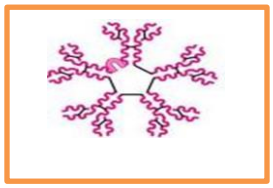
3- **Neutralization:** Neutralization of toxins released by bacteria e.g. tetanus toxin is neutralized when specific IgG antibody binds, thus preventing the toxin binding to it's receptor. In the case of viruses, antibodies can hinder their ability to attach to receptors on host cells.

4- **Complement activation (classical pathway):** especially by IgM and IgG, leads eventually to death of bacteria by the terminal complement components which make holes in the cell wall, leading to an osmotic death.
5- **Precipitation:** Precipitation of soluble antigens by immune complex formation. They can be removed by phagocytic cells. and can fix complement.
6- **Antibody dependent cell mediated cytotoxicity (ADCC):** Antibodies bind to organisms via their Fab region. Large granular lymphocytes NK cells, attach via Fc receptors, and kill these organisms not by phagocytosis but by release of toxic substances called perforins.
7- **Mucosal protection:** This is provided mainly by IgA. IgA acts chiefly by inhibiting pathogens from gaining attachment to mucosal surfaces.





Antibody Classes



IgM:

- occurs as a monomer & a pentamer .
- Occurs on the B-cell surface (Monomer).
- The Ig of early primary plasma cell response, circulating antibody.
- a potent agglutinator. Complement binding (Pentamer).

IgE:

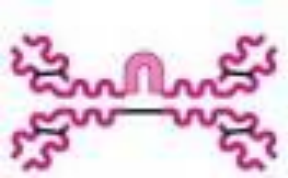
- the Ig associated with allergies (Monomer)..
- Stem binds to mast cells & basophils.
- Receptor binding results in histamine release & inflammation.

IgG:

- the most abundant circulating Ig. The dominant circulating Ig of the primary & the secondary response. Crosses the placenta. Complement binding (Monomer).

IgA:

- the Ig of secretions. Helps prevent antigen penetration of membranes (Dimer).



IgD:

- the Ig of B-cell activation. Found on B-cell surface (Monomer).

Organs and Cells of Immune System

Organs concerned with immune reactions are called lymphoid organs. They contain lymphoid cells.

Lymphoid organs are of 2 types.

1. Primary lymphoid organs
2. Secondary lymphoid organs

Primary lymphoid organs

Are the major site of lymphopoiesis. The lymphoid cells proliferate, differentiate and mature in to immune competent cells in the absence of antigenic stimulation. The primary lymphoid organs are large at birth and they atrophy with age progression; major primary lymphoid organs are

1. Thymus (site of T-cell maturation in human)
2. Bone marrow (site of B cell maturation in human)
3. Bursa of fabricious (site of B-cell maturation in bird)

Thymus:

- is the site of T cell differentiation and maturation, consist of the cortex and the medulla, cells found in thymus are; stroma cells, epithelial cells, macrophages, dendritic cells and thymocytes (the cells migrate from the bone marrow to the thymus and then become thymocytes).
- In cortex any thymocyte acquire receptors for self Ag will be killed by apoptosis (programmed cell death) this process called **negative selection**
- In medulla **positive selection** occur when cells acquire molecules (receptors) by which recognized Ags in association with class I MHC and class II MHC molecules.

Bone marrow: is the site of generation of all circulating blood cells in the adult, including immature lymphocytes, and is considered as the site of B cell maturation

Secondary lymphoid organs

Lymphocytes are made functional in the secondary lymphoid organs. The secondary lymphoid organs are small and poorly developed at birth and they grow progressively with age. The secondary lymphoid organs include:

1. **Lymph nodes:** are the organs in which immune responses to lymphoid-borne antigens are initiated, they have many functions.
2. **Spleen:** is the major site of immune responses to blood-borne antigens
3. **Mucosal associated lymphoid tissues (MALT):** The MALT of the gastrointestinal and respiratory tracts is colonized by lymphocytes and antigen presenting cell that initiate immune responses to ingested and inhaled antigens.

Cells of the immune system

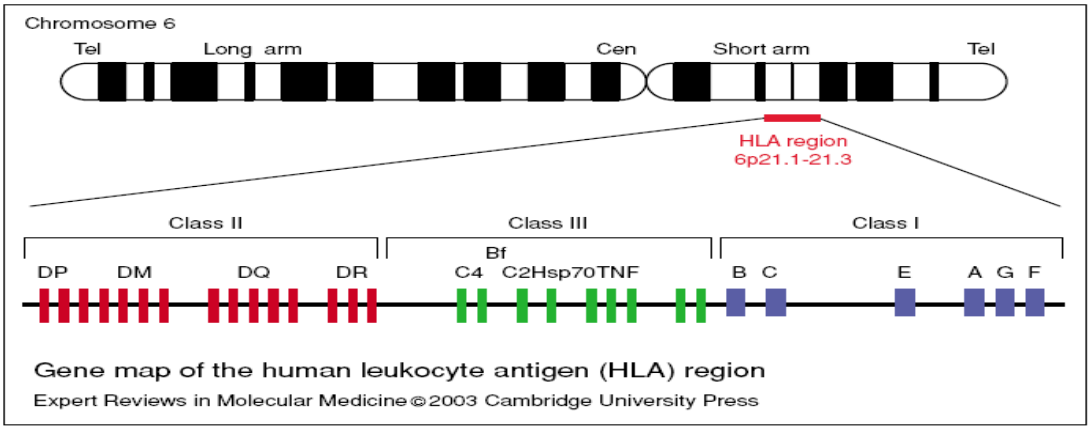
- All the cells of the immune system are derived from self-regenerating **haematopoietic stem cells** present in bone marrow and liver.
- These differentiate along either the **myeloid** or the **lymphoid** pathway.
- Myeloid precursor cells give rise to mast cells, erythrocytes, platelets, dendritic cells, polymorphs (eosinophils, basophils, neutrophils) and mononuclear phagocytes (monocytes in the blood, macrophages in the tissues).
- Lymphoid precursor differentiation gives rise to T (thymus-dependent) lymphocytes, B (bone marrow-derived) lymphocytes and NK lymphocytes.
- During post-natal life, B cell genesis takes place in the bone marrow. Each newly formed B cell expresses a unique B cell receptor (BCR) on its membrane for antigen-binding. Although T lymphocytes also arise in the bone marrow, they migrate to the thymus to mature.

- The B lymphocytes are responsible for secreting Ig antibodies and can also function as highly efficient **antigen-presenting cells** (APCs) for T lymphocytes.
- During its maturation, the T lymphocyte expresses a specific antigen-binding molecule known as the T cell receptor (TCR) on its membrane.
- The latter are divided into two major subsets: **T-helper cells**, which usually bear the 'cluster of differentiation' marker CD4, and **T-cytotoxic cells**, which usually carry CD8.
- The T-helper cells are required for activating the effector function of B cells, other T cells, NK cells and macrophages.
- They do this by transmitting signals via cell-to-cell contact interactions and/or via soluble hormone-like factors called lymphokines.
- The T-cytotoxic cells kill target cells such as virus-infected host cells. Another functional property of some T lymphocytes is to downregulate immune responses.
- These **T-suppressor** cells are usually CD8-positive. Dendritic cells and monocytes/macrophages play key roles in the immune system as APCs.

Lec.7

Major histocompatibility complex

- In all vertebrates there is a genetic region that has a major effect on graft survival. This region is referred to as the MHC.
- In human MHC is called human leukocyte antigen is a cluster of genes located on the short arm of chromosome 6, and encoding cell-surface molecules (class I and class II) that are involved in interactions with T-cells. HLA genes are the most polymorphic genes in man.
- Their function is to bind APC-processed short antigenic peptides and present them on the APC surface to T cells
- HLA phenotype is responsible for tissue transplant rejection when the recipient and donor are not HLA-matched.



The HLA genes

- The HLA genes are divided into 3 regions, one encode for class I, other encode class II, the last encode for class III .
- Class I region: encodes to class I molecules: HLA-A, -B and –C molecules.
- Class II region: previously known as immune response (Ir) genes. This encodes to class II molecules HLA-DR and DQ molecules which control the immune response to various antigens.
- The HLA class III region located between class I and class II genes and encodes components of the C system(C2, C4 and factor B).

The HLA molecules

Three types of molecules referred to as class I, II and III antigens are expressed by this gene complex.

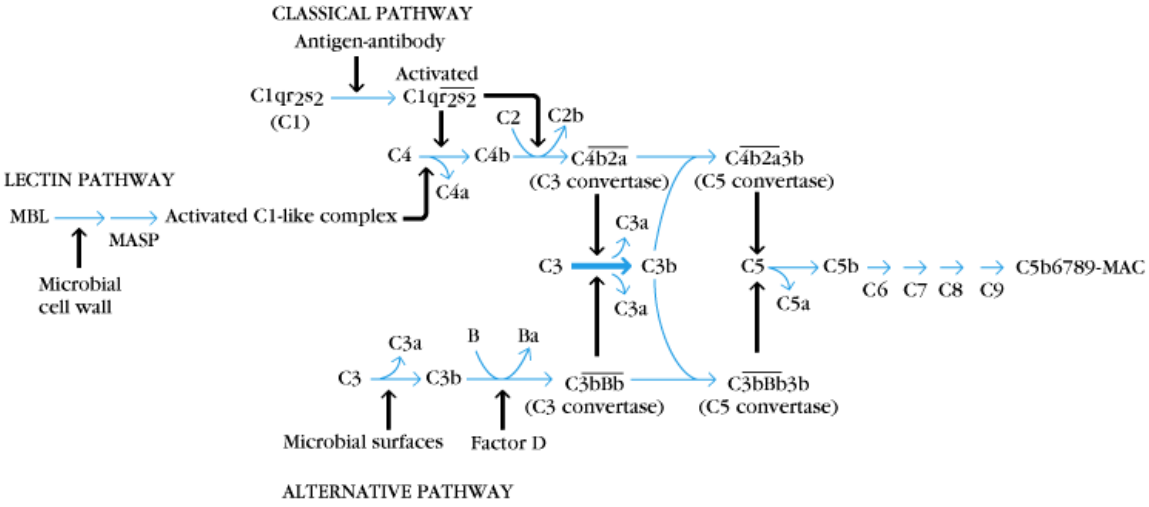
1- **The HLA-class I molecule:** The HLA class I molecules are expressed on most nucleated cells (but not RBCs), and play a central role in the activation of cytotoxic T-lymphocytes (CD8) by presenting short peptides of Ag to these cells.
2- **The HLA-class II molecule:** Class II molecules interacts with CD4-T cells, have a helper function. They are expressed on antigen presenting cells (APC): Dendritic cells, Macrophages, B cells.
3- **The HLA-class III molecule:** Class III molecules are not membrane proteins, but they are serum proteins and have no role in antigen presentation; however, they play some role in immune response.

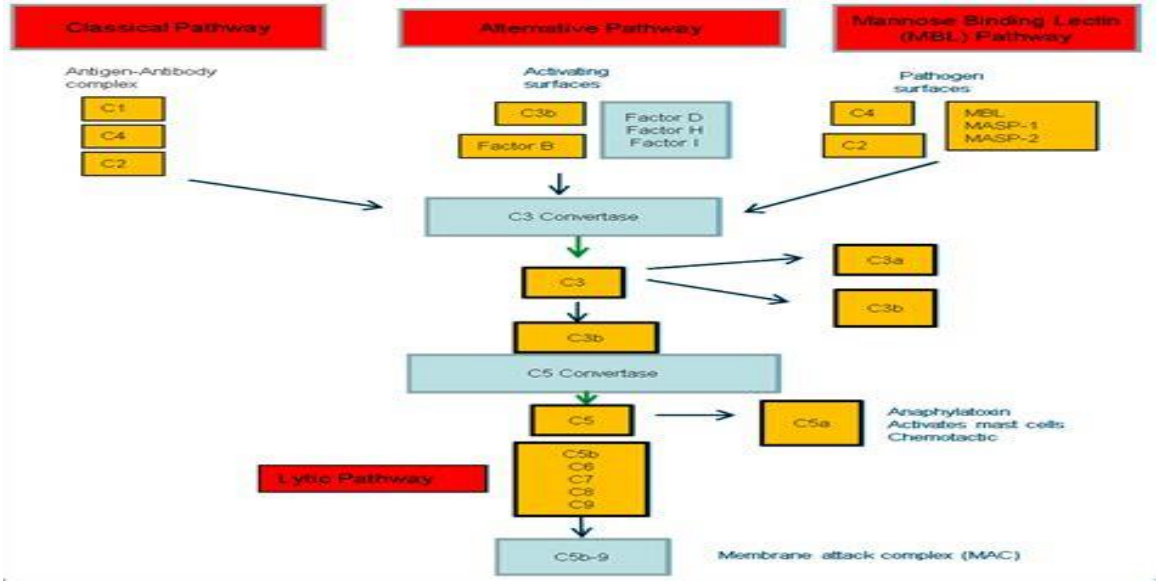
Complement

- The complement system is very much involved in the inflammatory response and is one of the key effector mechanisms of the immune system. It consists of at least 30 components—enzymes, regulators and membrane receptors—which interact in an ordered and tightly regulated manner to bring about phagocytosis or lysis of target cells.
- Complement components are normally present in body fluids as inactive precursors.
- The **alternative pathway** of complement activation can be stimulated directly by microorganisms and is important in the early stages of the infection before the production of antibody. It is part of the innate immune system.
- The **classical pathway** requires antibody, which may take weeks to develop. Both pathways can lead to the lytic or membrane attack pathway. During the course of complement activation, numerous split products of complement components, with important biological effects, are produced.

Alternative pathway

- Alternative activation Complement factor C3 is the central component of both the classical and alternative pathways .
- Products of C3 activation, C3b and inactivated C3b (iC3b) bind to microorganisms and are recognized by complement receptors (CRs) on phagocytes.
- If any C3b molecules bind to a normal host cell surface, they can then bind the next component in the sequence, factor B. Factor D (the only complement factor present in body fluids as an active enzyme) splits off a small fragment, Ba, leaving an active C3 convertase, C3bBb, on the cell surface.
- The C3bBb is a very unstable and quickly inactivated by control proteins, unless its bound to activating surface and stabilized by P (properdin), the C3bBbP enzymatic complex can cleave additional molecules of C3.
- If a second C3b is inserted into the C3-convertase, it become C3bBb3bP, this becomes a C5-convertase that can cleave C5 into C5a and C5b.
- The membrane attack unite for the alternative pathway begins with C5b and progresses through C6,7,8 and C9 in exactly the same sequence as it does for the classical pathway.





Disorders of the immune system

Hypersensitivity, also called an allergic reaction, is an exaggerated reaction of the immune system to an antigen to which there has been prior exposure (sensitized). Types include:

- **anaphylactic reactions (type I)**: e.g., IgE antibody on basophils and mast cells binds with antigens causing release of histamine, prostaglandins and other effectors. These types of reactions can be localized, respiratory or gastrointestinal related, systemic, or associated with shock
- **cytotoxic reactions (type II):** e.g., activation of complement and lysis of red blood cells (RBC) (main Ig: IgM), which can involve drugs (haptens) binding to RBC and inducing antibodies against them

- **immune complex reactions (type III):** e.g., complement fixing antigen–antibody complexes (main Ig: IgA). These are usually phagocytosed, but if the complexes are too small for phagocytosis, they can attach to the basement membrane of blood vessels and trigger inflammation
- **cell-mediated reactions (type IV, delayed hypersensitivity)**: e.g., contact allergy in the skin. This involves delayed hypersensitivity T cells and activation of memory cells.

Autoimmune reactions are damaging immunological reactions between the host and its own tissues as a result of breakdown in the mechanisms regulating immune tolerance. Types include:

1- Tissue destruction:-In diabetes, cytotoxicT-cell (CTLs) destroy insulin-producing β-cells in pancreas.
2- Antibodies block normal function:-In myasthenia gravis, antibodies binds to acetylcholine receptors.
3- Antibodies stimulate inappropriate function:-In Graves' disease (thyrotoxicosis), antibodies binds thyroid stimulating hormone (TSH) receptor and mimics thyroid-stimulating hormone, then activates unregulated thyroid hormone production.
4- Antigen-antibody complexes affect function:-In rheumatoid arthritis: IgM specific for IgG produced, and lead to deposition IgM-IgG complexes in joints and cause inflammation.

Classification of Autoimmunity.

- Organ specific autoimmune diseases : in which the immune response is directed against antigen associated with the target organ e.g., Diabetes mellitus,Coeliacdiseaseand Thyroiditis.
- Systemic(non-organ-specific)autoimmune diseases :in which the immune response is directed against antigen not associated with the target organ e.g., SLE, Sjögren'ssyndrome and rheumatoid arthritis

Immune deficiency is caused when there is a defect in one or more of the various points along the differentiation pathways of immunocompetent cells. Considering the complex cellular interactions involved in immune responses and the central role of T cells, immune deficiencies primarily involving T cells are also associated with abnormal B cell function. Immunodeficiency syndromes are associated with unusual susceptibility to infections and often associated with autoimmune disease and cancer. The types of infection occurring in patients with an immune deficiency can often provide the first clue as to the nature of the immune defect. Types include:

- congenital immune deficiency: these can involve humoural or cell-mediated immune components and are inherited as recessive traits
- acquired immune deficiency: these can involve humoural or cell-mediated immune components and often result from drugs, illness, cancer or viruses.

Lec. 8

Defense Mechanism Of Oral Cavity

Two major mechanisms of innate immunity in the oral cavity are immune exclusion and inflammation. **Immune exclusion** refers to the inactivation and clearance of microbes from the oral mucosal epithelium and enamel surfaces. **Inflammation** occurs when there is a need to remove infectious agents at sites of mucosal penetration and encompasses phagocytes, detection of PAMPs by PRRs and various inflammatory mediators. Acquired immune mechanisms are also important in the oral cavity;

The oral mucosal epithelium

- The oral mucosa is an anatomical **barrier** that prevents entry of potentially harmful microbes.
- Oral health depends on the integrity of the mucosal barrier, which also provides a **habitat** for normal oral flora.
- Continuous sloughing (desquamation) of the oral mucosal epithelium continuously removes microbes that colonize the mucosa, and this minimizes the microbial biomass in the oral cavity.
- Stable colonization therefore requires a continual process of microbial attachment, growth and reattachment to exposed epithelial cells, or growth of microbes in saliva at a rate exceeding the salivary flow or dilution rate.
- When the oral mucosa is compromised (e.g., during chemotherapy), infections frequently develop.
- Constituents of the oral mucosa that prevent penetration of microbes into deeper tissues include saliva, keratin in some areas of the mouth (on the free and attached gingiva, hard palate, areas of the dorsum of tongue), a granular layer, which discharges membrane-coating granules, and a basement membrane that provides barrier function for immune exclusion.
- Evidence of an intracellular lifestyle of some periodontal pathogens including *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* within buccal epithelial cells suggests that host cells may be used as a protective niche by some microbes to avoid extracellular defences such as antibodies, phagocytes and salivary antimicrobial components, as well as antibiotics

Antigen-non-specific defence chemicals in oral secretions

Various antigen-non-specific defence chemicals promote innate immune defence in the oral cavity. These include calprotectin, defensins, saliva (and the enamel pellicle), gingival crevicular fluid (GCF) and mucins. Non-cellular mediators of antimicrobial defence help to protect the oral mucosa through potent antibacterial, antiviral, and antifungal activities, which can affect oral microbes in several ways:

- they can aggregate or agglutinate microbes,
- they can promote or inhibit microbial adhesion,
- they can directly kill or inhibit the growth of microbes, and/or

- they can contribute to microbial nutrition.

Saliva

A. Antibacterial Factors in Saliva:

It contains numerous inorganic and organic factors that influence bacteria and their products in the oral environment.

- **Inorganic factors include:** bicarbonate, sodium, potassium, phosphates, calcium, fluoride….
- **Organic components include:** Lysozymes**,** Lactoferrin**,** Myeloperoxidase**,** Lactoperoxidase**,** Agglutinins(glycoprotein, mucins, fibronectin)

1. **Lysozymeis** a hydrolytic enzymes that cleaves the linkage between structural components of the cell wall of certain bacteria (both gram-negative and –positive) leading to cell lysis, its targets include *Veilonella spp*.and *Actinobacillus actinomycetemcomitans*.
2. **Lactoferrinit** binds the free iron in saliva causing bactericidal or bacteriostatic effects on various organisms requiring iron for their survival. It also provides fungicidal, antiviral, anti-inflammatory and immunomodulatory functions.
3. **The Lactoperoxidase-thiocyanate** system in saliva is bactericidal to some strains of Lactobacillus and Streptococcus by preventing the accumulation of lysine and glutamic acids essential for bacterial growth, also it is effective against Actinobacillus speceis.
4. **Myeloperoxidase**: an enzyme similar to salivary peroxidase. It is released by **leukocytes**and is bactericidal for Actinobacillus
5. **The histatins:** a family of histidine-rich peptides have antimicrobial activity against some strains of *Streptococcus mutans* and inhibit hemoagglutination of the periodonto pathogen *P. Gingivalis.* Neutralize lipopolysaccharides of G-ve bacteria. Potent inhibitors of *Candida albicans*

B. Salivary Antibodies

- Salivary immunoglobulins include sIgA which is important defense substance in saliva, it inhibits bacterial adherence
- in addition to small amounts of IgMand IgG

C. **Antiviral components** in saliva include the secretory leukocyte protease inhibitor (SLPI) and several other proteins that have been demonstrated to possess activity against human immunodeficiency virus (HIV). SLPI inhibits viral entry and/or uncoating in host cells secreted during inflammation. SLPI also displays some bactericidal and fungicidal activity.

Gingival cervical fluid (GCF)

- is a vehicle by which blood components including leukocytes can reach the oral cavity via flow of fluid through the junctional epithelium of the gingivae (gingival margin) into the gingival crevice.
- Normally, the flow of GCF is low but flow increases with inflammation to flush oral surfaces that are vulnerable to penetration by microbes.
- The composition of GCF also changes during inflammation from a transudate to a plasma-like inflammatory exudate, which can be collected from patients with oral disease.
- Various constituents of innate and acquired immunity reach sites of plaque accumulation from the blood via the GCF including neutrophils, plasma proteins (e.g., albumin and fibrin), monocytes, T and B lymphocytes, and Igs (IgG, IgM and IgA).
- Other enzymes including lysozyme and proteases (a mixture of host and bacterial) have also been detected in GCF, and these have been shown to inactivate IgA.
- The functional significance of GCF is related to the antimicrobial properties of its constituents that impact oral microbial colonization and survival.

Epithelial cells

- They plays an important role in innate host defense by responding to bacterial infections.
- This epithelium protect the deep structures and allow a selective interchange with the oral environment by its proliferation and differentiation
- The principle cell type of gingival epith. is the keratinocytes
- The role of their defense is by the degree of keratinization

Adaptive immunity in oral health and disease

- Acquired immunity in the oral cavity comprises both humoural and cellular mechanisms that involve GCF Igs (IgM, IgG and IgA) derived from plasma cells in the gingivae, effector T lymphocytes and, principally, **S-IgA**.
- The normal resident oral flora appears to be important in inducing a self-limiting humoural mucosal immune response that provides defence against potential pathogens.
- **Mucosal associated lymphoid tissues** that lies beneath the oral mucosal epithelium contains phagocytes for killing microbes and APCs, which sample antigens in the oral mucosa and provide the link between innate and acquired immune responses.
- Lymphoid cells around the basement membrane also help to eliminate any potential pathogens that overcome innate immune exclusion and pass through the intact oral mucosal epithelium.

Lec.9

Host-parasite Relationship

When microorganism first associated with a host, the host is said to be **"contaminated"**. If the microorganisms establish themselves and grow and multiply for period time, the host is said to be "**infected"**. If infection causes damage, the host is said to have an" **infectious disease".**

Ecological Interactions between Organisms in a Community:

Dynamic interrelationships based on **nutrition** and **shared habitat**

***SYMBIOSIS*:** neutral, antagonistic or synergistic relationship between two dissimilar organisms living in close association with each other.

1. ***MUTUALISM* (+/+):** mutually beneficial relationship between two species.e.g. certain indigenous enteric microorganisms produce large amount of the B & K vitamins which absorbed through the intestine wall of the human body and used in metabolism. In the same time the intestine provides the microorganisms with favorable Temp., moisture and nutrients for growth.
2. ***COMMENSALISM* (+/0):** relationship between two species in which one is benefited and the other is not affected, neither negatively or positively. e.g. *Veillonella* in the dental plaque require lactate for growth which provided by other dental plaque bacteria fermenting glucose to produce lactic acid (such as lactobacilli & Streptococi) the lactic acid used for growth of *Veillonella* while lactobacilli & Streptococci still unaffected.
3. ***PARASITISM* (+/-):** relationship between two species in which one benefits (parasite) from the other (host); usually involves detriment to the host.
   - ***Amphibiosis* (opportunistic pathogens):** Commensal microorganism of the human body that possess the potential for causing infection disease when conditions becomes favor for their invasion of tissue.
   - ***Antibiosis*:** is a relationship of antagonism. The antagomism among microorganisms is important to the host because it helps control the microbial population and thus helps prevent the over growth of certain microorganism.(e.g. some bacteria produce lethal substances called colicins or bacteriocins which inhibit the growth of other bacteria, also production of antibiotics is an example of antagonism relationship(.
   - ***Synergism*:** two usually independent organisms cooperate to break down a nutrient neither one could have metabolized alone (This is relationship in which different organisms produce a reaction that none can produce by individual growth.). (e.g. the relationship of *Proteus vulgaris* and *Staph.aureus* when growing separately both organisms ferment glucose resulting in the production acid only. When the species are grown together they produce acid and gas).

Source of infection

1- Exogenous infection:

- Infections due to some microbial species are acquired from **ill persons** with active or manifest infection (e.g. T.B, leprosy. Whooping cough)
- Healthy carrier:
  - Convalescent carrier: are persons limits localized infection continues for a period of week or months after clinical recovering from manifest infection.
  - Contact carrier: those of them who acquire the pathogen from patient.
  - Paradoxical carrier: those of them who acquire the pathogen from other carriers.
- **Infected animal**s: some pathogens that are primarily parasites of different animal species spread from the infected animal to man and cause human disease such infection are called **zoonoses** (e.g. anthrax, Brucellosis)

- ❖ **Soil**: a few infection disease of man are caused by microbes derived from soil (e.g. tetanus, gas-gangrene).

2- Endogenous infections: the source of endogenous infection are microorganisms grow as a commensal in the certain site of patient's body and under abnormal condition, these microorganisms cause disease in the other site of the body, e.g. *E.coli* have a commensalisms relationship and grow in the intestine as a normal flora but can caused urinary tract infection when invade the urinary tract.

KOCH'S POSTULATES:

Four criteria that were established by **Robert Koch** to identify the **causative agent of a particular disease**, these include:

1. The microorganism (pathogen) must be **present in all cases of the disease**
2. The pathogen can be **isolated** from the diseased host **and grown in pure culture**
3. The pathogen from the pure culture must cause the **same disease when inoculated** into a healthy, susceptible laboratory animal
4. The pathogen must be **reisolated** from the new host and **shown to be the same** as the originally inoculated pathogen.
   Currently, these four postulates are complemented by another:
5. The antibody to organism should be detected in the patient's serum

Types of bacterial pathogens:

1. **Oppertunistic pathogens**: these rarely cause disease in individual with intact immunological and anatomical defenses. Only when such defenses are impaired or compromised, as a result of congenital or acquired disease or by the use of immune-suppressive therapy or surgical techniques, are these bacteria able to cause disease. Many opportunistic pathogens (e.g. coagulase-negative staphylococci & E.coli) are part of the normal human flora and are carried on the skin or mucosal surface where they cause no harm and may actually have a beneficial effect by preventing colonization by other potential pathogens. However, introduction of these organisms into anatomical sites in which they are not normally found, or removal of competing bacteria by the use of broad-spectrum antibiotics, may allow their localized multiplication and subsequent development of disease.
2. **primary pathogens**: these are capable of establishing infection and causing disease in previously healthy individuals with intact immunological defenses.

General aspects of infection

Virulence

- Virulence is a quantitative measure of pathogenicity and is related to an organism's **toxigenic potential** and **invasiveness**.
- Virulence can be measured by the number of organisms required to cause disease

Communicable diseases

- Infections are called 'communicable diseases' if they are spread from host to host.
- Many, but not all, infections are communicable; for example, tuberculosis is communicable, as it is spread by airborne droplets produced by coughing, but staphylococcal food poisoning is not, as the exotoxin produced by the organism and present in the contaminated food affects only those eating that food.
- If a disease is highly communicable, it is called a 'contagious disease' (e.g., chickenpox).
- Depending on the degree of incidence and prevalence of an infectious disease in a community, it may be called an endemic, an epidemic or a pandemic infection:

- ❖ An **endemic** infection is constantly present at a low level in a specific population (e.g., endemic malaria in some African countries).
- ❖ An infection is an **epidemic** if it occurs much more frequently than usual (e.g., an epidemic of influenza in the winter).
- ❖ An infection is a **pandemic** if it has a worldwide distribution (e.g., human immunodeficiency virus (HIV) infection).

Natural history of infectious disease

An acute infection generally progresses through four stages:

1- The incubation period: time between the acquisition of the organism or the toxin and the commencement of symptoms (this may vary from hours to days to weeks).
2- The prodromal period: non-specific symptoms such as fever, malaise and loss of appetite appear during this period.
3- The acute specific illness: the characteristic signs and symptoms of the disease are evident during this period.
4- The recovery period: the illness subsides and the patient returns to health during this final phase.

Pathogenesis of bacterial disease

The major steps are transmission, adherence to host surfaces, invasiveness and toxigenicity.

Transmission

Most infections are acquired by transmission from external sources; that is, they are exogenous in origin. Others are caused by members of the normal flora behaving as opportunist pathogens; that is, they are endogenous in origin. Transmission can be by:

- inhalation: the airborne route
- ingestion: faecal contamination of food and water
- inoculation: by sexual contact, contaminated needles, skin contact, blood transfusions or biting insects.

There are four important portals (or gates) of entry of pathogens:

1- skin
2- respiratory tract
3- gastrointestinal tract
4- genitourinary tract.

Adherence to host surfaces

- Adherence is the first step in infection. Unless organisms have the ability to stick or adhere to host surfaces, they will be unable to cause infection.

- Some bacteria and fungi have specialized structures or produce substances that facilitate their attachment to the surface of human cells or prostheses (e.g., dentures, artificial heart valves), thereby enhancing their ability to colonize and cause disease.
- These adherence mechanisms are critical for organisms that attach to mucous membranes; mutants that lack these mechanisms are often non-pathogenic (e.g., the hair-like pili of *Neisseria gonorrhoeae* and *Escherichia coli* mediate their attachment to the urinary tract epithelium; the extracellular polysaccharides of *Streptococcus mutans* help it adhere to enamel surfaces).

Invasion of tissue (invassivenss)

The ability of organisms to penetrate tissues. The invasion of a host by a pathogen may be aided by the production of bacterial extracellular substance which acts against the host by breaking down primary or secondary defenses of the body.

Examples:

- Hyaluronidase (spreading factor)….. produce by Staph., Strept.,,, *Clostridium tetani.*
- Collagenase…… produce by Clostridium, Bacteroides
- Lecithinase…. produce by Clostridium
- Catalase…. Produce by T.B, Brucella
- Hemolysins…. Produce by Staph., Strept.

Toxigenicity

Toxin production or toxigenicity is another major mediator of bacterial disease. Toxins are of two categories: endotoxins and exotoxins. Their main features are shown in Table

1- **ENDOTOXIN:** a complex bacterial toxin that is composed of protein, lipid, and polysaccharide (LPS) which is released only upon lysis of the cell. Endotoxins - part of the Gram (-) Bacterial cell wall. Lipid A - Toxin portion of the LPS.
2- **EXOTOXINS:** a potent toxic substance formed and secreted by species of certain bacteria. Mostly seen in Gram (+) Bacteria. Most genes that code for exotoxins are located on plasmids or phages.

Table 5.3 **Comparison of the main features of exotoxins and endotoxins**

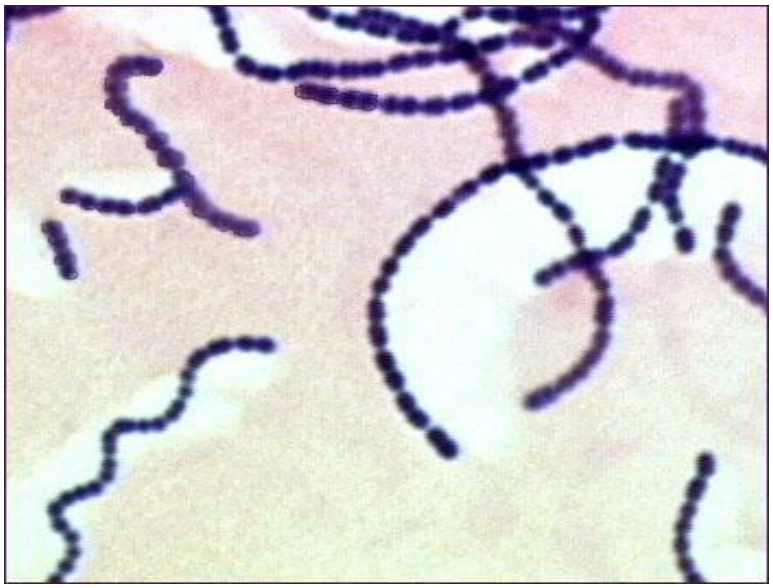
Property	Exotoxin	Endotoxin
Source	Some species of some Gram-positive and Gram-negative bacteria	Cell walls of Gram-negative bacteria
Origin	Secreted from cell	Cell wall constituent
Chemistry	Polypeptide	Lipopolysaccharide
Toxicity	High (fatal dose of the order of 1 μg)	Low (fatal dose in the order of hundreds of micrograms)
Clinical effects	Variable	Fever, shock
Antigenicity	Induces high-titre antibodies called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccines	No toxoids formed and no vaccine available
Heat stability	Most are thermolabile (destroyed rapidly at 60°C)	Thermostable at 100°C for 1 h
Typical diseases	Cholera, tetanus, diphtheria	Sepsis by Gram-negative rods, endotoxic shock

Lec.10

Streptococci

Characteristics

They are catalase-negative, Gram-positive spherical or oval cocci in pairs and chains; 0.7–0.9 μm in diameter. Chain formation is best seen in liquid cultures or pus.



Culture

These cocci grow well on blood agar, although enrichment of media with glucose and serum may be necessary. Typical haemolytic reactions are produced on blood agar :

- **α-haemolysis**: narrow zone of partial haemolysis and green (viridans) discolouration around the colony, e.g. viridans streptococci
- **β-haemolysis**: wide, clear, translucent zone of complete haemolysis around the colony, e.g. *Streptococcus pyogenes*
- **no haemolysis** (γ-haemolysis), e.g. non-haemolytic

Serology

The carbohydrate antigens found on the cell walls of the organisms are related to their virulence. Hence, serogrouping, termed **Lancefield grouping**, is useful in the identification of the more

virulent β-haemolytic species. Currently, 20 Lancefield groups are recognized (A–H and K–V) but not all are equally important as human pathogens. The following are worthy of note:

- **group A** includes the important human pathogen *Streptococcus pyogenes*
- **group B** contains one species, *Streptococcus agalactiae*, an inhabitant of the female genital tract; it causes infection in neonates
- **group C** mainly causes diseases in animals
- **group D** includes the enterococci (*Enterococcus faecalis*, etc.) and ranks next to group A in causing human disease.

***Streptococcus pyogenes* (group A)**

Habitat and transmission

The normal habitat of this species is the human upper respiratory tract and skin; it may survive in dust for some time. Spread is by airborne droplets and by contact.

Characteristics

It is found as a commensal in the nasopharynx of a minority of healthy adults, but more commonly (about 10%) in children. It grows well on blood agar, with a characteristic halo of β-haemolysis. Some strains produce mucoid colonies as a result of having a hyaluronic acid capsule. This may contribute to virulence by offering resistance to phagocytosis.

Exotoxins and enzymes

Produces a large number of biologically active substances, such as:

- **streptokinase**: a proteolytic enzyme that lyses fibrin
- **hyaluronidase**: attacks the material that binds the connective tissue, thereby causing increasing permeability (hence called the ‘spreading factor’)
- **DNAases** (streptodornases): destroy cellular DNA
- **haemolysins** (streptolysins, leukocidins): phage mediated and are responsible for the characteristic erythematous rash in scarlet fever. also cause the translucency around colonies in blood agar due to breakdown of hemoglobin

- **pyrogenic exotoxins (erythrogenic toxin):** associated with **streptococcal toxic shock syndrome** and **scarlet fever.**

Pathogenicity

Streptococcus pyogenes causes a number of infections; the most notable are:

- tonsillitis and pharyngitis
- peritonsillar abscess (now rare)
- scarlet fever
- mastoiditis and sinusitis
- otitis media (middle-ear infection)
- wound infections leading to cellulitis and lymphangitis
- impetigo (a skin infection).

Complications

After an episode of infection, some patients develop complications، such as rheumatic fever, glomerulonephritis and erythema nodosum, which may have long-lasting effects. Note that:

- in cellulitis, hyaluronidase ('spreading factor') mediates the subcutaneous spread of infection
- erythrogenic toxin causes the rash of scarlet fever
- post-streptococcal infection, manifesting as rheumatic fever, is caused by immunological cross-reaction between bacterial antigen and human heart tissue, and acute glomerulonephritis is caused by immune complexes bound to glomeruli

Oral streptococci

Oral streptococci, which live principally in the oropharynx, are a mixed group of organisms with variable characteristics. New typing techniques, particularly those based on molecular biology, have revealed the complex nature of the origin and the taxonomy of this group. Hence, the nomenclature of oral streptococci is in a constant state of flux. They typically show α-haemolysis on blood agar, but this is not a constant feature as some strains are non-haemolytic and others β-haemolytic. Oral streptococci can be divided into four main **species groups** as follows:

1. ***mutans* group**

2. ***salivarius* group**
3. ***anginosus* group**
4. ***mitis* group.**

Group	Species	
mutans-group*	*S. mutans* *S. sobrinus* *S. criceti* *S. ratti*	serotypes *c, e, f, k* serotype *d, g* serotype *a* serotype *b*
salivarius-group	*S. salivarius* *S. vestibularis*	
anginosus-group	*S. constellatus* *S. intermedius* *S. anginosus*	
mitis-group	*S. sanguinis* *S. gordonii* *S. parasanguinis* *S. oralis* *S. mitis* *S. cristatus* *S. oligofermentans* *S. sinensis* *S. australis* *S. peroris* *S. infantis* *S. dentisani* *S. tigurinus*	

Habitat and transmission

Streptococci make up a large proportion of the resident oral flora. It is known that roughly one-quarter of the total cultivable flora from supragingival and gingival plaque and half of the isolates from the tongue and saliva are streptococci. They are vertically transmitted from mother to child. Infective endocarditis caused by these organisms (loosely termed viridans streptococci) is generally a result of their entry into the blood stream during intraoral surgical procedures (e.g. tooth extraction), and sometimes even during tooth-brushing.

Pathogenicity

The *mutans* group of streptococci are the major agents of dental caries (but in the absence of predisposing factors, such as sucrose, they cannot cause caries). They have a characteristic ability to produce voluminous amounts of sticky, extracellular polysaccharides in the presence of dietary

carbohydrates ; these help tenacious binding of the organisms to enamel and to each other. They are also important agents of infective endocarditis, and some 60% of cases are due to this organism. Usually, bacteria released during dental procedures settle on damaged heart valves, causing infective endocarditis

Streptococcus mutans

potential role in the a etiology of dental caries. *Streptococcus mutans* was originally isolated from carious human teeth by Clarke in 1924 and shortly afterwards, was recovered from a case of infective endocarditis. Mutans streptococci are recovered almost exclusively from hard, non-shedding surfaces in the mouth, such as teeth or dentures, Mutans streptococci are regularly isolated from dental plaque at carious sites and can act as opportunistic pathogens, being isolated from cases of infective endocarditis. is generally a result of their entry into the blood stream during intraoral surgical procedures (e.g. tooth extraction)

Treatment and prevention

In patients at risk of infective endocarditis (e.g. those with damaged or prosthetic heart valves), prophylactic antibiotic cover should always be given before dental procedures.

Gram-positive anaerobic cocci

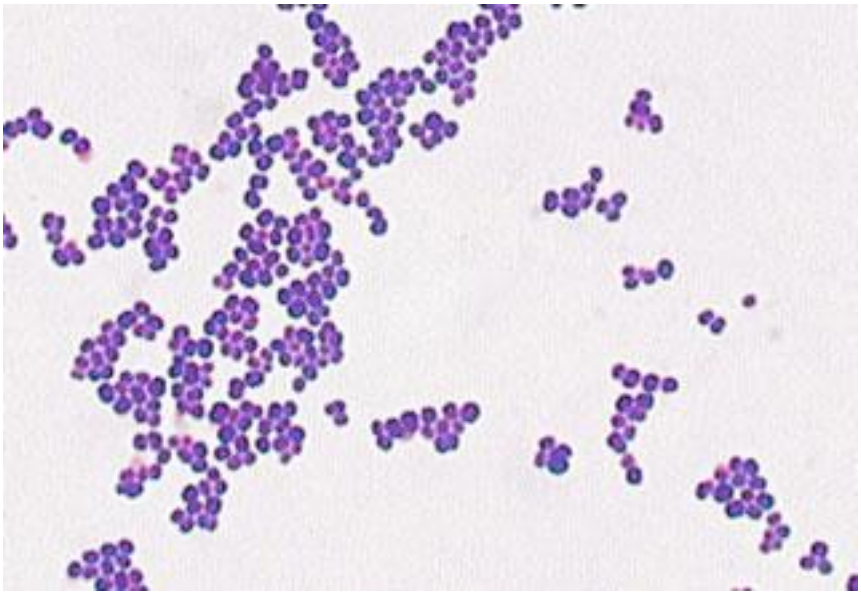
Gram-positive anaerobic cocci (GPAC) all belonged to the genus *Peptostreptococcus* until recently. However, they now comprise three genera: *Peptostreptococcus*, *Micromonas* and *Finegoldia*. The representative species are *Peptostreptococcus anaerobius*, *Finegoldia magnus* (previously *Peptostreptococcus magnus*) and *Micromonas micros* (previously *Peptostreptococcus micros*).

These GPAC can often be isolated from dental plaque and the female genital tract. They are also found in carious dentine, subgingival plaque, dentoalveolar abscesses and in advanced periodontal disease, usually in mixed culture. Their pathogenic role is still unclear**.**

Staphylococci

- Staphylococci are also Gram-positive cocci, but, unlike the chains of streptococci, they are arranged in characteristic grape-like clusters.
- The *Staphylococcus* genus contains more than 15 different species, of which the following are of medical importance: *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*.

- Staphylococci cause a variety of both common and uncommon infections, such as abscesses of many organs, endocarditis, gastroenteritis (food-poisoning) and toxic shock syndrome.
- They are not infrequent isolates from the oral cavity.
- Higher proportions of *Staphylococcus aureus* are found in the saliva of healthy subjects older than 70 years.
- Catalase positive (meaning it can produce the enzyme catalase), so is able to convert hydrogen peroxide into O_2 and water, the catalase test useful to distinguish staphylococci from streptococci



Grouping of Staphylococci

Staphylococci are divided in to two groups based on the presence or absence of the enzyme coagulase. This enzyme converts fibrinogen into fibrin causing blood plasma to clot.

- **Coagulase positive Staphylococci**
  - ***Staphylococcus aureus***
- **Coagulase negative Staphylococci**
  - ***Staphylococcus epidermidis***
  - ***Staphylococcus saprophyticus***

Staphylococcus aureus

Habitat, transmission, culture and identification

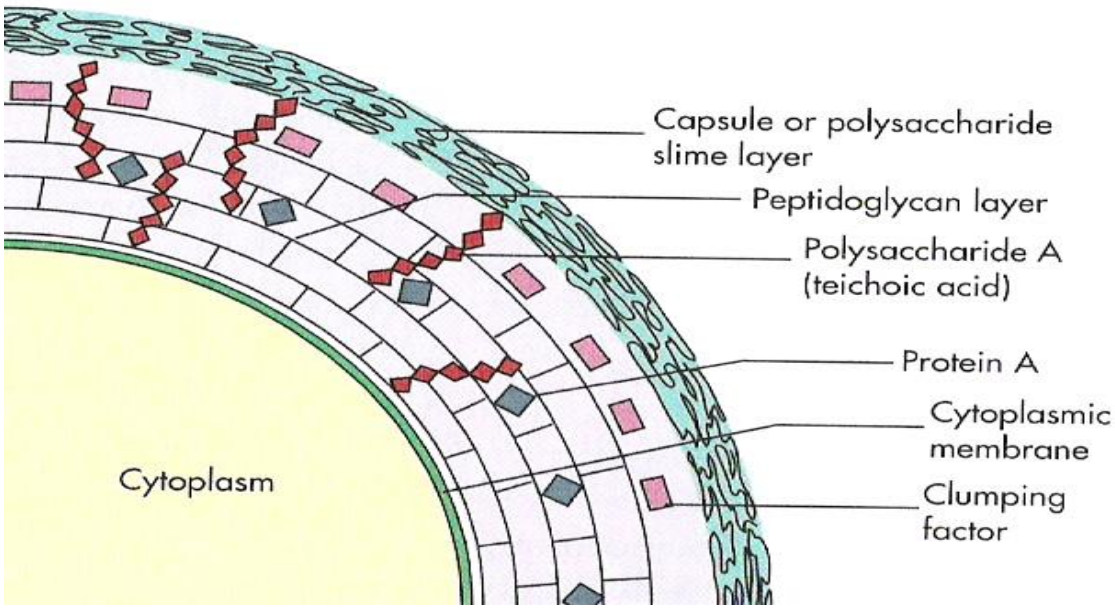
- The habitat is the human skin, especially the anterior nares and the perineum. Domesticated animals also carry staphylococci.
- Higher carriage rates are seen in hospital patients and staff.
- These bacteria are disseminated through air and dust and are always present in the hospital environment. The usual transmission route is via the hands and fingertips

- Grows aerobically as yellow or gold colonies on blood agar, catalase-positive (this differentiates them from the catalase-negative streptococci).
- *Staphylococcus aureus* coagulates dilute human serum or rabbit plasma (i.e., it is coagulase-positive), whereas *Staphylococcus epidermidis* does not (coagulase-negative).

Virulence factors

A- **Cell-Associated Virulence Factors**

1. Capsule or slime layer
2. Teichoic acid
3. Protein A.
4. Clumping factor (bound coagulase)



B- Extracellular Enzymes

1. Catalase: Affects bactericidal activity of polymorphs
2. Coagulase (bound or free): Clots plasma
3. Hyaluronidase : Connective tissue breakdown
4. Nuclease: DNA hydrolysis
5. Lipases: Breaks lipids of cell membranes
6. Beta-lactamase or Penicillinase: confers antibiotic resistance

C- Exotoxins

1- **Cytolytic (cytotoxins; cytolysins), they cause cytolysis as a result of plasma membrane damage**.

a. Alpha toxin
b. Beta toxin
c. Gamma toxin
d. Delta toxin: Cytopathic for RBCs, macrophages, lymphocytes, neutrophils and platelets.

e. Leukocidin: Kills neutrophils

2- **Enterotoxin**
3- **-Exfoliative toxin (epidermolytic toxin(**
4- **Pyrogenic exotoxins**

Pathogenicity

A variety of enzymes and toxins are produced by *Staphylococcus aureus*, although no one strain produces the whole range. The two most important are coagulase and enterotoxin. Coagulase is the best correlate of pathogenicity. Some of the diseases caused by *Staphylococcus aureus* are:

- **superficial infections:** common agent of boils, carbuncles, pustules, abscesses, conjunctivitis and wound infections; rarely causes oral infections; may cause angular cheilitis (together with the yeast Candida) at the angles of the mouth
- **Deep infections;** Osteomylitis, endocarditis, septicemia and pneumonia
- **food poisoning (vomiting and diarrhoea)** caused by enterotoxins
- **toxic shock syndrome,** also caused by an enterotoxin

Treatment and prevention:

- (80%) of strains are resistant to β- Lactam drugs and some to a number of antibiotics
- The latter phenomenon (**multiresistance**) is common, particularly in strains isolated from hospitals; these cause hospital (**nosocomial**) infection.
- Antibiotic active against *Staph. aureus* include: penicillin for sensitive isolate, flucloxacillin (stable against β- Lactamase), erythromycin, fusidic acid (useful for skin infections), cephalosporins and vancomycin.
- Hand – washing and aseptic management of lesions reduce Staphylococci spreading.

Staphylococcus epidermidis

Characters

- This species is found on the skin surface and is spread by contact.
- It is a cause of nosocomial infections.
- It is a major component of the skin normal flora and thus commonly a contaminant of cultures media.
- Important character of *S. epidermidis* is the **biofilm formation**. This ability to form a biofilm on the surface of a prosthetic device is probably a significant determinant of **virulence** for these bacteria
- Grows as white colonies on blood agar, hence the earlier name *Staphylococcus albus*;
- catalase-positive; coagulase negative

Pathogenicity

Being a normal commensal of the skin, this bacterium causes infection only when an opportunity arises (it is an opportunist pathogen). Common examples are catheter-related sepsis, infection of artificial joints and urinary tract infections.

Treatment

Staphylococcus epidermidis exhibits resistance to a number of drugs (multiresistance), including penicillin and methicillin. It is sensitive to vancomycin

Lec.11

Neisseria

The genus Neisseria contains two important pathogens:

- ***Neisseria gonorrhoeae*** **(the gonococcus)**
- ***Neisseria meningitidis*** **(the meningococcus).**

There are a number of non-pathogenic species, such as *Neisseria sicca*, *Neisseria mucosa* and *Neisseria lactamica*, which are members of the indigenous flora, including the oral mucosa. Hence it is important to differentiate these from the pathogenic species isolated from oral samples.

N. gonorrhoeae is the agent of gonorrhoea, the most frequently diagnosed venereal disease in Western Europe and the USA. Gonococci frequently cause pelvic inflammatory disease (PID) and sterility in women, in addition to arthritis and sometimes septicaemia. *N. meningitidis* is the aetiological agent of meningococcal meningitis, a highly contagious disease associated with a mortality rate approximating 80% when untreated.

Neisseria gonorrhoeae

Habitat and transmission

The human urogenital tract is the usual habitat; oral, nasopharyngeal and rectal carriage in healthy individuals is not uncommon. Spread is by both homosexual and heterosexual intercourse or intimate contact.

Characteristics, culture and identification

Non-motile, Gram-negative, non-capsulate diplococci. Specimens are usually inoculated onto an enriched medium (lysed blood or chocolate agar normally) and incubated under 5%–10% carbon dioxide (as the species is capnophilic). Small, grey, oxidase-positive colonies initially become large and opaque on prolonged incubation. Gram-stained smears (of urethral exudate from men and the cervix in women) usually reveal Gram-negative, kidney-shaped intracellular cocci in pairs.

Pathogenicity

Gonococci possess a number of virulent attributes:

- **pili** allow gonococci to adhere and colonize epithelial surfaces and thus cause infection
- **immunoglobulin A (IgA) proteases** produced by some gonococci break the heavy chain of IgA, thereby inactivating it (IgA is a major defence factor universally present on mucosal surfaces)
- some isolates of *N. gonorrhoeae* produce **β-lactamase**, which is plasmid mediated
- a **tracheal cytotoxin** damages the ciliated cells of the fallopian tube, leading to scarring and sterility.

Treatment and prevention

The majority of gonococci are resistant to β-lactam drugs and hence the choice is β-lactamase-stable cephalosporins. Prevention of gonorrhoea requires the practice of 'safe sex', health education and contact tracing.

Neisseria meningitidis

Habitat and transmission

The main reservoir is the nasopharynx in healthy individuals (10%–25%). Droplet spread is the most common transmission mode.

Characteristics Culture and identification

This organism resembles the gonococcus but *N. meningitidis* cells are capsulate. As for *N. gonorrhoeae*. Presumptive identification is made by observing Gram-negative cocci in pairs in nasopharyngeal discharge, cerebrospinal fluid or blood smears. Selective media are not required as the organism is found pure in cerebrospinal fluid. Identified by the carbohydrate utilization test: produces acid from the oxidation of glucose and maltose. Serology is useful.

Pathogenicity

In susceptible individuals, meningococci spread from the nasopharynx into the blood stream (septicaemia), and then to the meninges. Septicaemia is accompanied by a rash. Eventual death may be due to meningitis or adrenal haemorrhage (Waterhouse–Friderichsen syndrome). The antiphagocytic properties of the capsule help dissemination, whereas the toxic effects are mainly due to the meningococcal endotoxin.

Treatment and prevention

Penicillin or preferably ceftriaxone (or equivalent cephalosporin) due to wide prevalence of penicillin-resistant strains

Veillonella

Veillonella species are obligate anaerobic, Gram-negative cocci frequently isolated from oral samples. Three oral species are recognized: *Veillonella parvula*, *Veillonella dispar* and *Veillonella atypica*.

Veillonella parvula

Gram-negative, small anaerobic cocci. Found in the human oral cavity, mostly in dental plaque, they are considered as 'benevolent organisms' in relation to dental caries as they metabolize the lactic acid produced by cariogenic bacteria into weaker acids (acetic and propionic) with a reduced ability to solubilize enamel. No known pathogenic potential.

Lactobacilli

Lactobacilli are saprophytes in vegetable and animal material (e.g., milk). Some species are common animal and human commensals inhabiting the oral cavity and other parts of the body. They have the ability to tolerate acidic environments and hence are believed to be associated with human and animal caries.

The taxonomy of lactobacilli is complex. They are characterized into two main groups: **homofermenters**, which produce mainly lactic acid (65%) from glucose fermentation (e.g., *Lacto-bacillus casei*), and **heterofermenters**, which produce lactic acid as well as acetate, ethanol and carbon dioxide (e.g., *Lactobacillus fermentum*). *L. casei* and *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and the newly described species, *Lactobacillus oris*, are common in the oral cavity. It should be noted that the taxonomy of lactobacilli is under constant revision.

Habitat and transmission

Lactobacilli are found in the oral cavity, gastrointestinal tract and female genital tract. In the oral cavity, they constitute less than 1% of the total flora. Transmission routes are unknown.

Characteristics

Gram-positive coccobacillary forms (mostly bacillary), α- or non-haemolytic, are facultative anaerobes. These organisms ferment carbohydrates to form acids (i.e., they are **acidogenic**) and can survive well in acidic milieu (they are **aciduric**); they may be homofermentative or heterofermentative. The question as to whether they are present in carious lesions because they prefer the acidic environment, or whether they generate an acidic milieu and destroy the tooth enamel, has been debated for years (the classic ‘chicken and egg’ argument). Lactobacilli are also major constituents of the vaginal flora and help maintain its low pH equilibrium.

The beneficial role of lactobacilli in maintaining the homoeostasis of the intestinal microbiome has been recognized, and ‘lactobacillus-laced’ food items, sold as **probiotics**, have gained popularity among the health-conscious public.

Pathogenicity

Lactobacilli are frequently isolated from deep carious lesions where the pH tends to be acidic, and they are commonly isolated from the advancing front of the dentinal caries lesions. Indeed, early workers believed that lactobacilli were the main cariogenic agent (a theory that has been disproved), so much so that the number of lactobacilli in saliva (the **lactobacillus count**) was taken as an indication of an individual’s caries activity. Although this test is not very reliable, it is useful for monitoring the dietary profile of a patient because the level of lactobacilli correlates well with the intake of dietary carbohydrate

Aggregatibacter actinomycetemcomitans

This relatively new genus *Aggregatibacter* (formerly called *Actinobacillus*) includes species isolated from humans and mammals. (Latin *aggregare*: to come together, aggregate; bacter: bacterial rod; *Aggregatibacter*: rod-shaped bacterium that aggregates with others.)

The only species of this genus routinely isolated from the oral cavity is *Aggregatibacter actinomycetemcomitans*, so named because it is frequently isolated with *Actinomyces* spp. from actinomycotic lesions. The reason for this association is unknown. Multiple biotypes and up to six serotypes (*a*–*e*) have been described. This species is a major infective agent in particularly aggressive forms of periodontal disease in adolescents (localized aggressive periodontitis) and rapidly destructive periodontal disease in adults**.**

Habitat and transmission

Primary habitat is unknown but is likely to be subgingival sites of humans and mammals. Infection is endogenous**.**

Characteristics

Small, short (0.4–1 μm), straight or curved rods with rounded ends. Electron microscopic studies have revealed bleb-like structures on the cell surface, which appear to be released from the cells. Fresh isolates possess fimbriae (lost on subculture).

Pathogenicity

A number of virulence factors, including lipopolysaccharide (**endotoxin**), a **leukotoxin, collagenase, cytolethal distending toxin (cdt), epitheliotoxin-bone resorption inducing factor and a protease-cleaving IgG**, have all been isolated from *Aggregatibacter actinomycetemcomitans*. The leukotoxin, in particular, is thought to play a significant role in subverting the host immune response in the gingival crevice. It also has the potency to invade epithelial and vascular endothelial cells in vitro and buccal epithelial cells in vivo. Together with other coagents, *Aggregatibacter actinomycetemcomitans* is involved in localized aggressive periodontitis and destructive periodontal disease in adults. Also isolated from cases of infective endocarditis, and from brain and subcutaneous abscesses.

Treatment

This species is sensitive to tetracycline

Lactobacilli

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Small, short (0.4–1 μm), straight or curved rods with rounded ends. Electron microscopic studies have revealed bleb-like structures on the cell surface, which appear to be released from the cells. Fresh isolates possess fimbriae (lost on subculture).

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Treatment

This species is sensitive to tetracycline

Actinomyces spp.

- Although most *Actinomyces* are soil organisms, the potentially pathogenic species are commensals of the mouth in humans and animals. They are a major component of dental plaque, particularly at approximal sites of teeth, and are known to increase in numbers in gingivitis. An association between root surface caries of teeth and *Actinomyces* has been described. Other sites colonized are the female genital tract and the tonsillar crypts.

- A number of Actinomyces species are isolated from the oral cavity. These include *Actinomyces israelii*, *Actinomyces gerencseriae*, *Actinomyces odontolyticus*, *Actinomyces naeslundii*, *Actinomyces meyeri* and *Actinomyces georgiae*. A close relationship between *Actinomyces odontolyticus* and the earliest stages of enamel demineralization, and the progression of small caries lesions have been reported. The most important human pathogen is *A. israelii*.

Actinomyces israelii

Habitat and transmission

- This organism is a commensal of the mouth and possibly of the female genital tract. It is a major agent of human **actinomycosis**

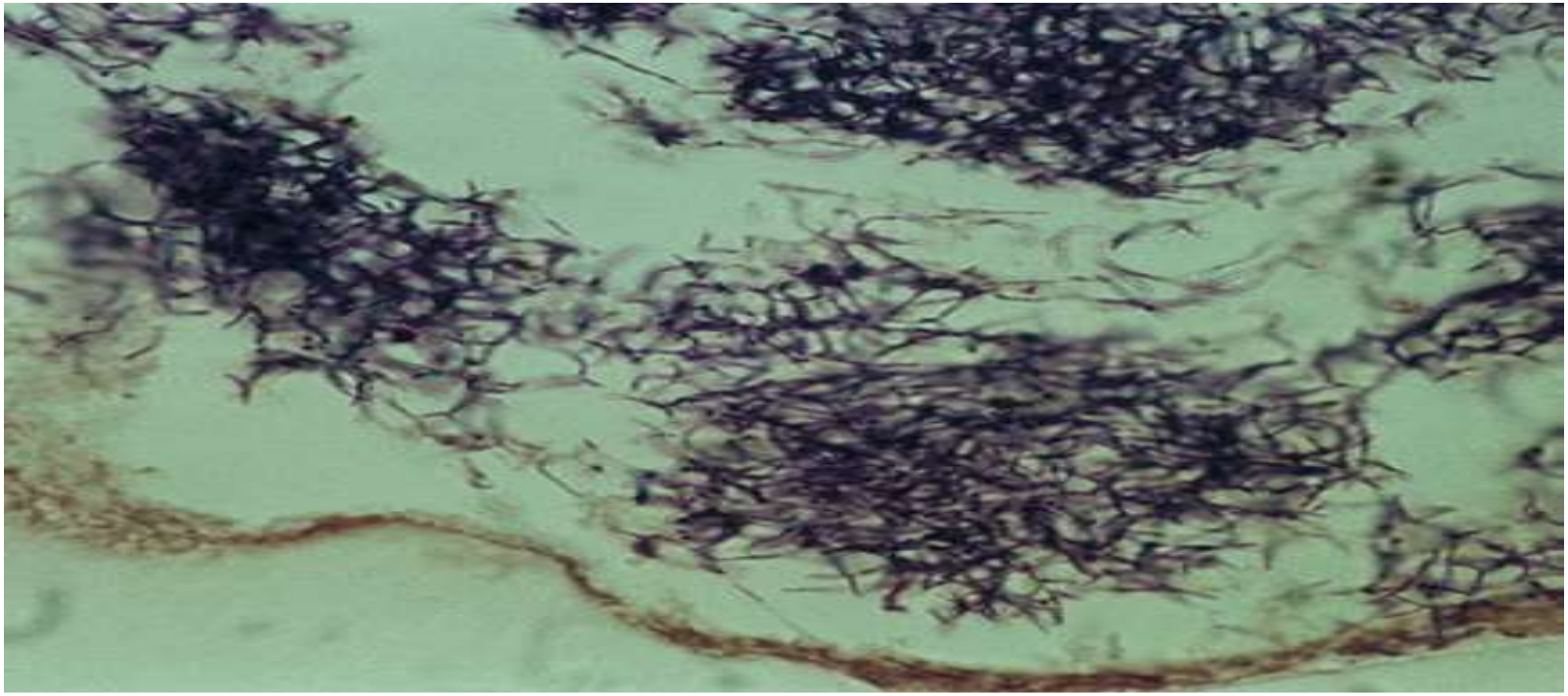
Characteristics

- Gram-positive filamentous branching rods. Non-motile, non-sporing and non-acid-fast.
- Grows slowly under anaerobic conditions, on blood or serum glucose agar at 37°C.
- Because of the exacting growth requirements and the relatively slow growth, isolating this organism from clinical specimens is difficult, particularly because the other, faster-growing bacteria in pus specimens tend to obscure the slow-growing actinomycetes. . .

Pathogenicity

- Most (70%–80%) actinomycotic infections are chronic, granulomatous, endogenous infections of the orofacial region . Typically, the lesions present as a chronic abscess, commonly at the angle of the lower jaw, with multiple external sinuses. There is usually a history of trauma such as a tooth extraction or a blow to the jaw. Actinomycetes are also isolated from infections associated with intrauterine devices, but their pathogenic role is unclear.

Ahistopathological section from an actinomycetic lesion of the mandible showing a branching filamentous mass of *Actinomyces* spp. infiltrating the bony cortex



- While the majority of the lesions (60%–65%) are in the **cervicofacial** region, some 10%–20% are **abdominal** and others are in the lung (**thoracic**) or skin. Although most infections are **monomicrobial** in nature (i.e., with *Actinomyces* alone causing the disease), a significant proportion of infections could be **polymicrobial**, with other bacteria such as *Aggregatibacter actinomycetemcomitans*, *Haemophilus* spp. and anaerobes acting as co-infecting agents.

Treatment and prevention

- Sensitive to penicillin, but prolonged courses up to 6 weeks are necessary for chronic infections. Oral penicillins such as amoxicillin are now popular. Recalcitrant lesions respond well to tetracycline because of its good bone penetration. Surgical intervention may be necessary in chronic jaw lesions.

Ecology of the oral flora

Lec.13

The oral ecosystem

- Ecology is the study of the relationships between living organisms and their environment. An understanding of oral ecology is essential to comprehend the pathogenesis of diseases, such as caries and periodontal disease, caused by oral bacteria

The oral ecosystem

- The mouth, being an extension of an external body site, has a natural microflora. This commensal (or indigenous, or resident) flora exists in harmony with the host, but disease conditions supervene when this relationship is broken.
- The predominant dental diseases in humans (caries and periodontal disease) are caused in this manner.
- In addition to the commensal flora, there are others (such as coliforms) that survive in the mouth only for short periods (transient flora).
- These transient species of flora cannot get a foothold in the oral environment due to the ecological pressure, that is, the colonization resistance exerted by the resident flora.
- Indeed, the latter are considered critical in defending the key portal of entry into the digestive system, by offending pathogens.
- The oral ecosystem comprises the oral flora, the different sites of the oral cavity where they grow (i.e., habitats) and the associated surroundings.

Factors modulating microbial growth in the oral cavity:

1- Anatomical factors

- The shape of the teeth.
- The topography of the teeth (e.g. occlusal fissures).
- malalignment of the teeth
- Poor quality of restorations (e.g. fillings and bridges)

2- Saliva

- Adsorbing to oral surfaces, especially teeth, to form a salivary pellicle to which microorganisms can attach.
- acting as a primary sources of nutrients (carbohydrates and proteins)
- Aggregating microorganisms and there by facilitating their clearance from the mouth by swallowing or deposition on surfaces, contributing to plaque formation.
- Inhibiting the attachment and growth of some exogenous microorganisms by non-specific defense factors (e.g. lysozyme, lactoferrin and histatins) and specific defense factors (sIgA).

3- Gingival crevicular fluid (GCF)

- acting as a primary source of nutrients: proteolytic and saccharolytic bacteria in the crevice can utilize the crevicular fluid to provide peptides, amino acids and carbohydrates for growth.
- maintaining pH conditions.
- providing specific and non-specific defense factors (IgG and IgM) phagocytosis (neutrophils)

4- Microbial factors

Microbes in the oral environment can interact with each other both in promoting suppressing the neighboring bacteria. Mechanisms that accomplish this include:-

- competition for receptors for adhesion
- production of toxins, such as bacteriocins that kill cells of the same or other bacterial species e.g.: *Strept. salivarius* produces an inhibitor (enocin) that inhibits *Strept. pyogens*.
- Production of metabolic end-products such as short-chain carboxylic acids.
- Use of metabolic end-products of other bacteria for nutritional purpose (e.g.: *Veillonella* use acids produced by *Strept. mutans*).

5- Miscellaneous factors:

- pH ……. Many microbes require a neutral pH for growth the acidity of most oral surfaces is regulated by saliva (mean pH=6.7). Depending on the frequency of intake of dietary carbohydrates, the pH of plaque can fall to as low as (5) as a result of bacterial metabolism. Under these conditions acidophilic bacteria can grow well (e.g: lactobacilli).

- *Antimicrobial therapy* : Systemic or topical antibiotics and antiseptics affect the oral flora.

- *Oxidation-reduction potential:* The oxidation-reduction potential of the environment (Eh) varies in different locations of the mouth. Redox potential falls during plaque development from an initial Eh of over +200mv (highly oxidize) to -141mv (highly reduced) after 7days. Such fluctuations favor the growth of different groups of bacteria.

- Diet. :Fermentable carbohydrates are the main class of compounds that alter the oral ecology they acts as a major source of nutrients, promoting the growth of acidogenic flora

Acquisition of the normal oral flora

1. The infant mouth is sterile at birth, except perhaps for a few organisms acquired from the mother's birth canal.

2. A few hours later, the organisms from the mother's (or the nurse's) mouth and possibly a few from the environment are established in the mouth.

3. These pioneer species are usually streptococci, which bind to mucosal epithelium (e.g. *Streptococcus salivarius*).

4. Only *S.mitis*, *S.oralis*, *S.salivarius* are able to become establish in the mouth of infant, other bacteria appear as transients. Lactobacillus, *S.mutans* can not establish the oral cavity prior to the eruption of teeth (mothers are the main source of *S.mutans* in the mouth of infants).

5. One of the major changes in the oral environment which occur at around the age 6 months is the eruption of the 1st deciduous teeth. The appearance of hard enamel surfaces in the mouth are suitable for establishment of *S.sanguinis* , *S.mutans* ,*Actinomyces* ,*Prevotella* , *Porphyromenas* , and Spirochaete

6.oral flora on the child's first birth usually consist of Streptococci, Staphylococci, Nesseria, Lactobacillus, Fusobacterium, and Prevotella (all these bacteria are transients…..go to number 5 above).

7. The anaerobes (Prevotella, Porphyromonas, Spirochaetes) do not appear in significant number until adolescence. (For e.g.:- only 18-40% of 5-years old have Spirochaetes and black-pigmented anaerobes compared with 90% of 13-16 years old).

8. A second childhood (in terms of oral bacterial colonization) is reached if all teeth are lost as a result of senility. Bacteria that colonize the mouth at this stage are very similar to those in a child before tooth eruption.

9. Introduction of prosthetic appliance at this stage changes the microbial composition once again. Candida species is particularly increased after the introduction of acrylic dentures (also *Staph.aureus* and lactobacilli are high in age 70 or over).

Nutrition of oral bacteria:-

A. Host resources

1. Remnants of the host diet always present in the oral cavity (e.g.: sucrose, starch)
2. Salivary constituent (e.g.: glycoproteins, minerals, vitamins)
3. Crevicular exudates (e.g. proteins)

B. Microbial resources

1. Extra cellular microbial products

- Intracellular food storage (glycogen) granules

The plaque biofilm

- The plaque biofilm is a tenacious microbial community embedded in an extracellular polysaccharide matrix, and attached to either the soft- or hard-tissue surfaces of the mouth, comprising living and dead bacteria and their extracellular products, together with host compounds, mainly derived from the saliva.

Dental plaque formation & caries

- Adherence to a surface in the mouth is essential for survival of oral bacteria. In the case of supragingival plaque formation, organisms do not colonize clean enamel but interact with a layer of material on the tooth surface called the "pellicle" comprises mucins, salivary glycoproteins, minerals and immunoglobulins. Pellicle formation occurs in seconds on cleaned enamel and reaches a maximum thickness in 90-120 minutes.

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Plaque biofilm formation is a complex process comprising a number of different stages:

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2. Transport. Bacteria approach the vicinity of the tooth surface prior to attachment, by means of natural salivary flow, Brownian motion or chemotaxis.

3- Initial adhesion (low affinity interactions)

Two types of forces are involved at these stages; Van der Waal's and electrostatic forces, then hydrogen bonds between hydroxyl groups in the pellicle and phosphate groups in the bacterial cell wall play a role in the adhesion of bacteria.

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In order for the bacteria to remain attached to the tooth surface for long periods they must establish higher affinity interactions between specific receptors on the host surface(ligands), and components on the bacterium called adhesins (such as fimbriae of bacteria).

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Once bound to a surface, the bacterium can divide and remain attached. Extracellular products are formed (polysaccharide) and daughter cells repeat the process so that microcolonies develop. And co-aggregation between the microorganisms occur, either between similar species (intrageneric) e.g. among streptococci or aggregation between different bacterial species (intergeneric) e.g.: between *S.sanguinis* and *Actinomyces naeslundii* or between Streptococcus spp. and Porphyromonas spp. The resultant biofilm is called "dental plaque":-soft, non-mineralized bacterial deposit which form on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin

Specific and non-specific plaque hypothesis

Although mutans streptococci have been recognized as the major group of organisms involved in caries, there is some controversy as to whether one or more specific groups of bacteria are principally involved in caries – the specific plaque hypothesis – or whether the disease is caused by a heterogeneous mixture of non-specific bacteria – the nonspecific plaque hypothesis

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Therefore, it seems likely that combinations of bacteria other than mutans streptococci and lactobacilli may be able to initiate carious lesions, and the plaque flora may be non-specific in nature. The current evidence implies that some bacteria (mutans streptococci, Lactobacillus spp. and Actinomyces spp.) may be more important than others in the initial as well as subsequent events leading to both enamel and root surface caries.

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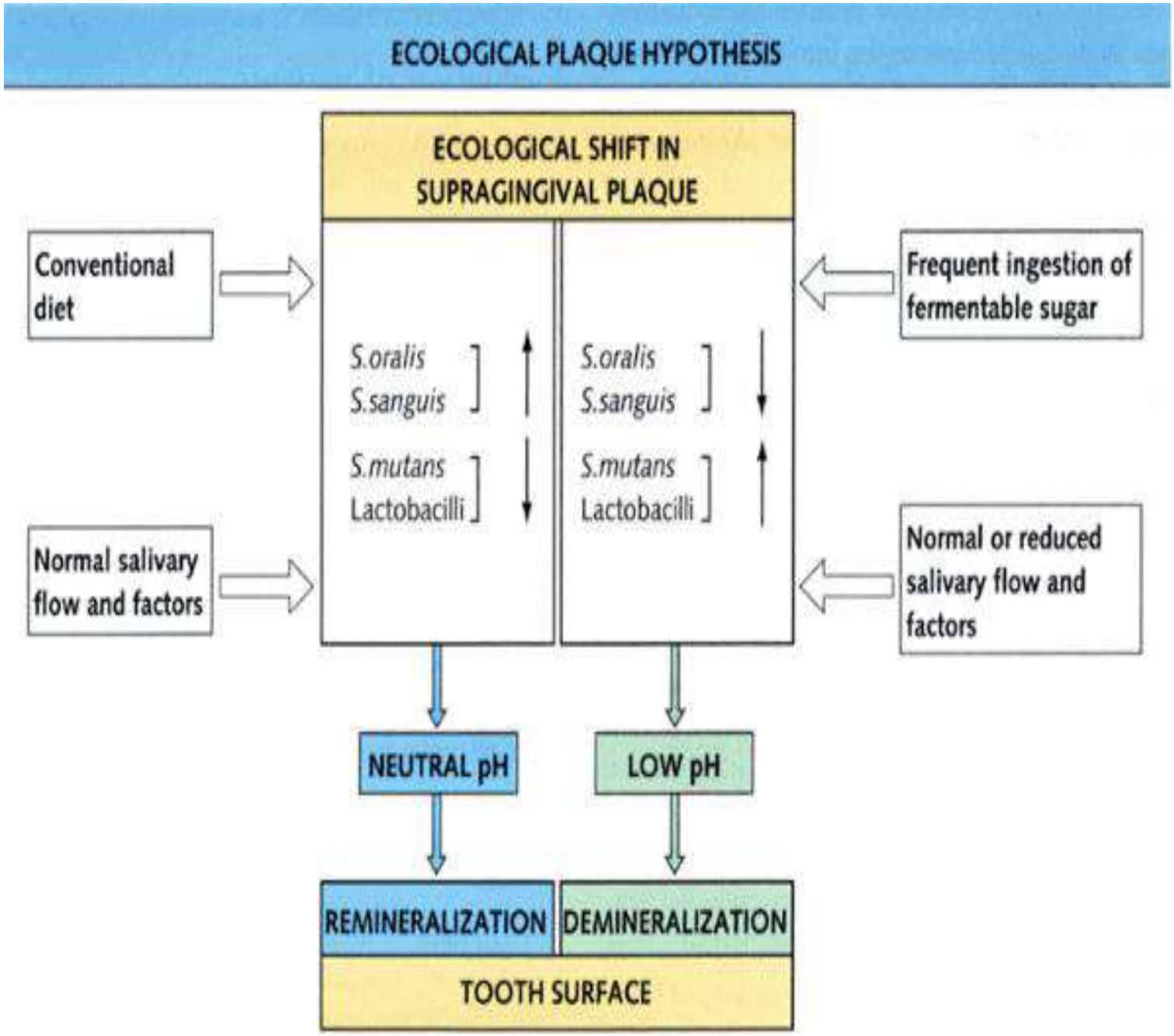
According to ecological plaque hypothesis. Cariogenic flora found in natural plaque are weakly competitive and comprise only a minority of the total community. With a conventional diet, levels of such potential cariogenic bacteria are clinically insignificant, and the processes of remineralization and demineralization are in equilibrium. But when the frequency of intake of fermentable carbohydrate increases, then the plaque pH level falls and remains low for prolonged periods, promoting the growth of acid tolerant bacteria(aciduric) and decrease in level of acid-labile bacteria. This conditions leads to initiate demineralization.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TOOTH SURFACE

Dental Caries

- Dental caries is a progressive irreversible microbial disease affecting the hard parts of tooth exposed to the oral environment, resulting in demineralization of the inorganic constituents and dissolution of the organic constituent, thereby leading to a cavity formation.

Classification

Dental caries can be classified with respect to the site of the lesion

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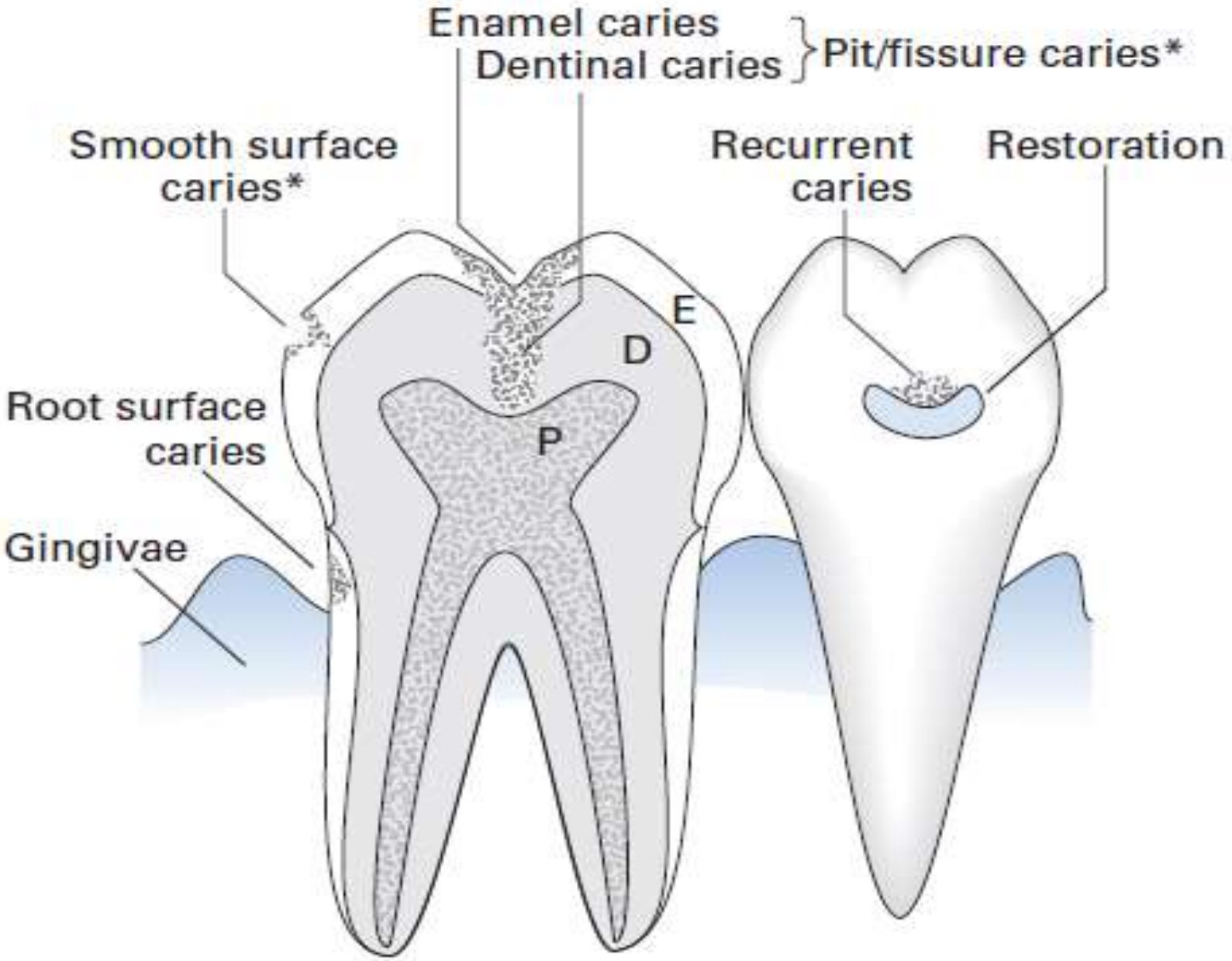
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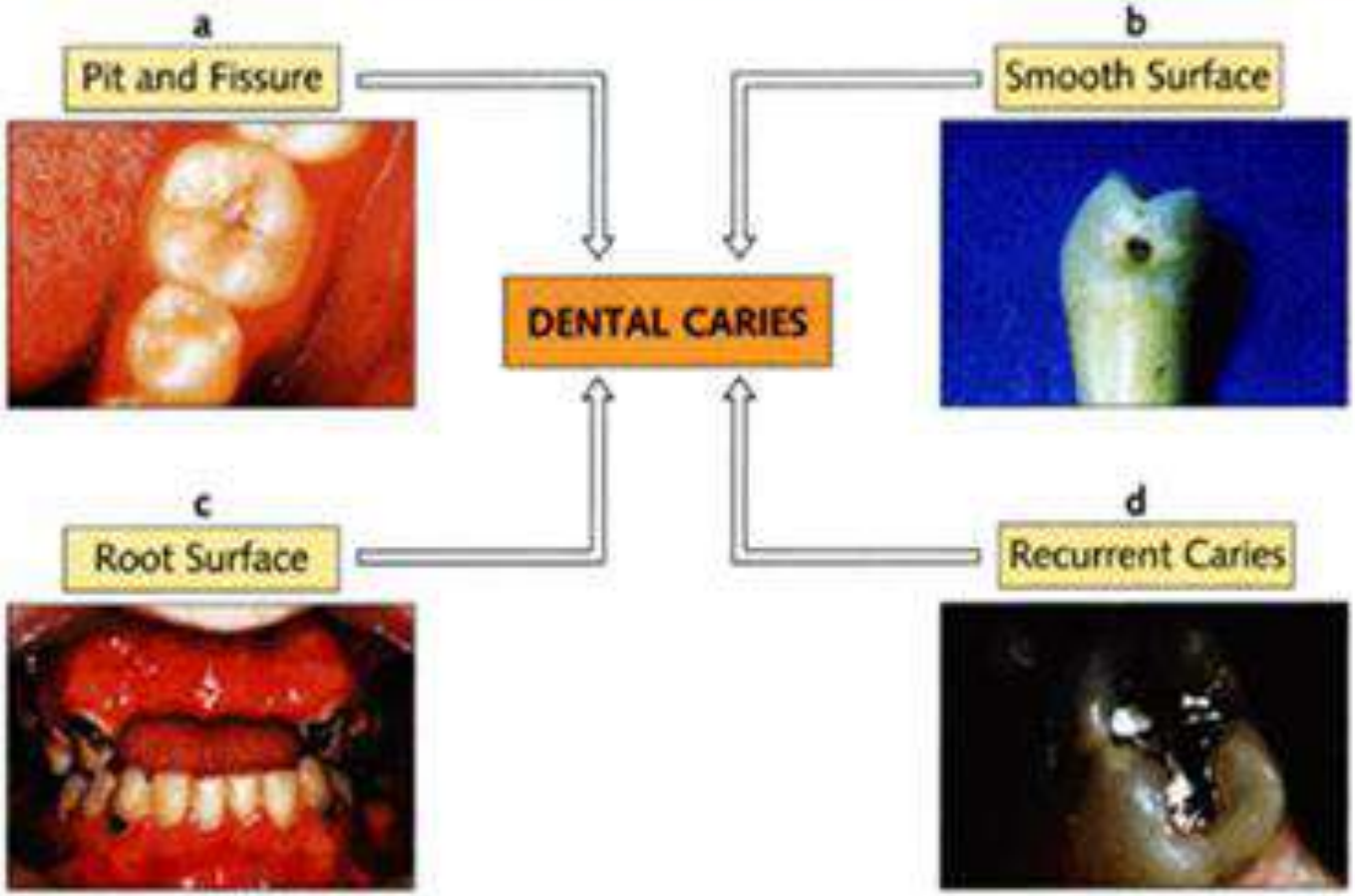
TYPES OF CARIES



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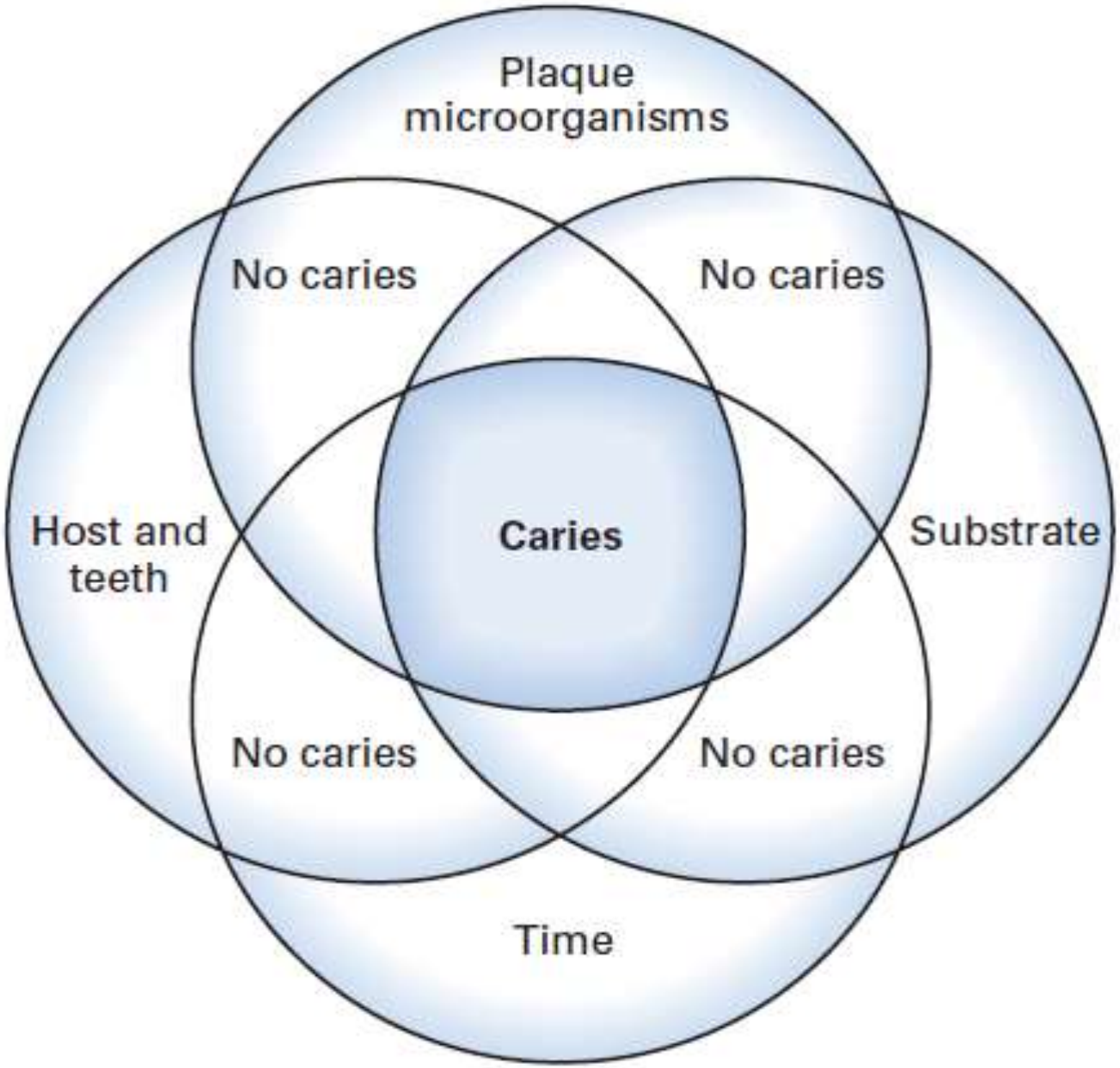
The main factors involved in dental caries are the tooth, saliva, supragingivalplaque, the diet (especially carbohydrate intake) and the time necessary for caries development These complex factors can interact in numerous different ways of interrelationships to result in the initiation and progression of carious lesions or healing of an early white spot lesion


Plaque
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Prevention of dental caries

The major approaches to prevention of caries are:

1. sugar substitutes: stopping or reducing between-meal consumption of carbohydrates, or substituting noncariogenic artificial sweeteners
2. fluorides: making the tooth structure less soluble to acid attack by using fluorides
3. sealants: to protect susceptible areas of the tooth (e.g. pits and fissures) that cannot easily be kept plaque-free by routine oral hygiene measures
4. reducing cariogenic flora: so that even in the presence of sucrose, acid production will be minimal (e.g. oral hygiene aids, antimicrobial agents and possibly immunization)
5. probiotics replacement of cariogenic bacteria by organisms with low or no cariogenic potential.

Treatment

Chlorhexidine as a 0.2% mouthwash is by far the most effective antimicrobial in plaque control

Ecology of the oral flora

Lec.13

The oral ecosystem

- Ecology is the study of the relationships between living organisms and their environment. An understanding of oral ecology is essential to comprehend the pathogenesis of diseases, such as caries and periodontal disease, caused by oral bacteria

The oral ecosystem

- The mouth, being an extension of an external body site, has a natural microflora. This commensal (or indigenous, or resident) flora exists in harmony with the host, but disease conditions supervene when this relationship is broken.
- The predominant dental diseases in humans (caries and periodontal disease) are caused in this manner.
- In addition to the commensal flora, there are others (such as coliforms) that survive in the mouth only for short periods (transient flora).
- These transient species of flora cannot get a foothold in the oral environment due to the ecological pressure, that is, the colonization resistance exerted by the resident flora.
- Indeed, the latter are considered critical in defending the key portal of entry into the digestive system, by offending pathogens.
- The oral ecosystem comprises the oral flora, the different sites of the oral cavity where they grow (i.e., habitats) and the associated surroundings.

Factors modulating microbial growth in the oral cavity:

1- Anatomical factors

- The shape of the teeth.
- The topography of the teeth (e.g. occlusal fissures).
- malalignment of the teeth
- Poor quality of restorations (e.g. fillings and bridges)

2- Saliva

- Adsorbing to oral surfaces, especially teeth, to form a salivary pellicle to which microorganisms can attach.
- acting as a primary sources of nutrients (carbohydrates and proteins)
- Aggregating microorganisms and there by facilitating their clearance from the mouth by swallowing or deposition on surfaces, contributing to plaque formation.
- Inhibiting the attachment and growth of some exogenous microorganisms by non-specific defense factors (e.g. lysozyme, lactoferrin and histatins) and specific defense factors (sIgA).

3- Gingival crevicular fluid (GCF)

- acting as a primary source of nutrients: proteolytic and saccharolytic bacteria in the crevice can utilize the crevicular fluid to provide peptides, amino acids and carbohydrates for growth.
- maintaining pH conditions.
- providing specific and non-specific defense factors (IgG and IgM) phagocytosis (neutrophils)

4- Microbial factors

Microbes in the oral environment can interact with each other both in promoting suppressing the neighboring bacteria. Mechanisms that accomplish this include:-

- competition for receptors for adhesion
- production of toxins, such as bacteriocins that kill cells of the same or other bacterial species e.g.: *Strept. salivarius* produces an inhibitor (enocin) that inhibits *Strept. pyogens*.
- Production of metabolic end-products such as short-chain carboxylic acids.
- Use of metabolic end-products of other bacteria for nutritional purpose (e.g.: *Veillonella* use acids produced by *Strept. mutans*).

5- Miscellaneous factors:

- pH ……. Many microbes require a neutral pH for growth the acidity of most oral surfaces is regulated by saliva (mean pH=6.7). Depending on the frequency of intake of dietary carbohydrates, the pH of plaque can fall to as low as (5) as a result of bacterial metabolism. Under these conditions acidophilic bacteria can grow well (e.g: lactobacilli).

- *Antimicrobial therapy* : Systemic or topical antibiotics and antiseptics affect the oral flora.

- *Oxidation-reduction potential:* The oxidation-reduction potential of the environment (Eh) varies in different locations of the mouth. Redox potential falls during plaque development from an initial Eh of over +200mv (highly oxidize) to -141mv (highly reduced) after 7days. Such fluctuations favor the growth of different groups of bacteria.

- Diet. :Fermentable carbohydrates are the main class of compounds that alter the oral ecology they acts as a major source of nutrients, promoting the growth of acidogenic flora

Acquisition of the normal oral flora

1. The infant mouth is sterile at birth, except perhaps for a few organisms acquired from the mother's birth canal.

2. A few hours later, the organisms from the mother's (or the nurse's) mouth and possibly a few from the environment are established in the mouth.

3. These pioneer species are usually streptococci, which bind to mucosal epithelium (e.g. *Streptococcus salivarius*).

4. Only *S.mitis*, *S.oralis*, *S.salivarius* are able to become establish in the mouth of infant, other bacteria appear as transients. Lactobacillus, *S.mutans* can not establish the oral cavity prior to the eruption of teeth (mothers are the main source of *S.mutans* in the mouth of infants).

5. One of the major changes in the oral environment which occur at around the age 6 months is the eruption of the 1st deciduous teeth. The appearance of hard enamel surfaces in the mouth are suitable for establishment of *S.sanguinis* , *S.mutans* ,*Actinomyces* ,*Prevotella* , *Porphyromenas* , and Spirochaete

6.oral flora on the child's first birth usually consist of Streptococci, Staphylococci, Nesseria, Lactobacillus, Fusobacterium, and Prevotella (all these bacteria are transients…..go to number 5 above).

7. The anaerobes (Prevotella, Porphyromonas, Spirochaetes) do not appear in significant number until adolescence. (For e.g.:- only 18-40% of 5-years old have Spirochaetes and black-pigmented anaerobes compared with 90% of 13-16 years old).

8. A second childhood (in terms of oral bacterial colonization) is reached if all teeth are lost as a result of senility. Bacteria that colonize the mouth at this stage are very similar to those in a child before tooth eruption.

9. Introduction of prosthetic appliance at this stage changes the microbial composition once again. Candida species is particularly increased after the introduction of acrylic dentures (also *Staph.aureus* and lactobacilli are high in age 70 or over).

Nutrition of oral bacteria:-

A. Host resources

1. Remnants of the host diet always present in the oral cavity (e.g.: sucrose, starch)
2. Salivary constituent (e.g.: glycoproteins, minerals, vitamins)
3. Crevicular exudates (e.g. proteins)

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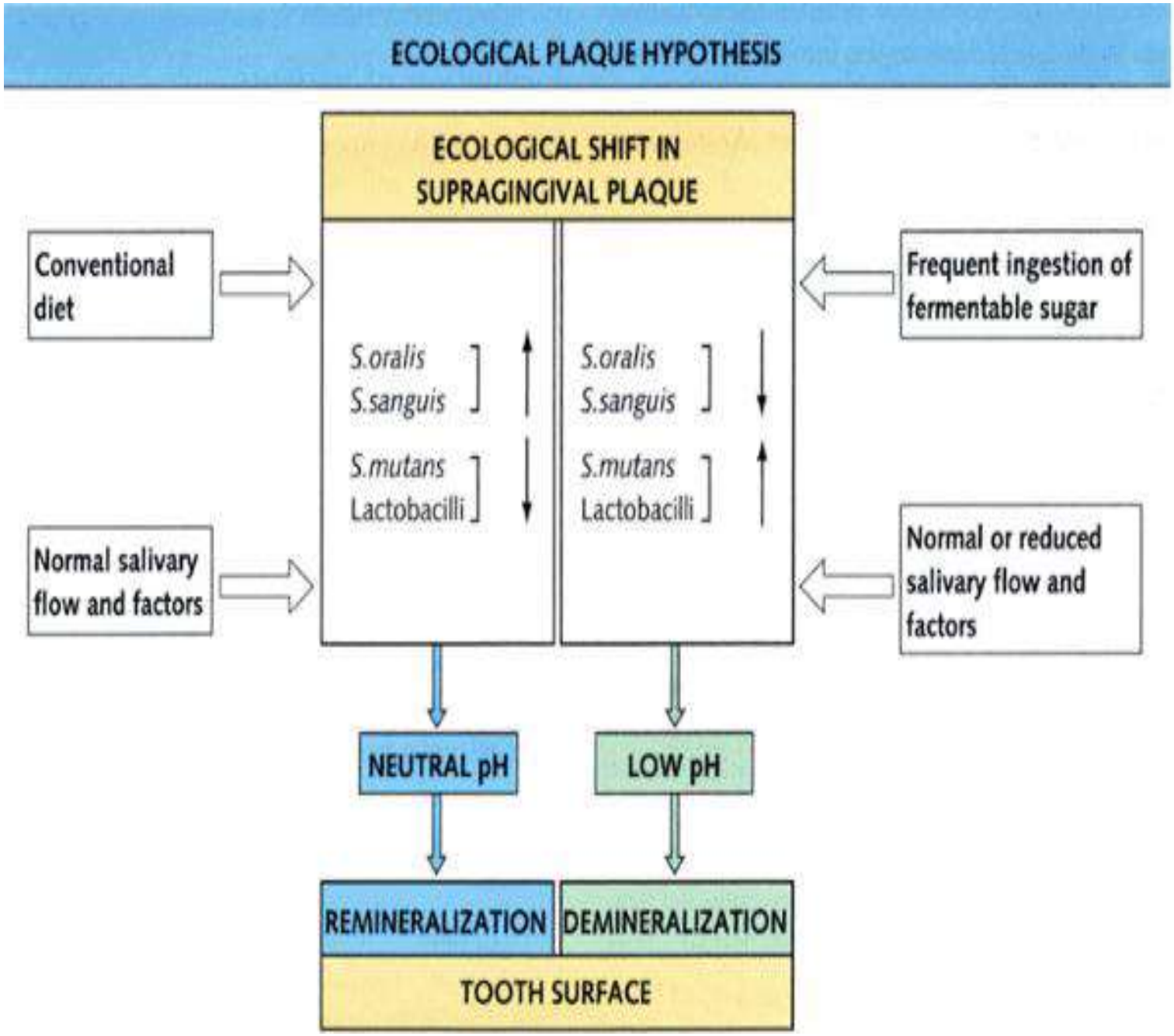
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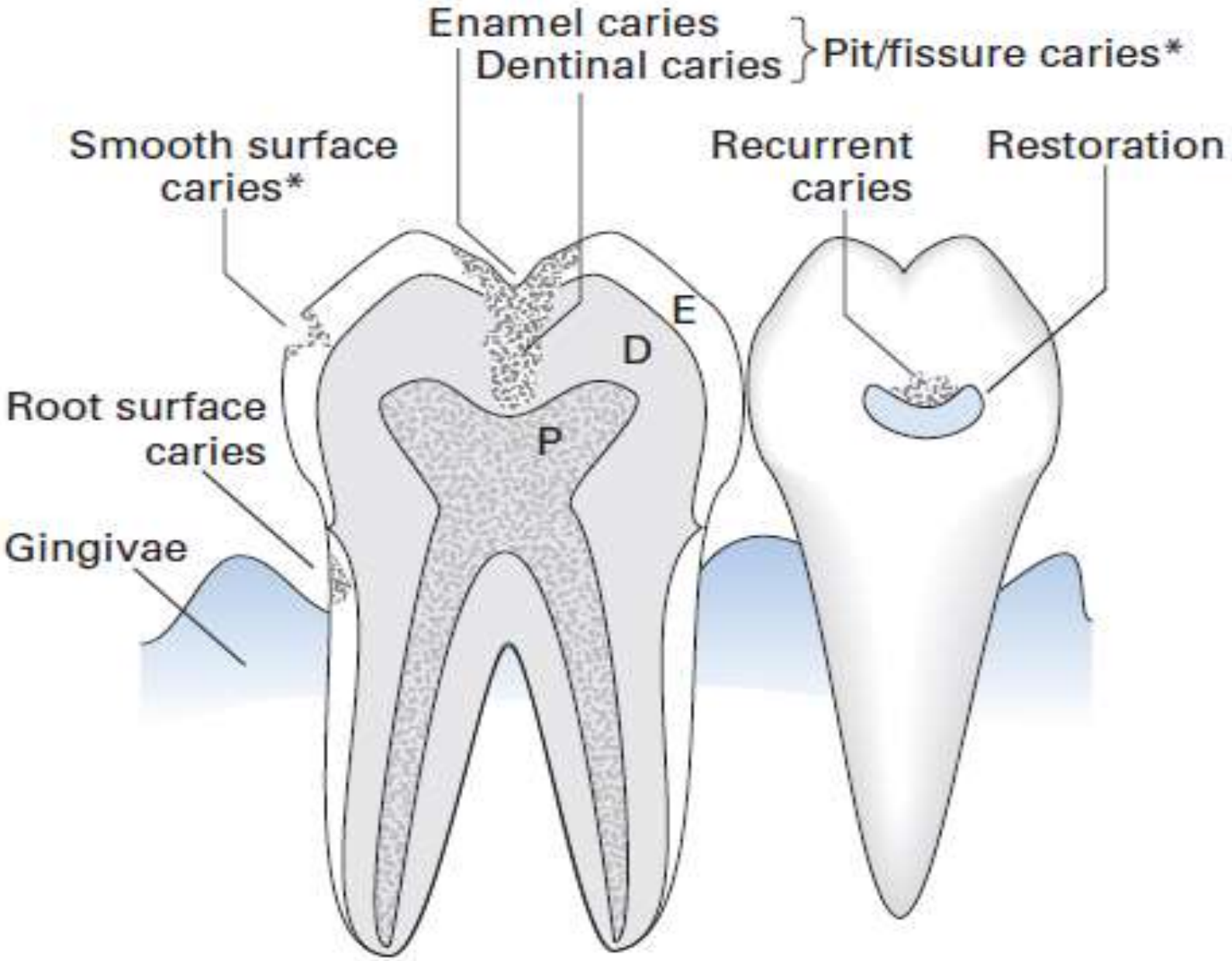
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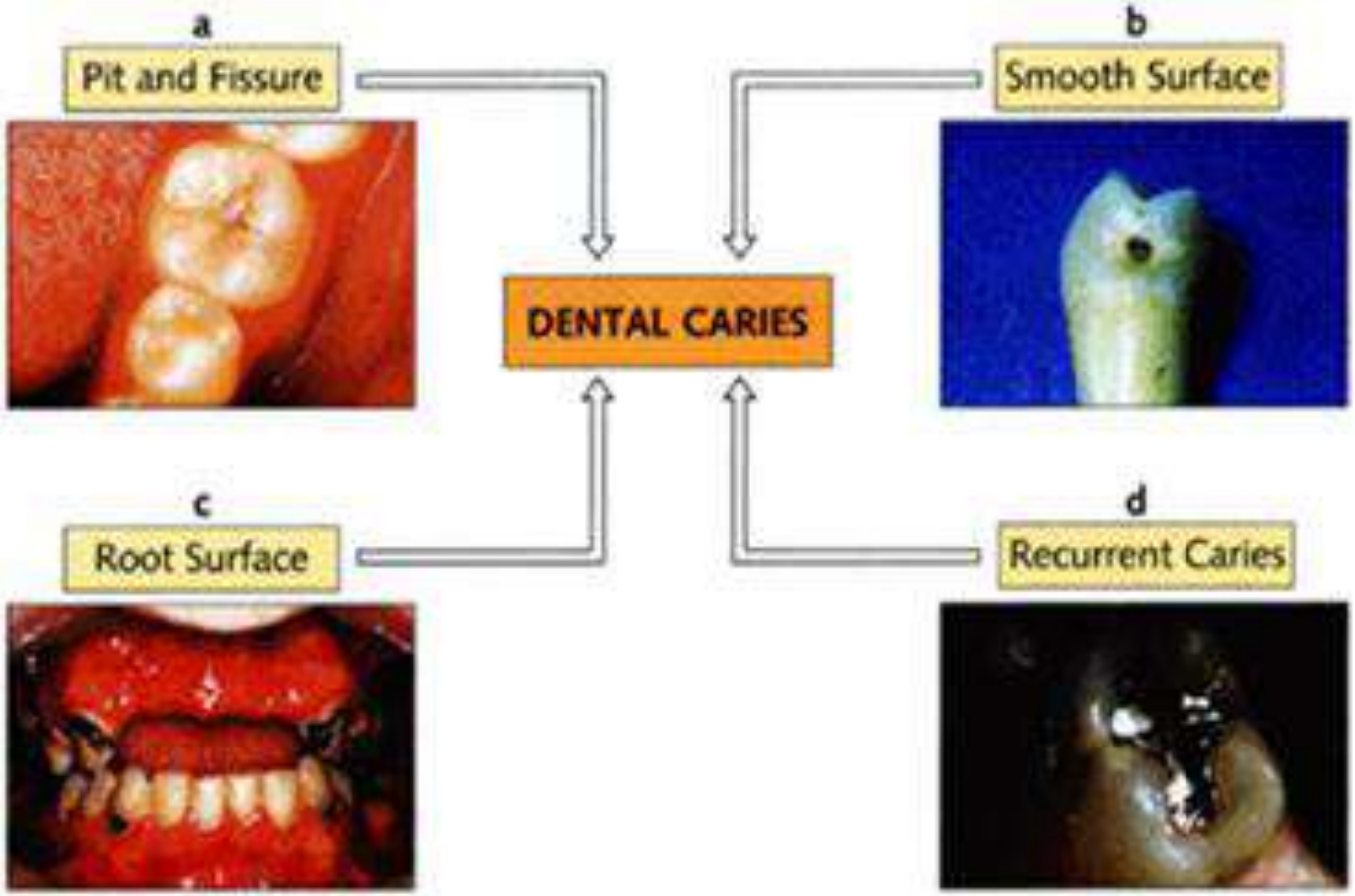

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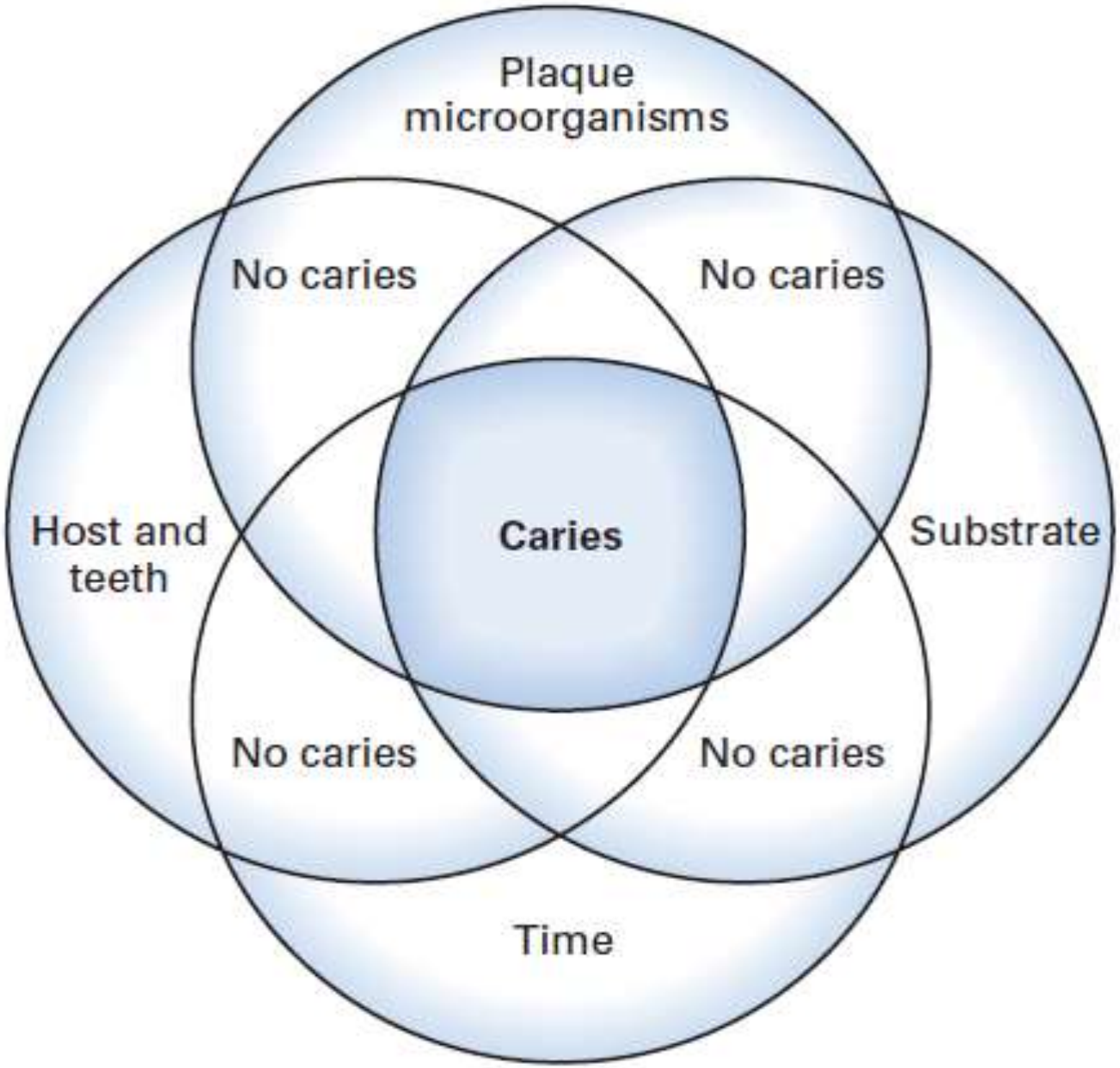
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Periodontal diseases

- Bacteria in combination with the host inflammatory response are responsible for most forms of periodontal disease.
- Periodontal diseases are mixed infections and a variety of bacteria or groups of bacteria are required for the initiation and progression of the disease in a susceptible host.
- These bacteria are often present in healthy individuals, and thus periodontal diseases can be considered opportunistic infections (most of the putative pathogens are also present in healthy mouths and disease requires a shift in the balance between host and organism that allows the pathogenic bacteria to increase in number and express their virulence factors at susceptible sites) .

Gingivitis

- The organisms involved in gingivitis tend to also be commonly found in mature supragingival plaque in healthy individuals.
  - Actinomyces spp: Gram-positive facultative that comprise a large proportion of the supragingival and subgingival plaque microbiota.
  - Prevotella intermedia: a Gram-negative black pigmenting anaerobe.
  - Bacteroides species: Gram-negative anaerobes.
  - Fusobacterium nucleatum: a Gram-negative anaerobe present in high numbers in supra- and sub-gingival plaque both in health and disease.

Chronic periodontitis

- Organisms associated with chronic periodontitis often anaerobic, colonizers of mature subgingival plaque that possess tissue destructive properties.
- *Porphyromonas gingivalis:* a highly proteinaceous, asaccharolytic, Gram-negative black pigmenting anaerobe.
- *Tannerella forsythia*: a Gram-negative anaerobe with an outer S-layer.
- *Treponema denticola* and other medium and large size spirochetes. Prevotella intermedia, *Campylobacter rectus*, *Fusobacterium nucleatum*: *Eikenella corrodens*, *Peptostreptococci*
- Herpes viruses: may work synergistically with bacteria.

Localized aggressive periodontitis

- LAP is almost exclusively associated with *Aggregatibacter actinomycetemcomitans*, a Gram-negative capnophile.

Generalized aggressive periodontitis

- GAP is associated with a subset of organisms involved in chronic periodontitis: *P. gingivalis, Tannerella forsythia,* and Selenomonas

Virulence factors of periodontal bacteria

- Fimbriae, pili
- Capsule
- Peptidiglycan
- Endotoxin
- Proteolytic enzymes (collagenase, gelatinase, hyaluronidase…)
- Leukotoxin
- Protease
- LPS
- Butyric acid
- Volatile sulfur compounds
- Ammonia & indole

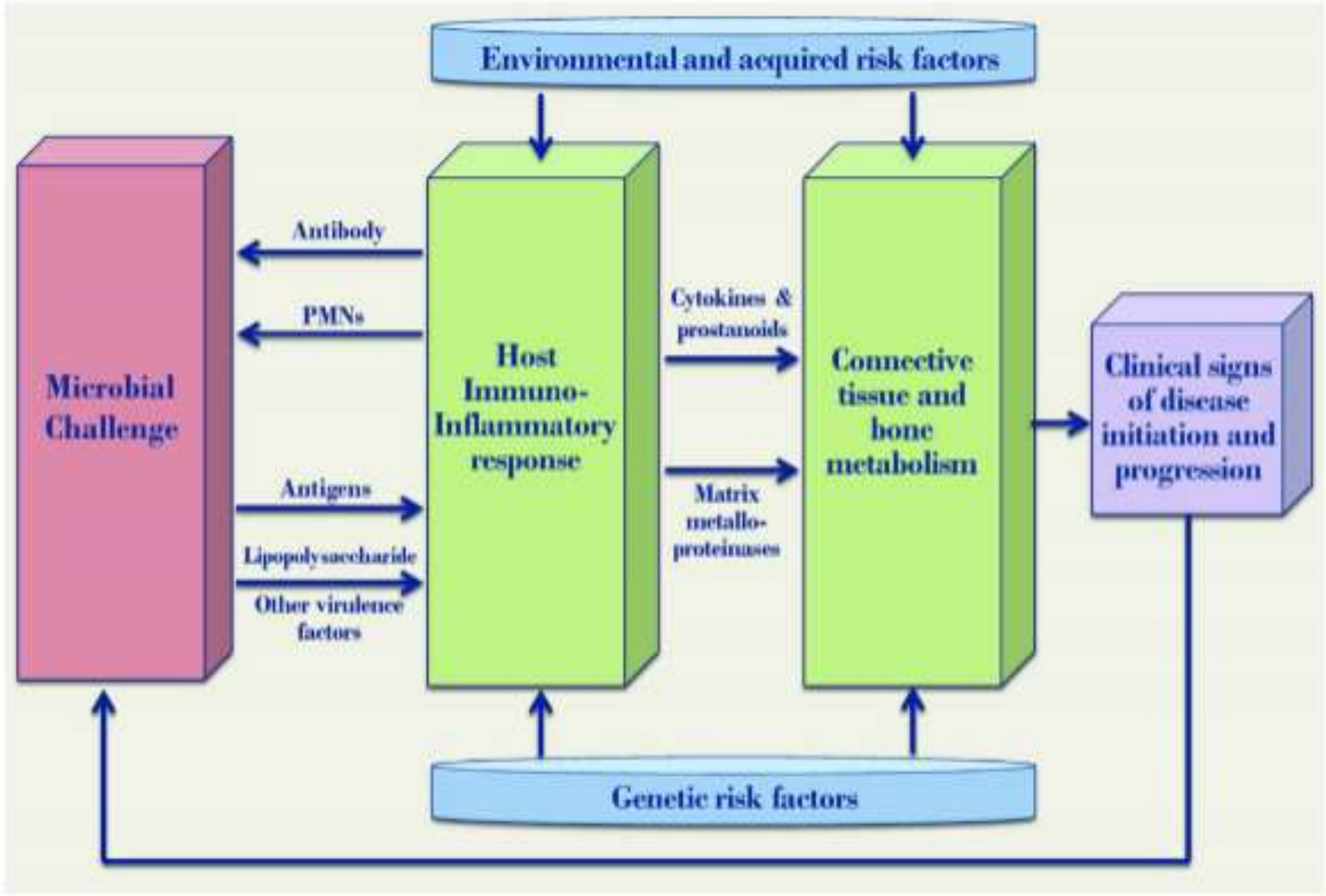


FIGURE 6 Overview of the pathogenesis of human periodontitis. The bacteria of the dental plaque challenge the host, which reacts by mounting a local inflammatory response. Under the influence of certain environmental, acquired, and genetic risk fac-

Endodontic Microbiology

- Routes of root canal infection: -

a) Dental Tubules

b) Direct Pulp Exposure

c)Periodontal Disease

d) Anachoresis (is a process by which microorganisms are transport in the blood or lymph to an area of tissue damage, where they leave the vessel, enter the damaged tissue, and establish an infection.

*Bacterial Genera Represented in Endodontic Infection:-

G+ve Bacteria		G+ve Bacteria		G-ve Bacteria		G-ve Bacteria	
Anaerobes		Facultatives		Anaerobes		Facultatives	
Cocci	Rods	Cocci	Rods	Cocci	Rods	Cocci	Rods
Peptostreptococci *Gemella*	*Actinomyces* *Pseudoramibacter* *Eubacterium*	*Enterococcus* *Streptococcus*	*Lactobacillus* *Actinomyces*	*Veillonella*	*Dialister* *Porphyromonas* *Prevotella* *Tannerella*	*Neisseria*	*Eikenella* *Capnocyto-phaga*

Ecology of the Endodontic Microbiota:-

root canal with necrotic pulp provides a space for bacterial colonization and provides bacteria a moist, warm, nutrition, and anaerobic environment. Selective pressure must occur in the root canal system that favor the establishment of some species and inhibit others. The key ecologic factors that influence the composition of the microbiota in the necrotic root canal include oxygen tension and redox potential type and amount of available nutrients, and bacterial interactions.

a**) Oxygen Tension and Redox Potential:-** In the very initial phases of the pulpal infectious process, facultative bacteria predominate. After a few days or week, oxygen is depleted within the root canal as a result of pulp necrosis and consumption by facultative bacteria and then provide suitable environment for obligate anaerobic bacteria.

b) Available Nutrients:-

- In the root canal system, bacteria can utilize the following as sources of nutrients:-

1. The necrotic pulp tissue.

2. Proteins and glycoproteins from tissue fluids and exudates that seep into the root canal system via apical and lateral foramens.

3. Components of saliva that may coronally penetrate the root canal.

4. Products of the metabolism of other bacteria.

c) Bacterial Interactions:-

- They are several bacterial Interactions in the root canal such as: Mutualism and Commensalism or Competition and Antagonism.

Oral mycology

Oral fungal disease

- Fungal infections in the oral occurs either as primary localized lesions or as manifestication of systemic mycoses. The most common group of fungal infections that dental pracitioners diagnose and treat are caused by Candida spp. Some of the rarer mycoses such as histoplasmosis.

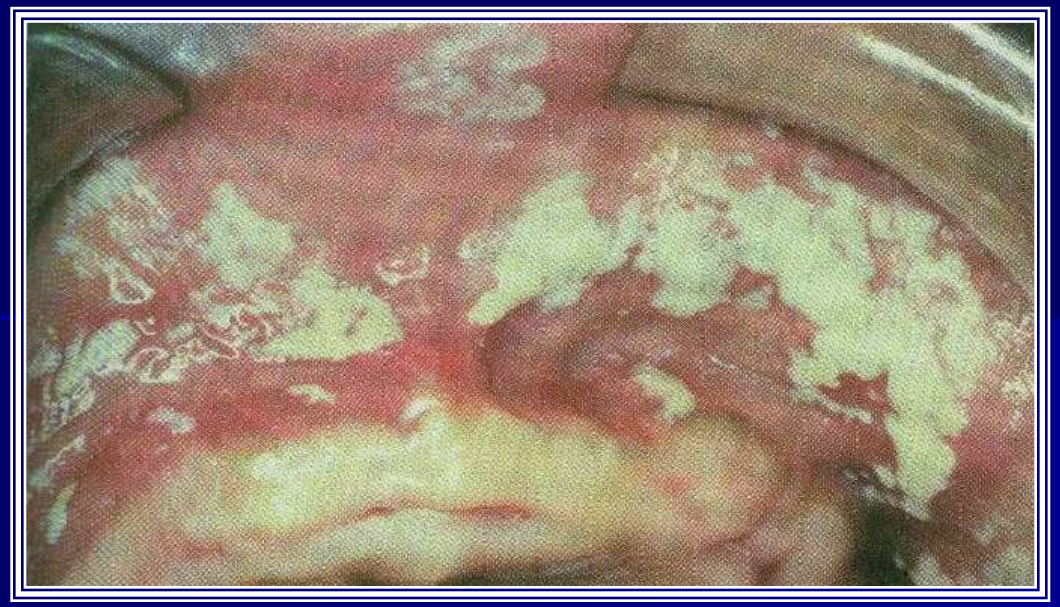
Candidiasis

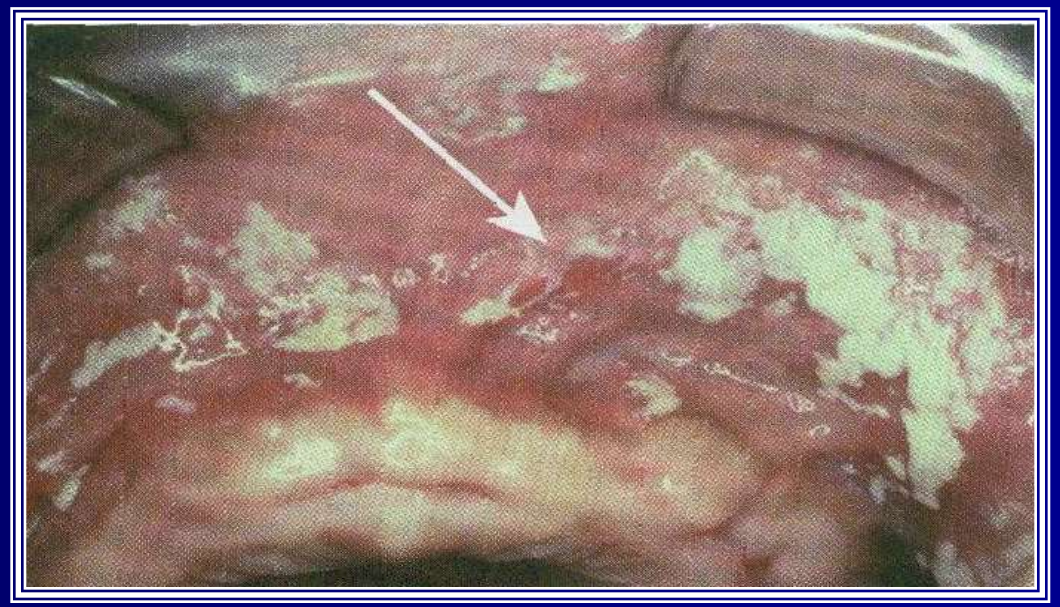
- Candidiasis is a primary or secondary mycotic infection caused by members of the genus Candida, the clinical manifestations may be acute, sub- acute or chronic to episodic and involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditic and meningitis .
- Systemic candidiasis is usually seen in patients with cell-mediated immune deficiency, and those receiving aggressive cancer treatment, immunosuppression, or transplantation therapy .

Clinical manifestations:

Thrush

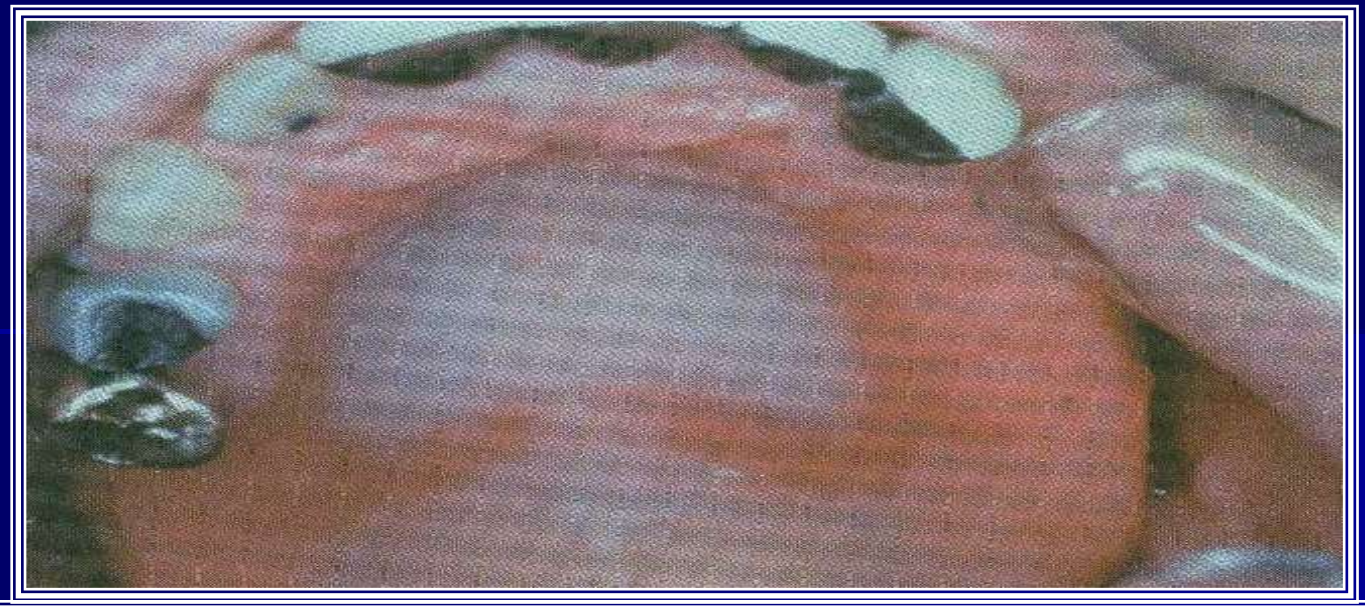
- It is an infection of mouth caused by Candida and the common sign of thrush is the presence of creamy white, slightly raised lesion of the mouth.
- The lesion, may have a "cottage cheese " appearance, in severe cases, may spread into the esophagus , or swallowing tube , causing pain or difficulty swallowing .

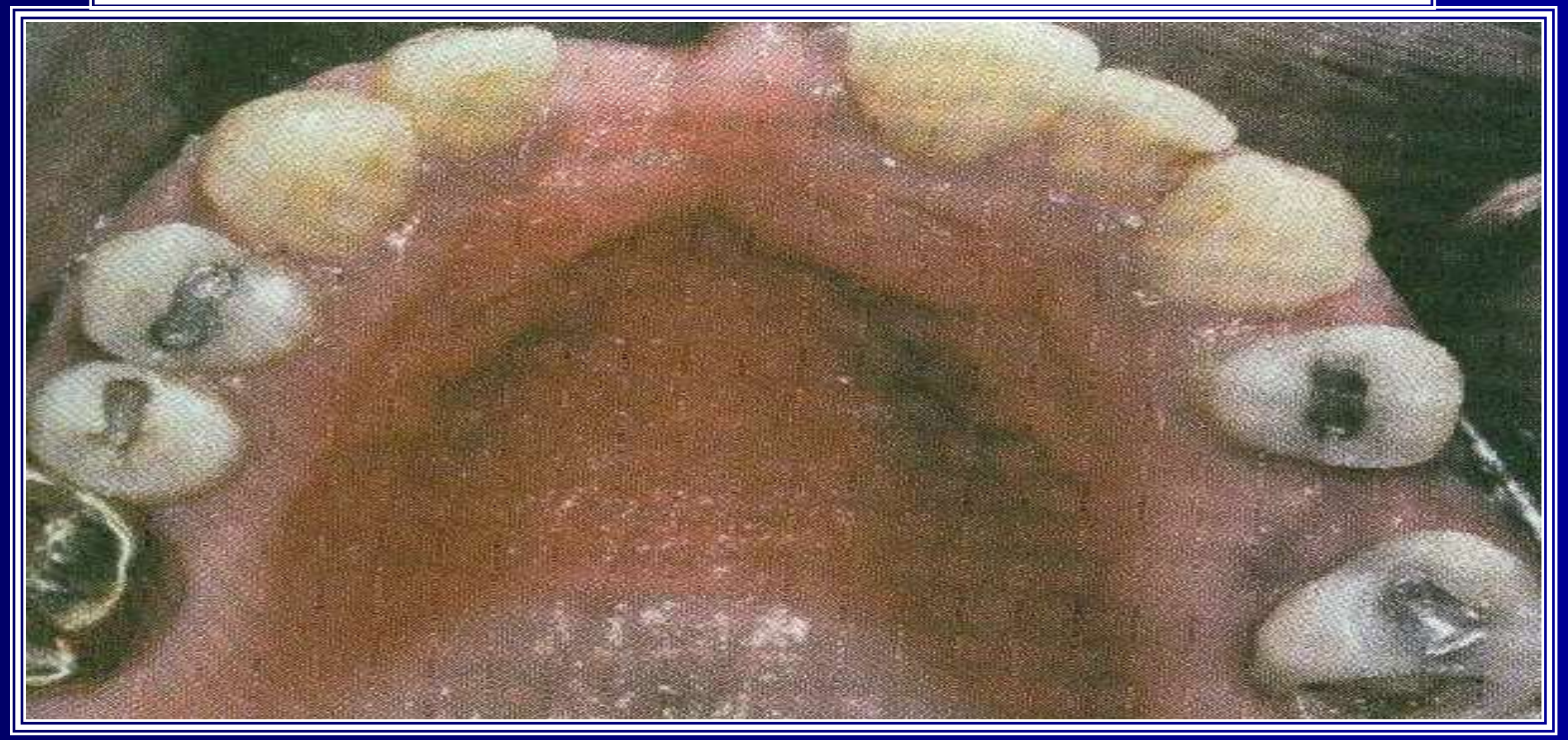




Stomatitis

- Stomatitis is an inflammation of the mucus lining of any of the structures in the mouth which may involve the cheeks , gums , tongue , lips , throat .
- Erythematous candidosis related to dentures is the most common form of oral candidosis in about 50 % of denture wearer





Angualr cheilitis

- Angular cheilitis is an inflammatory lesion at the labial commeasure , or corner of the mouth and often occurs bilaterally .
- However , nutritional deficiencies such as vitamin B12 and iron also some underlying causes for angular cheilitis .
- It also reported that angular cheilitis is one of the common presentations of oral candidiasis in HIV infected patients .

Pathogenesis

- The ability of Candida to adhere to the mucosa and dentures plays an important role in the pathogenesis of oral yeast infections. Adherence is achieved by specific and nonspecific mechanisms.
- However, the mechanisms of are still not fully understood. Local defense mechanisms have a key role in preventing yeast colonization in the oral cavity.
- These include the physical local barrier of the epithelia, antimicrobial peptides, secretory immunoglobulin A, and salivary factors such as flow rate and specific.

- molecules (lysozyme, histatin and lactoferrin). Secreted aspartic proteinase (SAP), phospholipases and lipases are extracellular enzymes that facilitate adherence and/or tissue penetration.
- SAPs efficiently degrade extracellular matrix and host surface proteins (laminin, fibronectin, and mucin).

Examples risk patients for oral candidiasis

- Patients with dental prostheses
- Patients with reduced salivary flow rate
- Patients with oral mucosal diseases
- Asthmatic patients on corticosteroid therapy
- Diabetic patients
- Patients with rheumatic diseases

- HIV infected and AIDS patients
- Patients with malignant disease
- Patients receiving immunosuppressive drugs
- Patients receiving radiotherapy to the head and neck
- The elderly

Antifungal therapy:

- Antifungal can be grouped into three classes based on their site of action: azoles, which inhibit the synthesis of ergosterol (the main fungal sterol); polyenes , which interact with fungal membrane sterols physicochemically; and 5-fluorocytosine, which inhibits macromolecular synthesis.
- Many different types of mechanisms contribute to the development of resistance to antifungal. These mechanisms include alteration in drug target, alteration in sterol biosynthesis, reduction in the intercellular concentration of target enzyme, and over expression of the antifungal drug target

- The first group, the Polyenes represented by amphotericin B, target ergosterol , a sterol present in the fungal cell membrane, and make pores causing cell death
- The second group of antifungal drugs is ergosterol biosynthesis inhibitors, which include azoles, morpholines and allylamines. They can inhibit the late pathway of ergosterol biosynthesis and cell division, causing loss of membrane structure and function. Azoles are the most popular drugs from this group, and they can be divided into two classes: imidazoles, which include ketoconazole and clotrimazole used for superficial infections, and triazoles, which include fluconazole, voriconazole and itraconazole used for systemic infections .

- The third group includes inhibitors of nucleic acid synthesis, i.e. 5-flucytosine which is converted by to 5-fluorouracil(5-FU) by the enzyme cytosine deaminase , subsequently ,5-FU is converted by UMP pyrophosphorylase into 5- fluorouridylic acid , which is further phosohorylated and incorporated into RNA , resulting in disruption of protein synthesis .
- A serious problem in treatment of fungal infections is the resistance to azoles and 5-flucytosine through a mechanism dependent on alternations in the target enzyme and in drug efflux pumps

Viral infectious diseases

Herpes simplex virus(HSV)

A. Primary herpetic gingivostomatitis (type I H.S.V)

In a person without circulating antibodies. It’s developed in children and young adult, rarely in children under 6 months and mainly transmitted by saliva, incubation period is 5 days.

In the oral cavity is characterized by multiple vesicular eruptions located in the all attached oral mucosa, gingival and movable mucosa, chiefly lips. Buccal mucosa, pharynx and tonsils may be involved .

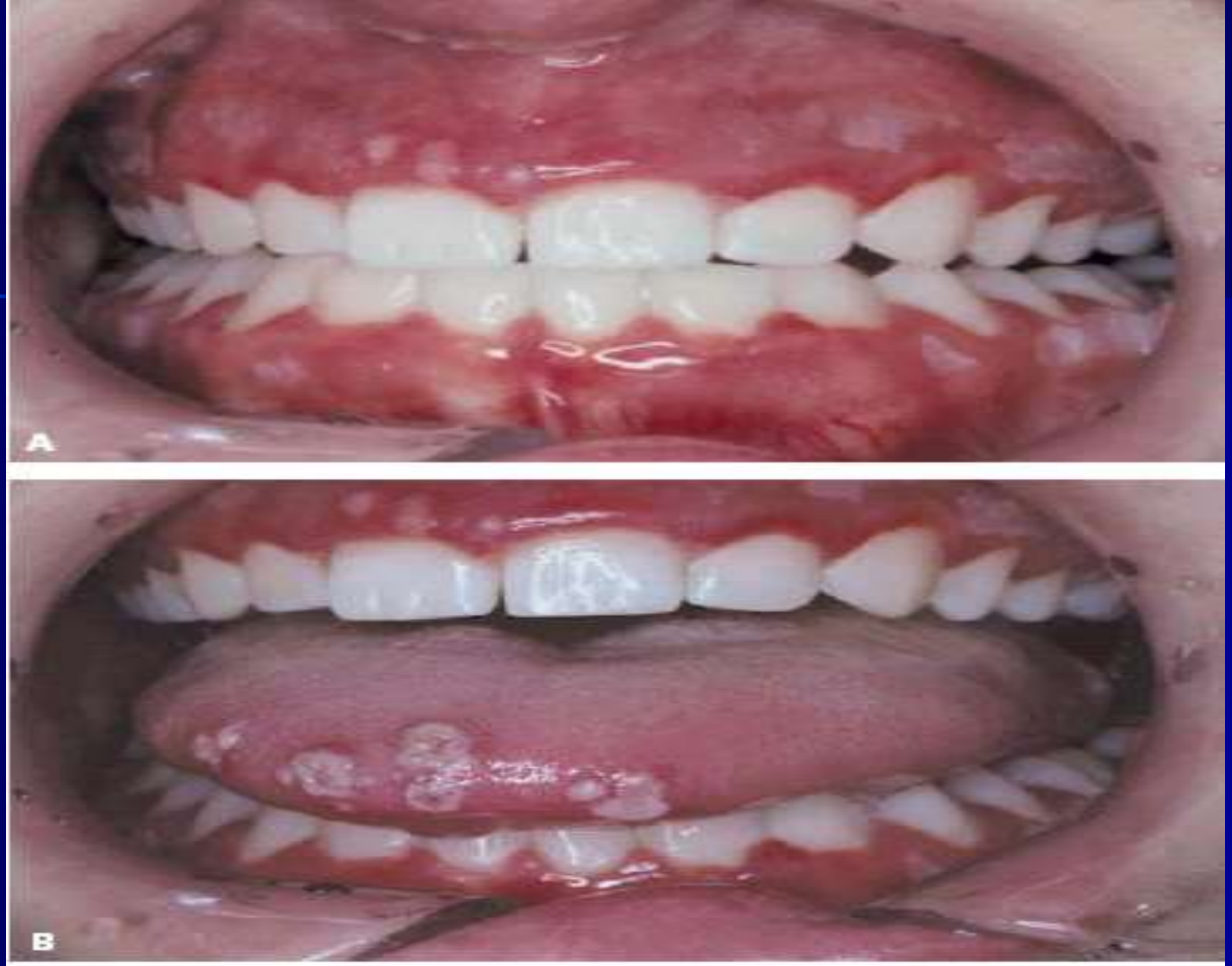


Figure 13–2 Primary herpetic gingivostomatitis in a child. Painful gingival (*A*) and tongue (*B*) lesions had been present for 4 days with associated fever and malaise. This was a first-time attack. In 10 days with only supportive care, the patient was asymptomatic and well.

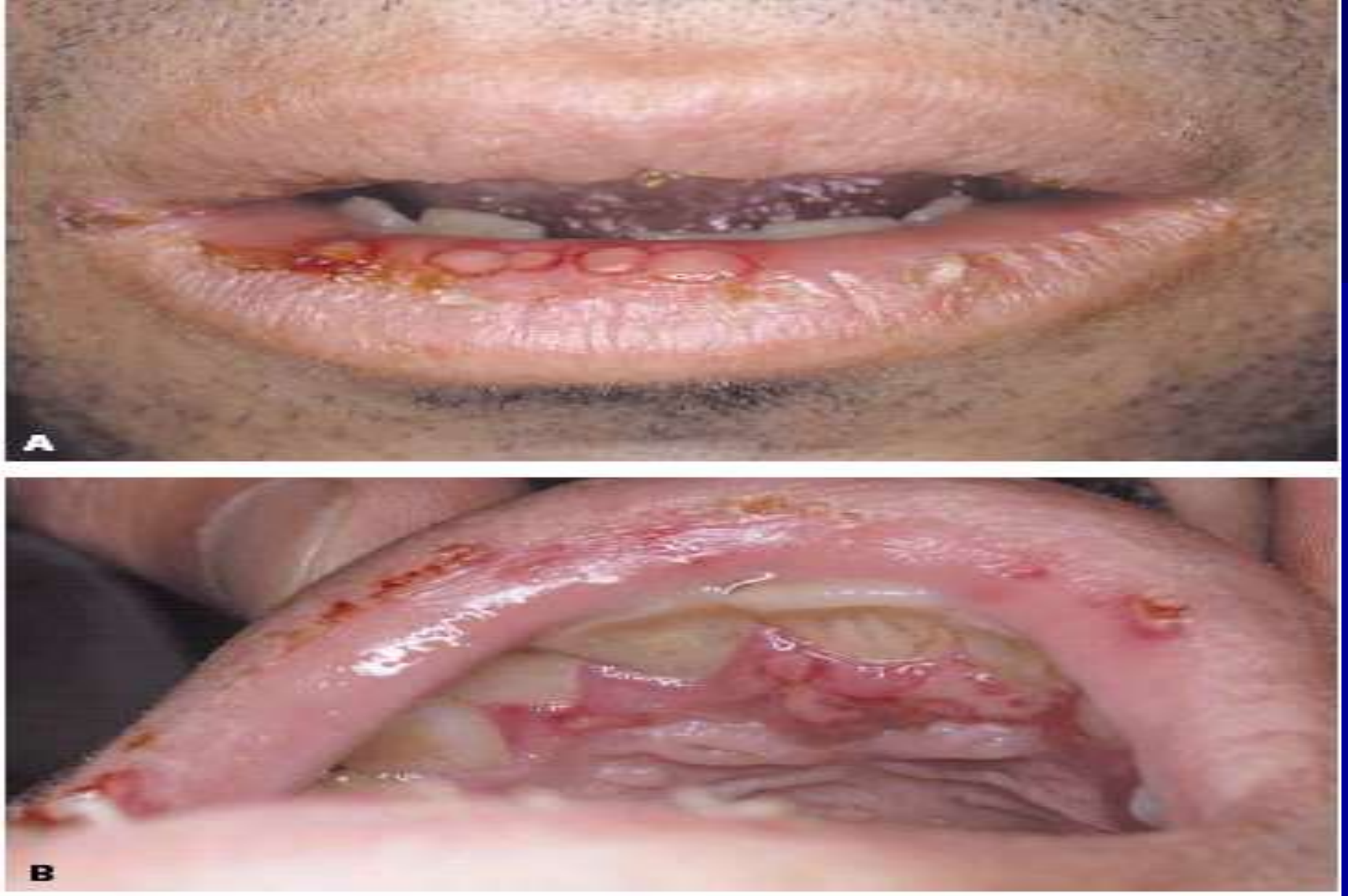


Figure 13–4 *A* and *B*, Adult onset primary herpetic gingivostomatitis in a 32-year-old male with spontaneous severe oral ulcers and gingivitis. There was acute pain and fever with no other findings. Following supportive care only, he was well in 2 weeks, and since then, has had no further similar attacks.

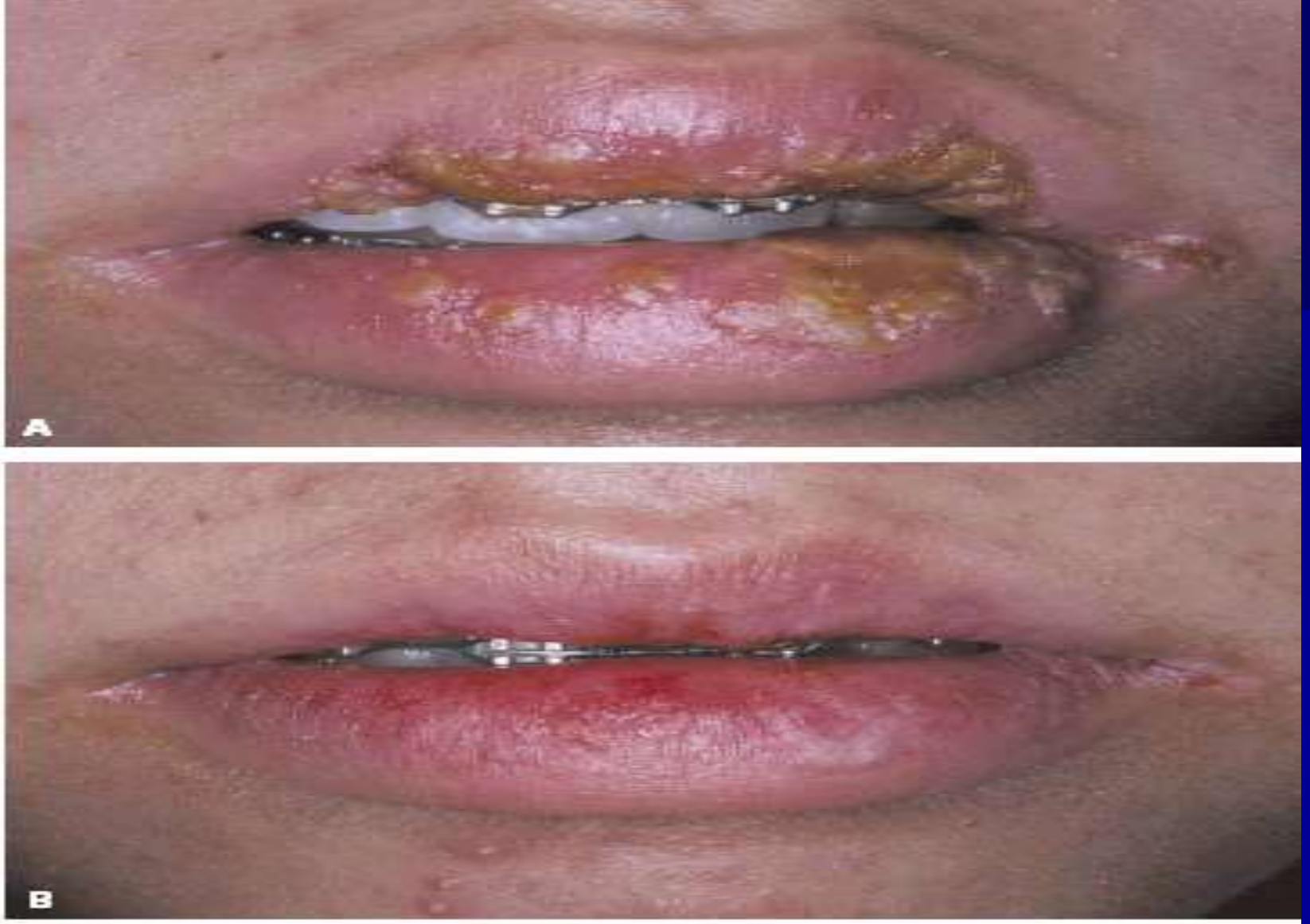


Figure 13–3 Primary herpetic gingivostomatitis in an adolescent. *A*, Intraoral and labial ulcers of 5 days duration associated with sudden onset, fever, pain, and lymphadenopathy. There were no other medical findings or associated causative factors. *B*, One week later, the patient was essentially without signs or symptoms; and at the end of 2 weeks from the onset, she was completely healed. Treatment included acyclovir for 5 days, fluids, and anti-inflammatory-antipyretic analgesics.

Treatment

supported care consist of the prescribing a soft diet, analgesic to reduce pain and fever with antibiotic to prevent secondary infection, soothing mouth wash to wet the lesion.

B. Secondary herpes simplex infection:

1.Recurrent (Herpes Labialis)

Occurs mostly on the firmly attached mucosa such as hard palate and attached gingiva and not movable mucosa.

Heals in 10 days and no treatment is applied, with reassurance of patient.

2. Herpetic whitlow infection:

is viral dermatitis of finger and also called terminal pulp infection.

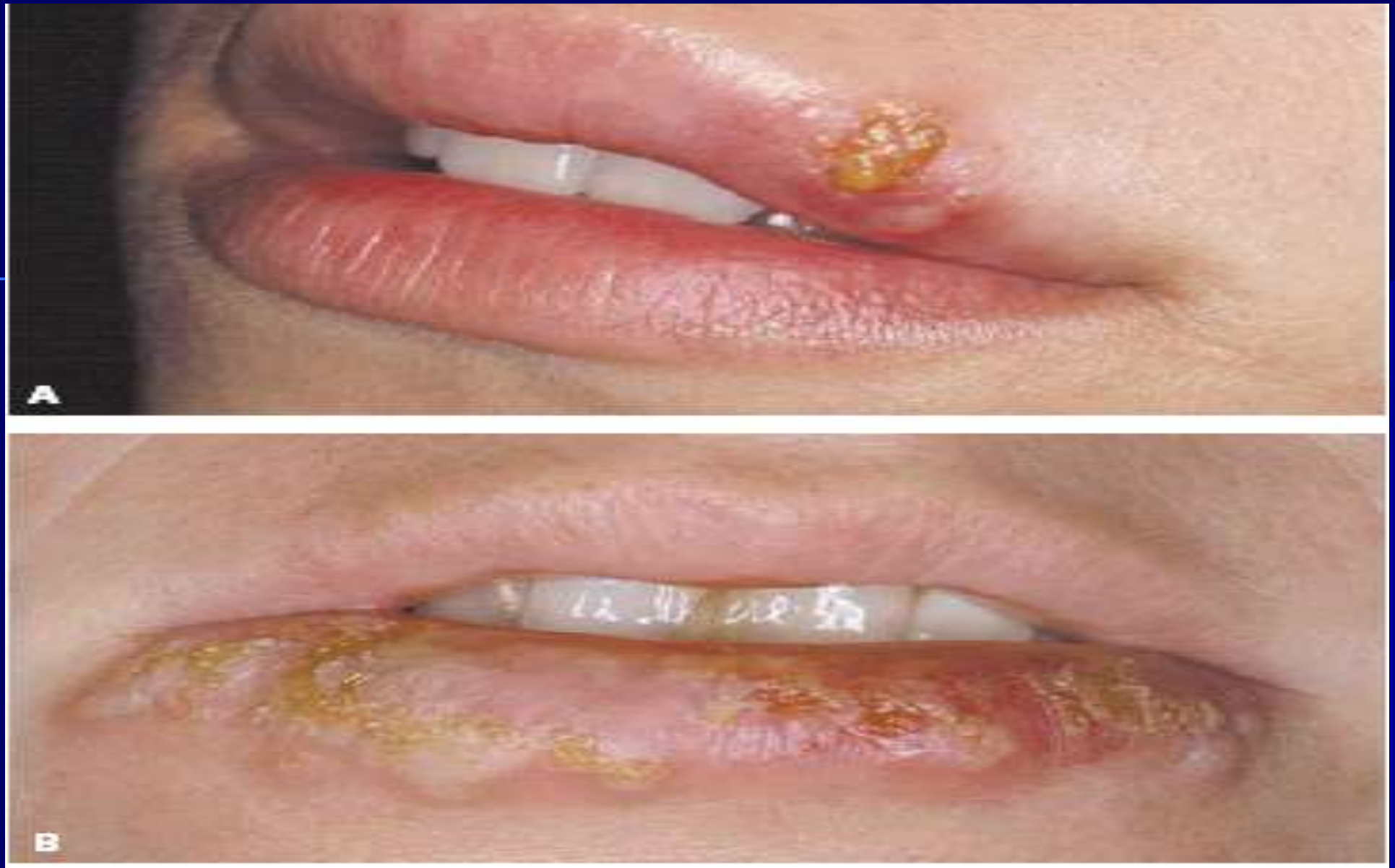


Figure 13–9 Recurrent herpes labialis (cold sores). *A*, Single lesion at day 7 in a 12-year-old boy; *B*, multiple lesions in an adult female at day 10. The lesions spontaneously healed in another 10 days, with only application of lubricant. The sores recurred periodically and would shed virus for about 4 days.

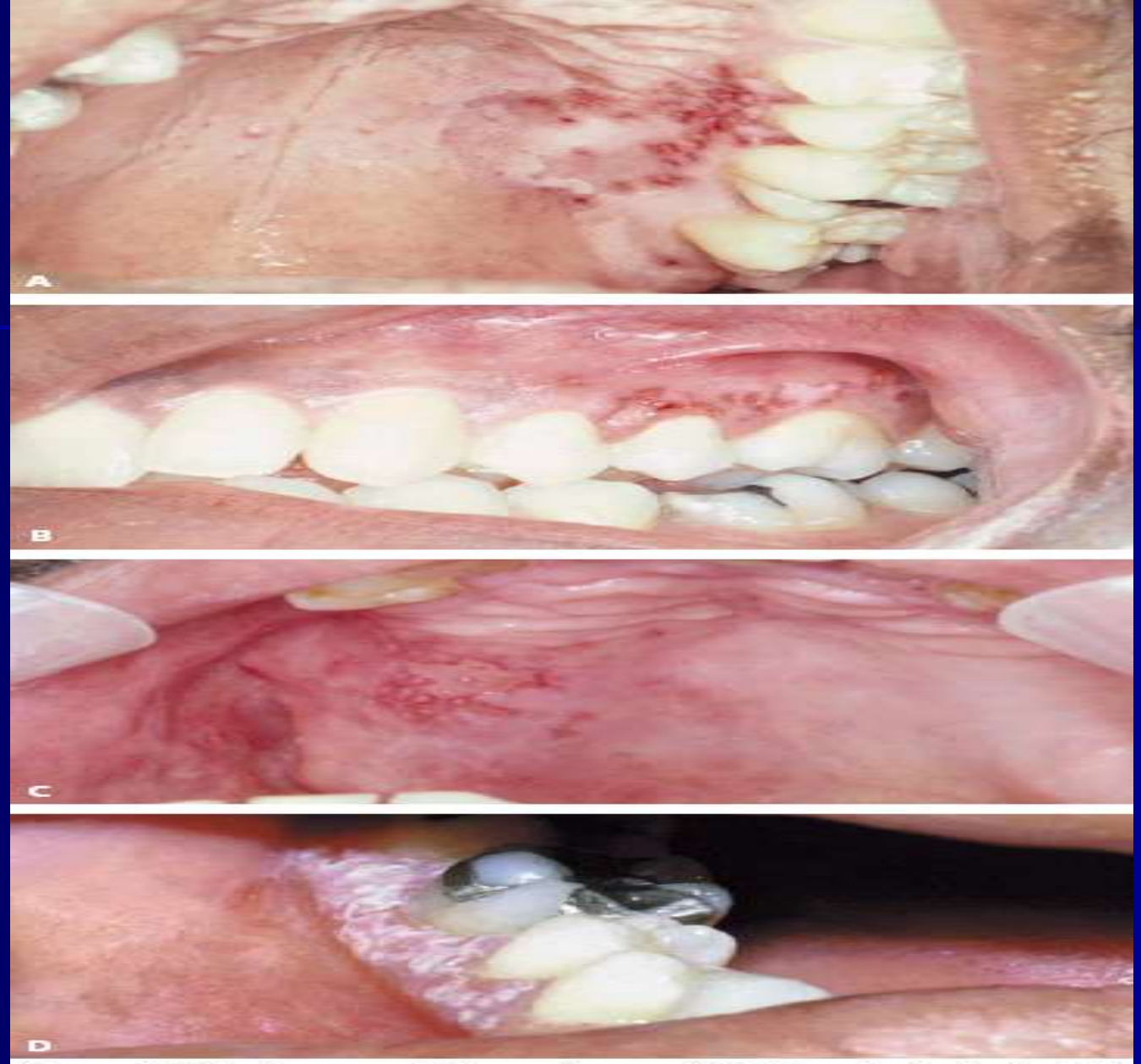


Figure 13–10 Recurrent intraoral herpes infections. *A,* Typical palatal herpes that would occur periodically, last less than 10 days, and were not associated with any specific event. *B,* Classic gingival herpes. These are often mistaken for a response to trauma. *C,* This patient would have occasional outbreaks under a denture. Note how the small herpetic ulcers tend to coelesce. Healing usually occurs within 2 weeks without any treatment. *D,* Long buccal distribution of human herpes virus type 1.

Figure 13–6 Characteristic herpetic whitlow contracted by contact between this patient's finger cuticle and herpes simplex virus type 1-infected saliva.

Varicella-zoster infection

- Primary Varicella zoster**(chicken pox)**

It’s an acute infectious viral disease the incubation period last for 2-5 weeks, appears as vesicles with an erythematous area on boundary and are extremely pruritic, fever and mild generalized lymphadenopathy are also present.

Resolved within 4-8 days.

- **Secondary varicella zoster infection**

along the peripheral nervous system commonly from dorsal root ganglia.

It starts as painful vesicular eruption on skin and also may affect oral cavity when the trigeminal ganglia is infected with varicella.

Patient gets malaise, fever and enlarged lymph node at area and runs in a course of about 2 weeks.

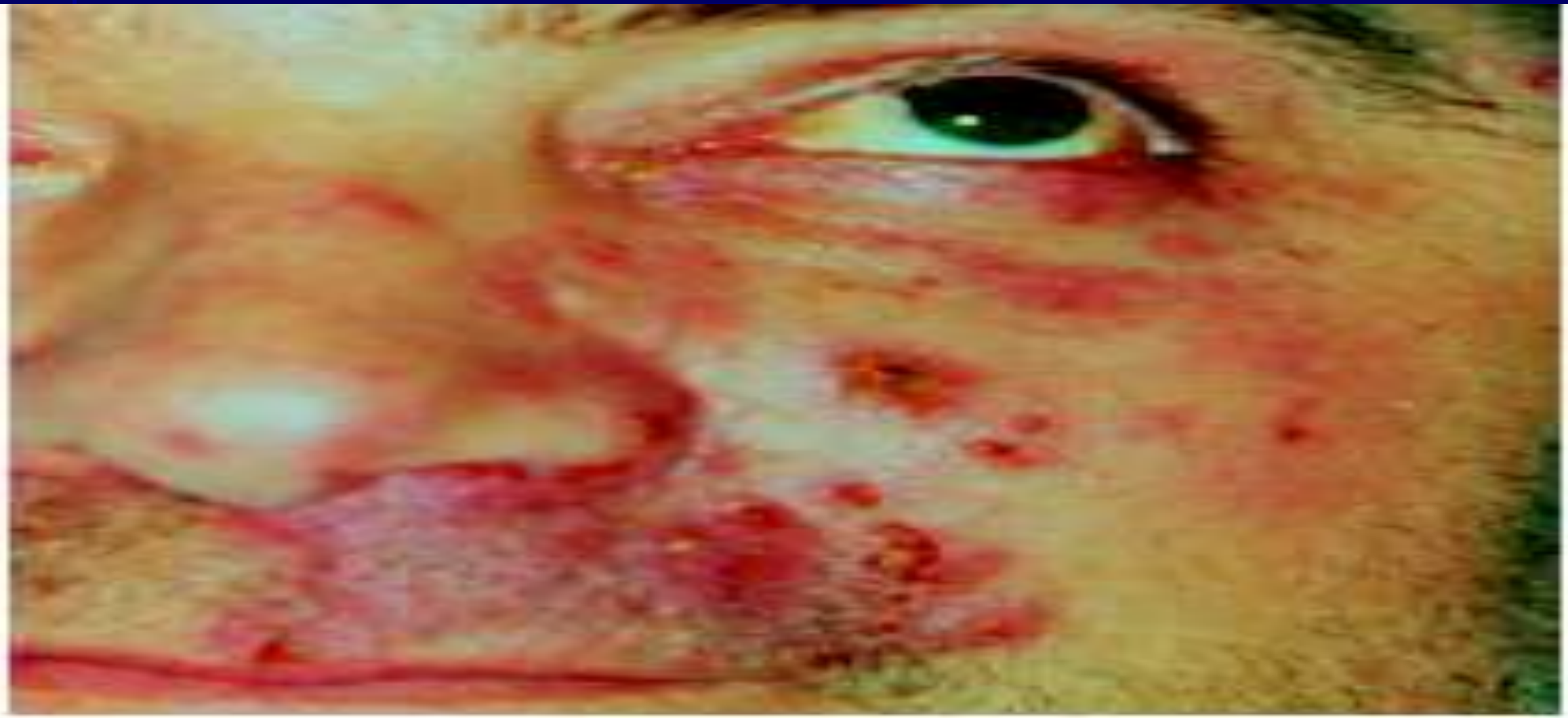


FIGURE 4-7 Facial lesions of herpes zoster involving the second division of trigeminal nerve.

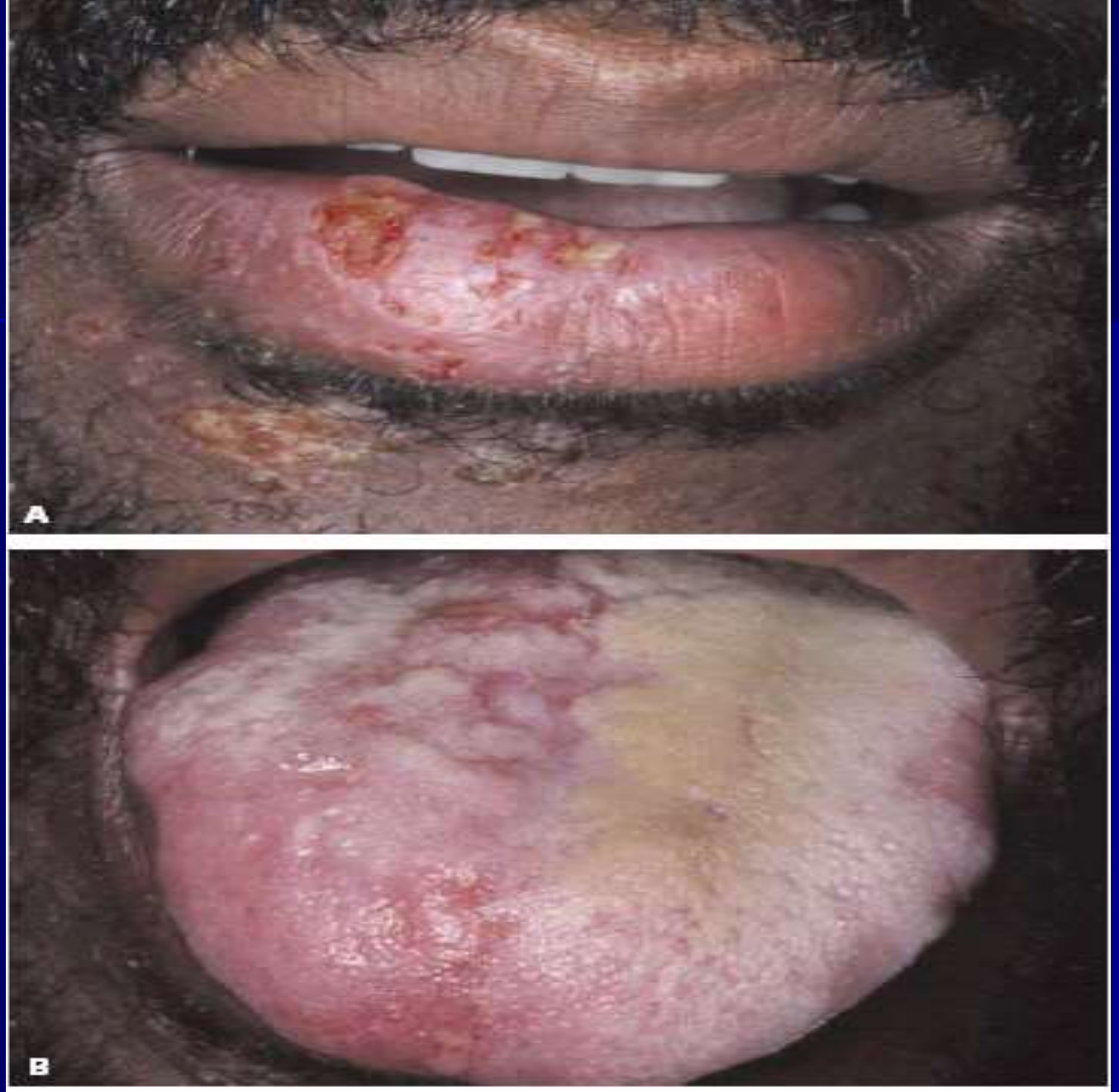


Figure 13–11 *A* and *B*, Herpes zoster (shingles) due to reactivation of the varicella (chicken pox) virus. Note the unilateral distribution of lesions.

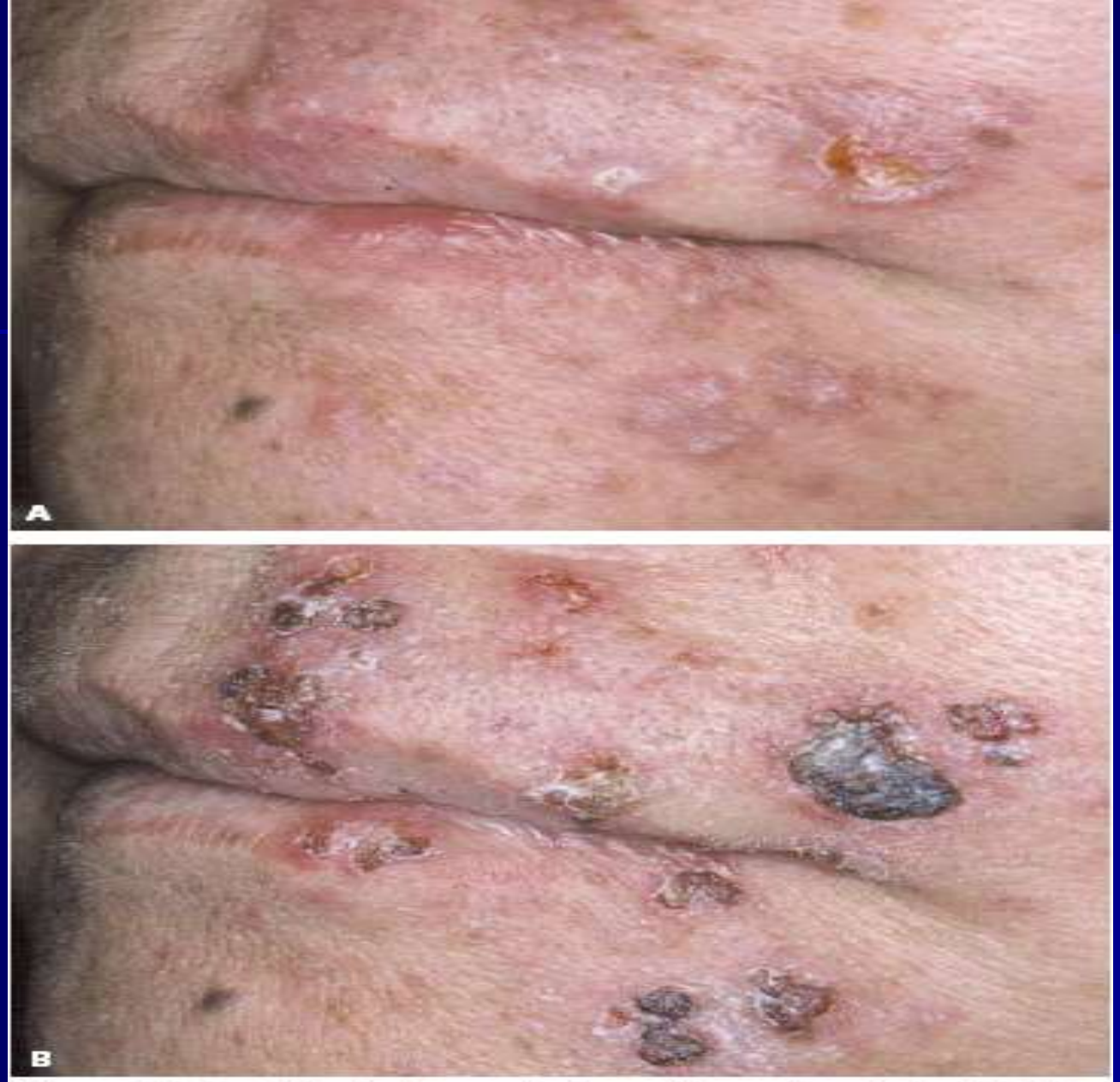


Figure 13–12 Shingles in an elderly and stressed patient. *A,* Painful unilateral ulcers present for 1 week. *B,* At day 11 the ulcers reverted from pain to mainly itching. Note the presence of scabs and healing process. Clinical healing was complete by day 20; but then vague neuropathy in that area began to bother the patient.

Herpangina

Viral disease caused by “coxsackie group A virus”

there will be fever and ill-feeling followed by vesicle rupture, lesion occurs mainly in soft palate and tonsilar area.

The disease run a course of (7-10) days.

Measles

a highly contagious viral infection caused by a member of the paramyxovirus family. Typically, oral eruptions consist of early pinpoint elevations over the soft palate that combines with ultimate involvement of the pharynx with bright erythema.

German measles share some clinical features with measles, such as fever, respiratory symptoms

Clinical Featurs:

After an incubation period of 7 to 10 days, prodromal symptoms of fever, malaise, conjunctivitis, photophobia, and cough develop.

In 1 to 2 days small erythematous macules with white necrotic centers appear in the buccal mucosa, these lesion spots, known as **Koplik's spots**. precede the skin rash by 1 to 2 days.

HUMAN IMMUNODEFICIENCY VIRUS INFECTION (HIV)

is transmitted by exchange of blood or body fluids principally through sexual contact. The injection of blood or from mother to child.

Transmission of the virus may be followed by infection which is detected by the appearance of HIV antibodies in the blood.

This generally occurs within 3 months of exposure. A few patients have an acute HIV infection at this time, the clinical features of which include pyrexia, skin rash, headache, diarrhea, sore throat, and erythema of the buccal and palatal mucosa.

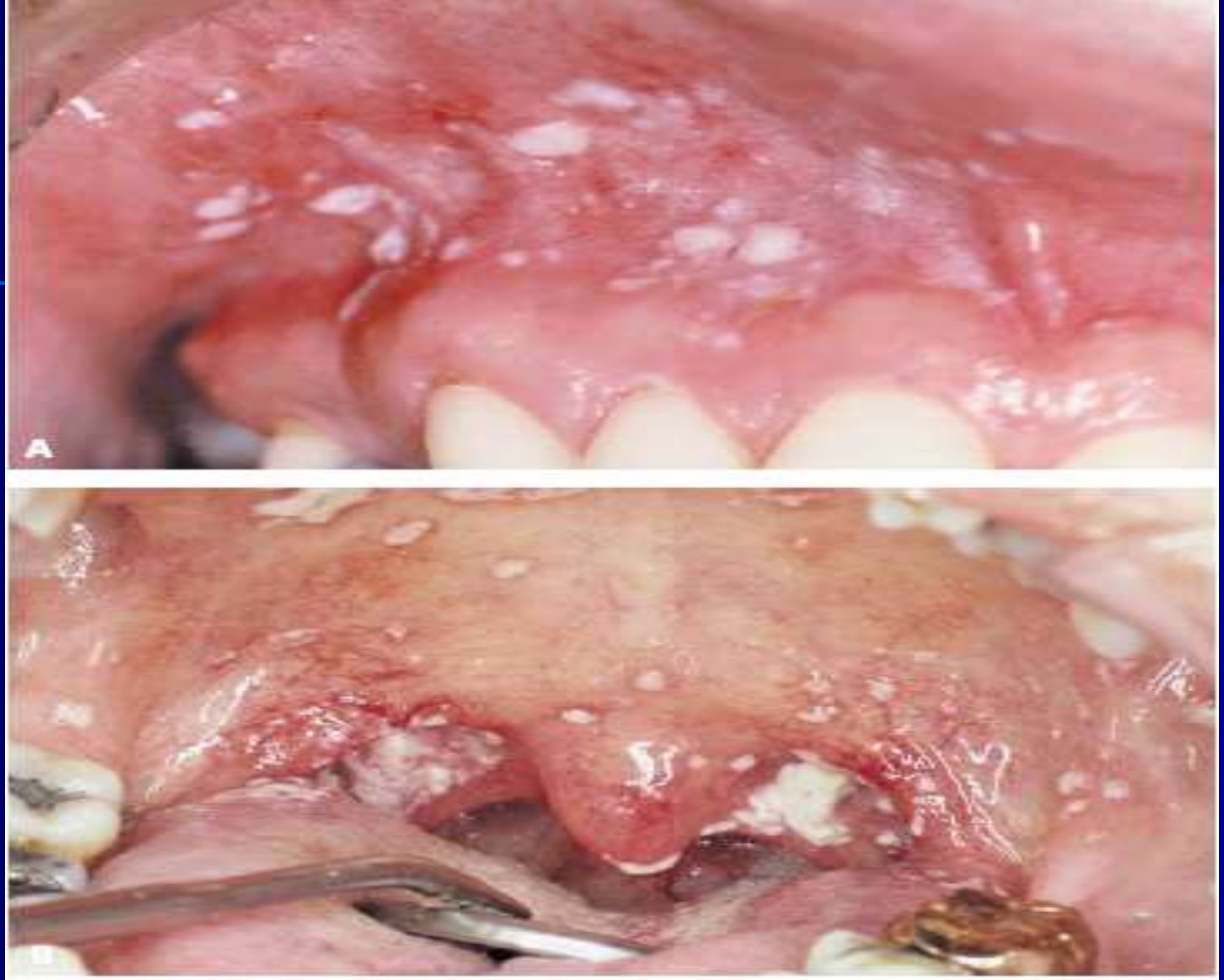


Figure 14–3 *A* and *B*, Pseudomembranous candidiasis (thrush) in HIV-positive patients.

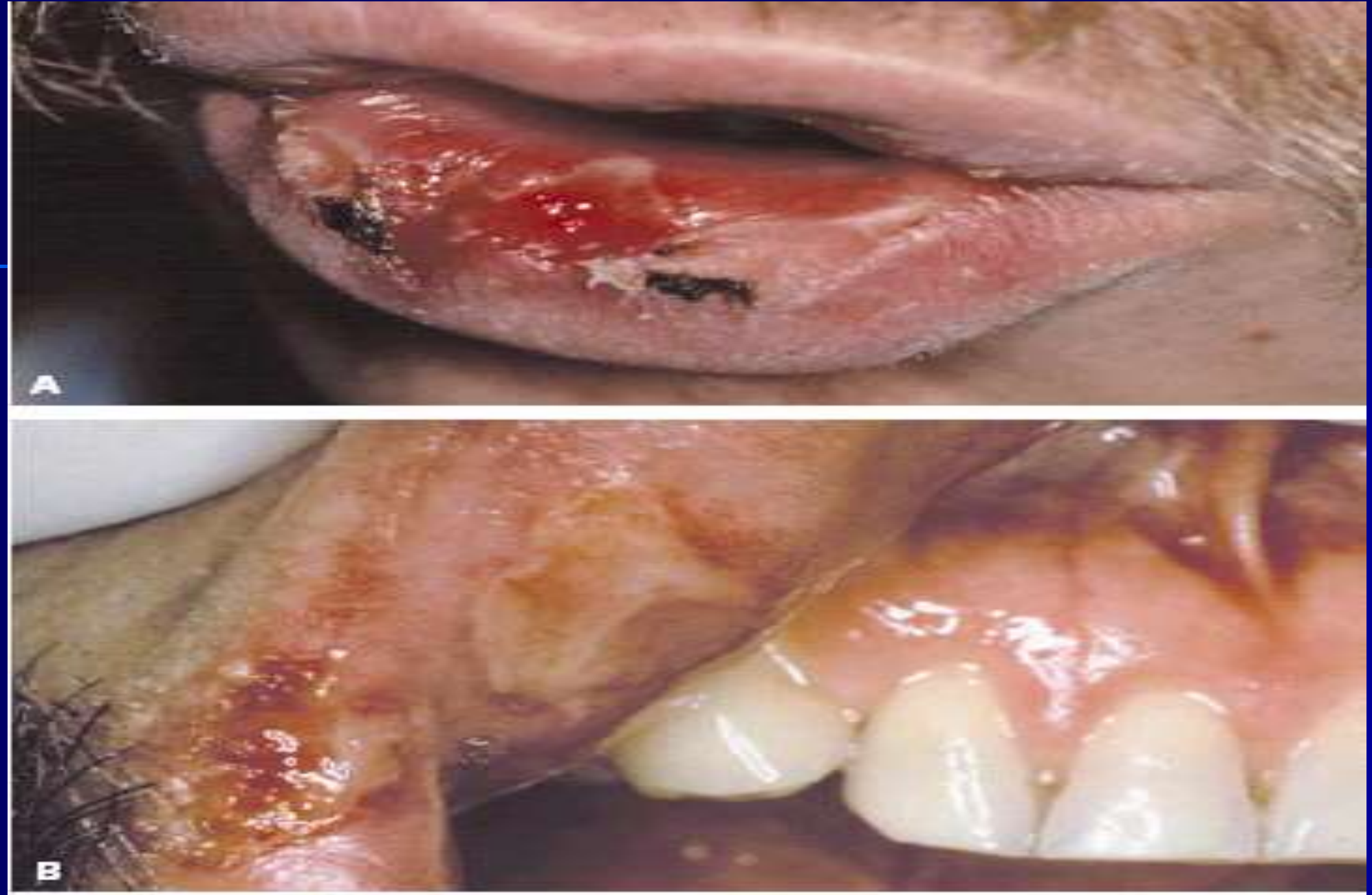


Figure 14–6 *A* and *B*, Herpes simplex-1 infections in HIV-positive patients. These painful ulcerations required antiviral drugs for control.

Kaposi sarcoma