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MICROBIOLOGY

[201]: BASICS OF BIOCHEMISTRY AND MICROBIAL CONTROL

UNIT 5
ANTIBIOTICS AND THEIR MODE OF ACTION

PREPARED BY

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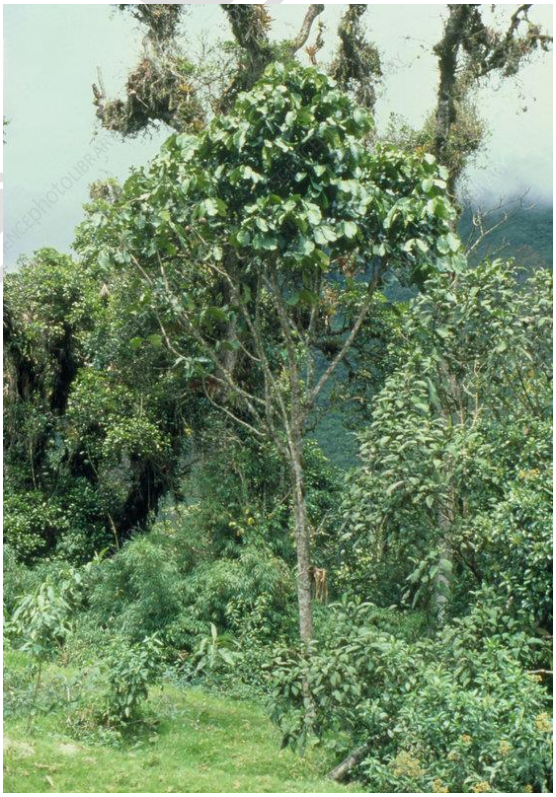
CHEMOTHERAPEUTIC AGENTS AND CHEMOTHERAPY

- The treatment of a disease with a chemical substance is known as chemotherapy and the chemical substance used is called a chemotherapeutic agent.
- Some of these chemicals are synthesized in laboratories and are called synthetic therapeutics. (e.g., Sulfonamides)
- While others are produced by microorganisms and are known as a natural chemotherapeutics. (Commonly known as antibiotics)
- Some antibiotics may be prepared synthetically, but most of them are prepared by microbial synthesis.
- Antitoxins and other substances which are produced from bodies of infected animals, and chemical agents used for killing of inhibiting microbial growth in vitro are not considered as chemotherapeutic agents.
- Natural quinine from bark of Cinchona tree to treat malaria was used as early as in 1630 by Europeans.
- South American Indians used bark of Cinchona tree for relieving symptoms of malarial fever even before.



- Systematic chemotherapy started in 1910, after search of antimicrobial compound called **Salvarsan** (arsenal compound) by **Paul Ehrlich**. This compound was effectively used in treating Syphilis without causing any harm to the patient.
- This was first systematic search for a compound having potent microbial properties, good chemical stability and low toxicity for humans and animals.
- He was awarded Nobel prize for it in 1908 with Elie Metchnikoff. Although now Ehrlich's compound for treatment of Syphilis has been replaced by other arsenical compound and antibiotics.
- **Domagk & Discovery of Sulfonamides:** In 1935, Domagk showed therapeutic value of sulfonamides. These substances are effective against large variety of pathogenic organisms. This compound was known as Prontosil.
- The sulfonamides are useful in treatment of infections caused by *Meningococci* and *Shigella*, *Streptococci* and *Staphylococci* (respiratory infection), gram negative organisms causing urinary tract infections.
- **Discovery of Penicillin:** Antibiotic is a metabolic product of one organism, which in very small amount is detrimental or harmful to other microorganisms.
- Vuillemin in 1989 define antibiosis condition in which one creature destroys the life of other organism to protect itself.
- Antibiotic had been known for their activities since long before, the term was given to them. Moldy soyabean was used for treatment of boils by Chinese. They controlled the foot infections by wearing sandals furry with mold.
- In 1929, Sir Alexander Fleming noticed agar plate inoculated with *Staphylococcus aureus*, which was contaminated with mold. The mold colony showed a clear zone around it indicating, inhibition of bacterial growth or lysis of bacterial cells.
- He isolated and identified the mold, the inhibitory substance present in contaminant mold became a wonder drug for treatment of infection of war wound and preventing death of the soldiers.
- As the mold was identified *Penicillium* species, Fleming called the antibiotic Penicillin.
- This was followed by discovery of *Bacillus brevis* which produced a substance that killed many gram-positive bacteria. This substance contains two active principles now known as gramicidin and tyrocidine.

- Thereafter was discovered Streptomycin by Waksman. Since 1940, thousands of antibiotics have been discovered. Many of them are not of any practical importance and few have changed the complete concept of chemotherapy.
- The antibiotics are popular because they are capable of destroying many kinds of pathogens, and are non-toxic to the host.

CHARACTYERISTICS OF IDEAL CHEMOTHERAPEUIC AGENT

- Chemotherapeutic agent must have selective toxicity for parasite, i.e., should not be or less toxic to host cells and highly toxic to the parasites. Substance should damage the parasite, without causing any damage to host cells.
- Therefore, antiseptics and germicides like phenols and heavy metals are not satisfactory as chemotherapeutic agents.
- Germicides are inactivated by the proteins thereby their effectiveness are destroyed by body fluids which are rich in proteins.
- The body tissue or cells which are damaged or killed by germicides serve as an excellent medium for the growth of the microorganisms.
- Therefore, all chemical compounds cannot be satisfactory used as chemotherapeutic agents. To act as chemotherapeutic agent a chemical compound should fulfill certain criteria:
    - Ability to destroy o prevent activity of parasites, without causing any damage or only minor damage to the body.
    - Should be capable of penetrating the cells and tissues of host, so that it can come in contact with parasite.
    - Not alter the host's natural defense mechanism.
- **Characteristics of Antibiotics:** The antibiotics must fulfill certain qualities to useful as chemotherapeutic agent.
    - They should be of broad spectrum, able to destroy or inhibit many different species of pathogenic microorganisms.
    - They should prevent the development of resistant form of pathogens.
    - They should not produce undesirable side effects in host e.g., sensitivity, allergic reaction, damage to nerve, irritation to body systems.
    - They should not eliminate the normal microbial flora of host.

ANTIBIOTICS AND THEIR MODE OF ACTION

Antibiotics can be classified in different ways:

- **MECHANISM OF ANTIMICROBIAL ACTION:**
  1. Their effect on microorganisms Bacteriostatic or Bactericidal
  2. On the basis of their chemical structure
  3. On the basis of their mode of action i.e., how they damage microorganisms
- **ANTIBIOTICS MAY ALSO BE CLASSIFIED AS:**
  1. Affecting cell wall peptidoglycan synthesis
  2. Affecting cell membrane
  3. Inhibiting protein and DNA synthesis
  4. Inhibiting the specific enzyme system

ANTIBIOTIC AFFECTING CELL WALL SYNTHESIS:

Antibiotic that inhibits cell wall synthesis are non-toxic to human host as cell wall is absent in mammalian tissues. Peptidoglycan present in cell wall is responsible for giving rigidity to cell wall. Peptidoglycan is composed of repeating units of N-acetyl glucosamine and N-acetyl muramic acid cross linked with small peptides. Biosynthesis of peptidoglycan is a complex process, involving several numerous steps.

Interference of any of these step results in inhibition of cell wall synthesis thereby, preventing a bacterial cell from surviving due to lack of cell wall. Certain antibiotics like Penicillin, Cephalosporin, Cycloserine, Vancomycin and Bacitracin interfere in Peptidoglycan synthesis thereby inhibiting cell wall synthesis.

However, bacteria like *Mycoplasma*

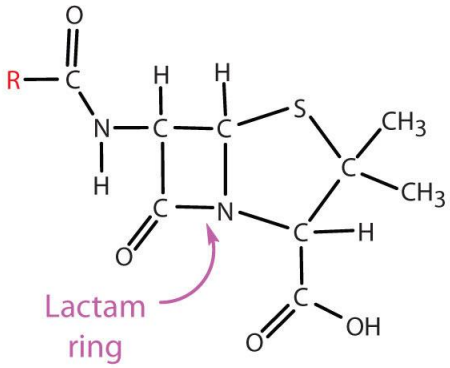
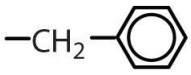
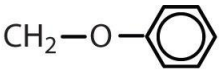
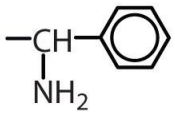
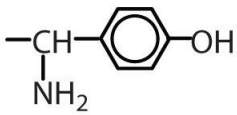
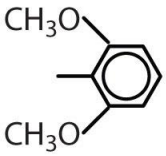
1. Which lack peptidoglycan are not affected by Penicillin.
2. Concentrations of penicillin, below which kills bacteria results in accumulations of precursors of peptidoglycan synthesis.

3. Bacterial cells susceptible to penicillin can be protected from destruction by exposing them in a medium of high osmotic pressure. It will prevent cell from bursting. Due to lack of peptidoglycan cell with become spherical. Cells without cell wall are called spheroplast.

PENICILLIN:

Produced by Penicillium notatum was discovered by Alexander Fleming in 1929. It is selective for gram positive bacteria, some spirochetes and gram-negative Diplococci. Generally, it is not toxic to human patients, but in some cases may give rise to sensitivity reactions rqanging from mild skin reaction to severe anaphylaxis.

Penicillin and Semi-synthetic penicillin are class of β-lactam antibiotics. Structure is characterized by containing 4 membered ring of beta-lactam ring composed of three Carbon atoms and one Nitrogen atom.

Penicillin Structure	R Group	Drug Name
		penicillin G
		penicillin V
		ampicillin
		amoxicillin
		methicillin

One of the first semisynthetic penicillins to be produced for clinical use was phenethicillin. It is more readily absorbed than penicillin V and just as effective as penicillin C. Another of the semisynthetic penicillins, methicillin, is more resistant to penicillinase and therefore is less likely to be inactivated.

AMPICILLIN:

Another semisynthetic penicillin, acts against a broad spectrum of bacteria. It is strongly bactericidal and lacks toxicity, but it is not resistant to penicillinases. It is relatively stable to gastric acid and hence can be administered orally.

Mode of Action: Penicillins interfere with the final stages of peptidoglycan biosynthesis. The penicillins inhibit the transpeptidase reaction, namely, the cross-linking of the two linear polymers. The penicillins are bactericidal to growing cells.

CEPHALOSPORINS:

Cephalosporins are a group of antibiotics produced by a species of marine fungus, *Cephoiosporium acremonium*, which bears considerable resemblance to *Penicillium spp*. They are effective against Gram-positive and Gram-negative bacteria. The cephalosporins have antibacterial properties similar to those of the semisynthetic penicillins. They are effective therapeutically and have a low toxicity. As with penicillin, several semisynthetic cephalosporins have been manufactured commercially for therapeutic use.

Mode of Action: As would be anticipated from the similarity in chemical structure of penicillin and cephalosporin, the mode of action of the cephalosporins is that of inhibition of the cross-linking transpeptidase. They are bactericidal to growing cells.

CYCLOSERINE:

Cycloserine, a relatively simple compound, is related in structure to alanine. It was originally discovered as an antibiotic produced by *Streptomyces* and is now manufactured through chemical synthesis. The main use of this antibiotic is in tuberculosis therapy. However, because of potential undesirable side effects, its utilization is limited.

Mode of Action: Cycloserine manifests its inhibitory effect on peptidoglycan synthesis by interference with synthesis of the peptide moiety of the peptidoglycan. Specifically, it inhibits both alanine racemase and n-alanyl-o-alanine synthetase, the enzymes involved in the synthesis of the pentapeptide side chains.

BACITRACIN:

A product of *Bacillus subtilis* and chemically is a polypeptide. Because of its toxicity to animal and human cells it cannot be used for systemic chemotherapy. It does have application for topical treatment of infections caused by Gram-positive bacteria.

Mode of Action: Bacitracin interferes with regeneration of the monophosphate form of bactoprenol from the pyrophosphate form.

VANCOMYCIN:

Vancomycin is an antibiotic produced by *Streptomyces orientalis*. It is a complex chemical entity consisting of amino acids and sugars. It is a narrow spectrum antibiotic which is effective against gram-positive *Cocci*. It is poorly absorbed from gastrointestinal tract and so must be injected. It is used to treat *Staphylococcal* infections caused by *Methicillin resistant Staphylococci* (MRSA).

Mode of Action: Vancomycin inhibits peptidoglycan synthesis by binding the D-alanyl-D-alanine group on the peptide side chain of one of the membrane-bound intermediates. It causes harm to kidney.

DAMAGE TO CELL MEMBRANE:

Several polypeptide antibiotics produced by *Bacillus spp.* have the ability to damage cell-membrane structure. They adversely affect the normal permeability characteristics of the cell membrane. Included in this category are the polymyxins, gramicidins, and tyrocidines.

The polymyxins are particularly effective against Gram-negative organisms, while the tyrocidines and gramicidins are more effective against Gram-positive organisms. These agents are bactericidal; they cause a leakage from the cytoplasmic content of the cell. Because of their toxicity to tissue they have limited application in chemotherapy.

Another category referred to as polyene antibiotics are large ring structures with many double bonds. Examples are nystatin, produced by *Streptomyces noursei*, and amphotericin, produced by *Streptomyces nodosus*. Polyene anti- biotics act upon cells which have sterols in their

cytoplasmic membrane. They act upon fungi (including yeasts) and animal cells but do not affect bacteria. Their antimicrobial action is attributed to their ability to increase cell permeability.

INHIBITING PROTEIN SYNTHESIS:

STREPTOMYCIN:

Streptomycin is produced by *Streptomyces griseus*, a soil organism isolated by Schatz, Bugie, and Waksman, who reported on its antibiotic activities in 1944. It is particularly important because it inhibits many organisms resistant to sulfonamides and penicillin. Its antibacterial spectrum includes many Gram-negative bacteria, including *Francisella tularensis* and some organisms in the *Salmonella* group. It is inhibitory for several species of *Mycobacterium*, including *Mycobacterium tuberculosis*.

Highly purified streptomycin is nontoxic to humans and other animals when given in small doses, but it appears to have a cumulative detrimental effect on a specific region of the nervous system when given as a medication over long periods of time.

Streptomycin is characterized chemically as an aminoglycoside antibiotic. Other aminoglycoside antibiotics are kanamycin, produced by *Streptomyces kanamyceticus*, and neomycin, produced by *Streptomyces fradiae* and other species of *Streptomyces*.

Streptomycin and other aminoglycoside antibiotics inhibit protein synthesis by combining irreversibly with the 30S subunit mRNA Thus the normal synthetic sequence is disrupted.

TETRACYCLINES:

Chlortetracycline, *oxytetracycline*, *tetracycline*, *doxycycline*, and *minocycline* are generic names for five antibiotics having similar biological and chemical properties. As a group they are commonly called tetracyclines. Note that the antibiotic produced by *Streptomyces aureofaciens* is chlortetracycline. while *Streptomyces rimosus* produces oxytetracycline. They are broad-spectrum antibiotics with similar antimicrobial spectra, and cross resistance of bacteria to them is common.

Hydrochlorides and bases of the tetracyclines are extremely stable as dry powders. In solutions, tetracycline retains its activity for 3 weeks or more, whereas chlortetracycline and oxytetracycline are less stable. Tetracycline, oxytetracycline, chlortetracycline, minocycline, and

doxycycline are chemically very similar. It is not surprising, therefore, that there are no great differences in their activity. The antimicrobial spectra are similar, and all are bacteriostatic in their action. Organisms that are resistant to one are likewise resistant to the others. Tetracycline has a low order of toxicity in laboratory animals. It is readily absorbed from the intestinal tract; hence it is effective when given orally.

The tetracyclines inhibit protein synthesis through interference with the binding of aminoacyl-tRNA to the 30s subunit ribosome.

CHLORAMPHENICOL:

Chloramphenicol is a broad-spectrum antibiotic active against many Gram-positive and Gram-negative bacteria produced by *Streptomyces venezuelae*. Its antimicrobial spectrum is similar to that of tetracycline. It is also bacteriostatic. Chemically, it is a nitrobenzene ring with nonionic chlorine. The possibility of serious side effects such as blood dyscrasias have limited the use of this antibiotic as a general antibacterial agent.

Chloramphenicol inhibits protein synthesis by combining with the 50s subunit ribosome. The transpeptidation and translocation functions associated with this site are blocked.

ERYTHROMYCIN:

Erythromycin is produced by a strain of *Streptomyces erythraeus* isolated from soil collected in the Philippines. Erythromycin is active against the Gram-positive bacteria, some Cram-negative bacteria, and pathogenic spirochetes. With regard to antimicrobial spectrum and clinical usefulness, it resembles penicillin, but it is also active against organisms that become resistant to penicillin and streptomycin. It is, therefore, often prescribed to those patients with allergies when penicillin is indicated.

Erythromycin belongs to the chemical class of antibiotics known as macrolides. Structurally it contains a large lactone ring linked with amino sugars through glycosidic bonds. Erythromycin inhibits protein synthesis as a result of binding on the 50s subunit ribosome; the steps of transpeptidation and translocation in protein synthesis is blocked.

INHIBITION OF SPECIFIC ENZYME SYSTEM:

The sulfonamides, which were discussed earlier in this chapter for their role in Enzyme Systems the development of chemotherapy, represent a category of compounds whose antibacterial attack is directed toward a specific essential enzyme. There are numerous sulfonamides. All of them have the same basic structure.

This structure is related to the compound p-aminobenzoic acid. Many bacteria require p-aminobenzoic acid (PABA) as a precursor to their synthesis of the essential coenzyme tetrahydro folic acid (THFA). PABA is a structural part of the THFA acid molecule.

The selective action of sulfonamides is explained by the fact that the PABA molecule and a sulfonamide molecule are so very similar that the sulfonamide may enter the reaction in place of the PABA and block the synthesis of an essential cellular constituent, which in this case is THFA. The cellular functions of the THFA coenzyme include amino acid synthesis, thymidine synthesis, etc. Lack of this coenzyme will quite obviously disrupt normal cellular activity. Sulfonamides will inhibit growth of those cells which synthesize their THFA from PABA and will not interfere with the growth of those cells (including mammalian host cells) which require the vitamin folic acid and reduce it directly to THFA. This accounts for the selective antibacterial action of sulfonamides and makes them useful in the treatment of many infectious diseases.

This mode of action is an example of competitive inhibition between an essential metabolite (PABA) and a metabolic analog (a sulfonamide). After the antimicrobial activity of sulfonamides was discovered, D. D. Woods an English bacteriologist observed that its effect could be reversed by PABA. Although, in 1940, PABA was unknown as a bacterial metabolite, Woods predicted the mode of action described above.

ANTIFUNGAL, ANTIVIRAL AND ANTITUMOR CHEMOTHERAPEUTIC AGENTS

Drugs that control growth of viruses and eukaryotic pathogens such as fungi and parasites are available, but they often affect eukaryotic host cells as well. As a result, selective toxicity for eukaryotic pathogenesis very difficult to attain; only agents that preferentially affect pathogen

specific metabolic pathways or structural components are useful. There are a limited number of these drugs, and discuss some important ones that affect viruses and fungi here.

ANTIVIRAL DRUGS: Because viruses use their eukaryotic hosts to reproduce and perform metabolic functions, most antiviral drugs also target host structures, resulting in host toxicity. However, several compounds are more toxic for viruses than for the host, and a few agents specifically target viruses. Largely because of efforts to find effective measures to control infections with the Human Immunodeficiency Virus (HIV), the cause of AIDS, significant achievements have been made in the development and use of antiviral agents.

ANTIVIRAL CHEMOTHERAPEUTIC AGENTS:

Antibiotics such as those that we have discussed are generally not effective against viruses. You will recall that viruses are intracellular, and hence the chemotherapeutic agent, in order to attack the virus, must enter the host cells. Also, the agent must not be toxic to the host cell while exerting an inhibiting action on the virus. This demands a high level of selective toxicity. In cases of infection by bacteria, fungi, or protozoa the infectious agent is acted upon outside the host cells. Additionally, there are many more metabolic processes that can be interrupted with these microorganisms.

Among the more promising of the chemotherapeutic agents for treating viral diseases is Interferon. Interferons are small glycoprotein substances of which two types are leukocytic interferon and fibroblast interferon. Cells exposed to interferon develop antiviral properties. The antiviral action of interferon is attributed to interference of protein synthesis.

Natural interferons are in very short supply and are expensive. Recent advances in recombinant DNA techniques (genetically engineered bacteria like *Escherichia coli* to produce interferon on a large scale commercially) have increased the availability of interferon for both chemotherapeutic and experimental use.

Acycloguanosine is a nucleoside analog which is active against the herpes virus in animals. Its mode of action appears to be that of inhibition of nucleotide utilization. A synthetic nucleotide analog, 5'-iododeoxyuridiue has been shown to have antiviral activity and promise as an antiviral

chemotherapeutic agent Its mode of action is most likely that of inhibition of nucleic acid synthesis-preventing the incorporation of thymidine into DNA.

Amantadine is a tow-molecular-weight compound which is very effective against influenza A virus; it is not effective against influenza B. The incidence of influenza A infections is greatly reduced by use of this drug. The mode of action of amantadine is that of interfering with the uncoating of virus particles and the subsequent release of their nucleic acids.

ANTIFUNGAL ANTIBIOTICS:

Nystatin is an antifungal agent useful in the therapy of nonsystemic fungal infections. It is produced during fermentation by a strain of *Streptomyces noursei*. This antibiotic was discovered in 1950 by Elizabeth Hazen and Rachel Brown.

NYSTATIN:

The antimicrobial activity of nystatin is restricted to yeasts and other fungi, e.g., *Candida*, *Aspergillus*, *Penicillium*, and *Botrytis*; it is fungicidal in action. Chemically, nystatin is a polyene with an empirical formula of $C_{47}H_{75}NO_{17}$.

GRISEOFULVIN:

Griseofulvin is obtained from *Penicillium griseofulvin*. It is used in the treatment of many superficial fungus infections of the kin and body surfaces and is also effective in the treatment of some systemic (deep-seated) mycoses. The drug is administered orally.

ANTITUMOR ANTIBIOTICS:

Some antibiotics have been found to possess antitumor activity. The anthramycin group (anthramycin, sibromycin, tomaymycin, and neothramycin) is an example of potent antitumor agents. One of the complicating factors associated with the potential use of these anticancer agents is that they are also cardiotoxic, a fact that illustrates the need for a high level of specificity in a chemotherapeutic agent. The antitumor action of these antibiotics is directed toward DNA

structure and function. One of the problems is that of determining whether, through the manipulation of the structure of an antibiotic, e.g., anthramycin, one can cut out the cardiotoxic property without destroying the antitumor property.