Med.Microbiology Lect:2 2 /10/2014 Dr.Hassan A.Al-Saadi

Bacterial Physiology

cultivation of microorganisms

requirements for growth

Most of the dry weight of microorganisms is organic matter containing the elements carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. In addition, inorganic ions such as potassium, sodium, iron, magnesium, calcium, and chloride are required to facilitate enzymatic catalysis and to maintain chemical gradients across the cell membrane.

sources of metabolic energy

Fermentation

Fermentation is characterized by **substrate phosphorylation** , an enzymatic process in which a pyrophosphate bond is donated directly to ADP (adenosine diphosphate) by a phosphorylated metabolic intermediate

respiration

Aerobic bacteria that grow and get energy in the presence of O2.Anerobic bacteria which grow without O2. Chemical reduction of an oxidant (electron acceptor) through a specific series of electron carriers in the membrane establishes the proton motive force across the bacterial membrane.

Nutrition

Carbon Source

As already mentioned, some bacteria are able to use hotosynthetic energy to reduce carbon dioxide at the expense of water. These organisms belong to the group of **autotrophs**, creatures that do not require organic nutrients for growth. Other autotrophs are the **chemolithotrophs**, organisms that use an inorganic substrate such as hydrogen or thiosulfate as a reductant and carbon dioxide as a carbon source. **Heterotrophs** require organic carbon for growth, and the organic carbon must be in a form that can be assimilated.

Nitrogen Source

Nitrogen is a major component of proteins, nucleic acids, and other compounds, accounting for approximately 5% of the dry weight of a typical bacterial cell. Inorganic dinitrogen (N2) is very prevalent, comprising 80% of the earth's atmosphere. It is also a very stable compound, primarily because of the high activation energy required to break the nitrogen–nitrogen triple bond.

Sulfur Source

Similar to nitrogen, sulfur is a component of many organic cell substances. It forms part of the structure of several coenzymes and is found in the cysteinyl and methionyl side chains of proteins.

Phosphorus Source

Phosphate (PO_4^{3-}) is required as a component of ATP; nucleic acids; and such coenzymes as NAD, NADP, and flavins. In addition, many metabolites, lipids (phospholipids, lipid A), cell wall components (teichoic acid), some capsular polysaccharides, and some proteins are phosphorylated. Phosphate is always assimilated as free inorganic phosphate (Pi).

Mineral Sources

Numerous minerals are required for enzyme function. Magnesium ion (Mg^{2+}) and ferrous ion (Fe^{2+}) are also found in porphyrin derivatives: magnesium in the chlorophyll molecule, and iron as part of the coenzymes of the cytochromes and peroxidases. Mg^{2+} and K^+ are both essential for the function and integrity of ribosomes. Ca^{2+} is required as a constituent of gram-positive cell walls, although it is dispensable for gram-negative bacteria. Many marine organisms require Na^+ for growth.

Environmental factors affecting growth

Nutrients

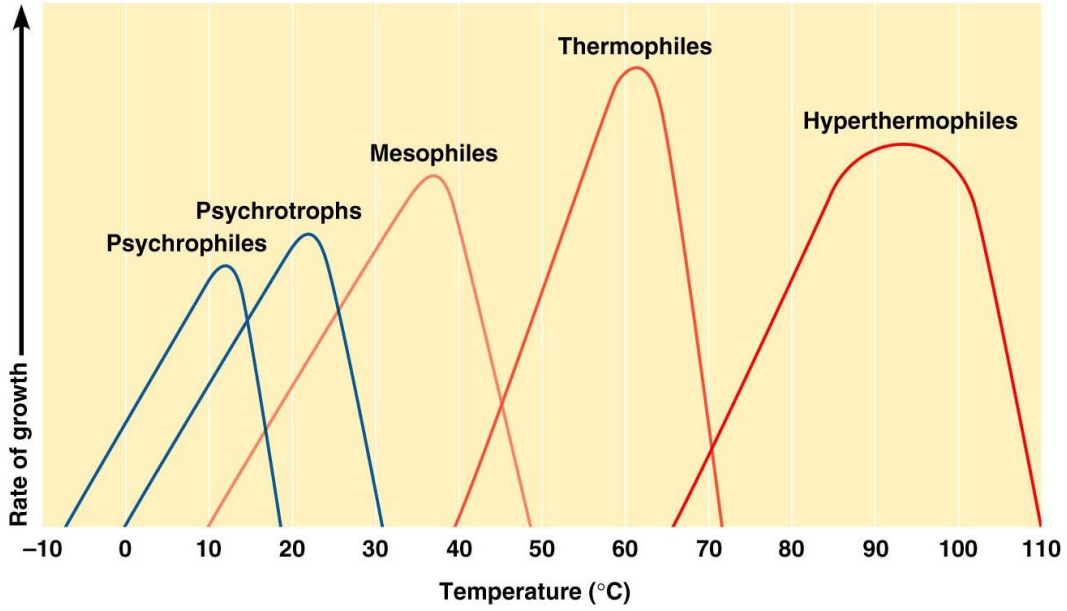
The function of each type of nutrient is described, and a list of suitable substances presented. (1) hydrogen donors and acceptors, about 2 g/L; (2) carbon source, about 1 g/L; (3) nitrogen source, about 1 g/L; (4) minerals: sulfur and phosphorus, about 50 mg/L of each, and trace elements, 0.1–1 mg/L of each; (5) growth factors: amino acids, purines, and pyrimidines, about 50 mg/L of each, and vitamins, 0.1–1 mg/L of each.

Hydrogen Ion Concentration (pH)

Most organisms have a fairly narrow optimal pH range. The optimal pH must be empirically determined for each species. Most organisms **(neutralophiles)** grow best at a pH of 6.0– 8.0, although some forms **(acidophiles)** have optima as low as pH 3.0, and others **(alkaliphiles)** have optima as high as pH 10.5.

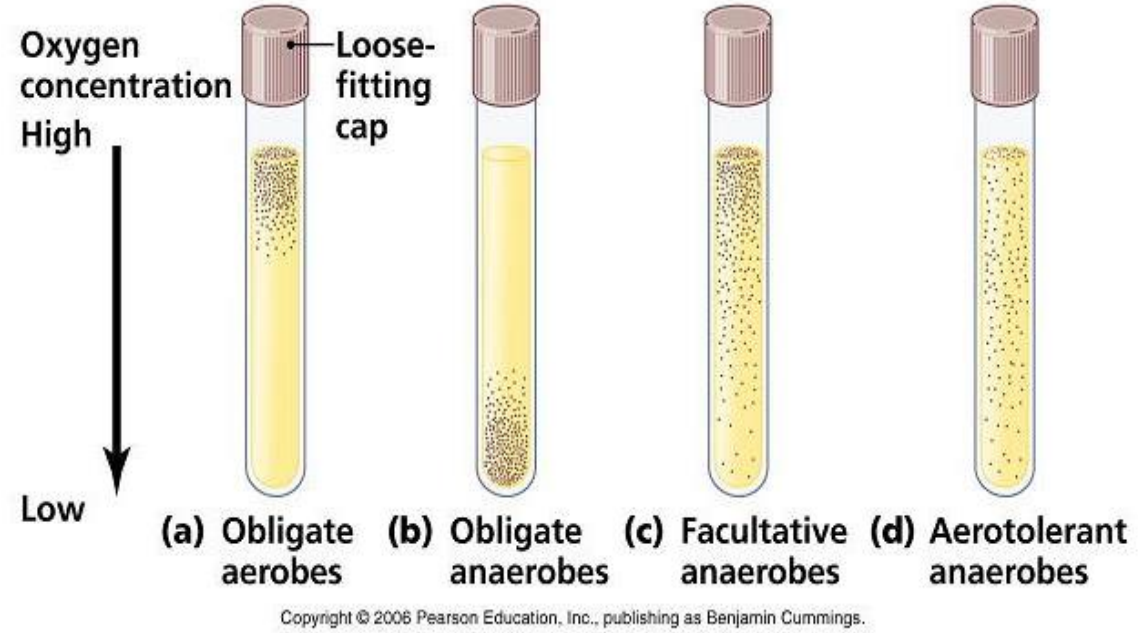
Temperature

Different microbial species vary widely in their optimal temperature ranges for growth (Figure 5-2): **Psychrophilic** forms grow best at low temperatures (-5–15°C) and are usually found in such environments as the Arctic and Antarctic regions; **psychrotrophs** have a temperature optimum between 20°C and 30°C but grow well at lower temperatures. They are an important cause of food spoilage. **Mesophilic** forms grow best at 30–37°C, and most **thermophilic** forms grow best at 50–60°C. Some organisms are **hyperthermophilic** and can grow at well above the temperature of boiling water, which exists under high pressure in the depths of the ocean. Most organisms are mesophilic; 30°C is optimal for many free-living forms, and the body temperature of the host is optimal for symbionts of warm-blooded animals.



Aeration

The role of oxygen as hydrogen acceptor is discussed in Chapter 6. Many organisms are **obligate aerobes *Pseudomonas spp***, **anaerobes**, able to live aerobically or anaerobically; some are **obligate anaerobes *Clostridium spp*** requiring a substance other than oxygen as hydrogen acceptor and are sensitive to oxygen inhibition; some are **microaerophiles,** which require small amounts of oxygen (2%–10%) for aerobic respiration (higher concentrations are inhibitory); and others are **aerotolerant anaerobes,** which are indifferent to oxygen.



Ionic Strength and Osmotic Pressure

To a lesser extent, such factors as osmotic pressure and salt concentration may have to be controlled. For example, these factors must be considered. Organisms requiring high salt concentrations are called **halophilic**; those requiring high osmotic pressures are called **osmophilic**.

Physical and Chemical Growth determinates

Physical Agents

Heat

Application of heat is the simplest means of sterilizing materials, provided the material is itself resistant to heat damage. A temperature of 100 °C will kill all but spore forms of bacteria within 2–3 minutes in laboratory-scale cultures; a temperature of 121 °C for 15 minutes is utilized to kill spores. Steam is generally used, both because bacteria are more quickly killed when moist and because steam provides a means for distributing heat to all parts of the sterilizing vessel. At sea level, steam must be kept at a pressure of 15 lb/sq in (psi) in excess of atmospheric pressure to obtain a temperature of 121 °C; autoclaves or pressure cookers are used for this purpose. At higher altitudes, the pressure would need to be higher than 15 psi to reach 121 °C.

Radiation

Ultraviolet light and ionizing radiations have various applications as sterilizing agents.

Chemical Agents

The chemical structures and uses of biocides are shown in Table 4–3.

Alcohols

Ethyl alcohol, isopropyl alcohol, and *n*-propanol exhibit rapid, broad-spectrum antimicrobial activity against vegetative bacteria, viruses, and fungi but are not sporicidal. Activity is optimal when they are diluted to a concentration of 60–90% with water.

Aldehydes

Glutaraldehyde is used for low-temperature disinfection and sterilization of endoscopes and surgical equipment. It is normally used as a 2% solution to achieve sporicidal activity. Formaldehyde is bactericidal, sporicidal, and virucidal.

Biguanides

Chlorhexidine is widely used in handwashing and oral products and as a disinfectant and preservative. Mycobacteria are generally highly resistant.

Bisphenols

The bisphenols are widely used in antiseptic soaps and hand rinses. In general, they are broad-spectrum but have little activity against *Pseudomonas aeruginosa* and molds. Triclosan and hexachlorophene are bactericidal and sporostatic.

Halogen-Releasing Agents

The most important types of chlorine-releasing agents are sodium hypochlorite, chlorine dioxide, and sodium dichloroisocyanurate, which are oxidizing agents that destroy the cellular activity of proteins. Hypochlorous acid is the active compound responsible for the bactericidal and virucidal effect of these compounds. At higher concentrations, these compounds are sporicidal. Iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal. Iodophors (eg, povidone-iodine) are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active I_2.

Heavy Metal Derivatives

Silver sulfadiazine, a combination of two antibacterial agents, Ag^+ and sulfadiazine, has a broad spectrum of activity. Binding to cell components such as DNA may be responsible for its inhibitory properties.

Organic Acids

Organic acids are used as preservatives in the pharmaceutical and food industries. Benzoic acid is fungistatic; propionic acid is both bacteriostatic and fungistatic.

Peroxygens

Hydrogen peroxide has broad-spectrum activity against viruses, bacteria, yeasts, and bacterial spores. Sporicidal activity requires higher concentrations (10–30%) of H_2O_2 and longer contact times.

Phenols

Phenol and many phenolic compounds have antiseptic, disinfectant, or preservative properties.

Quaternary Ammonium Compounds

These compounds are useful antiseptics and disinfectants. QACs have been used for a variety of clinical purposes (eg, preoperative disinfection of unbroken skin) as well as for cleaning hard surfaces. They are sporostatic; they inhibit the outgrowth of spores but not the actual germination process. QACs are also mycobacteriostatic and have an effect on lipid-enveloped but not lipid-nonenveloped viruses.

Vapor-Phase Sterilants

Heat-sensitive medical devices and surgical supplies can be effectively sterilized by vapor-phase systems employing ethylene oxide, formaldehyde, hydrogen peroxide, or peracetic acid.

Definitions

The following terms are commonly employed in connection with antimicrobial agents and their uses.

Biocide

A general term describing a chemical agent, usually broad-spectrum, that inactivates microorganisms (Table 4–3). Biocides can be antiseptics, disinfectants, or preservatives. The activity of biocides against microorganisms depends on: 1) the external physical environment; 2) the nature, structure, composition, and condition of the microorganism itself; and 3) the ability of the microorganism to degrade or inactivate the biocide

Bacteriostatic

A specific term referring to the property by which a biocide is able to inhibit bacterial multiplication; multiplication resumes upon removal of the agent. (The terms "fungistatic" and "sporostatic" refer to biocides that inhibit the growth of fungi and spores, respectively.)

Bactericidal

A specific term referring to the property by which a biocide is able to kill bacteria. Bactericidal action differs from bacteriostasis only in being irreversible; ie, the "killed" organism can no longer reproduce, even after being removed from contact with the agent. In some cases, the agent causes lysis (dissolution) of the cells; in other cases, the cells remain intact and may even continue to be metabolically active. (The terms "fungicidal," "sporicidal," and "virucidal" refer to the property whereby biocides are able to kill fungi, spores, and viruses, respectively.)

Sterilization

A physical or chemical process that completely destroys or removes all microbial life, including spores.

Disinfectants

Products or biocides used to kill microorganisms on inanimate objects or surfaces. Disinfectants are not necessarily sporicidal, but are sporostatic, inhibiting germination or outgrowth.

Septic

Characterized by the presence of pathogenic microbes in living tissue.

Antiseptic

A biocide or product that destroys or inhibits the growth of microorganisms in or on living tissue.

Aseptic

Characterized by the absence of pathogenic microbes.

Preservation

The prevention of multiplication of microorganisms in formulated products, including pharmaceuticals and foods.

Antibiotics

Naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentrations.

Growth and Growth curve

The Lag Phase

The lag phase represents a period during which the cells, depleted of metabolites and enzymes as the result of the unfavorable conditions that existed at the end of their previous culture history, adapt to their new environment. Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume.

If the cells are taken from an entirely different medium, it often happens that they are genetically incapable of growth in the new medium. In such cases a long lag may occur, representing the period necessary for a few mutants in the inoculum to multiply sufficiently for a net increase in cell number to be apparent.

The Exponential Phase

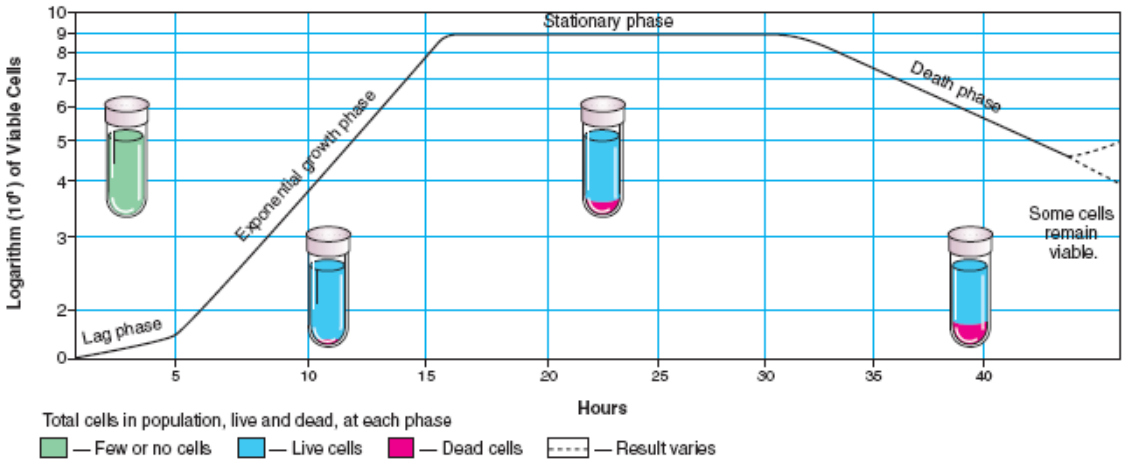
During the exponential phase, the mathematics of which has already been discussed, the cells are in a steady state. New cell material is being synthesized at a constant rate, but the new material is itself catalytic, and the mass increases in an exponential manner. This continues until one of two things happens: either one or more nutrients in the medium become exhausted, or toxic metabolic products accumulate and inhibit growth. For aerobic organisms, the nutrient that becomes limiting is usually oxygen. When the cell concentration exceeds about 1×10^7/mL (in the case of bacteria), the growth rate will decrease unless oxygen is forced into the medium by agitation or by bubbling in air. When the bacterial concentration reaches $4–5 \times 10^9$/mL, the rate of oxygen diffusion cannot meet the demand even in an aerated medium, and growth is progressively slowed.

The Maximum Stationary Phase

Eventually, the exhaustion of nutrients or the accumulation of toxic products causes growth to cease completely. In most cases, however, cell turnover takes place in the stationary phase: There is a slow loss of cells through death, which is just balanced by the formation of new cells through growth and division. Sporulation, exotoxins production. When this occurs, the total cell count slowly increases although the viable count stays constant.

The Phase of Decline: The Death Phase

After a period of time in the stationary phase, which varies with the organism and with the culture conditions, the death rate increases until it reaches a steady level. The mathematics of steady-state death is discussed below. In most cases the rate of cell death is much slower than that of exponential growth. Frequently, after the majority of cells have died, the death rate decreases drastically, so that a small number of survivors may persist for months or even years. This persistence may in some cases reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die and lyse.



Antimicrobial drugs

Mechanisms of Action of Antimicrobial Drugs

Selective Toxicity

An ideal antimicrobial agent exhibits selective toxicity, which means that the drug is harmful to a pathogen without being harmful to the host. Often,

selective toxicity is relative rather than absolute; this implies that a drug in a concentration tolerated by the host may damage an infecting microorganism.

Selective toxicity may be a function of a specific receptor required for drug attachment, or it may depend on the inhibition of biochemical events essential to the pathogen but not to the host. The mechanisms of action of antimicrobial drugs can be discussed under four headings:

(1) Inhibition of cell wall synthesis.
(2) Inhibition of cell membrane function.
(3) Inhibition of protein synthesis (ie, inhibition of translation and transcription of genetic material).
(4) Inhibition of nucleic acid synthesis.

Resistance to Antimicrobial Drugs

There are many different mechanisms by which microorganisms might exhibit resistance to drugs.

(1) Microorganisms produce enzymes that destroy the active drug. ***Examples:*** Staphylococci resistant to penicillin G produce a -lactamase that destroys the drug. Other -lactamases are produced by gram-negative rods. Gram-negative bacteria resistant to aminoglycosides .

(2) Microorganisms change their permeability to the drug. ***Examples:*** Tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Resistance to polymyxins is also associated with a change in permeability to the drugs. Streptococci have a natural permeability barrier to aminoglycosides.

(3) Microorganisms develop an altered structural target for the drug.

(4) Microorganisms develop an altered metabolic pathway that bypasses the reaction inhibited by the drug.

(5) Microorganisms develop an altered enzyme that can still perform its metabolic function but is much less affected by the drug.

Antibiotics and their actions:

(1) Inhibition of cell wall synthesis.Cephalosporins,Vancomycin,and Cycoserine.

(2)Inhibition of Cell Membrane Function. nalidixic acid**,** novobiocin

(**3)Inhibition of Protein Synthesis**. erythromycins, lincomycins, tetracyclines, aminoglycosides, and chloramphenicol.

(4)Inhibition of Nucleic Acid Synthesis

Examples of drugs acting by inhibition of nucleic acid synthesis are the quinolones, pyrimethamine, rifampin, sulfonamides, trimethoprim, and trimetrexate.

. Sulfonamides and trimethoprim each can be used alone to inhibit bacterial growth. If used together, they produce sequential blocking, resulting in a marked enhancement (synergism) of activity. Such mixtures of sulfonamide (five parts) plus trimethoprim (one part) have been used in the treatment of pneumocystis pneumonia, malaria, shigella enteritis, systemic salmonella infections, urinary tract infections, and many others.

Antimicrobial Activity In Vivo

Analysis of the activity of antimicrobial agents in vivo is much more complex than the circumstances in vitro. The activity involves not only the drug and parasite but also a third factor, the host.

Drug-Pathogen Relationships

Several important interactions between drug and pathogen have been discussed in the preceding pages. The following are additional important in vivo factors.

Environment

In the host, varying environmental influences affect microorganisms located in different tissues and in different parts of the body—in contrast to the test tube or Petri dish, where the environment is constant for all members of a microbial population. Therefore, the response of the microbial population is much less uniform within the host than in the test tube.

State of Metabolic Activity

In the body, the state of metabolic activity is diverse—undoubtedly, many organisms exist at a low level of biosynthetic activity and are thus relatively insusceptible to drug action. These "dormant" microorganisms often survive exposure to high concentrations of drugs and subsequently may produce a clinical relapse of the infection.

Distribution of Drug

In the body, the antimicrobial agent is distributed in tissues and fluids. Many drugs do not reach the central nervous system effectively. The concentration in urine is often much greater than the concentration in blood or other tissue. The tissue response induced by the microorganism may protect it from the drug.

Location of Organisms

In the body, microorganisms often are located within tissue cells. Drugs enter tissue cells at different rates. Some (eg, tetracyclines) reach about the same concentration inside monocytes as in the extracellular fluid.

Interfering Substances

The biochemical environment of microorganisms in the body is very complex and results in significant interference with drug action. The drug may be bound by blood and tissue proteins or phospholipids; it may also react with nucleic acids in pus and may be physically adsorbed onto exudates, cells, and necrotic debris. In necrotic tissue, the pH may be highly acid and thus unfavorable for drug action (eg, aminoglycosides).

Concentration

In the body, microorganisms are not exposed to a constant concentration of drug; in the test tube they are.

Absorption

The absorption of drugs from the intestinal tract (if taken by mouth) or from tissues (if injected) is irregular. There is also a continuous excretion as well as inactivation of the drug. Consequently, the levels of drug in body

compartments fluctuate continually, and the microorganisms are exposed to varying concentrations of the antimicrobial agent.

Distribution

The distribution of drugs varies greatly with different tissues. Some drugs penetrate certain tissues poorly (eg, central nervous system, prostate). Drug concentrations following systemic administration may therefore be inadequate for effective treatment.

Variability of Concentration

It is critical to maintain an effective concentration of a drug where the infecting microorganisms proliferate. This concentration must be maintained for a sufficient length of time to eradicate the microorganisms. Because the drug is administered and is absorbed and excreted irregularly, the levels constantly alteration at the site of infection. In order to maintain sufficient drug concentrations for a sufficient time, the time-dose relationship must be considered. The larger each individual drug dose, the longer the permitted interval between doses. The smaller the individual dose, the shorter the interval that will ensure adequate drug levels.

Postantibiotic Effect

The postantibiotic effect is the delayed regrowth of bacteria after exposure to antimicrobial agents. It is a property of most antimicrobials, except that most -lactams do not show the postantibiotic effect with gram-negative bacilli. The carbapenems do have a postantibiotic effect with the gram-negative bacilli.

Host-Pathogen Relationships

Host-pathogen relationships may be altered by antimicrobial drugs in several ways.

Alteration of Tissue Response

The inflammatory response of the tissue to infections may be altered if the drug suppresses the multiplication of microorganisms but does not eliminate them from the body. An acute process may in this way be transformed into a chronic one. Conversely, the suppression of inflammatory reactions in

tissues by impairment of cell-mediated immunity in recipients of tissue transplants or antineoplastic therapy or by immunocompromise as a result of disease (eg, AIDS)

Alteration of Immune Response

If an infection is modified by an antimicrobial drug, the immune response of the host may also be altered. One example illustrates this phenomenon: Pharyngeal infection with -hemolytic group A streptococci is followed frequently by the development of antistreptococcal antibodies, and if there is a hyperimmune response the infection may be followed by rheumatic fever. If the infective process can be interrupted early and completely with antimicrobial drugs, the development of an immune response and of rheumatic fever can be prevented (presumably by rapid elimination of the antigen). Drugs and dosages that rapidly eradicate the infecting streptococci (eg, penicillin) are more effective in preventing rheumatic fever than those which merely suppress the microorganisms temporarily (eg, tetracycline).

Alteration of Microbial Flora

Antimicrobial drugs affect not only the microorganisms causing disease but also susceptible members of the normal microbial flora. An imbalance is thus created that in itself may lead to disease. A few examples are of interest.

(1) In hospitalized patients who receive antimicrobials, the normal microbial flora is suppressed. This creates a partial void that is filled by the organisms most prevalent in the environment, particularly drug-resistant gram-negative aerobic bacteria (eg, pseudomonads, staphylococci). Such superinfecting organisms subsequently may produce serious drug-resistant infections.

(2) In women taking antibiotics by mouth, the normal vaginal flora may be suppressed, permitting marked overgrowth of candida. This leads to unpleasant local inflammation (vulvovaginitis) and itching that are difficult to control.

(3) In the presence of urinary tract obstruction, the tendency to bladder infection is great. When such urinary tract infection due to a sensitive microorganism (eg, *Escherichia coli*) is treated with an appropriate drug, the organism may be eradicated.

(4) In persons receiving antimicrobial drugs for several days, parts of the normal intestinal flora may be suppressed. Drug-resistant organisms may establish themselves in the bowel in great numbers and may precipitate serious enterocolitis (*Clostridium difficile,* etc).

First-Generation Cephalosporins

First-generation cephalosporins are very active against gram-positive cocci—except enterococci and nafcillin-resistant staphylococci—and moderately active against some gram-negative rods—primarily *E coli*, proteus, and klebsiella. Anaerobic cocci are often sensitive, but *Bacteroides fragilis* is not.

Cephalexin, cephradine, and cefadroxil are absorbed from the gut to a variable extent and can be used to treat urinary and respiratory tract infections.

Second-Generation Cephalosporins

The second-generation cephalosporins are a heterogeneous group. All are active against organisms covered by first-generation drugs but have extended coverage against gram-negative rods—including klebsiella and proteus but not *P aeruginosa.*

Third-Generation Cephalosporins

Third-generation cephalosporins have decreased activity against gram-positive cocci, except for *S pneumoniae*; enterococci are intrinsically resistant to cephalosporins and often produce superinfections during their use.

Fourth-Generation Cephalosporins

Cefepime is the only fourth-generation cephalosporin now in clinical use in the United States. It has enhanced activity against *Enterobacter* and *Citrobacter* species that are resistant to third-generation cephalosporins.