REVIEW OF MICROBIAL PHYSIOLOGY AND GENETICS

The first portion of this section will deal with bacteria and fungi, their structure, chemical composition, and metabolism. This will be followed by a discussion of chemotherapeutic agents that are active against bacteria, fungi and animal parasites. The second section will deal with Basic Virology (viral structure, classification, replication and the effects of antiviral agents of viral replication). The last portion will deal with Microbial Genetics (mutations, gene transfer, and recombinant DNA technologies).

BACTERIAL STRUCTURE

BACTERIAL MORPHOLOGY

Cell Wall

The cell wall of bacteria protects the cell against osmotic lysis. Both Gram positive and Gram negative bacteria have peptidoglycan (mucopeptide) as the innermost layer of the cell wall. They differ in amount, and in the nature of the surface layers.

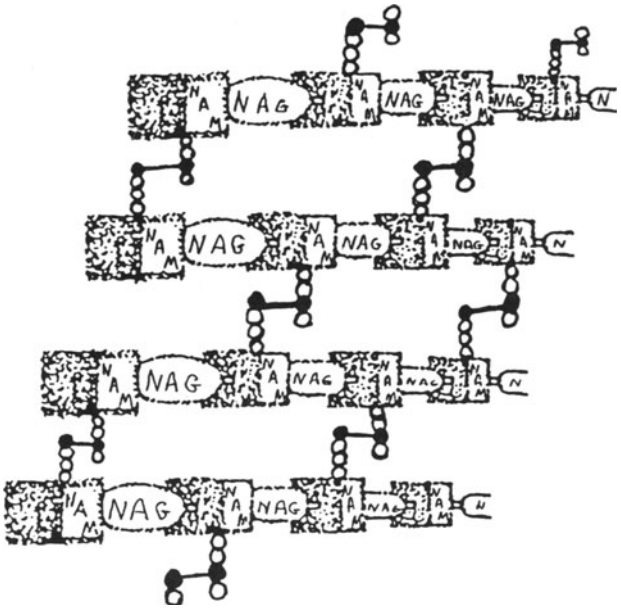
The cell wall of Gram positive bacteria contains from 40-90% peptidoglycan, while cell walls of Gram negative bacteria contains only 5-10% peptidoglycan. In Gram positives, the next layer is carbohydrate, composed of ribitol teichoic acid, and the outermost layer of the cell wall is composed of two or three kinds of protein. The lipoteichoic acids of group A streptococcal cell walls are involved in adherence to epithelial cells.

The cell walls of Gram negative bacteria are composed of an outer layer of lipoprotein-lipopolysaccharide (endotoxin) anchored to the peptidoglycan layer (in the periplasmic space) through protein and lipoprotein molecules. The periplasmic space enables bacteria to keep various proteins close to the cell in a concentrated form (e.g. beta lactamase, etc.). The cell membrane, which is not a part of the cell wall, appears as a double layered structure immediately below the cell wall.

The basic unit of peptidoglycan is a disaccharide-tetrapeptide containing N-acetylmuramic acid, N-acetylglucosamine, D-alanine, L-alanine, D-glutamic acid or its derivative, D-isoglutamine, and a basic amino acid, usually diaminopimelic acid. The basic units of mucopeptide are cross-linked to each other to form a tight meshwork which surrounds and protects the entire cell.

Lipopolysaccharides are composed of lipid A, core and O-antigen. Lipid A is the toxic moiety of endotoxin. The polysaccharide side chains are the O antigen epitopes.

The figure below is a representation of the basic unit of peptidoglycan: NAM = N-acetyl muramic acid, NAG = N-acetyl glucosamine; the circles are the amino acids where chain cross-linking occurs, between alanine and lysine in Gram positive cocci or alanine and diamino pimelic acid (DAPA) in other bacteria.



The mucopeptidase, lysozyme, hydrolyzes the linkage between N-acetylmuramic acid and N-acetyl glucosamine causing

A. the peptidoglycan layer to dissolve.
B. the cell to become osmotically-fragile.
C. Both
D. Neither

(Answer on next page)

Cytoplasmic Membrane

The ultrastructural appearance of membranes is bilayered, i.e., structures with the lipid oriented so that the non-polar (fat soluble) fatty acid side chains face the interior and the polar (water soluble) glycerol esters face the exterior of the membrane. Membrane proteins are embedded in the lipid bilayer.

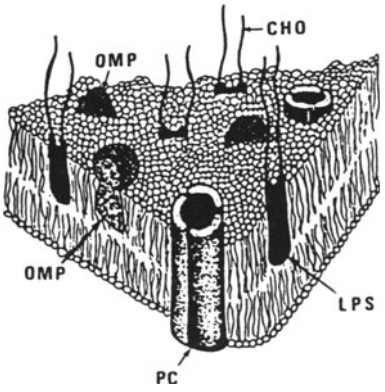
Isolated cytoplasmic membranes of both Gram positive and Gram negative bacteria are approximately one-third lipid (mostly phospholipid) and two-thirds protein; occasionally polysaccharide is also attached to the membrane. There are at least three kinds of proteins associated with the cytoplasmic membranes of bacteria;
1) biosynthetic enzymes which are responsible for the synthesis of the external layers of the cell, in particular the membrane, cell wall and capsule,
2) transport proteins responsible for the transport of water-soluble materials from the medium into the cell, and
3) the cytochrome enzymes and other enzymes of the electron transport system.

The cytoplasmic membrane is a complex biologically active structure; it acts as a permeability barrier. The lipid bilayer acts as a barrier to the passage of water-soluble chemicals. A second function of the cell membrane is to serve as a site for synthesis of peptidoglycan, lipopolysaccharide and capsule. The third important function of the cell membrane is to serve as the site of electron transport and oxidative phosphorylation in aerobic and facultative bacteria.

Mesosomes

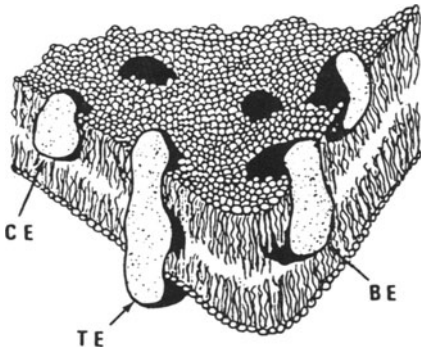
Mesosomes are invaginations of the membrane whose function is unclear. These sac-like structures are low in enzyme content. Septal mesosomes appear to be involved in cell division (they are associated with DNA).

The outer membrane of the cell wall of Gram negatives is somewhat selective, and is not as permeable to antibiotics as is the cell wall of Gram positives. Hence, the former organisms have become more important in human medicine during the antibiotic era.



OUTER MEMBRANE OF GRAM NEGATIVE CELL WALL

PC=PORIN PROTEIN CHANNEL
OMP=OUTER MEMBRANE PROTEIN
LPS=LIPOPOLYSACCHARIDE
CHO=CARBOHYDRATE SIDE CHAIN OF LPS



CYTOPLASMIC MEMBRANE

CE = CYTOCHROME ENZYMES
TE = TRANSPORT ENZYMES
BE = BIOSYNTHETIC ENZYMES

Answer to question about lysozyme's effect on bacterial cell wall = C.

INTRACYTOPLASMIC STRUCTURES

Bacterial Nucleus (Nucleoid)

The double-stranded DNA of bacteria is circular; there are usually 1-3 copies per cell. The highly coiled DNA strand is not enclosed in a nuclear membrane; DNA-associated histones are also absent. It is attached to the membrane and has associated a high mRNA content (20%).

Ribosomes

Ribosomes are the only structural organelle in the bacterial cytoplasm. They are numerous, and mostly grouped in chains (polysomes).

Cytoplasmic Inclusions

Several species of bacteria form lipid inclusions which appear to be a source of reserve energy for the organism. Metachromatic granules are composed of polymetaphosphate. The function of metachromatic granules is not clear, but may be related in some fashion to energy metabolism. Starch or glycogen granules have also been detected in bacteria.

Spores

Most spore-forming bacteria are Gram positive rods (Bacillus and Clostridium). They are composed of a bacterial nucleoid surrounded by a cell membrane and several layers known as the outer and inner coats which appear to be composed of highly stable proteins such as keratin, possibly with some phospholipoprotein containing Ca^{++} dipicolinate. The mature spore is a dormant organism characterized by a very low water content and metabolic rate. Spores are highly resistant to heat, light, and other deleterious agents. Spores contain the same catabolic and anabolic enzymes as do vegetative cells, but do not contain respiratory enzymes. They are formed by the parent vegetative cell in response to an adverse environmental condition such as depletion of C source.

PROKARYOTES VS EUKARYOTES

Bacteria (Prokaryotes) lack:

1. nuclear membrane
2. DNA-associated histones
3. introns in genes
4. steroids in cell membrane
5. phosphatidyl choline in membrane
6. mitochondria
7. endoplasmic reticulum

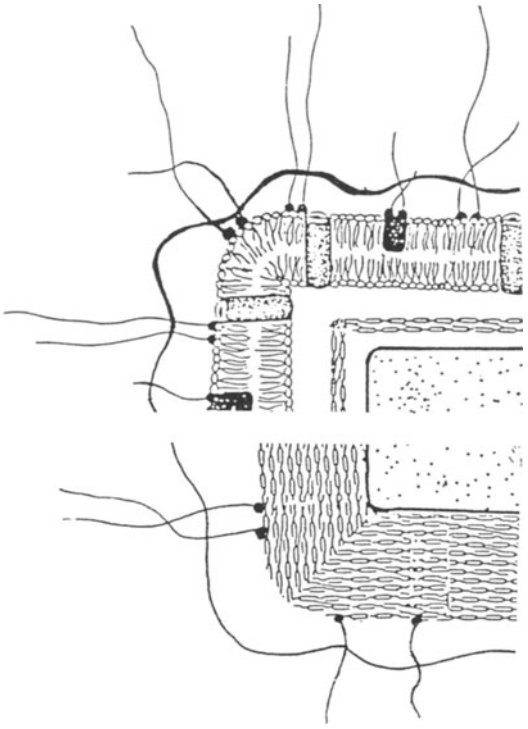
Bacteria have:

1. polygenic mRNA
2. formylmethionyl is initiator tRNA instead of methionyl
3. 70S ribosomes instead of 80S
4. Unique components of cell wall
   a. peptidoglycan
   b. diaminopimelic acid

Germination occurs in three stages.

1. Activation - caused by damage to the spore coat
2. Initiation - needs a nutritionally favorable environment. An autolysin is activated which degrades the cortex peptidoglycan. Water is taken up and calcium dipicolinate released.
3. Outgrowth - the spore protoplast emerges and active biosynthesis occurs.

Capsule

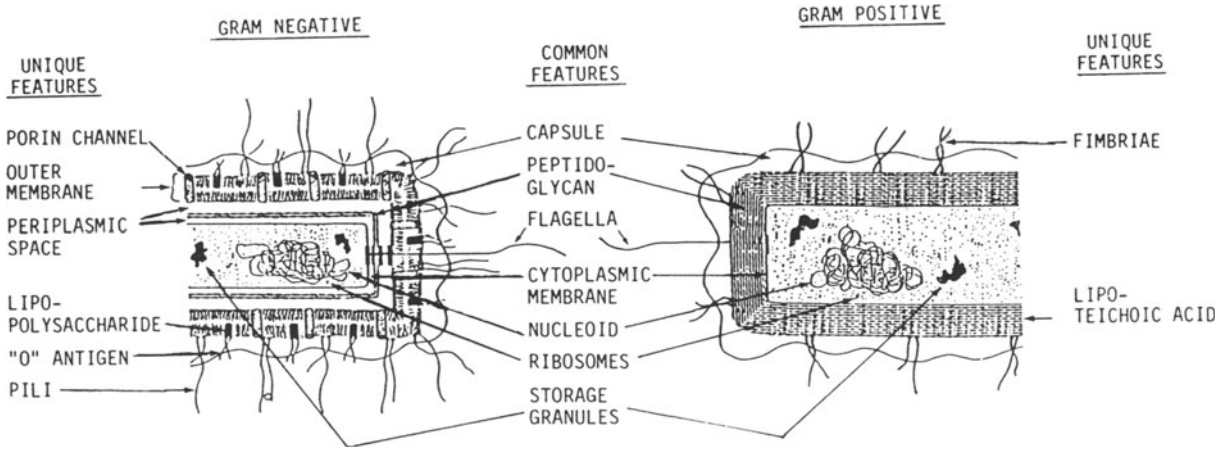


Capsules of most species of bacteria are polysaccharide, but in the genus Bacillus, there are capsules of poly D-glutamic acid. Polysaccharide capsules vary from relatively simple structures such as hyaluronic acid which is a linear polysaccharide composed of N-acetylglucosamine and glucuronic acid, to the highly branched polysaccharides formed by Streptococcus pneumoniae. Although there are usually no more than three or four sugars in capsules of S. pneumoniae, they are branched polysaccharides and are unique enough that specific antisera to the capsules can be formed. Some 80 strains of S. pneumoniae have been identified on the basis of the antigenic properties of their capsules.

Capsules of certain species of bacteria inhibit phagocytosis and thereby enhance the ability of the organism to establish an infection, i.e., they are antiphagocytic virulence factors.

COMPARATIVE ANATOMY OF BACTERIA

Cross sections of Gram negative (top) and Gram positive (bottom) bacteria are shown in the upper right quadrant. Note the striking differences in the cell wall composition; e.g., the large amount of peptidoglycan in the Gram positive vs. the presence of the periplasmic space in the Gram negative, etc. In the diagram below the common and unique features of both groups of organisms have been listed for your edification. Label the structures in the figure in the upper right hand corner.



EXTERNAL STRUCTURES

Flagella

Flagella, the organelles responsible for motility of bacteria, are located either at the ends of the cell (polar flagellation) or over the entire surface (peritrichous flagellation). There are three parts of the flagellum; filament, hook and basal body. The basal body arises in the membrane and is anchored in the cell wall; it is the motor.. The flagellar filament is composed of an elastic protein named flagellin; these are the H antigens of bacteria.

Axial Filaments

The organelles responsible for motility of spirochetes are called axial filaments. They are composed of protein and have a hook at the proximal end which is attached to the cell.

Pili (Fimbriae)

Pili are short, hair-like, protein structures which occur on a large number of bacterial species. Host cell selectivity may be directed by pili, e.g., the pili of the gonococcus has an affinity for the columnar epithelium of the urethra.

The sex pilus is found only on male strains of bacteria which are capable of donating their DNA by conjugation. These pili are usually named after the fertility agent carried by the strains such as F pilus and Hfr pilus. DNA of the donor is transferred to the recipient after the cells are brought into intimate contact by contraction of the sex pilus.

In streptococci, fimbriae are the site of the major surface antigen, the M protein. Lipoteichoic acid, associated with these fimbriae, is responsible for the adherence of group A streptococci to epithelial cells of their hosts.

Bacterial chemotaxis occurs when attractants (e.g. sugars) or repellents (e.g., toxic metabolites) react with chemoreceptors in the membrane or periplasmic space. Methyl-accepting proteins relay signals from the receptors to the flagellar apparatus. S-adenosyl methionine serves as the methyl donor. The signal influences flagellar rotation such that the organism moves toward, or away from, the attractant or repellent depending upon the direction of rotation of the flagella. This is an energy dependent event.

The basic mechanism of chemotaxis is hypothesized to function as follows: The chemotaxins arrive in the periplasmic space following passage through porin channels. Here they interact with periplasmic binding proteins which deliver them either to membrane transport proteins for passage into the cell or to chemoreceptor proteins of the chemotaxis system. When this occurs it exposes methylation sites on the cytoplasmic side of the receptor which are methylated by an enzyme called CheR. A series of methylation and phosphorylation events transmit the signals for directional rotation in the cytoplasm of the cell. At least five additional proteins are involved; CheY and CheZ control the direction of rotation. Phosphorylated CheY is thought to interact directly with the motor-switch complex to cause clockwise rotation. CheZ accelerates CheY dephosphorylation, thus favoring counterclockwise rotation and active "swimming" toward an attractant. The cells appear to "tumble" when the direction of flagellar rotation is reversed as would occur upon the addition of a repellant or the absence of an attractant.

BACTERIAL GROWTH

GROWTH OF BACTERIA

Source of Carbon

Autotrophs are organisms which are able to use carbon dioxide as the sole carbon source. Heterotrophs require the major portion of their carbon in the form of organic carbon although almost all heterotrophs require some carbon dioxide.

Microorganisms which are pathogenic for man are heterotrophs/autotrophs?
(Choose One)

Physical Requirements for Growth

The temperature for optimum growth of most bacteria is between 20-40 C; these are mesophiles. Bacteria which grow in association with warm blooded animals have optimum growth temperatures in the vicinity of 35-40 C.

Microorganisms which are pathogenic for man are mesophiles/psychrophiles?
(Choose One)

The growth rate is affected by the osmotic pressure of the medium. Most bacteria are able to tolerate 1-2% salt, but the growth drops off rapidly as the salt concentration increases above this level. Haloduric organisms (e.g., S. aureus) can grow in the presence of high salt concentrations

Certain pathogenic microorganisms are haloduric; this fact is used in the design of selective media for their isolation from clinical specimens. Name a medium used for Staphylococcus aureus:

_____

The effect of pH on the growth of bacteria is as might be predicted - bacteria grow best at pH values near neutrality

Bacteria which require oxygen for growth are aerobes; strict aerobes will grow only in the presence of oxygen. Bacteria which grow in the absence of oxygen are anaerobes. Strict anaerobes are unable to grow in the presence of oxygen and are apparently poisoned by it; they lack a functional electron transport system and are unable to produce energy by oxidative phosphorylation. They utilize organic compounds as H+ donors and acceptors. obligate anaerobes also lack catalase and superoxide dismutase. The latter converts toxic superoxide radicals to H2O2 which is then converted to H2O and O2 by catalase. Peroxidase also breaks down H2O2. in the presence of O_2 as more energy is available for growth.

What is the genus name of a strict anaerobe which is a sporeformer?

_____

What is the genus name of a strict anaerobe which predominates in the gut?

_____

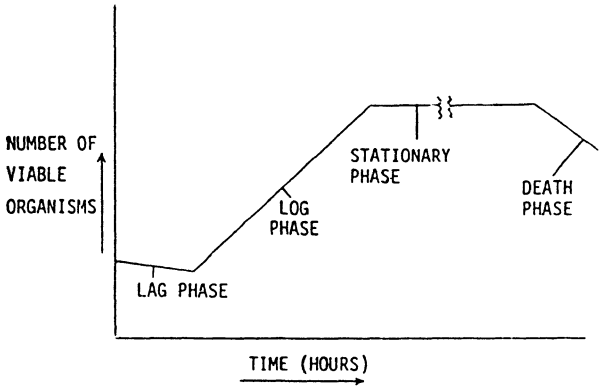
(See the Pathogenic Bacteriology section for answers to the above)

Measurement of Bacterial Populations

Bacteria reproduce by binary fission, an asexual process which results in two genetically identical daughter cells. Each division is a generation and it follows that each generation leads to a doubling in cell count. The growth rate is the number of generations per unit time and is obtained by dividing the number of generations by the time interval required to increase the cell count to that level. The generation time is the time required for one generation.

Phases of Growth

There are four distinct phases of growth. The first phase of growth is the lag phase. The second phase is the exponential or logarithmic phase of growth. During this phase, total and viable counts are very nearly equal. In the third phase of growth, the stationary phase, growth has stopped. Since growth is an exponential increase of protoplasm, the last generation will use as much as one half of the nutrients in the medium which means that the medium suddenly becomes unable to support growth. Usually, it is the energy source which becomes limiting, but it can also be a vitamin or amino acid which is necessary for growth. Growth may also stop because of an increase in toxic products such as acid.



A fourth phase of the growth curve, the death phase, can occur. There is a decline in the population of bacteria. The medium is no longer able to support growth and the cell cannot maintain life indefinitely.

Sterilization and Disinfection

Sterilization involves the use of physical or chemical agents to eliminate all viable organisms; disinfection refers to the use of germicidal agents to remove the potential for infectivity of a material.

Heavy metals such as mercury are commonly used as disinfectants. Their action can be blocked by proteins or other sources of -SH groups.

Halogens such as iodine combine irreversibly with proteins and inactivate them. They also act as oxidants to destroy vital cell constituents. H_2O_2 also kills via oxidizing cellular enzymes, etc.

Alkylating agents such as formaldehyde react with proteins, nucleic acids, and other compounds with labile H+ to block their activity. These disinfectants are also blocked by proteins.

BACTERIAL METABOLISM

Exoenzymes

Bacteria frequently secrete enzymes which digest large, insoluble molecules into small, soluble molecules which can pass through the cell membrane.

Nutrient Transport

Kinetics - pinocytosis does not occur in bacteria and nutrients must be transported into the cell in a soluble form. Velocity of transport follows kinetics described by the Michaelis-Menten equation for enzyme reactions.

Characteristics - Bacteria use facilitated diffusion, active transport, and group translocation to transport substrates. The stereoisomer which is biologically active is selectively transported. Transport systems responsible for transport of substrates which are catabolized are usually inducible (except glucose which is constituitive.) Active transport and group translocation require energy in order to concentrate substrate inside the cell and, in the case of group translocation, to phosphorylate the substrate. Transport proteins are located in the cell membrane. Facilitated diffusion and active transport are accomplished by binding proteins which reversibly, but selectively, absorb substrate from solution.

Transport of Amino Acids occurs by active transport without chemical modification. One system may be responsible for the transport of more than one amino acid.

Transport of sugars occurs mainly by active transport and group translocation and occasionally by facilitated diffusion. Group translocation occurs only with sugars and is affected by the phosphotransferase system.

Gases (such as oxygen) water, and some ions (such as sodium) are transported into the cell by passive diffusion.

Porin channels formed by protein trimers in the outer membrane of Gram negative cell walls allow passive diffusion of molecules < 600 daltons (trisaccharides or tetrapeptides).

The four types of transport seen in bacteria are

1. _____
2. _____
3. _____
4. _____

Which of the above

- require energy?
- require a specific binding protein?
- is involved in amino acid transport?
- is involved in sodium transport?

ENERGY METABOLISM

Fermentation

Fermentation is the anaerobic metabolism of a substrate. The Embden-Meyerhof pathway is the most common pathway used for sugar fermentation. Energy is mobilized during fermentation in phosphoryl groups of molecules such as adenosine triphosphate and phosphenol-pyruvate. Some anaerobic and facultative bacteria also ferment amino acids for energy.

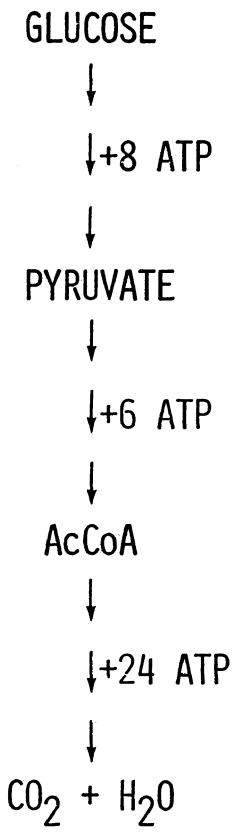
Aerobic Energy Metabolism

The reduction of oxygen to water by NADH is a rich source of energy which takes place in aerobic and facultative bacteria through the action of the electron transport system. Some of the energy is trapped by oxidative phosphorylation and the rest is lost as heat.

The electron transport system of bacteria is located in the cell membrane and is composed of cytochrome enzymes, lipid cofactors such as vitamin K and coenzyme Q, and coupling factors, the latter being involved in oxidative phosphorylation.

Aerobic Carbohydrate Metabolism

The most common mechanism for aerobic metabolism of carbohydrates in bacteria is a combination of the Embden-Meyerhof pathway and the tricarboxylic acid cycle. The latter is used for the aerobic oxidation of products of the metabolism of carbohydrates, amino acids and lipids. Hexoses are oxidized to pyruvate, which is oxidized to acetyl-CoA which then enters the tricarboxylic acid cycle by condensation with oxaloacetic acid to citrate. Fatty acids formed by the hydrolysis of triglycerides are also oxidized to acetyl-CoA. Amino acids may be oxidized to pyruvate, acetyl-CoA, oxaloacetate, fumarate or succinate.



Which type of metabolism produces more energy: aerobic or anaerobic?

How much more?

CHEMOTHERAPEUTIC AGENTS

CHEMOTHERAPEUTIC AGENTS-ANTIMETABOLITES

Sulfonamides

Action
Sulfonamides are bacteristatic, that is, they inhibit growth but do not kill; these drugs depend on the immune system of the host to remove and kill the infecting bacteria. Para-aminobenzoic acid and many other natural products such as thymine, purines, serine and methionine may overcome the action of sulfa drugs, thus sulfonamides are often found to be ineffective in sites of extensive tissue destruction.
Sulfonamides inhibit the condensation of 2-amino-4-hydroxy-6-dihydropteridinyl-pyrophosphate with para-aminobenzoic acid. Sulfonamide condenses with pteridine pyrophosphate forming an analogue of dihydropteroic acid. They act as allosteric inhibitors of dihydropteroate synthetase. The drug is most effective against those bacteria which are able to synthesize their own folic acid.

The low toxicity of sulfonamides for man is understandable since man is unable to synthesize folic acid. The concentration of folic acid in tissue is either too low to reverse the action of sulfonamides or sulfa sensitive bacteria are impermeable to folic acid.

Resistance
The most frequently observed naturally occurring resistance to sulfonamides is associated with the presence of an R factor. Resistance appears to be due to the production of an altered dihydropteroate synthetase.

Clinical Use
Sulfonamides are used for urinary tract infections, some upper respiratory tract infections, for shigellosis, and for trachoma and inclusion conjunctivitis. They are also used in combination with a dihydrofolate reductase inhibitor, trimethoprim, which gives enhancement of antibacterial action.

Drugs that inhibit microbial growth but do not kill the organism are _____.

Sulfonamide drugs are not toxic to humans because we do not synthesize _____.

Compounds that compete with sulfa drugs and may reverse their bacteristatic action include:

1. _____
2. _____
3. _____
4. _____
5. _____

Other Antimetabolites

Para-aminosalicylic acid is also an analogue of para-aminobenzoic acid with many actions similar to sulfonamide. Para-aminosalicylic acid is bacteristatic and inhibits the condensation of 2-amino-4-hydroxy-6-dihydropteridinyl-pyrophosphate and para-aminobenzoic acid. The action of para-aminosalicylic is also reversed by para-aminobenzoic acid. The most important use of para-aminosalicylic acid is for the treatment of tuberculosis.

Isoniazid (INH) is a bactericidal agent which is also used for treatment of tuberculosis, frequently in combination with para-aminosalicylic acid. The mode of action of isoniazid is to inhibit synthesis of mycolic acids, an important component of the mycobacterial cell wall. INH toxicity is manifest as peripheral neuritis; it also has nephrotoxicity.

Sulfone derivatives such as diaminodiphenylsulfone (Dapsone) have been the drugs of choice for treatment of leprosy; however, recently rifampin has been used with promising results. These drugs also act as PABA antagonists.

Trimethoprim is a competitive inhibitor of microbial dihydrofolate reductase; it has little activity against the mammalian enzyme. When combined with sulfonamides the sequential blockade of the pathway to tetrahydrofolate makes for effective synergy. Trimethoprim has broad spectrum activity, including Plasmodia and Pneumocystis carinii; it is widely used for enteric and urinary tract infections.

Which antimetabolite(s)

- inhibit dihydrofolate reductase?

- inhibit dihydropteroic acid synthetase?

- inhibit mycolic acid synthesis?

--In the flow chart below--
Insert the antimetabolites on the numbered lines and the enzymes in the margins at the steps of their activity.

2-amino-4-hydroxy-6-dihydropteridinyl-pyrophosphate

+

Glutamic acid

+

Para-aminobenzoic acid

1. _____

2. _____

3. _____

↓

Dihydrofolic acid

4. _____

↓

Tetrahydrofolic acid

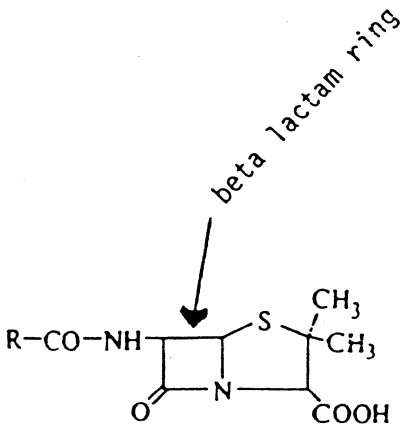
CHEMOTHERAPEUTIC AGENTS - ANTIBIOTICS

INHIBITORS OF CELL WALL SYNTHESIS

Penicillins and Cephalosporins

Structure

These antibiotics have a similar chemical structure, the common element being a beta lactam ring. They also have a similar mode of action. Benzylpenicillin, or penicillin G, has the disadvantages that it is hydrolyzed by acid, which limits its oral use, and is inactivated by penicillinase. Semisynthetic penicillins have substituted acyl groups which make them stable to acid, resistant to penicillinase, or both.



Mode of action

These are bactericidal agents which inhibit peptidoglycan synthesis. Their molecular configuration is similar to that of the D-alanyl-D-alanine terminus of the pentapeptide side chain and they react with the transpeptidase forming an inactive complex.

R = Side chains which are added to the 6-aminopenicillanic acid core in the semisynthetic penicillins; differences here determine degree of activity, spectrum, resistance to enzymatic cleavage, and other pharmacological properties.

Resistance

Bacterial resistance to penicillins is usually the result of a lactamase whose production is governed by plasmids. Beta lactamases hydrolyze the B-lactam ring. Penicillinases are active against penicillins but relatively inactive against cephalosporins.

Clavulanic acid is a β lactam compound without significant antimicrobic activity which can bind irreversibly to the enzyme and inactive it. When given at the same time as the antibiotic, this drug protects the penicillin from enzymatic degradation.

Clinical Use

Benzylpenicillin is used for infections caused by penicillin-sensitive organisms such as streptococci, Neisseria, and Treponema. Methicillin, oxacillin and nafcillin are used for infections caused by bacteria which form penicillinases Oxacillin has the advantage that it can be used orally. Ampicillin and Carbenicillin are more effective against Gram negative bacteria than are the other penicillins. The cepholosporins are used mostly against penicillin-resistant organisms since some penicillinases do not inactivate second and third generation cepholosporins. Cephalosporins can also be used in some allergic patients although cross reactivity does occur.

Allergy to the penicillin antibiotics is against the 6-aminopenicillanic acid portion of the molecule or, more commonly, to its degradation products.

Resistance to penicillin is usually due an enzyme, _____ the production of which is governed by independently replicating genetic unit known as a _____.

Other Inhibitors of Wall Formation

D-Cycloserine owes its antibacterial action to the fact that it is an analogue of D-alanine and competes with it for transport by the D-alanine-glycine transport system. D-Cycloserine is a competitive inhibitor of the alanine racemase and D-alanyl-D-alanine synthetase, both of which are important enzymes in the synthesis of peptidoglycan. The most important use of cycloserine is as a second line drug in the treatment of tuberculosis.

D-cycloserine is a competitive inhibitor of two enzymes, _____, and _____, both of which are important in _____ _____ synthesis.

Vancomycin is an antibiotic which inhibits the transfer of the disaccharide-pentapeptide from the phospholipid carrier to the cell wall acceptor. It binds to D-alanyl-D-alanine. Vancomycin is more active against Gram positive bacteria than Gram negative bacteria.

Vancomycin inhibits the transfer of disaccharide-pentapeptide from the _____ carrier to the _____ acceptor.

Bacitracin is a polypeptide antibiotic produced by Bacillus species which is active against Gram positive bacteria and Neïsseria. Bacitracin is used only topically. Bacitracin inhibits the hydrolysis of lipid pyrophosphate to lipid phosphate thereby preventing its reuse in mucopeptide synthesis. It inhibits production of monophosphate carrier protein.

ANTIBIOTICS WHICH INHIBIT PEPTIDOGLYCAN SYNTHESIS (BACTERICIDAL ACTION)

CYTOPLASM	MEMBRANE	CELL WALL
D cycloserine a competitive inhibitor of alanine racemase and D-alanyl-D-alanine synthetase.	Bacitracin inhibits the lysis of lipid pyrophosphate thereby limiting available substrate for mucopeptide synthesis.	Penicillins and Cephalosporins react with transpeptidase forming an inactive complex Vancomycin inhibits transfer of disaccharide-pentapeptide to the cell wall.

CELL MEMBRANE INHIBITORS

These antibiotics interact with the membrane of the cell and alter its osmotic properties. The membrane becomes "leaky" and allows the escape of K+ ions and vital metabolites, e.g. glucose. Some are also able to react with mammalian cell membranes, and hence are quite toxic.

Microbial and mammalian membranes are quite similar, hence drugs which affect their antibacterial action via membrane action are likely to be toxic. They cause _____ _____.

Polymyxins are a family of decapeptides. They are active against Gram negative bacteria only. Because of the extreme toxicity, they are used primarily in the treatment of antibiotic resistant Pseudomonas infections.

Polymyxin B is used in the treatment of infections caused by _____.

Polyenes are macrolide antibiotics. The two most important are nystatin and amphotericin B. These agents selectively inhibit organisms that have ergosterol in their membranes hence they are active against the FUNGI but have no toxicity for prokaryotic forms such as bacteria due to the absence of sterols in the bacterial cell membrane. They disrupt the integrity of the sterol-containing cell membrane.
Nystatin is highly toxic and is only used for topical fungal infections (e.g., candidiasis). Amphotericin B is used parenterally; nephrotoxicity is a major complication of its use.

Amphotericin B reacts with fungal membranes due to their content of _____.

Imidazoles are synthetic agents which exhibit anti-fungal activity. Miconazole and ketoconazole are clinically the most useful. The former is used topically or intravenously; ketoconazole is effective when administered orally. Both of these compounds interfere with ergosterol synthesis.

Ketoconazole interferes with the synthesis of _____.

REVIEW

Penicillin and other inhibitors of cell wall formation are bactericidal/bacteristatic?

Antifungal antibiotics include _____, _____, and _____.

Antifungal imidazole compounds include _____, and _____, both of which interfere with the synthesis of _____.

INHIBITORS OF PROTEIN SYNTHESIS

Streptomycin

Structure
Streptomycin is an aminoglycoside antibiotic.

Aminoglycoside antibiotics bind to the _____ ribosome, reacting with a ribosomal protein designated _____. They inhibit protein synthesis by _____ _____.

Action
Streptomycin is a bactericidal drug with several effects on growing bacteria. The lethal effect seems to be a result of its inhibition of protein synthesis by preventing initiation. Streptomycin binds to the 30S ribosome; the binding site has been identified as a ribosomal protein designated S12. Chromosomal mutations may alter this binding site, producing streptomycin resistant forms.

Resistance
One mode of resistance is an altered S12 ribosomal protein that no longer binds streptomycin. Resistance is also associated with the presence of R factors (plasmids) which carry genes for enzymes that cause a chemical modification of streptomycin making it ineffective as an antibiotic, i.e. streptomycin-spectinomycin adenyl transferase, streptomycin phosphotransferase and acetyl transferase.

Resistance to streptomycin is most often due to enzymes produced by the organism, namely _____, or _____.

Clinical use
Streptomycin is bactericidal for most Gram negative bacilli but not for most Gram positive bacteria. It is a first line drug for the treatment of tuberculosis, usually with ethambutol, rifampin, para-aminosalicylic acid and isoniazid. Streptomycin is also used frequently in the treatment of genitourinary tract infections caused by Gram negative bacilli. Streptomycin is the drug of choice for tularemia and plague and is used for brucellosis in combination with one of the tetracyclines. Streptomycin, when used for long periods of time, will cause damage to the eighth cranial nerve, resulting in hearing loss.

Streptomycin is most commonly used vs Gram _____. It is not effective on these bacteria if they are growing anaerobically (drug transport into cell requires aerobic growth).

Aminoglycoside antibiotics are bactericidal/bacteriostatic?

OTHER AMINOGLYCOSIDES

Kanamycin, Amikacin, and Gentamicin

Activity
These are also bactericidal antibiotics with an action similar to streptomycin. They also cause misreading in protein synthesis.

Resistance
Resistance to these antibiotics is associated with the presence of an R factor and is a result of the production of antibiotic modifying enzymes.

Clinical use
These antibiotics have pharmacological properties similar to streptomycin. They are bactericidal for Gram negative bacilli, mycobacteria and staphylococci.

Amikacin is used in treatment of Proteus and Pseudomonas infections. It is highly resistant to enzymatic inactivation. Gentamicin has a similar spectrum but is more readily inactivated by microbial enzymes.

The aminoglycosides are not absorbed from the intestine, hence treatment of systemic disease requires injection. They are commonly used in conjunction with B-lactam antibiotics for severe infections such as Gram negative sepsis. The basis for this synergy is the cell wall damage done by the lactam-active antibiotic facilitates aminoglycoside uptake by the offending pathogen.

Resistance to the aminoglycoside antibiotics is usually associated with a plasmid called an _____ factor. It is the result of enzymes which inactivate the antibiotic by enzymatic modifications such as

_____ ,

_____ ,

and _____.

[see table on antibiotic resistance mechanisms at end of this section]

Aminoglycoside antibiotics have toxicity for the _____ cranial nerve, causing _____.

Spectinomycin, although not an aminoglycoside, has some structural similarities. However, its action is bacteriostatic. It does not inhibit initiation, but does cause the formation of unstable initiation complexes. Spectinomycin is used in the treatment of gonorrhea to by-pass the problems of PPNG (see Pathogenic Bacteriology section for the meaning of this abbreviation).

TETRACYCLINES

Structure
The tetracyclines are a family of antibiotics with a four ring structure.

Activity
Tetracyclines are active against a wide variety of microorganisms. Sensitive organisms include not only Gram positive and Gram negative bacteria, but also rickettsia, mycoplasma and chlamydia. Tetracyclines are bacteristatic drugs which inhibit protein synthesis. They bind to the 30S ribosome and inhibit binding of aminoacyl-tRNA to the acceptor site of this ribosome.

Tetracycline antibiotics bind to the 30S ribosome and inhibit binding of _____.

Tetracyclines are bactericidal/static?

Resistance
Resistance is associated with the presence of an R factor in Gram negative bacilli which confers resistance against all tetracyclines. Unlike other R factor-mediated resistance, however, resistance to tetracyclines appears to be due to an impaired ability to transport the drug into the cell through the cytoplasmic membrane.

Bacterial resistance to tetracyclines is due to _____ _____.

Clinical use
Tetracyclines are absorbed from the gastrointestinal tract, and therefore can be used orally. They are first line drugs for treatment of infections caused by rickettsia, mycoplasma and chlamydia. Tetracyclines are also used for the treatment of cholera and brucellosis and for treatment of infections caused by bacteria which have become resistant to other antibiotics.

Tetracycline drugs are active against many bacteria, including _____, _____, _____, and _____.

A PRECAUTION ABOUT THE USE OF ANTIBIOTICS

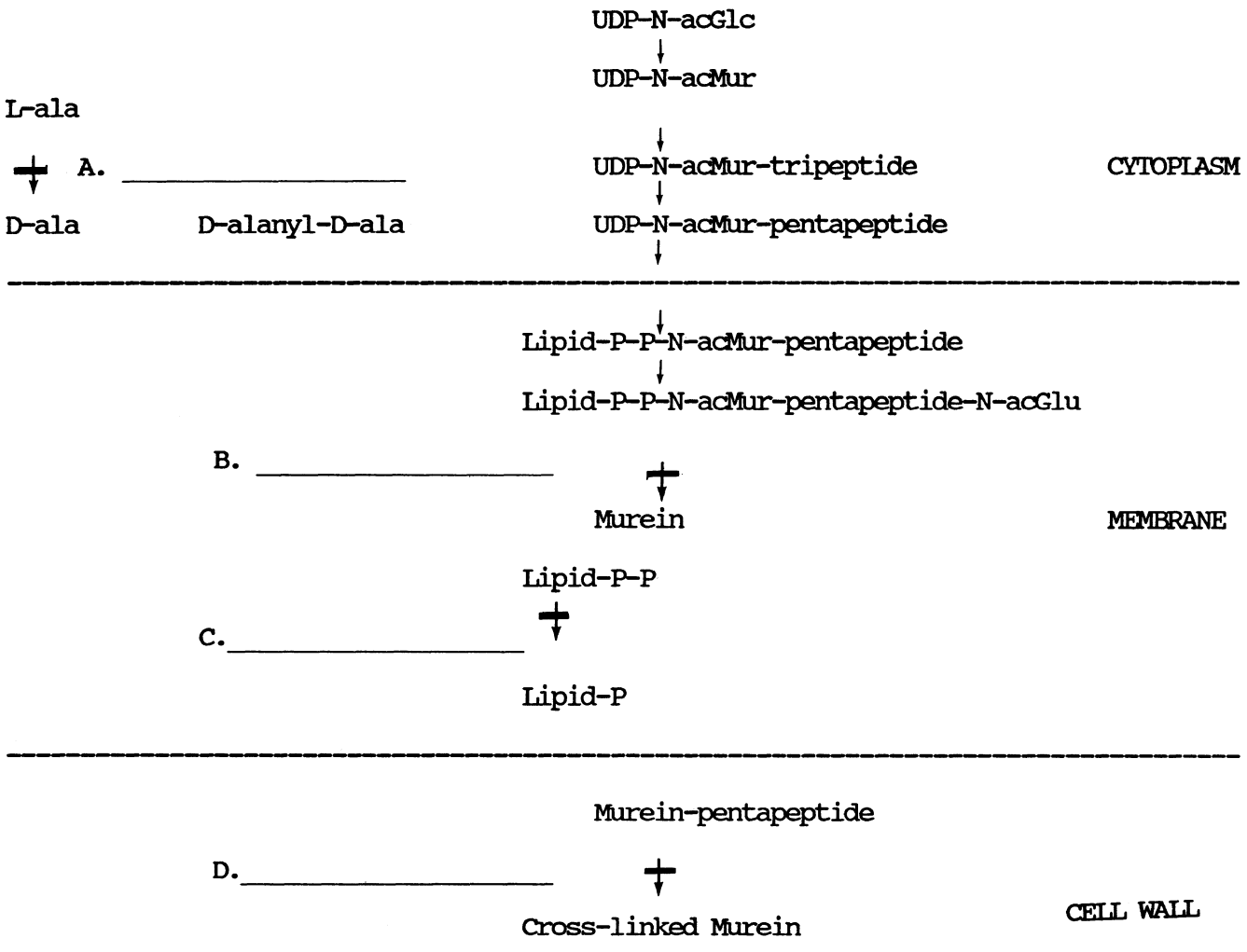
One of the serious side effects of therapy with the tetracyclines and to a lesser extent with penicillin and the aminoglycosides is superinfection by resistant organisms. Superinfection occurs in the gastrointestinal tract, oral cavity, and vagina, usually after oral administration of antibiotics. Resistant organisms which predominate in these cases are Staphylococcus aureus, Candida albicans, Pseudomonas and Proteus. Tetracyclines are also deposited in teeth during calcification and may produce a yellow stain when used in large doses in children.

REVIEW OF ANTIBIOTICS WHICH AFFECT CELL WALL SYNTHESIS

Fill in the blanks with the appropriate antibiotics. (Answers on next page)

A. _____ B. _____

C. _____ D. _____



CHLORAMPHENICOL

Activity
Chloramphenicol is a broad spectrum antibiotic which is bacteristatic for both Gram positive and Gram negative bacteria, rickettsia and chlamydia. Chloramphenicol is one of several antibiotics which bind to the 50S ribosome, others being the macrolide antibiotics such as erythromycin and the lincomycins.. Chloramphenicol's effect on protein synthesis appears to be the result of the interference with peptide bond formation; it inhibits peptidyl transferase of the 50S Ribosome.

Resistance
Resistance to chloramphenicol in Gram negative bacilli is also associated with the presence of an R factor which is responsible for the formation of an enzyme, chloramphenicol acetyl transferase, which catalyzes the formation of the mono- and diacetyl derivatives of chloramphenicol with acetyl coenzyme A resulting in inactivation of the drug.

Clinical use
It should be used primarily for typhoid fever, H. influenzae, meningitis, anaerobic infections and for those bacterial infections resistant to other drugs. Aplastic anemia associated with the use of chloramphenicol has restricted the use of this drug.

Clindamycin, although chemically unlike chloramphenicol, has a similar mechanism of action, i.e. it inhibits peptidyl transferase of the 50S ribosome and blocks petide bond formation. The major use for clindamycin is in therapy of infections caused by anaerobes.

REVIEW OF ANTIBIOTICS THAT INTERFERE WITH PROTEIN SYNTHESIS

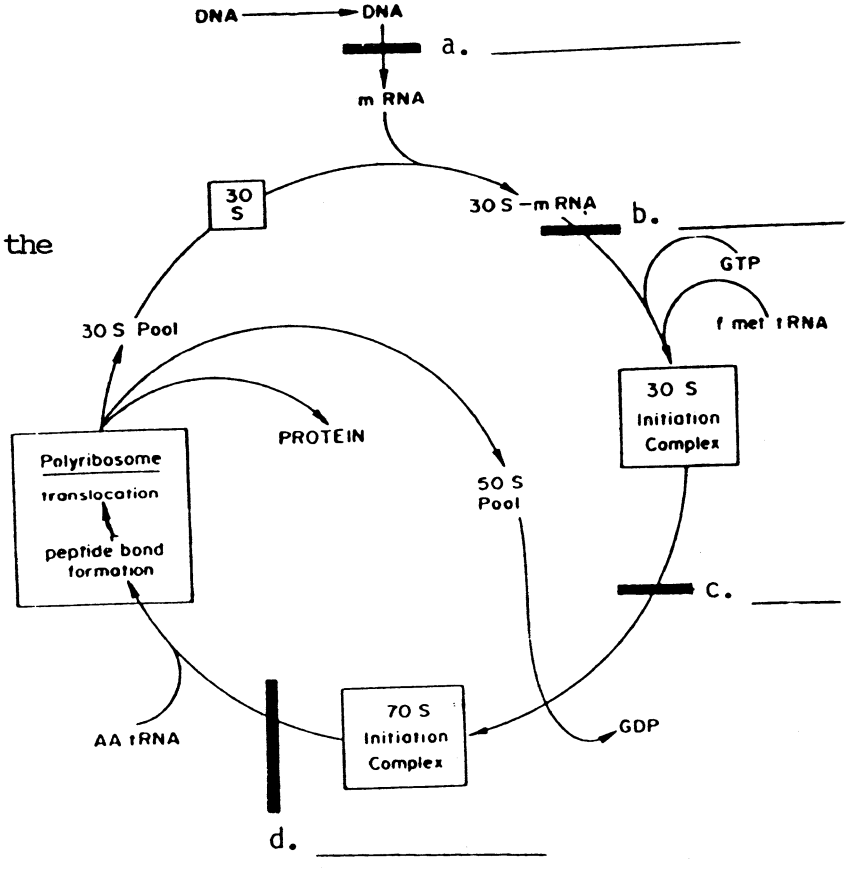
In the blank write the name of the appropriate antibiotic.
(answers on next page)

A. _____

B. _____

C. _____

D. _____



ANSWERS TO CELL WALL QUESTIONS
A=cycloserine
B=vancomycin
C=bacitracin
D=penicillin or cephalosporin

ERYTHROMYCIN

Structure
This is the most important of the macrolide antibiotics. These agents contain a macrocyclic lactone ring to which 1 or more sugars are attached.

Activity
Erythromycin reacts with the 50S ribosomal subunit and seems to block the translocation step in protein synthesis by inhibiting the release of charged tRNA from the donor site.

Resistance
May be either mutational or plasmid mediated. The chromosomal change which imparts resistance is due to a conformational change in one of the ribosomal proteins resulting in a decrease in drug binding. Plasmid-mediated resistance is due to methylation of an adenine residue in the 23S subunit, which reduces its affinity for the antibiotic. The modified ribosomes are cross-resistant to lincomycin and clindamycin, suggesting that these two non-macrolide antibiotics have a similar site of action to that of erythromycin.

Clinical use
This bacteristatic antibiotic is used as the primary drug for M. pneumoniae infections and for Legionella as well. It is also used against streptococci in patients allergic to penicillin.

GRISEOFULVIN

This is a fungistatic agent which is active against mycotic agents. It is used primarily in the treatment of dermatophyte infections. Treatment must be for a period of time (usually months) sufficient for skin or other infected tissue to be sloughed. Griseofulvin inhibits the assembly of proteins, thus it inhibits cell division by blocking the assembly of microtubules which are essential for chromosome movement during mitosis. It concentrates in keratinized layers of skin.

Chloramphenicol = bactericidal/static.

Erythromycin = bactericidal/static.

Fusidic acid is a steroid produced by the mold Fusarium. It binds to elongation factor G and blocks the growth of the peptide chain. It is used against Gram positive cocci especially penicillin-resistant Staphylococcus aureus.
[Where would Fusidic acid go in the table of page 23?]

Most antibiotics are bactericidal. Those that are not include clindamycin, chloramphenicol, tetracyclines, the sulfas and erythromycin.

Answers to protein synthesis antibiotics questions

A=rifamycin
B=tetracycline
C=aminoglycosides
D=chloramphenicol

INTERFERENCE WITH RNA SYNTHESIS

Rifampin

Activity
Rifampins are bactericidal for many species of Gram positive bacteria, Gram negative bacteria and Mycobacterium tuberculosis. Rifampins cause RNA synthesis to decrease. They inhibit the action of DNA-dependent RNA polymerase by binding to the polymerase which makes the enzyme inactive. They inhibit initiation of RNA synthesis.

Resistance
Resistance to rifampins is due to an altered B-subunit of RNA polymerase with a decreased ability to bind rifampin.

Clinical use
Rifampin is the most widely used of the rifamycins since, unlike the other rifamycins, it is readily absorbed from the intestinal tract. Its principal use is in the treatment of tuberculosis.

REVIEW QUESTIONS

Antibiotics which interfere with protein synthesis include:

1. _____
2. _____
3. _____
4. _____

The major bacteristatic antibiotics include:

1. _____
2. _____
3. _____

REVIEW OF PLASMID (R FACTOR) MEDIATED ANTIBIOTIC RESISTANCE

Group	Antibiotic	Mechanism of Resistance*
1.	Erythromycin Clindamycin	Methylation of ribosomal RNA keeps antibiotic from binding to ribosome
2.	Sulfonamides Tetracycline	Alteration of cell membrane decreases permeability to the antibiotic
3.	Chloramphenicol	Acetylation of the antibiotic inactivates it
4.	B-Lactams	B-lactamase hydrolysis breaks down antibiotic to an inactive form
5.	Aminoglycosides	Modifying enzymes cause a) acetylation b) adenylation c) phosphorylation

* In addition chromosomal mutations decrease susceptibility to erythromycin, streptomycin and rifampin by altering host cell proteins to decrease drug binding. Methicillin resistance is due to a change in a penicillin-binding protein (e.g. a transpeptidase) in the cell membrane.

Antibiotic Actions

Antibiotic	Synthetic Step Blocked	Level of Blockade
Bacitracin	Cell Wall Synthesis	Inhibits hydrolysis of lipid pyrophosphate
Penicillins		Blocks Transpeptidase
Cephalosporins		
Cycloserine		Competes with D-alanine
Nalidixic Acid	DNA Synthesis	Inhibits DNA gyrase
Fluoroquinolones		
Rifampin	RNA Synthesis	Inhibits DNA-dependent RNA polymerase
Aminoglycosides	Protein Synthesis	30S ribosomal subunit -blocks initiation
Amikacin		
Gentamicin		
Streptomycin		
Tobramycin		
Tetracycline		-blocks binding of aminoacyl tRNA
Chloramphenicol		50S ribosome: inhibits peptidyl transferase
Clindamycin		
Erythromycin		

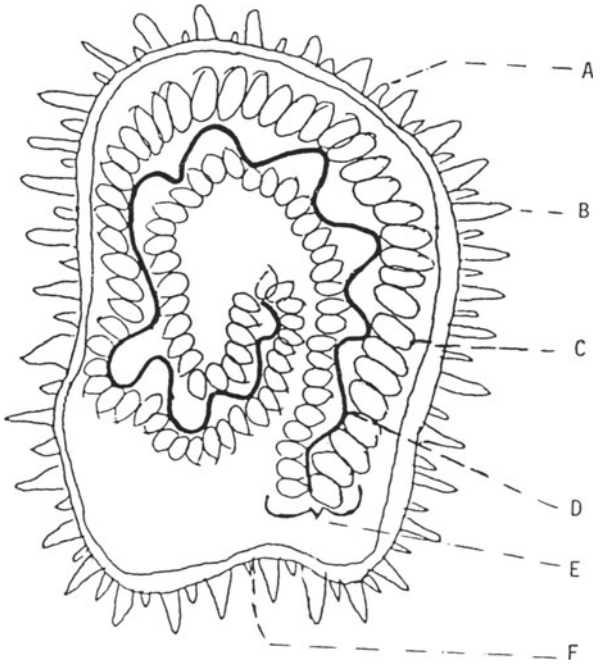
VIRAL STRUCTURE

The outermost component of a virion is the capsid, made up of protein subunits called capsomers. The capsid serves four important functions: (1) it protects the viral genome, (2) it aids in infection by attaching the virion to susceptible cells, (3) it is the stimulus for antibody production, (4) it serves as the antigen in serologic tests and (5) it is responsible for tissue tropism in naked viruses such as polio.

The viral genome, the other major component of every virion, is found inside the virus particle and may be either double-stranded or single stranded DNA, or single-stranded or double-stranded RNA. Once introduced into a susceptible cell, the viral genome provides the genetic information needed for production of new virions in a cell. The cell contributes cellular structures (ribosomes), energy, and enzymes for the synthesis of viral macromolecules. Since viruses lack most of these essential components, they must invade and make use of living cells in order to be replicated.

Animal virions are either naked or enveloped. A naked virion consists of nucleic acid enclosed in a protein shell known as the capsid; nucleic acid and capsid together are termed nucleocapsid. An enveloped virion in turn consists of a nucleocapsid surrounded by a structure called the envelope. The envelope consists of viral protein components (usually glycoproteins), and host cell-derived lipids and lipoproteins. Lipid solvents (e.g., ether) and detergents solubilize the envelope and inactivate the virus. The capsid and the envelope contribute antigens useful in vaccine development and in serologic tests.

label the components of the virus.



A. _____

B. _____

C. _____

D. _____

E. _____

F. _____

VIRAL CLASSIFICATION

All naked animal virus particles resemble icosahedra. Enveloped animal virions exhibit a large variety of shapes (symmetry). In many cases, a nucleocapsid that is distinctly icosahedral or helical, depending on the virus, is surrounded by an envelope which gives the particle the appearance of a sphere, e.g., influenza virus. Other enveloped animal viruses are shaped like a bullet, e.g., rabies virus; and still others look like bricks, e.g., poxviruses.

Taxonomic classification of viruses is based on the relatively constant physical and chemical properties of virions. Some of the criteria used for classification are (1) type of nucleic acid found in the virion (DNA or RNA) and whether the nucleic acid is single-stranded or double-stranded, (2) shape of the viral nucleocapsid (icosahedral or helical), (3) nature of the outermost viral component (naked or enveloped), and (4) antigenic properties.

Viruses are classified on the basis of

1. _____
2. _____
3. _____
4. _____

MAJOR HUMAN VIRUS GROUPS

<u>DNA viruses</u>

1. Herpes
   a. simplex I & II
   b. varicella
   c. cytomegalo
   d. Epstein-Barr
2. Hepadna (Hepatitis B)
3. Adeno
4. Pox
5. Papova
6. Parvo

The viral capsid has four functions:

1. _____
2. _____
3. _____
4. _____

Many viruses (e.g., the Herpes group, are inactivated by detergents and ether. This is because their outer membrane is rich in _____.

RNA viruses

1. Picorna
   a. Entero
      1. Polio
      2. Coxsackie
      3. ECHO
      4. Hepatitis A
   b. Rhino

2. Toga
   a. WEE, EEE, VEE
   b. Rubella

3. Flavi
   a. Dengue
   b. Yellow fever
   c. St. Louis

4. Arena (LCM)

5. Bunya

6. Rhabdo

7. Orthomyxo

8. Paramyxo
   a. Parainfluenza
   b. Mumps
   c. Measles
   d. Respiratory syncytial

9. REO
   a. Reo
   b. Rota
   c. Orbi

10. Corona

11. Retro
   a. Oncorna (HTLV-1)
   b. Lenti (HIV)

Uncertain NA type
1. Slow Viruses
2. Prions (protein only)
3. non A/non B Hepatitis
   [Hepatitis C is RNA;
   may be a Flavivirus]

The Enteroviruses are so called because they grow in the intestinal tract; however, they cause CNS, cardiovascular, hepatic, and muscular diseases. They get to the target tissues by hematogenous dissemination through blood hence circulating antibodies will protect against disease but have no effect on infection.

The German word "pech" can be translated "bad luck". It can also serve as a mnemonic for the Enteroviruses:

P = _____

E = _____

C = _____

H = _____

Non A/non B hepatitis is the most common cause of transfusion-induced hepatitis; this is because most of the blood used in hospitals has been screened for Hepatitis B. Hepatitis A is the most common hepatitis virus; why isn't it the number 1 cause of post-transfusion hepatitis?
[see hepatitis section for answer]

The table below is a helpful way to group the viruses. There are 6 families of DNA-containing viruses, Herpes, Hepadna, Adeno, Papova, Pox and Parvo (The HHAPPPy viruses). If one can remember these and the generalities associated with the DNA viruses, then any other virus will be the opposite. As nothing is ever that simple, the table also contains the exceptions to that rule, e.g., if all DNA viruses are Double stranded and Naked, then the RNA viruses should all be single stranded and enveloped; and they are with the exception of the REO (dsRNA) and Picorna (naked RNA) as noted in the table. The nucleic acid of all viruses is linear except for two Hepatitis viruses, B and D, and the Papovaviruses.

VIRUS CLASSIFICATION

Virus Type	Generalities	Exceptions
DNA Viruses	Double Stranded	Parvo
Herpes Hepadna Adeno Papova Pox Parvo	Naked	Herpes, HBV and Pox
	Nuclear site for Replication	Pox
	Icosahedral (cubic) symmetry	Pox (complex)
RNA Viruses*	Single Stranded	REO
Paramyxo Toga Picorna Corona Rhabdo Orthomyxo Arena Bunya Retro REO Flavi	Enveloped	REO and Picorna
	Helical symmetry	REO, Picorna, Retro and Toga
	Cytoplasmic site for replication	Orthomyxo and Retro (nuclear + cytoplasmic)

* The first 5 viruses listed are polycistronic single RNA strands; the remaining viruses have segmented genomes

VIRAL REPLICATION

STEPS	ACTIVITY
1. Adsorption	-virus attaches to specific receptors on cell membrane -interaction is, at first, reversible, then becomes irreversible
2. Penetration	-virus particle is actively taken up by cell through a process called pinocytosis or phagocytosis
3. Uncoating	-takes place at cell membrane or vesicles -viral nucleic acid is released inside of cell by cell host enzymes
4. Intracellular replication of viral components	
A. RNA viruses	-picorna and toga-viruses - the genome is +RNA and serves as mRNA -others (e.g., myxo [ortho & para], rhabdo) carry viral RNA dependent RNA polymerase which synthesizes +mRNA from viral -RNA strands. -early proteins are enzymes for viral RNA synthesis or inhibitors of cellular synthetic events. -late proteins are viral structural proteins and assembly proteins synthesized in response to the viral genome
B. DNA viruses	
1. RNA synthesis	-host cell supplies transcriptase, pox has its own DNA dependent RNA polymerase
2. Early protein synthesis	-synthesis of enzymes for DNA synthesis, tumor antigens, etc.

The first step in viral replication is _____.

Antibody to viral capsid/nucleic acid blocks this process.

Early viral proteins are _____ _____ _____;

late proteins are _____ _____ _____.

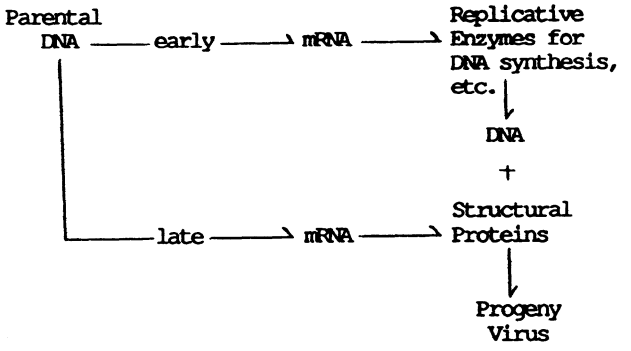
3. Viral DNA -many new copies of viral DNA synthesis

4. Late protein synthesis -viral capsid proteins(structural)

5. Assembly:
   - -newly synthesized viral nucleic acid and protein assembled inside cell
   - -viral envelope added (usually from cell membrane)
   - -new viruses released from cell by budding or lysis

6. Effects of Viruses on Cells
   - -lytic viruses inhibit cell RNA, DNA, protein synthesis
   - -tumor viruses transform cells
   - -latent viruses (herpes) probably do not alter host cell greatly

Replication of DNA viruses

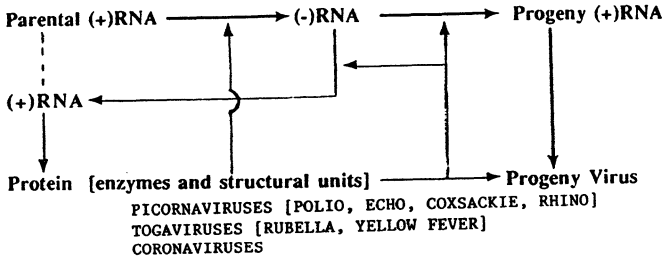


The template for early proteins in a Herpes infected cell is provided by <u>viral RNA/viral DNA via newly synthesized RNA.</u>

REPLICATION OF SPECIFIC RNA VIRUSES

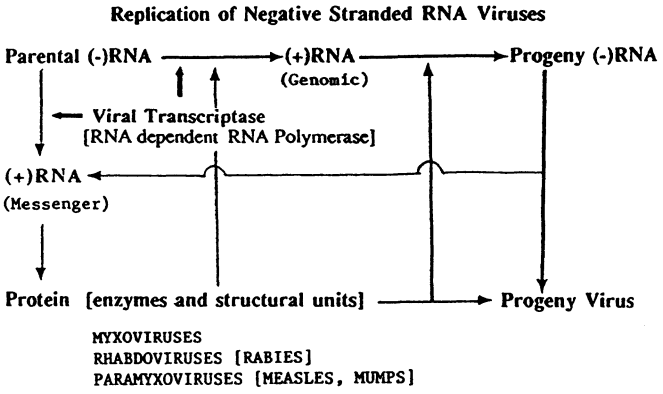
1. <u>Picorna</u>
   <u>Corona</u>
   <u>Togavirus</u>
   ex. polio
   rubella
   WEE
   - -viral RNA is single stranded piece of messenger RNA (+mRNA)
   - -naked viral RNA can infect cells
   - -viral RNA (+mRNA) gets to polysomes to make new viral proteins
   - -new proteins made as one long protein, then cleaved to viral specific proteins
   - -replicase (RNA-dependent RNA polymerase), an enzyme which copies +mRNA and makes a negative strand of mRNA
   - -the -mRNA is then used as template to make new viral RNA (+mRNA)

Replication of Positive Stranded RNA Viruses



The template for poliovirus RNA is <u>+mRNA/-mRNA</u>.

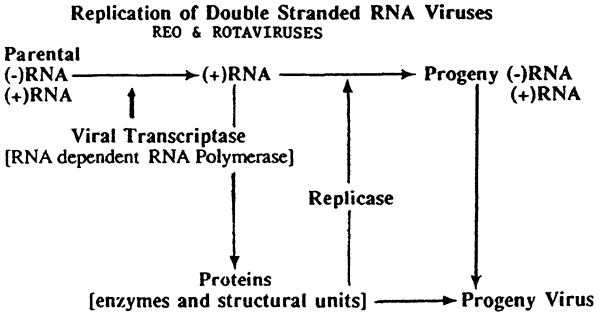
2. Orthomyxovirus
(influenza virus)
-viral RNA is fragmented into 8 pieces of (-mRNA)
-virus carries into cell a transcriptase enzyme (RNA dependent RNA polymerase)
-transcriptase copies (-mRNA) to make (+mRNA) for viral proteins
-(+mRNA) is also template for new viral (-mRNA)



3. Paramyxoviruses and Rhabdoviruses
(mumps, measles, rabies)
-viral RNA is single-stranded (-mRNA), not segmented
-virus carries transcriptase like the influenza virus; similar replication cycle

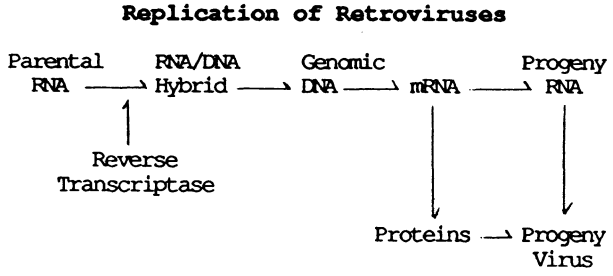
The RNA dependent RNA polymerase found in influenza virus is also called _____;
it converts -RNA into +RNA template for protein synthesis.

4. Diplornavirus
(reovirus and rotovirus)
-viral RNA is double-stranded and composed of ten fragments
-virus carries transcriptase



5. Retroviruses
(RNA tumor virses)
-viral RNA is single stranded
-virus carries reverse transcriptase (RNA-dependent DNA polymerase)
-transcriptase copies viral RNA into DNA
-DNA is integrated into host genome and serves as template for new viral RNA

The template for mumps capsid is provided for by viral RNA/newly synthesized RNA.



REPLICATION OF SPECIFIC DNA VIRUSES

1. Herpesvirus
   - -replicates inside nucleus
   - -viral envelope obtained from nuclear membrane

2. Adenovirus
   - -replicates inside nucleus

3. Poxvirus
   - -replicates in cytoplasm; complex
   - -virus carries many enzymes into cell

4. Papovavirus
   - -replicates in nucleus, circular DNA

RNA dependent RNA polymerase is a part of the following viruses

1. _____
2. _____
3. _____
4. _____

VIRAL ENZYMES

VIRUS	ENZYMES
Negative RNA ex orthomyxo paramyxxo rhabdo	RNA polymerase
Retrovirus	Reverse transcriptase RNAse H [breaks down RNA in RNA-DNA strand] Polynucleotide ligase [closes DNA breaks]
Pox	More than a dozen e. g., DNA-dependent RNA polymerase RNA methylase DNAse
Herpes	Thymidine kinase* DNA polymerase*
Reoviruses including Roto	RNA polymerase
Hepatitis B	DNA-dependent DNA polymerase Protein kinases

* produced in cell not brought in with virion

ANTIVIRAL AGENTS

INTERFERENCE WITH VIRAL REPLICATION

1. Interferon (IFN)
   - -a group of proteins made by cells in response to viruses, synthetic nucleotides (poly r:IC), foreign cells that interferes with viral replication

Interferon produced by a macrophage would be an alpha/beta interferon

IFN species
leukocyte (alpha), fibroblast (beta), lymphocyte (gamma/immune)

- -cell genome has information for IFN: if one inhibits cell metabolism with actinomycin D, no IFN produced
- -IFN induces cells to make products which inhibit viral or foreign cell replication
- -IFN released from cells, spreads to other cells and induces new IFN

Interferon is effective against

A. DNA viruses

B. RNA viruses

C. Both

D. Neither

(answer at bottom of page)

IFN induced products

1. **2'5'A synthetase** which polymerizes ATP into an oligonucleotide that activates RNAse L which degrades viral RNA
2. **eIF-2α kinase** which autophosphorylates in the presence of dsRNA. The modified kinase then phosphorylates eukaryotic initiation factor-2 (eIF-2) which inhibits viral mRNA translation

Biologic Activities of interferon

1. antiviral
2. Inhibition of cell growth
3. Immune modulation
   a. NK
   b. ADCC
   c. macrophage activation

Interferon inhibits viral replication by inducing cellular production of

1. _____
2. _____

both of which interfere with viral mRNA activity.

2. Specific Antibody
   1. IgG, A, M
   2. Neutralize by interfering with the functions of viral capsid or envelop proteins. The antibodies block
      - absorption
      - penetration
      - uncoating (rare)

(answer = C)

3. CHEMICAL INHIBITORS

a. **Amantidine** and **rimantidine** both inhibit viral uncoating that follows viral entry into the cytoplasm of the cell. They may also interfere with transcription of viral RNA. They are used against influenza strain A viruses; strain B is resistant.
b. **Idoxuridine** and **trifluorothymidine** are halogenated pyrimidines that are able to block DNA synthesis because they are incorporated into DNA in the place of thymidine. They are very toxic and used mainly against herpetic keratitis.
c. **Adenine arabinoside** (Ara-A) inhibits DNA viruses, especially Herpes. It must be phosphorylated intracellularly before it becomes an inhibitor for **DNA polymerase**. Less toxic than idoxuridine.
d. **Dideoxycytidine** and **dideoxyinosine** are phosphorylated intracellularly to their triphosphate form and are then able to block viral reverse transcriptase, thus interfering with **HIV** replication.
e. **Acyclovir** is very active against Herpes simplex, less so against zoster and Epstein Barr viruses and inactive vs cytomegalovirus. It is phosphorylated intracytoplasmically by **viral thymidine kinase** (which explains its spectrum of activity) into a monophosphate which is further phosphorylated by cellular kinase to the active triphosphate form which binds to and inhibits viral **DNA polymerase**.
f. **Gancylovir** is an analog of acyclovir that is active against all herpes viruses. It is used primarily for **cytomegaloviral infections**; this virus does not encode a thymidine kinase and hence is resistant to acyclovir. The cellular kinases phosphorylate gancyclovir to its active form that blocks DNA polymerase. There is significant toxicity associated with gancyclovir; it suppresses spermatogenesis, bone marrow precursors, and gut epithelium.
g. **Azidothymidine** inhibits **HIV** replication by inhibiting reverse transcriptase and causing termination of viral DNA strand elongation. It is phosphorylated by cellular kinases to its active triphosphate form.
h. **Ribavirin** is active against both DNA and RNA viruses. It interferes with viral nucleic acid synthesis by unclear mechanisms. It is used in aerosol form to treat **Respiratory Syncytial Viral infections** in infants.

Antiviral drugs activated in vivo by cellular kinase enzymes that phosphorylate the drug to an active form include

1. _____
2. _____
3. _____
4. _____
5. _____

The DOC (drug of choice) for RSV is _____.

The DOC for Herpes simplex is _____.

Amantadine is used to treat _____.

HIV infections can be treated with

1. _____

VIRAL IMMUNOTHERAPY AND PROPHYLAXIS

I. Active, Artificial Immunity

A. Recommended for all persons in US

Disease	Condition of Vaccine
1. Rubella*	live attenuated
2. Measles*	live attenuated
3. Mumps*	live attenuated
4. Polio	
(Sabin)**	live attenuated
(Salk)**	inactive

Live viral vaccines are NOT recommended for immunosuppressed individuals

B. Recommended for special conditions (epidemic, military, travel, exposure)

Disease	Condition of Vaccine
1. Rabies***	inactive
2. Yellow Fever	live attenuated
3. Influenza**	inactive
4. Adenovirus**	active
5. Togavirus	inactive
6. Chickenpox	live attenuated
7. Hepatitis B	inactive

MMR vaccine combination consists of

1. _____
2. _____
3. _____

*May be combined; all are a single antigenic type
**May be polyvalent
***Usually given with immune serum

Live viral vaccines induce immunity of long duration.

II. PASSIVE, ARTIFICIAL IMMUNITY-use of immune serum or gamma globulin. Used only under special circumstances.

1. Rabies
2. Rubella
3. Hepatitis A and B
4. Rubeola
5. Mumps
6. Chicken Pox
7. Polio

This type of treatment may be effective in disease prevention; it is of little value after onset of disease.

Polyvalent viral vaccines include

1. _____
2. _____
3. _____

III. New approaches to acquired immunity is the use of
1. viral subunit particles
2. synthesis of viral peptides:
   a. in bacteria via cDNA technology
   b. by other, non-pathogenic hybrid viruses (e.g. vaccinia)

REVIEW STATEMENTS

These should be used to strengthen and expand you understanding of Microbial Physiology. The statements are "factoids" that may help in the STEP 1 exam. You may wish to develop your own list to expand your knowledge base. If you have a spare sheet of paper available, white down the correct statement for every question you miss in going through review exams. This way you can avoid marking on the review exam (so you can use it again) and still have captured that fact for future review.

Flagella are the organelles responsible for motility of bacteria. Their direction of rotation controls movement; counterclockwise=forward (positive) chemotaxis.

Pili are short hair-like protein structures which occur on the surface of bacteria. Some, for example, those that occur on the gonococcus, are thought to be associated with the **adherence** of the organism to host cells. The sex pilus, which is found only in male strains of bacteria, is involved in **conjugation**.

Capsules of most species of bacteria are composed of **carbohydrate**, with the exception of *Bacillus anthracis*, which has a capsule composed of poly-D glutamic acid. In many pathogenic microorganisms the capsule **inhibits phagocytosis.**

Three kinds of proteins are associated with the **cytoplasmic membrane** of bacteria. They include **biosynthetic enzymes, transport proteins** and **cytochrome enzymes,** which serve the following functions in the cell: synthesis of external layers of the cell, transport of water-soluble materials into the cytoplasm, and electron transport activities, respectively.

Spores are highly resistant to deleterious agents in the environment, probably because of the content of **keratin-like proteins** in the spore coat, and their low metabolic rate.

The **periplasmic space** and outer layer are unique to the Gram negative bacteria, as are **porin channels** in the outer membrane. **Teichoic acids** are unique to Gram positive microorganisms.

Bacterial resistance to antibiotics is usually conferred by **plasmids** (R factors) which induce **production of enzymes** with modify the antibiotic.

A complication of **clindamycin** therapy is **enterocolitis** caused by *Clostridium difficile.*

Chloramphenicol is useful in the treatment of typhoid fever and *Haemophilus influenzae* meningitis.

Penicillin and **cephalosporin** inhibit **transpeptidation**, the final reaction of mucopeptide synthesis. Bacterial resistance to these two antibiotics is usually the result of a **β-lactamase**, whose production is governed by a **plasmid.** Penicillin-destroying enzymes from Gram positive bacteria are relatively inactive against 2nd and 3rd generation cephalosporins.

Rifampin inhibits the action of DNA dependent RNA polymerase by binding to the polymerase.

Sulfonamides are **bacteriostatic** drugs which inhibit the condensation of pteridine pyrophosphate with PABA: they block dihydrofolate synthetase; **trimethoprim** blocks the action of dihydrofolate reductase. An analog of PABA which has a similar mechanism of action is **para-amino salicylic acid**, which is used in the treatment of tuberculosis. Sulfanilamide is relatively non-toxic for humans because we are unable to synthesize **folic acid.**

Streptomycin causes damage to the eighth cranial nerve.

Tetracyclines are broad spectrum agents that bind the 30S ribosome and inhibit binding of aminoacyl tRNA.

Chloramphenicol and the **tetracyclines** are both broad specrum antibiotics which inhibit protein synthesis; they differ in that chloramphenicol attaches to the 50S ribosomal subunit while tetracyclines attach to the 30S subunit. Chloramphenicol interferes with protein synthesis by inhibiting peptide bond formation (**peptidyl transferase).**

Cessation of bacterial growth is caused by toxic metabolic end products, unfavorable pH, and nutrient exhaustion.

Methicillin and **oxacillin** are used for penicillin-resistant strains of bacteria.

Lipid A, core and O antigen are all parts of lipopolysaccharide(endotoxin); **lipid A is toxic.**

Drugs which affect the 30S ribosome are the **aminoglycosides** and **tetracyclines.**

D-Cycloserine, vancomycin and **bacitracin** are all inhibitors of peptidoglycan synthesis.

Surfactants, particularly **cationic detergents** (e.g., quaternary compounds such as zephiran) act by **disrupting the cell membrane** or viral envelop.

Phenol is both a detergent and an effective protein denaturant. **Alcohols** such as ethanol and isopropyl alcohol also are bactericidal due to **protein denaturant** activity.

Pseudomonads are highly **resistant to antibiotics** because 1) their porin channels limit the passage of water soluble molecules, and 2) highly active antibiotic inactivating enzymes are produced by these organisms.

Metronidazole is useful against *Giardia, Trichomonas* and anaerobic bacteria. It is coverted to its active form inside the parasite by low redox potential compounds such as ferredoxin.

The **protein coat** (capsid) of true viruses functions to **maintain infectivity** of nucleic acid in the extracellular state, serves as an antigen in **vaccines,** and **aids in the penetration** of virions into susceptible cells. **Cell susceptibility** range of a virus is determined by surface protein units of the capsid or envelope

One of the first events which occurs after a virulent virus infects a cell is **cessation of host cell macromolecular biosynthesis.**

IUDR (5 iodo-2' deoxyuridine) is incorporated into viral DNA to produce faulty nucleic acid.

The **picornaviruses** are single strands of (+) RNA. During replication replicative intermediates (RI=double stranded RNA; a +RNA and a -RNA) are formed by the enzyme **replicase.** Orthomyxoviruses and paramyxoviruses also have an RI; however, these viruses have a (-) RNA as the parental type. Viral transcriptase (**RNA dependent RNA polymerase**) makes (+) RNA used as mRNA for protein synthesis. **Orthomyxoviruses** have eight distinct (-) RNA strands; **paramyxo's** only have one. **Reovirus** RNA is double stranded RNA which exists in 10 distinct segments.

The capsid proteins in the **progeny of a hybrid virus** (e.g.polio RNA in a coxsackie capsid) will be encoded by the genetic information of the nucleic acid donor.

Replication of **HIV** would be blocked by inhibitors of **DNA-dependent RNA polymerase.**

Varicella virus gets its envelop from the **nuclear** membrane.

Picornaviruses use specific **viral proteases** to produce mature **viral proteins** from precursor polyproteins.

Viral envelopes are important for viral **adsorption and entry** into cells.

Viral uncoating occurs after the virus has gained entry into the cell.

Acyclovir is effective against several **Herpes viruses**; it is activated in the cell by **phosphorylation** catalyzed by a **virally-encoded enzyme.**

Ganciclovir is the drug of choice for **cytomegalovirus**

Ribavirin by aerosol route is the recommended therapy for **Respiratory Syncytial Virus** infection in neonates.

MICROBIAL PHYSIOLOGY REVIEW EXAM

SELECT THE SINGLE BEST COMPLETION FOR EACH QUESTION .

1. If a composite virus contains the RNA from Hepatitis A and the capsid of Hepatitis C

   A. only Hepatitis C progeny would be formed
   B. the composite virus would reproduce itself to form new composite virus
   C. the host-cell range of the composite virus would be the same as that of Hepatitis A
   D. the host-cell range of the progeny would be the same as that of Hepatitis A
   E. the host cell range of the progeny would be the same as that of Hepatitis C

2. Cytomegalovirus infections are treated most efficiently with

   A. acyclovir.
   B. amantadine.
   C. azidothymidine
   D. ganciclovir.
   E. ribavirin.

3. *Staphylococcus aureus* isolated from hospitals is usually penicillin resistant whereas *S. aureus* isolated from the community at large is usually penicillin sensitive. The reason for this difference is

   A. hospital personnel usually are colonized with penicillin-sensitive strains.
   B. hospital strains mutate forming penicillin-resistant strains which survive better than sensitive strains.
   C. hospital strains acquire a plasmid which carries the structural gene for penicillinase.
   D. non-hospital strains do not form β-lactamase because it is an inducible enzyme which is only formed in the presence of penicillin.

4. A structure which contains lipid A, core polysaccharide and O antigen would occur in an organism

   A. with a periplasmic space.
   B. with ribitol teichoic acid in its cell wall.
   C. which forms spores.
   D. which is Gram positive.
   E. with flagella.

5. Ribavirin

   A. is best used together with AZT to treat AIDS.
   B. inhibits viral attachment.
   C. must must be phosphorylated intracellularly to be active.
   D. is used in aerosol form to treat respiratory syncytial virus infections in infants.

6. A β–lactamase inhibitor that can be co-administered with penicillin to render a penicillin resistant Staphylococcus species susceptible to the antibiotic is

   A. lysozyme.
   B. clavulanic acid.
   C. cephalosporinase.
   D. penicillin-binding protein.

7. What is the basis of the specific toxicity of ketoconazole?

   A. Bacteria lack sterols in their membranes
   B. The primary sterol in fungal membranes is ergosterol
   C. The presence of the endo-plasmic reticulum in mammalian cells
   D. Humans require folic acid preformed

8. N-acetyl glucosamine and N-acetylmuramic acid are fundamental building blocks for growth. They are a part of which component of the bacterial cell?

A. outer membrane.
B. inner membrane.
C. lipopolysaccharide.
D. peptidoglycan.
E. capsule.

9. Most disinfectants eliminate infectious organisms by

A. bacteristatic mechanisms
B. reducing the microbial flora to a federally accepted level.
C. killing the vegetative cells.
D. killing vegetative cells and spores.

10. Tetracyclines are useful drugs which

A. prevent RNA synthesis.
B. are bactericidal.
C. prevent binding of aminoacyl tRNA to the 30S ribosome.
D. bind to the L12 subunit of the 50S ribosome.

11. Introduction of a naked viral genome in the cytoplasm is infectious (leads to a productive infection) with which virus?

A. adenovirus
B. HIV
C. poliovirus
D. parvovirus
E. mumps virus

12. Several antivirals are nucleotide analogs, including both:

A. IUdR and acyclovir
B. acyclovir and interferon
C. amantadine and azidothymidine
D. cyclophosphamide and vidarabine

13. Interferon

A. interferes directly with translation of viral RNA.
B. blocks penetration of viruses into susceptible cells.
C. induces host cells to produce antiviral proteins that interfere with translation of viral messenger RNA.
D. interferes with viral absorption onto cell membranes.

14. Which three antibiotics bind the bacterial 50s ribosome?

A. Tetracycline, streptomycin and bacitracin
B. Erythromycin, chloramphenicol and lincomycin
C. Tobramycin, gentamicin and amikacin
D. Bacitracin, vancomycin and D-cycloserine

15. A unique feature of eucaryotic cells that is not found in procaryotic cells is a

A. nuclear membrane.
B. periplasm.
C. peptidoglycan layer.
D. 70s ribosome.

16. Incorporation of uracil into bacterial nucleic acids would be inhibited by which of the following antimicrobials?

A. Penicillin
B. Streptomycin
C. Chloramphenicol
D. Rifampin
E. Tetracycline

17. The outer membrane of a Gram negative bacteria is resistant to the action of detergents largely due to the presence of

A. lipoteichoic acids.
B. phosphotidyl inositol.
C. cholesterol and ergosterol.
D. lipoprotein and lipopolysaccharide.
E. glycoproteins and the capsule.

18. A patient develops a fever, goes into shock and dies in the hospital. Upon performing a blood culture you find that the paitent had a Gram negative sepsis. What is the likely cause of the paitent's fever and subsequent demise?

A. Peptidoglycan components that are released into the blood
B. The presence of pili on the bacterial cell
C. The presence of lipopolysaccharide in the outer membrane
D. The release of lipoprotein by the bacterial cell on autolysis

19. The membrane bound chemoreceptors of the chemotactic system undergo what type of modification which is the trigger for chemotaxis?

A. phosphorylation
B. methylation
C. acetylation
D. dephosphorylation
E. proteolytic cleavage

20. What antifungal agent interferes with the biosynthesis of ergosterol?

A. Amphotericin B
B. Ketoconazole
C. Polymyxin
D. Nystatin
E. potassium Iodide

21. Cycloserine is a water soluble antibiotic with a molecular weight of 102. You would predict that it would

A. arrive in the periplasmic space by diffusion through the porin pores of the outer membrane.
B. arrieve in the periplasmic space by diffusion through the phopholipid region of the outer membrane.
C. be transported through the cell membrane by the phosphotransferase system.
D. Pass through the cell membrane by simple diffusion.
E. probably not pass through the cell membrane

22. Chloramphenicol is more effective against prokaryotes than eukaryotes because it

A. inhibits prokaryotic DNA-directed RNA polymerase.
B. inhibits protein synthesis by 70S, but not by 80S ribosomes.
C. activates a cell wall autolysin.
D. causes misreading of UUU codon in mRNA.
E. inhibits DNA synthesis by prokaryotes.

23. Streptomycin

A. inhibits peptidyl transferase.
B. prevents initiation of protein synthesis by ribosomes of prokaryotes.
C. disrupts cell membranes which contain sterols.
D. binds to 50S ribosomes such that it inhibits peptide bond formation.
E. prevents binding of mRNA to the ribosome.

24. One important reason why trimethoprim and sulfamethoxazole together are more effective against bacteria than the sum of their individual activities is because

A. both inhibit dihydropteroic acid synthetase.
B. both inhibit dihydrofolate reductase.
C. trimethoprim is bacteristatic and sulfamethaxazole is bactericidal.
D. trimethoprim inhibits cell wall synthesis and sulfamethoxazole inhibits folic acid synthesis.
E. both inhibit folic acid synthesis, but inhibit different steps.

25. During herpesvirus replication:

A. Viral capsid proteins mediate cell attachment
B. Viral regulatory proteins and enzymes are made in immediate early phase
C. Late transcription takes place in the cytoplasm
D. DNA is replicated by host cell DNA polymerase

26. Prokaryotes contain which of the following structures?

A. Nuclear membrane
B. Histones
C. Endoplasmic reticulum
D. Ribosomes
E. Mitochondria

27. Virus envelopes

A. Provide protection for viruses during tansit through the stomach and intestine.
B. contain the transcriptases necessary for production of mRNA in host cells.
C. contain subunits called capsomeres and protomers.
D. are important for virus adsorption and entry into cells.

28. During adenovirus replication

A. Capsid proteins attach to receptors on the nuclear membrane
B. Viral DNA is replicated by cellular DNA polymerase
C. Late and early transcription takes place in the cytoplasm
D. Capsid proteins are made during immediate early phase

29. A structure which contains lipid A, core polysaccharide and O antigen would occur in an organism

A. with a periplasmic space.
B. with ribitol teichoic acid in its cell wall.
C. which forms spores.
D. which is Gram positive.
E. with flagella.

30. Actinomycin D selectively blocks DNA dependent RNA polymerases. This drug would be expected to inhibit which of the following viruses?

A. Hepatitis A virus.
B. Hepatitis E virus.
C. Retrovirus.
D. Togavirus.

DIRECTIONS (ITEMS 31-38): Each of the numbered items or incomplete statements in this section is negatively phrased, as indicated by a capitalized word such as **NOT, LEAST**, or **EXCEPT**. Select the **ONE** lettered answer or completion that is **BEST** in each case and fill in the circle containing the corresponding letter on the answer sheet.

31. All of the following antiviral agents would be effective against DNA viruses **EXCEPT**

A. interferon.
B. amantadine.
C. iododeoxyuridine.
D. ribavirin.
E. adenosine arabinoside.

32. All of the following are properties of acyclovir **EXCEPT**

A. effective in inhibiting Herpesvirus replication.
B. can be administered orally and intravenously.
C. blocks penetration of virus into host cells.
D. activity based upon viral thymidine kinase.

33. Resistance to tetracycline is a common trait in bacteria largely due to the fact that tetracycline is used to promote weight gain in beef cattle and it is an over-the-counter drug in most third world countries. Therefore many different mechanisms of resistance to tetracycline have evolved in the bacteria. What is **NOT** a mechanism of resistance to tetracycline?

A. Efflux of tetracycline from the cell by a specific transport protein
B. Modification of ribosomes so the drug will not bind
C. The presence of porin channels in the outer membrane
D. Modification of tetracycline by a specific enzyme that inactivates it

34. If you were treating a patient with a Mycoplasma pneumonia what antibiotic would you clearly **NOT** use?

A. streptomycin
B. erythromycin
C. tetracycline
D. penicillin

35. Actinomycin D selectively blocks DNA dependent RNA polymerases. This drug would be expected to inhibit all of the following **EXCEPT**

A. Polyomavirus.
B. Hepatitis B virus.
C. Togavirus.
D. Retrovirus.

36. All of the following are true about the effect of penicillin G on *Streptococcus pneumoniae* **EXCEPT**

A. most pronounced when added during the exponential phase of growth.
B. results in death of the streptococci.
C. prevent formation of peptidoglycan cross links.
D. depends on the presence of an autolysin.
E. dissolves the outer membrane of the cell.

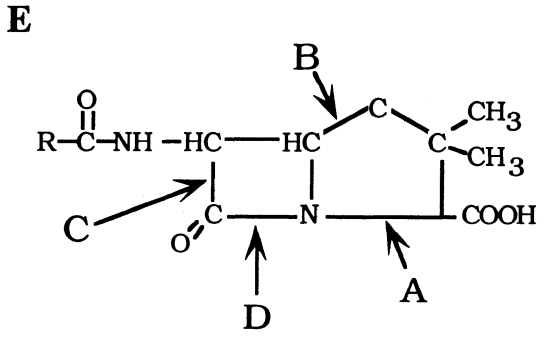
37. If you were treating a patient with a *Mycoplasma* pneumonia, what antibiotic would you clearly **NOT** use?

A. Streptomycin
B. Erythromycin
C. Rifampicin
D. Chloramphenicol
E. Penicillin

38. All of the following antiviral agent would be effective against DNA viruses **EXCEPT**

A. interferon.
B. amantadine.
C. iododeoxyuridine.
D. ribavirin.
E. adenosine arabinoside.

Questions 39-40
The antibiotic pictured is penicillin. Select the area of the molecule that best matches each statement below



39. In a penicillin resistant *Staphylococcus aureus*, the antibiotic is inactivated by hydrolysis at which point?

40. Resistance to the action of penicillinase, as well as changes in the spectrum of sensitivity of microbes to the antibiotic, is conferred by modification of the above molecule at what site?

MATCH THE THERAPEUTIC AGENT WITH THE PATIENT DESCRIBED BELOW.
AN ANSWER MAY BE USE MORE THAN ONCE, OR NOT AT ALL.

A.	Penicillin	G.	Isoniazid
B.	Streptomycin	H.	Erythromycin
C.	Oxacillin	I.	Trimethoprim-sulfamethoxazole
D.	Metronidazole	J.	Clavulanic acid
E.	Ribavirin	K.	Azidothymidine
F.	Acyclovir	L.	Flucytosine

41. This 12 year old young lady has cardiac abnormalities consistent with Rheumatic carditis. Three weeks following a routine visit to her oral hygienist, she develops fever and chills. She develops splinter hemorrhages in her nail beds, and is hospitalized with a diagnosis of subacute bacterial endocarditis.

42. A Marine sargent reports to the Infirmary with a low grade fever and a non-productive cough. Her serum agglutinates Streptococcus strain MG.

43 A young kindergarten student develops otitis media after a ski trip with her family. Culture yielded a gram positive coccus in short chains which grew on blood agar with alpha hemolysis. Growth was inhibited by optochin.

44. A 24 year old third year Medical student came to the student health service for a check-up after a serious exposure to an AIDS patient with tuberculosis. His lungs were clear to auscultation; no lesions were seen on X-ray. His ppd skin test was negative; however on re-testing 3 weeks later a reaction measuring 14 mm of induration was observed.

45. A 14 week old infant is admitted to the hospital for a severe respiratory infection. Histological examination of exfoliated cells in repiratory secretions reveals numberous syncytial masses.

46. A 12 year old girl scout has been hospitalized with a diagnosis of meningitis. The spinal fluid contains numerous PMNs and gram negative coccal forms. The organism is cultured on Thayer Martin medium and is found to ferment both glucose and maltose.

47. This three year old presents with a high fever and a stiff neck. Spinal fluid analysis reveals an elevated opening pressure, pleocytosis, a decrease in glucose content (20% of blood glucose levels), increased levels of immunoglobulins, and *H. influenzae* type b capsular carbohydrate antigen.

48. This 19 year old pom pom girl has moderate flu-like symptoms and a sore throat. Her serum contains antibodies that agglutinate sheep erythrocytes.

49. The child brought to the hospital Emergency room is in great respiratory distress. She has a fever of 40.2 C and is producing blood tinged sputum. A gram stain of the sputum reveals numerous neutrophils and gram positive cocci in random clusters.

50. This 49 year old pidgeon breeder has been experiencing intermittent frontal headaches and malaise. His wife has noted that he seems disoriented and irrational at times. Cerebrospinal fluid contains numerous lymphocytes and a few phagocytic cells containing budding yeast cells.

KEY

1. D	11. C	21. A	31. B	41. A
2. D	12. A	22. B	32. C	42. A
3. C	13. C	23. B	33. C	43. A
4. A	14. B	24. E	34. D	44. E
5. D	15. A	25. B	35. C	45. E.
6. B	16. D	26. D	36. E	46. A
7. B	17. D	27. D	37. E	47. B
8. D	18. C	28. A	38. B	48. F
9. C	19. B	29. A	39. D	49. C
10. C	20. B	30. C	40. E	50. L

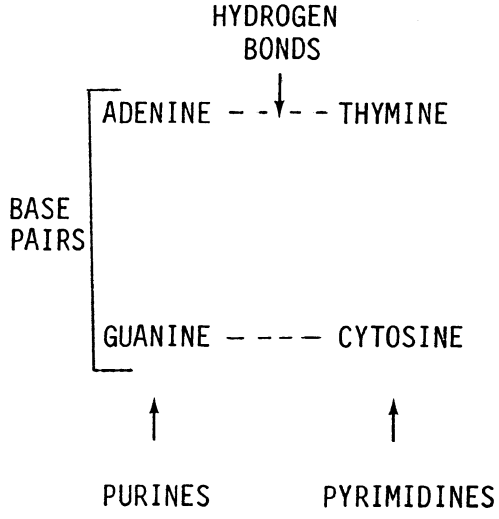
MICROBIAL GENETICS

MUTATIONS

The term mutation refers to an abrupt and usually stably inherited change in properties of an organism. Mutations can occur either spontaneously, or may be induced by a mutagen. (see table, pg. 47)

Mutation in Populations

The proportion of mutants in a given population of cells is called the mutant frequency. The probability that a mutation will occur during a particular time interval, such as the generation time, is the mutation rate, and is expressed as a mutation per cell, per division.



Selection of Mutants

Wild type is the designation given to strains as they are found in nature, or to certain laboratory standard strains. Mutants, called auxotrophs, have an additional nutritional requirement. Markers are other detectable mutations with a distinctive observable effect on the organism serving to mark the chromosome at the locus at which it occurs. Such markers include colony morphology, fermentation patterns and antibiotic resistance.

Point Mutations

Point mutations may occur as a result of a base pair substitution in the specific nucleotide sequence of a gene. When one purine on a chain replaces another purine, and when the pyrimidine on the other chain is replaced by a different pyrimidine, the substitution is called a transition.

Transversions occur when a purine in a DNA chain is replaced by a pyrimidine, and when the pyrimidine on the other chain is replaced by a purine.

Transitions may be produced by agents such as 5-bromouracil or 2-aminopurine. Alkylating agents such as ethylethane-

DEFINE THE FOLLOWING POINT MUTATIONS (Answers on next page)

1. GC changes to AT
2. GC changes to TA
3. CG changes to AT
4. TA changes to GC

sulfonate also cause transitions. Nitrous acid oxidatively deaminates the amino-substituted bases; adenine, guanine, and cytosine.

A missense mutation occurs when the above substitution occurs and the triplet code is altered such that a different amino acid is inserted into the protein. The product may be inactive or only partially active.

A nonsense mutation occurs when the above substitution occurs and the triplet code is altered such that a chain termination codon (UAG, UAA, or UGA) appears.

Frameshift mutations are those mutations which result in an addition or deletion of a nucleotide into a sequence of mRNA cause a reading frameshift of the trinucleotide sequences. Frameshift mutations may result in an addition or deletion of one, two, four or five nucleotides, all of which result in a shift of the reading frame. Frameshift mutations are known to be caused by a group of polycyclic compounds called acridines (e.g., proflavin). Acridines are capable of strong binding to DNA by intercalation between adjacent base pairs.

Deletions

Mutations resulting in the loss of large segments of DNA, covering from one to several genes, are referred to as deletions. Deletions can occur spontaneously, or may be induced by X-rays, UV Light, or treatment with nitrous acid.

Carcinogenic compounds have been found to be mutagens once they are activated by liver microsomal oxidases. In the activated state, as epoxides, these agents act preferentially as frameshift mutagens. Chemical mutagens, particularly those of the frameshift variety, may be important factors in carcinogenesis.

Define the following mutations, identify a suitable mutagen, and describe the consequences of the mutation. Remember, nonsense codons are UAG, UAA, UGA.

(answers on next page)

Wild type gene=AUG-ACC-UGG-UCA-CCA-TTT-AAT-

Auxotroph #1=AUG-ACT-UGG-UCA-CCA-TTT-AAT-

Auxotroph #2=AUG-ACC-UGA-GUC-ACC-ATT-TAA-T

Auxotroph #3=AUG-ACC-UUC-ACC-ATT-TAA-T

Auxotroph #4=UAG-ATT

Answers to point mutations question

1 = transition
2 = transversion
3 = transversion
4 = transversion

RECOMBINATION

Recombination is the formation of a new genotype by reassortment of genes following a genetic cross. It involves a structural change due to the crossing over, or exchange of genetic material between two different, but homologous chromosomes; significant sequence homology is required. Only a portion of the donor chromosome is added to the recipient to form a partial diploid. Significant sequence homology must exist before recombination can take place.

Model of Recombination: The Breakage and Reunion model of recombination proposes that the chromosomes break and are reunited such that each progeny contains genetic material from both parents. This model is supported by the observation that recombination can occur without DNA synthesis. Reciprocal recombination is the rule, however, on occasion, the recombinant is unidirectional (progeny contains genes from one parent but the 2nd reunion does not occur).

COMPLEMENTATION

Complementation is the process by which two recessive mutations can supply each other's deficiency to produce a wild type phenotype. It is another method of genetic analysis used to determine whether two mutants, apparently defective in the same way, are defective in the same gene. It should not be confused with recombination which deals with structural changes between genes. Two types of complementation are known to occur.

Intergenic complementation occurs with genes specifying proteins consisting of two or more nonidentical polypeptides.

Intragenic complementation occurs with genes specifying multimeric proteins consisting of two or more identical polypeptides.

Transferred chromosomal fragments cannot replicate unless they are integrated into the recipient DNA (e.g. in transformation and some conjugation events).

Types of Recombination

A. Legitimate (Generalized)

Host cell enzymes that are involved include:

- Rec A = Proteolytic and ATPase activities
- Rec BC = Endonuclease, exonuclease
- Nucleases
- DNA polymerase
- DNA ligase

B. Nonhomologous (or illegitimate)

Independent of Rec A gene function
e.g. = Transposon insertion, and certain phage mediated events.

Answers to Auxotroph questions

1= Transition; 5-bromouracil; missense mutation-protein may or may not lose function
2= Frameshift by insertion; acridine orange or proflavin; nonsense mutation-protein loses function due to premature chain termination
3= Frameshift by deletion; acridine dyes; missense mutation-usually loss of protein function
4= Deletion of larger segment of DNA:irradiation, nitrous acid or bifunctional alkylating agent; protein loses function

SUPPRESSION

The effects of a harmful mutation in an organism may be reversed to yield the wild type phenotype. When this occurs there may be a true back mutation to the original genotype, or the genetic code may be misread leaving the original mutation unchanged. When the effects of a primary mutation are eliminated by altering the translation process, the phenomenon is called suppression. Two types of suppression are known: genotypic suppression in which a second mutation results in permanent alteration of the translation process; and phenotypic suppression in which added substances allow temporary nonheritable alterations in the interactions of translation components to occur.

Genetic suppression occurs when _____

_____

_____.

Genotypic suppression

When the effects of a primary mutation are eliminated by a secondary mutation the latter is called a suppression mutation. Such suppressor mutations are classified as intragenic suppressors if they are located in the same gene as the original mutation; or extragenic suppressors if they are located in a different gene, or even a different chromosome, than the original mutation.

In intragenic suppression the secondary mutation is found in the same gene and cancels the deleterious effect of the primary mutation. Several examples can be cited: (1) a missense mutation followed by another missense mutation; (2) a frameshift mutation followed by another frameshift mutation of opposite sign; and (3) a nonsense codon which reverts not back to the original codon, but to another codon which allows a functional protein to be made.

Extragenic (or intergenic) suppression occurs when the suppressor is located on a gene other than the one containing the primary mutation. These suppressors must, therefore, influence the expression

CODON	AMINO ACID SIGNAL
UGG =	Tryptophan

Missense ↑ ↓ Nonsense

UAG = Stop signal

Nonsense ↑ ↓ Missense

UAU = Tyrosine

Genotypic/phenotypic suppression occurs when a second mutation "corrects" harmful effects of an earlier mutation.

of the primary mutation by virtue of an alteration in a second functional unit of the translation process. The biochemical basis of the type of extragenic suppression that has been described in most detail involves synthesis of specific species of tRNA's.

Examples of intragenic suppression

1. _____

_____

2. _____

_____

3. _____

_____

Phenotypic Suppression

Streptomycin and other antibiotics belonging to the aminoglycoside family of antibiotics are known to cause "ambiguities" in amino acid incorporation; i.e., codon recognition of other amino acids at low frequencies. A translation error of this sort is sufficient to allow insertion of a proper, or at least compatible, amino acid at a position that is otherwise mutant. This sort of change results in a translation error which allows the synthesis of a small amount of active protein.

Effects of Some Common Mutagens on DNA

Mutagen	Mechanism	Induced DNA Alteration
5-Bromouracil	Base analogue	T C transition
2-Aminopurine	Base analogue	A G transition
Nitrous acid	Base deamination	A G and C T transitions
Ethyl ethanesulfonate	Alkylation of quanine	Transition or transversion
Acridine dyes	Intercalation between bases	Frame-shift
Ultraviolet irradiation	Pyrimidine dimerization	Frame-shift, transversion CT transition, deletion
Ionizing Radiation	Free radical formed	Chromosomal strand breakage

GENE TRANSFER

Three types of gene transfer take place in bacteria. All involve a unidirectional transfer of genetic material from donor to recipient cells.

TRANSFORMATION

Transformation is the DNA-mediated transfer of a limited amount of genetic information from a disrupted to an intact cell. DNA is obtained from the donor cell either naturally by cell lysis, or artificially by a chemical extraction procedure, and is added to the recipient cells. Once DNA is taken up by the recipient cells, recombination of any marker can take place, and the cell is said to be transformed. Native double stranded DNA is the form most effective in transformation.

The Recipient

For successful transformation the recipient cells must be in a particular physiological state, called competence. The duration of the state is restricted to the late logarithmic phase of growth. Only competent cells can trap or bind the donor DNA to the recipient cells. Once the donor DNA is taken into the cell, integration of the donor DNA is accomplished by recombination. The DNA forms a partial diploid with the bacterial chromosome. When DNA replication occurs, 1 daughter will be a transformant.

Once donor DNA is taken into the competent recipient cell, integration occurs by _____.

Enzymes involved include

1. _____
2. _____
3. _____
4. _____
5. _____

(refer to "recombination" p. 45 for answer).

Gene transfer between bacteria is accomplished by 3 different processes:

1. _____
2. _____
3. _____.

Competence Factor is an extracellular protein that binds DNA to receptors on the cell; DNAse at cell surface degrades 1 strand and the other enters the cell.

TRANSDUCTION

Transduction is the type of gene transfer mediated by a bacteriophage, and involves the transfer of a limited amount of genetic information from a lysed donor cell to an intact recipient cell.

Generalized transduction occurs when a bacteriophage has the capacity to transfer any of the genes of the bacterial chromosome. Following infection of phage into bacteria, the virus particles multiply, making new enzymes, DNA, and coat protein (the assembly process is called encapsidation). Occasionally, a mistake is made during the assembly of the phage and a piece of bacterial DNA is packaged into the phage coat protein. This is the transducing particle, and since it contains little or no phage DNA, it cannot replicate further. Upon lysis of the bacteria, the phage particles are released, with the transducing particles making up only a small percentage of the total virus particles. A generalized transducing phage can pick up genes from any region of the bacterial chromosome.

Specialized transduction occurs when a particular phage strain can transduce only a few restricted genetic markers. These bacteriophages are temperate phages which during lysogeny integrate into a specific site on the bacterial chromosome. Upon induction of the prophage into the lytic cycle, genes adjacent to the prophage insertion point are occasionally carried along with the phage chromosome.

Abortive transduction occurs when the added piece of chromosome from a transducing phage fails to recombine or replicate, but still functions.

Gene transfer mediated by bacteriophage is called _____.

It is a generalized process if _____ _____.

It is a specialized transfer if _____ _____.

If the added DNA fails to replicate the process is called _____ _____.

BACTERIOPHAGES

Viruses that specifically infect bacteria are called bacteriophages, or simply phages. The life cycle of a virulent bacteriophage consists of infection, intracellular development and assembly into a complete phage, and lytic release of new progeny phage; this is called the lytic cycle. Bacteriophages called temperate phages can provoke one of two responses upon infection into a host cell; the lytic cycle (as above) or lysogeny. In lysogeny, the phage chromosome is integrated and replicates in concert with the host chromosome. The integrated phage chromosome is called a prophage, and the bacterial cell containing the prophage is called a lysogen. Lysogenic bacteria continue cell division indefinitely, and may lose the prophage in one of two ways; by the return of the prophage to the lytic cycle, or by spontaneous loss of the prophage. In the former case, bacteria are lysed and progeny phage are released, whereas in the latter case, bacteria retain their viability.

The Lytic Cycle

The lytic cycle of phage multiplication consists of the following steps:

1) absorption
2) penetration
3) intracellular development
4) maturation
5) lysis

1) Absorption. This process involves the presence of both an absorption organ on the phage and a highly specific receptor site on the bacteria. Phage resistance occurs if the host cell modifies its receptor molecule.

2) Penetration. The attachment of the tail fibers and pins to the cell surface triggers a contraction of the tail sheath. This results not only in the penetration of the tail core into the cell wall, but also a syringe-like action discharging the DNA into the host cell.

3) Intracellular Development. Intracellular development begins immediately after infection, with transcription of the phage DNA by host RNA polymerase. The phage mRNA is translated by the protein synthesizing machinery of the host and forms a number of new proteins called "early" proteins.

The next step is replication of nucleic acid. In dsDNA phages replication proceeds by the general mechanism of DNA synthesis. In ssDNA phages a complementary strand of DNA is synthesized (the dsDNA replicative form) which serves as a template for the synthesis of both mRNA and new phage DNA.

Shortly after replication begins, the "late" proteins begin to appear. Among the late proteins synthesized are subunits for phage components, as well as the enzyme lysozyme. This enzyme attacks the mucopeptide of the host cell wall and is primarily responsible for lysis and release of progeny phage.

4) **Assembly.** Once all the structural components are synthesized, maturation begins. The initial step involves the condensation of DNA, possibly with the aid of positively charged proteins, called internal proteins. The capsid subunits assemble around the condensed DNA to form the head. The tail and tail fibers are also formed independently and assemble to form the complete phage.

5) Lysis. The final step in phage multiplication is the lytic release of progeny phage. Lysis involved two or more gene products; at least one involved with an action on the membrane, and the other the action of lysozyme on the mucopeptide portion of the cell wall.

LYSOGENY

Definition

The stable (integrated) association of a temperate phage chromosome, in the prophage state, with the bacterial chromosome is called lysogeny. The prophage contains all the genetic information of the phage; however these genes are repressed.

Lysogen Formation

Following temperate phage infection, there is a critical period in which the viral particle either enters the lytic cycle or the lysogenic state. If lysogeny is to occur, this will be initiated by the synthesis of an mRNA which codes for a repressor protein. The repressor binds to the phage DNA in a region called the immunity region. This binding results in repression of virtually all phage directed syntheses and prevents the phage from entering the lytic cycle. The repressed phage chromosome becomes integrated in the host chromosome and remains in the lysogenic state.

Integration and Excision

The next step is the attachment of the phage chromosome to the bacterial DNA. The phage chromosome circularizes by joining single stranded cohesive ends, which are linked together by DNA ligase. Reciprocal recombination occurs between the phage and bacterial DNA's resulting in a linear integration of the phage chromosome. Excision occurs by a reversal of the steps of integration.

1 = A; see p. 49
2 = B

Phage Conversion

Lysogeny often results in the expression of new characteristics by the bacterial population. These may be due to
1) expression of phage genes, or
2) the induction of previously silent bacterial genes.

A medically significant phage conversion system involves the relationship of C. diphtheria exotoxin (tox+). The gene responsible for the production of the diphtheria toxin is located on the chromosome of phage beta.

Phage conversion involving toxin production occurs in several other organisms. For example, erythrogenic toxin, the toxin responsible for the rash of scarlet fever, is produced by lysogenic strains of Streptococcus pyogenes. In addition, types C and D toxins of Clostridium botulinum are produced following infection of specific phages.

A. transduction
B. lysogeny
C. both
D. neither

1. Integration of donor bacterial DNA into host (recipient) cell
2. Integration of phage DNA into host (recipient) cell

(Answers at left)

CONJUGATION

Conjugation is a process of genetic exchange between two bacterial strains which is dependent upon cell to cell contact. It is the major means by which Gram negative bacteria acquire multiple drug resistance.

Cell contact is a prerequisite for _____.

In conjugation there are two mating types; the male is the donor and contains an F (for "fertility") factor, and is referred to as F+; the female is the recipient and because it lacks an F factor is referred to as an F-. When strains of opposite mating type are allowed to grow together for a short period of time, and undergo conjugation, the F factor of the male replicates, transferring one of its copies to the female recipient. The recipient cell is converted to an F+ cell, now being capable of serving as a donor. Thus, as long as growth continues, the conjugation process can continue in an infectious manner.

The <u>Male/Female</u> donor contains a _____ factor, called an _____ factor. During genetic exchange this factor replicates and one copy is transferred to the <u>Female/Male</u> recipient, which is then converted to an _____ cell. This is the primordial sex change operation.

The F factor is a circular piece of DNA of molecular weight 50×10^6 and codes for approximately 40-60 proteins. Certain clones of F+ cells are capable of transferring chromosomal genes with increased efficiency, resulting in a high frequency of recombination (Hfr). These cells are called Hfr's and arise from F+ cells in which the F factor becomes integrated into the bacterial chromosome. The F factor DNA contains a region of homology with the bacterial chromosome and a recombination event takes place between the two DNA's such that the F factor is inserted linearly into the bacterial chromosome.

<u>F' formation</u>

The integration of the F+ factor into the chromosome to form an Hfr is a reversible process. In some instances, the F factor brings along with it some of the chromosomal genes, and in this state is termed an F'. The chromosomal genes incorporated into the circular F factor are passed on during conjugation. This process is called sexduction and results

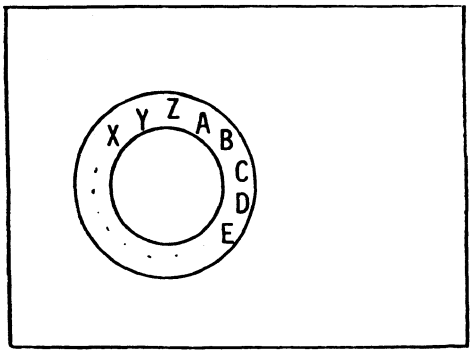
in a high frequency transfer of the F factor and linked chromosomal genes. The recipient cell in sexduction now possesses the same properties as the donor; i.e., it is F+ and contains the added genes attached to the F factor.

Physiology of Conjugation

Male strains have a small tubular appendage which forms a bridge between male and female strains. This appendage is called the F pilus and is synthesized under the control of F, with up to five pili per cell. The function of the F pilus is to form a specific attachment between the male and female that will allow conjugation to proceed. The pilus contracts bringing the cells into close contact to facilitate transfer of the F factor.

Cell-to-cell contact, which is essential for conjugation is accomplished via a specialized appendage called the _____ _____.

IDENTIFY THE CELLS BELOW (F-, Hfr, F' and F+)



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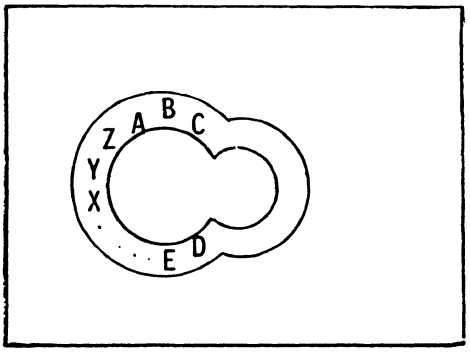

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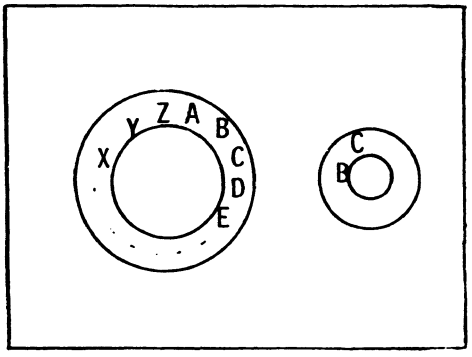

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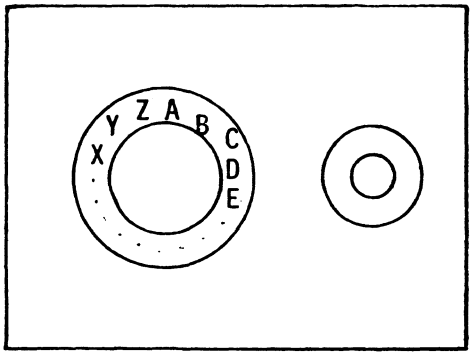


_____

EXTRACHROMOSOMAL GENETIC ELEMENTS

Extrachromosomal genetic elements are often found in bacteria in addition to the normal chromosomal DNA. They are referred to as plasmids and are capable of autonomous replication in the cytoplasm. When an extrachromosomal element is capable of replicating either autonomously or integrated into the bacterial chromosome it is called an episome. Thus, the F factor which can alternatively exist as an F+ or an Hfr, is an example of an episome.

Phenotype functions mediated by plasmids include resistance to antibiotics, heavy metals, ultraviolet light, and specific phages, as well as production of antibiotics, bacteriocins, some toxins and other virulence enhancing factors (such as hemolysins, coagulase, etc.).

Bacteriocinogenic factors are extrachromosomal elements which produce bactericidal substances called bacteriocins. Bacteriocins differ from antibiotics in that they are proteins which act on only the same or closely related species of bacteria. Organisms which produce the bacteriocin are resistant to its action, whereas sensitive cells readily absorb the bacteriocin. Once absorbed, the bacteriocin initiates a highly specific action which leads to the death of the cell. This action differs for each type of bacteriocin, and may involve inhibition of oxidative phosphorylation, or cessation of DNA, RNA, or protein synthesis.

Resistance Transfer Factors

Resistance to antibiotics and other chemotherapeutic agents has been found to exist in a variety of microorganisms. In the Enterobacteriaceae, individual strains may show resistance to several antibiotics. Such multiple drug resistance is specified by an extrachromosomal element, called a resistance factor or R factor.

Plasmids which are capable of integration into the host genome are called _____.

F factors are _____ or _____ depending upon whether or not they have integrated into the host genome (answer below).

REVIEW STATEMENTS

For successful transformation the recipient cell must be in a particular physiologic state, called _____.

The difference between generalized and specialized transduction is that in the former/latter the temperate bacteriophage integrates into the host genome at a specific site and, upon induction, genes adjacent to the prophage insertion site are carried along with the phage chromosome.

(answers = plasmids and episomes)

An R factor can be transferred by conjugation from one cell to another, and is often referred to as a resistance transfer factor (RTF). Genetic studies have shown that R factors consist of two distinct components, a transfer factor (RTF) and a resistance determinant (r determinant). The RTF is thought to be similar in function to an F factor, being responsible for both its own autonomous replication and conjugal transfer. The r determinant contains genes which specify resistance to various antibiotics. These two elements may exist independently, or associated together as an RTF:r determinant complex, i.e., R factor (perhaps similar to an F').

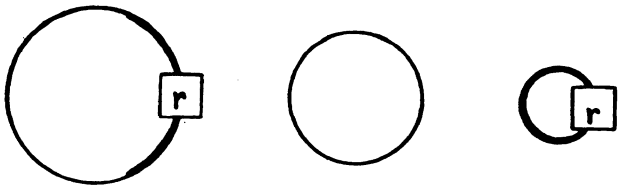
TRANSPOSONS - These are linear pieces of DNA, often containing r determinants, which promote their own transfer from 1 piece of DNA to another. For example, a transposon contained in the chromosome of a bacterium could transfer to a plasmid in the same cell; this complex may then be transferred to another bacteria by conjugation. The transposon might then dissociate itself from the plasmid and incorporate into the genome of the host cell. It can inactivate host genes during insertion.

The transposon has terminal repeating sequences of nucleotides on either side of the genes to be transferred which are mirror images of each other (they are palindromes). These are called INSERTION SEQUENCES which permit recognition of the correct area in the host genome, and insertion. The transposon has an endonuclease which will only "nick" DNA after a certain nucleotide sequence has occurred.

Transposon effects include mutations, and insertion of antibiotic resistance genes. Mutations can occur due to deletions or insertions, depending upon whether the transposon is coming or going.

Transposition occurs in the absence of recA function; therefore it is a type of _____ recombination (see p.45).

Two examples of episomes would be _____ and _____.



R factor = RTF + r determinant

TRANSPOSON

I	GENES TO BE TRANSFERRED	S
S	e.g. r dtmt.	I

Match the processes below with the components required for these processes to occur (answers on next page).

1. Sexduction _____
2. Transduction _____
3. Conjugation _____
4. Transformation _____

COMPONENTS:
A) Bacteriophage
B) Cell-to-cell contact
C) F' cell
D) Competent cell
E) Hfr cell
F) F- cell
G) F pilus
H) Host bacterium

Which properties are common to

5. Plasmids and episomes?

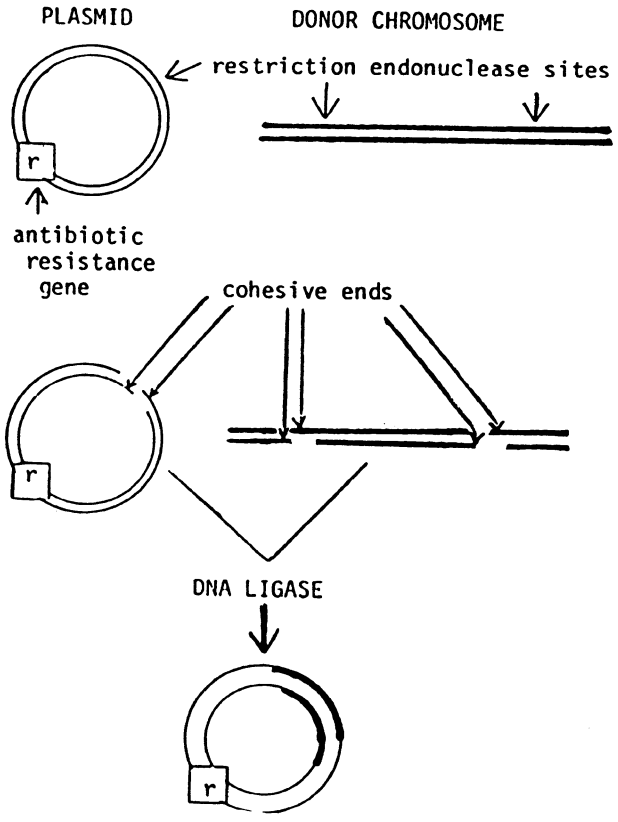
6. Episomes and transposons?

A) Autonomous replication
B) Integration into host DNA
C) Both
D) Neither

RECOMBINANT DNA TECHNOLOGIES

The essential features in the construction and transfer of plasmid recombinant DNA molecules include: a method of specifically cleaving and then joining together DNA molecules from different sources; a source of carrier DNA capable of replicating itself and any foreign DNA joined to it; a method of transferring the composite DNA molecule to recipient bacterial cells; and finally, a method of detecting whether the recipient cells contain the recombinant DNA molecule.

Several specific enzymes are involved in the construction of plasmid recombinant DNA molecules. The first step involves a cleavage of a circular plasmid DNA molecule into a linear open form. One way of producing such a molecule is with E. coli R factor 1 restriction endonuclease (Eco R1), which makes staggered nicks in complementary strands of DNA at sites separated by several nucleotides. Different endonucleases recognize specific nucleotide sequences hence the DNA can be split at different sites. The resulting single-strand ends contain complementary sequences (cohesive ends) capable of hydrogen bonding again to form a circular molecule. Hydrogen bonding may also occur with single-strand regions of another plasmid or segment of foreign DNA cleaved by Eco R1 endonuclease. In this case, a recombinant DNA molecule is formed which is made up of the original plasmid and the segment of foreign DNA. The final step, sealing of the DNA molecules, is accomplished by DNA ligase, which catalyzes phosphodiester bonds to re-unite the DNA strands.



Answers to genetic transfer questions

1. B, C, E, F, G, H
2. A, H
3. B, F, G, H
4. D, H
5. A
6. B

Summary of essential features for gene cloning:

1. **Vector** - A source of carrier DNA; e.g., a **plasmid** or bacteriophage capable of gene transfer
2. **Connector** - A method of specifically splitting **(restriction endonuclease)** and rejoining **(DNA ligase)** DNA strands
3. **Selector** - A method of selecting the recombinant; e.g., **antibiotic resistance** gene carried on the vector
4. **Detector** - A method of detecting the product of the cloned (desired) gene; e.g., **ELISA** test for HBsAg

Barriers to expression of cloned eukaryotic genes in prokaryotic hosts include:

1. Promoters of eukaryotes are different; must supply prokaryote promoter.

2. Translation initiation signals (ribosome binding sites) of eukaryotes are different; must supply prokaryote binding sites.

3. Introns must be removed; most easily done by using cDNA.

How are these overcome?

1. RNA polymerase does not recognize the promoters of eukaryotic DNA. **The eukaryotic DNA segment is placed behind a prokaryotic promoter in the plasmid** which will allow RNA polymerase to attach and read the exon.

2. Ribosome binding sites are different in eukaryotic cells. This is overcome by **placing a prokaryotic ribosome binding site in front of the promoter in the plasmid;** this will provide a mechanism for efficient transcription and translation.

3. The eukaryotic DNA carries introns that must be removed. To avoid these we must start with mRNA. After the eukaryotic DNA is processed to mRNA we can "capture" the mRNA by using an affinity column coated with antibody to the desired protein. Alternately, an oligo(dT) column can be used to isolate mRNA with poly (A) tails. **Then utilizing reverse transcriptase, a sDNA can be produced. Using other DNA polymerase enzymes, dsDNA can then be produced.** This dsDNA codes for the mRNA without introns. This is called **cDNA** which stands for **complementary DNA.** The cDNA is then reinserted into a plasmid and is capable of being transfected into a cell and replicated.

Techniques that are employed in nucleic acid analysis

A. Polymerase chain reaction (PCR)

PCR is a method of **gene amplification** which produces a large amount of DNA in a short period of time. Requirements for this reaction include the following:

1. **Template DNA** containing the gene to be amplified.

2. **Oligonucleotides** spanning the region to be amplified.

3. **Taq polymerase** isolated from the thermophile *Thermus aquaticus*, which withstands elevated temperatures (70-95C) without denaturation.

4. **Thermal cycling chamber** to alternately denature DNA, renature it, and allow polymerization to occur.

The principle of the PCR is simple; with each temperature cycle there is a doubling of DNA strands.

At the lower temperature the DNA polymerase synthesizes complementary strands with the supplied oligonucleotides. The temperature is then raised to denature the dsDNA and separate the strands. The Taq enzyme withstands this elevated temperature and hence is still active when the chamber is cooled. It then synthesizes another set of dsDNA strands from the oligonucleotides provided. Thus one strand of DNA amplified over 15 cycles will produce 220,000 copies.

PCR has been useful for cloning specific genes, rapid identification of pathogens, and a variety of other uses. Procedures in which the PCR reaction can be utilized include:

1. For use in **amplifying DNA** present in small quantities so that it can be used to produce hormones, etc for human use. Many useful products have come out of this line of research, for example humulin (human erythropoietin), many monokines (IL-!), many cytokines (IL-2), etc.

2. To detect **HIV infection**
It takes 1 month or more to mount an antibody response to the infection. With the use of PCR, one can detect the presence of HIV DNA in lymphocytes within 2-3 days post infection.

3. To **diagnose tuberculosis**
With the use of PCR, one can detect as few as 10 tubercle bacilli in a sputum sample in a day or less.

B. Nucleic Acid hybridization

This technique is used to compare nucleic acids and to quantitate their concentration. It depends upon the ability of **complementary nucleic acid strands** to specifically **align and form stable double stranded associations.** The strand that is used as the detecting probe can be labeled with a fluorescent molecule, or it may be chemiluminescent, or radioactive thus its presence can be quantitated.

C. Restriction fragment length polymorphism analysis (RFLP)

RFLP can be utilized to determined the relatedness of two samples of DNA or RNA. The procedure is performed as follows:

a. Digest a piece of DNA from one source (via restriction endonucleases) and prepare a gel electrophoretic analysis; then digest and analyze DNA from a second source and compare their RFLP patterns to see if they are related.

b. There are some **human diseases** we can specifically identify using RFLP.
   a. In a number of **thalassemias**, we can detect them with RFLP.
   b. RFLP can also be used to pick up **sickle cell anemias**.
      1. In this disease, there is a normal hemoglobin called HBA and an abnormal hemoglobin called HbS.
      2, HbS results from a glutamic acid to valine shift in amino acid position #6 which results from a single nucleotide change in the codon.

Utilization of Recombinant DNA Techniques in Gene Therapy

This has allowed us to take various kinds of retroviruses and use them to insert certain genes in target cells. If you take out the infectious genes of retroviruses, namely the **gag, pol** and **env** genes, the retrovirus becomes harmless in regard to changing or transforming cells of the host. These infectious genes are replaced with an innocuous gene and the virus becomes a **modified retrovirus**.

1. If this modified retrovirus is placed in lymphocytes, you can infect 1-10% of the cells and insert the desired gene.

2. This approach has been used in gene therapy experiments in humans. The first experiment involved insertion of the gene for adenosine deaminase (ADA), the enzyme found lacking in those with ADA deficiency. Over 90% of the individuals with ADA deficiency also have a severe immune deficiency (SCID) .

3. The experiment was performed by placing the ADA gene in a modified retrovirus, adding this retrovirus to T-lymphocytes removed from the patient, and then getting infection and recombination in the T-lymphocytes with inclusion of the ADA gene. The T lymphocytes are then reinjected into the individual who is found to have a functioning ADA gene and a relatively normal immune system.

4. There are currently over 60 different protocols that are available for gene therapy. These include hypercholesterolemia, cystic fibrosis, and cancer.

REVIEW STATEMENTS

These should be used to strengthen and expand you understanding of Microbial Genetics. The statements are "factoids" that may help in the STEP 1 exam. You may wish to develop your own list to expand your knowledge base. If you have a spare sheet of paper available, white down the correct statement for every question you miss in going through review exams. This way you can avoid marking on the review exam (so you can use it again) and still have captured that fact for future review.

A **transversion** occurs when a purine in DNA is replaced by a pyrimidine.

Acridines cause frameshift mutants.

Restriction endonucleases break the chromosome at specific sites determined by nucleotide composition.

A secondary mutation which eliminates the effects of a primary mutation is a **suppressor**.

Mutations in the codon, the mRNA, the tRNA or the ribosome would all **effect translation**.

General recombination requires extensive DNA sequence homology.

UV light can cause **thymidine dimers**; repair enzymes can correct this error.

Polypeptide chain termination is caused by **nonsense** codons.

The bacteriophage-mediated transfer of genetic information from one bacteria to another is termed **transduction**.

Temperate bacteriophages can enter either the lytic cycle or the lysogenic state.

Bacteriophages are made of two molecular components, nucleic acid and protein.

DNA ligase is a phosphodiesterase.

Abortive transduction occurs in general transduction when the donated DNA fails to recombine or replicate, but still functions. A term that describes this event would be **complementation**.

Only **lysogenic** *C. diphtheriae* produce diphtheria toxin (are **toxigenic**).

The type of gene transfer mediated by purified DNA is **transformation. Competence** is a physiological state of recipient cells which is required for DNA binding in transformation.

Bacteriophage mediated transfer of genetic material is called **transduction**; a phage chromosome that has integrated into the bacterial DNA is a prophage.

Genetic complementation tests are used to determine whether two different different mutants carry mutations in the same cistron.

The conjugation bridge in bacteria is formed by **F pili.**

The **R-factor** of Gram negative enteric bacteria carries genes for **resistance** to several **antibiotics**.

Episomal transfer of resistance to antibiotics (RTF) occurs by the genetic mechanism called **conjugation**.

Following injection into a bacterial cell, the life cycle of a **virulent bacteriophage** consists of multiplication, packaging of nucleic acid, and cell lysis (release).

Following infection of sensitive bacteria by certain bacteriophages, the phage DNA becomes integrated in the host cell DNA and may influence or convert the recipient cell to produce new antigens or new toxins. A bacterium is said to be **lysogenic** when it contains a bacteriophage chromosome integrated into its own chromosome.

Genetic exchange between two bacterial strains which is dependent on cell to cell contact is **conjugation.**

The **polymerase chain reaction** requires a DNA polymerase, appropriate nucleotides for assembly, oligonucleotide primers and a DNA template. It is a technique used to replicate specific short regions of DNA exponentially in vitro.

Prokaryotic genes do not have intron sequences of noncoding DNA. prokaryotes can not splice out unnecessary sequences in mRNA hence **cDNA** is used to synthesize some mammalian proteins in bacteria.

The transfer of hereditary characteristics via cell-free DNA to competent recipient bacteria is termed **transformation; DNAse** interferes with this process.

A **generalized transducing particle** of *E. coli* containing host DNA can transfer any of the bacterial genes to a sensitive bacterial cell.

A mutation in DNA resulting in a **transition** results in one pyrimidine replacing another, it may be caused by the analog 5-bromouracil.

Excision repair involves the removal of thymidine dimers from ssDNA leaving a gap, and then utilizes the complementary strand as a template to resynthesize a new portion of DNA, which is sealed by **ligase.**

A **missense mutation** occurs when one base pair in a codon is replaced by another (with resultant replacement of one amino acid in the protein). **Nonsense codons** cause polypeptide chain termination due to a nucleotide change in the code which results in a **termination signal** (e.g., UGA).

Extrachromosomal, autonomously replicating circular DNA segments are called **plasmids**. Episomes are plasmids that have the ability to also replicate as a part of the cell's own DNA.

Recombination occurs due to breakage and reunion of chromosome strands.

For successful **transformation** the recipient cell must be in a particular physiologic state called **competence.**

Conjugation is a process of genetic exchange between two bacterial cells which is dependent upon cell-to-cell contact.

The F+ cell is the male "donor", F- female cells are the recipients, which are converted to F+ as a consequence of **conjugation.** In **Hfr** strains the F factor DNA integrates into the chromosome of the host cell. **F'** cells are those which have a piece of donor chromosome DNA that has been transferred along with the F factor.

Transposons are linear pieces of DNA, often containing **r determinants**, which promote their own transfer from one piece of DNA to another. Transposons encode a protein that catalyzes transposition (**transposase**). They are flanked by **insertion sequences**, which are the unique nucleotide sequences which permit recognition and insertion of the genes into the chromosome.

The enzyme that splits the plasmid chromosome for recombination is a **restriction endonuclease**. DNA annealing of the "new" DNA into the plasmid is accomplished by **DNA ligase**.

Bromouracil induces mutations through mispairing when it is incorporated into DNA as a structural analog of thymidine.

Alkylating agents such as nitrogen mustard and nitrosoguanidine react with guanine residues and cause improper base pairing during DNA replication.

Endonucleases do not digest DNA in the cell of origin because these cells have a **methylase** in the cytoplasm that methylates the DNA and protects it.

Restriction enzymes are of a group of bacterial **endonucleases** that cleave DNA at or near specific recognition sequences. The **Restriction fragment** is the piece of DNA produced by cleaving a larger fragment with a restriction enzyme

MICROBIAL GENETICS REVIEW EXAM

SELECT THE SINGLE BEST COMPLETION FOR EACH QUESTION BELOW.

1. The abnormal excision of a prophage and the incorporation of adjacent genes into a phage is one of the events leading to

   A. abortive tranduction.
   B. generalized transduction.
   C. specialized transduction.
   D. lysogenic conversion.
   E transposition.

2. Sequence specific enzymes that cleave DNA to give sticky ends are

   A. DNA methylases.
   B. DNA ligases.
   C. restriction endonucleases.
   D. beta-lactamases.
   E. photolyases.

3. The expression of prophage genes that confer a new phenotype to the bacteria, such as the production of diphtheria toxin, is termed

   A. prophage immunity.
   B. superinfection immunity.
   C. prophage induction.
   D. lysogeny.
   E. lysogenic conversion.

4. Conjugation

   A. is a mechanism of plasmid transfer.
   B. occurs only with temperate bacteriophages.
   C. can be inhibited by the addition of DNAse to the gene transfer mixture.
   D. requires the participation of a competent recipient.
   E. occurs optimally when both donor and recipient strains are F minus.

5. The addition of DNAse to a donor-recipient mixture will inhibit the following type of gene transfer:

   A. conjugation.
   B. transformation.
   C. transduction.
   D. transposition
   E. sexduction.

6. Specialized transduction

   A. transfers all genes of the chromosome with equal frequency.
   B. requires competent cells.
   C. is accomplished with phage particles obtained by induction of lysogens.
   D. is always accomplished with virulent bacteriophages.
   E. requires cell to cell contact of donor and recipient.

7. Plasmid mediated resistance to antibiotics may result in

   A. the formation of an altered peptidoglycan in the cell wall.
   B. the production of enzymes capable of inactivating the antibiotic.
   C. the induction of new antibiotic synthesis by the cell.
   D. production of nucleases to cleave antibiotic-producing transposons.
   E. altered ability of a cell to repair damage to DNA.

8. Transposable elements are capable of causing mutations in cells by

A. the induction of thymidine dimers.
B. direct insertion into a gene.
C. the inhibition of SOS repair functions.
D. combining specifically with DNA polymerase I.
E. virtue of the fact that they possess a reverse transcriptase capable of synthesis of new DNA.

9. Thymidine dimers are formed in DNA due to the effect of

A. ionizing radiation.
B. ultraviolet radiation.
C. alkylating gents.
D. photoreactivation.

10. If DNA is damaged by an alkylating agent causing interstrand links, the most effective type of repair would be

A. direct repair.
B. photoreactivation.
C. excision repair.
D. post-replication repair.
E. suppression.

11. A genetic element capable of moving from one chromosome to another that is independent of recA function is a

A. transvertant.
B. transformant.
C. transposon.
D. transuctant.
E. transvestite.

12. A method of gene amplification which produces large amounts of DNA in a short period of time is

A. specialized transduction.
B. recombination.
C. complementation.
D. polymerase chain reaction.
E. lysogenic conversion.

13. In clinical settings the acquisition of multiple antibiotic resistance by enteric Gram-negative bacteria most often involves

A. conjugative plasmids.
B. transducing phage.
C. nonconjugative plasmids.
D. spontaneous mutation.
E. transformation of competent recipients.

14. Autonomously replicating DNA molecules that can serve as vehicles for DNA transfer are called

A. selectors.
B. bacteriogenic factors.
C. connectors.
D. vectors.

15. Restriction endonucleases are enzymes found in bacteria that

A. degrade DNA sequentially beginning at the 5' end.
B. cleave DNA at sequence-specific sites to yield either sticky (single stranded) or blunt ends.
C. form phophodiester bonds at the site of a single strand break in DNA.
D. are an integral part of genetic recombination.
E. cleave a portion of the gene that is transcribed but do not appear in the final mRNA transcript.

16. Eukaryotic DNA contains introns that present a barrier to successful transcription in prokaryotic organisms. To overcome this obstacle, we can employ what additonal reagent?

A. ECOR1 endonuclease.
B. bacteriocinogenic factor.
C. cDNA.
D. intron-recognizing primer oligonucleotides.
E. intron-specific endonucleases.

DIRECTIONS (ITEMS 17-28): Each of the numbered items or incomplete statements in this section is negatively phrased, as indicated by a capitalized word such as **NOT, LEAST**, or **EXCEPT**. Select the **ONE** lettered answer or completion that is **BEST** in each case

17. Which of the following is **NOT** true of transposons?

A. They are genetic units that move within and between different DNA molecules.
B. They require RecA for homologous recombination.
C. They are flanked by inverted repeat regions of DNA at their ends.
D. They may cause mutations by insertion into a gene.
E. They may contain genes which encode antibiotic resistance.

18. Restriction endonucleases recognize specific nucleotide sequences and may do all of the following **EXCEPT**

A. methylate the sequence so it can't be cleaved.
B. cleave the sequence to give sticky (cohesive) end.
C. cleave the sequence to give flush (blunt) end molecules.
D. join the nucleotide chains to effect repair.

19. Point mutations and base pair substitutions are caused by all of the following **EXCEPT**

A. alkylating agents.
B. nitrous acid.
C. pyrimidine analogs.
D. acridines.
E. sulfur mustards.

20. Bacteria are able to exchange genetic information by all of the following methods **EXCEPT**

A. conjugation.
B. transformation.
C. transposon insetion.
D. transduction.
E. complementation.

21. All of the following are genetic processes which enable cells to overcome a mutation **EXCEPT**

A. repair.
B. recombination.
C. suppression.
D. complementation.
E. lysogeny.

22. DNA vectors used in DNA technology should have all of the following properties **EXCEPT**

A. a selectable phenotype.
B. single sites for restriction enzymes.
C. autonomous replication.
D. ability to transfer genes to viable cells.
E. intervening sesequences.

23. Bacteria are simple genetic units with all of the following properties **EXCEPT**

A. they are haploid.
B. their genetic material is organized into a single circular chromosome.
C. they use the same genetic code as eukaryotic cells.
D. their introns are highly conserved genetic repeats.
E. their genotypes and phenotypes are the same

24. All of the following are true of recombinant DNA technology **EXCEPT**

A. utilizes enzymes such as restriction endonuclease.
B. allows the combining of prokaryotic and eukaryotic DNA in the same organism.
C. has the potential of cloning any single gene.
D can utilize DNA as a donor gene.
E. requires a heat labile DNA polymerase.

25. Mechanisms of plasmid specified antibiotic resistance include all of the following **EXCEPT**

A. detoxification, or alteration of the antibiotic to an inactive state.
B. interference with transport of the antibiotic into the cell.
C. alteration of the target site such that it no longer interacts with the antibiotic.
D. misreading of the genetic code by the antibiotic to insert missense amino acids in the protein.

26. Polymerase chain reaction techniques now make it possible to do all of the following **EXCEPT**

A. synthesize viral vaccine components
B. identify pathogens with specific probes
C. synthesize human hormone
D. prove identity of different DNA samples.

27. All of the following are true of recombinant DNA technology **EXCEPT**

A. is possible because of the utilization of enzymes such as restriction endonucleases, DNA polymerase, and DNA ligase.
B. allows the combining of prokaryotic and eukaryotic DNA in the same organism.
C. has the potential of cloning any single gene.
D. can not use cDNA as a primer.

28. All of the following are ingredients of the polymerase chain reaction **EXCEPT**

A. thermal cycling chamber.
B. Taq polymerase.
C. oligonucleotides spanning the region to be amplified.
D. template DNA containing the gene to be amplified.
E. plasmids with antibiotic resistance genes.

Directions (items 29-32) Match each statement describing a characteristic of genetic exchange with the transfer mechanism or mechanisms that demonstrate that feature. **AN ANSWER MAY BE USED MORE THAN ONCE, OR NOT AT ALL.**

A. Transformation
B. Transduction
C. Conjugation
D. Mutation
E. Education
F. Complementation
G. Suppression
H. Insertional inactivation

29. This process is sensitive to the presence of DNAse in the suspension medium

30. This process requires that the bacteria be in a state of competence

31. This process involves packaging and transfer of DNA by a bacteriophage

32. This process is the most common means whereby bacteria become resistant to antibiotics

KEY

1.	C	6.	C	11.	C	16.	C	21.	E	26.	D
2.	C	7.	B	12.	D	17.	B	22.	E	27.	D
3.	E	8.	B	13.	A	18.	D	23.	D	28.	E
4.	A	9.	B	14.	D	19.	D	24.	E	29.	A
5.	B	10.	D	15.	B	20.	B	25.	D	30.	A
										31.	B
										32.	C