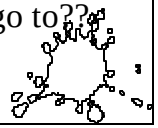
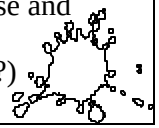
Why Are <u>YOU</u> Taking Microbiology???

- To learn about diseases you might encounter in Nursing??
- To understand the disease process??
- To understand the diagnostic process and to understand the treatment protocols??
- To be able to talk intelligently with Doctors?
- To be the highlight of any party you go to??



Learning Microbiology

- Learn the **<u>basics</u>** of microbiology so that you can intelligently discuss the nature and causes of disease
- Understand the mechanisms underlying bacterial and viral diseases
- Understand morphologic and biochemical sites of attack to kill bacteria and viruses without killing normal cells
- Memorize bacteria/viruses that cause disease and general characteristics of transmission, progression, and treatment (whole course??)

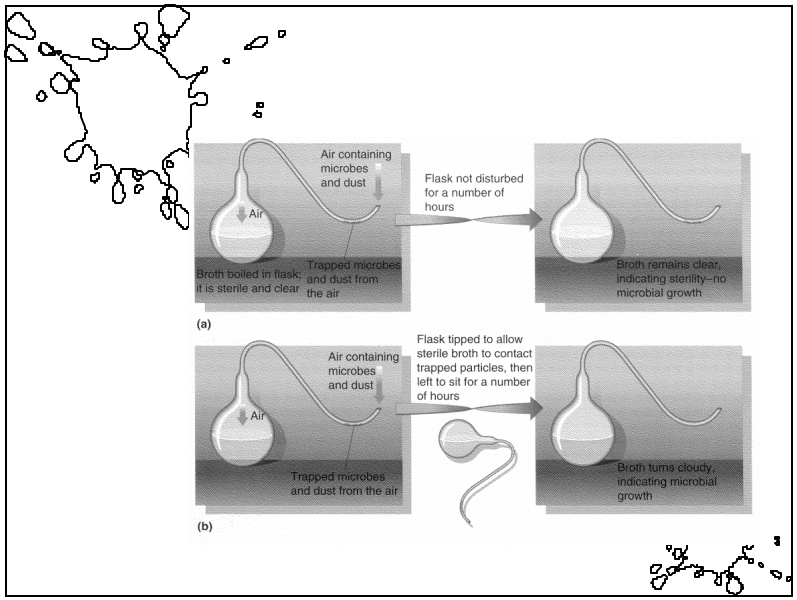


World of Microorganisms

- **Microbial world:**
  - **Prokaryotes (Bacteria)**
    - **description**
  - **Eukaryotes (Animals, Plants, Fungi, Protozoa)**
    - **description**
  - **Viruses (DNA, RNA, Retroviruses)**
    - **description**
- **Why are these important?**

Discovery of Microbes

- **1674-Antoni van Leeuwenhoek**
  - **amateur lens grinder: saw "animalcules"**
- **Generation of Life**
  - **Spontaneous generation: non-living substances converted into living organisms (abiogenesis)**
  - **Biogenesis - states "Life from Life"**
    - **Lazzaro Spallanzani (1765): boiled beef broth**
    - **Pasteur (swan necked flasks)**



Properties of Life

- **Metabolism-benefits include**
  - **alcoholic fermentation**
  - **antibiotic production**
  - **biopesticides**
  - **decomposition of dead organic matter**
  - **oxygen production**
  - **normal flora**
  - **destruction of toxic compounds**
  - **nitrogen cycling**

Properties of Life (continued)

- **Respiration-energy production**
- **Motility-movement by flagella/cilia or other**
- **Reproduction- asexual vs sexual**
- **Responsiveness to external and internal stimulation**
- **Ability to adapt to change**

Germ Theory of Disease

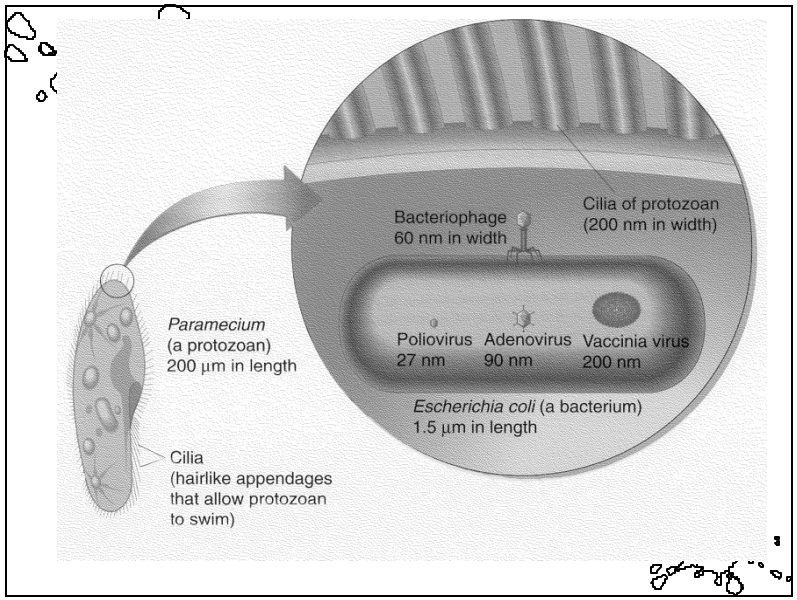
- **1876 Koch showed that *Bacillus anthracis* causes anthrax. 1st proof that microbe is able to cause disease.**
- **Koch's Postulates**
  - **suspected org. must always be found in diseased individual and never in healthy**
  - **must be cultivated in pure culture**
  - **pure cultures must cause same disease in susceptible animal**
  - **same organism must be re-isolated**

Immunization

- **Vaccination- 1st used successfully in 1798 by Edward Jenner who used cowpox virus to protect against smallpox (people who contracted cowpox did not develop smallpox).**
- **Active vs Passive immunization**
  - **active disease develops memory and long lasting protection**
  - **passive transfer from one individual to another is short lasting but quicker**

Defimtions

- **Chemotherapy-**
  - **antibiotics**
  - **chemicals**
- **Epidemiology**
  - **investigative science that provides information about the factors and conditions that contribute to the occurrence of diseases. ID source of outbreak**

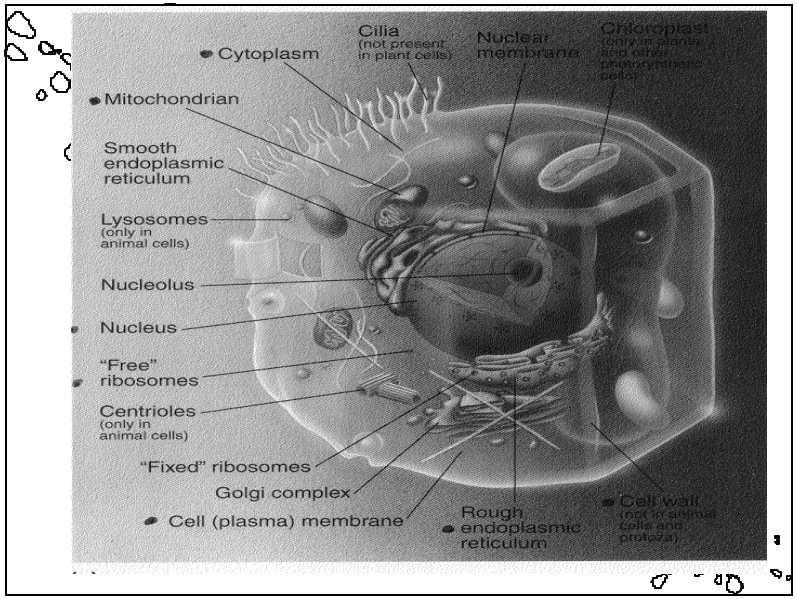


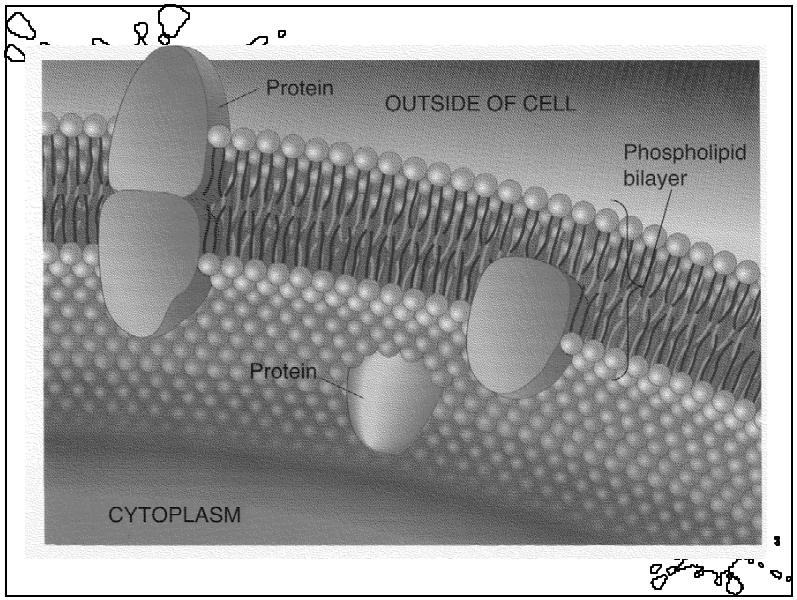
Introduction to Microbes

- **Eukaryotic cells**
  - **cell membrane (lipid bilayer)**
  - **cell wall (plants)- cellulose (polysaccharide)**
  - **nucleus (double membrane)**
  - **ribosomes- protein synthesis (80S)**
  - **mitochondria- "powerhouse" ATP synthesis**
  - **golgi apparatus-protein packaging**
  - **endoplasmic reticulum-transport**

(continued)

- **Fungi**
  - **cell wall, lack motility, absence of photosynthesis, depend on an external source of organic compounds to provide energy**
  - **yeasts and molds**
- **Protozoa**
  - **lack cell wall, non-photosynthetic, cilia, flagella**
- **Algae**
  - **cell wall, single celled to lg. multicellular, photosynthetic (generate 1/2 of earths O_2)**





Prokaryotic Structures

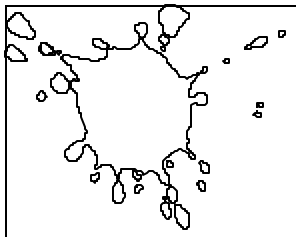
- **Prokaryotic cells**
  - **no nuclear membrane (nucleoid)**
  - **ribosomes (70S)**
  - **no mitochondria**
  - **cell membrane**
  - **cell wall (peptidoglycan)**
  - **no endoplasmic reticulum**
  - **Flagella (flagellin, noncontractile)**
  - **lipopolysaccharide membrane-endotoxin (LPS)**

Archaeobacteria

- **Archaeobacteria (ancient)-very different from eubacteria**
  - **plasma membranes contain lipids and protein, but lipids are not phospholipids, and the lipids contain ether bonds (rather than ester bonds)**
  - **cell walls lack peptidoglycan and may be composed entirely of protein**
  - **ribosomes sensitive to inhibitors that affect 80S of eukaryotic cells**

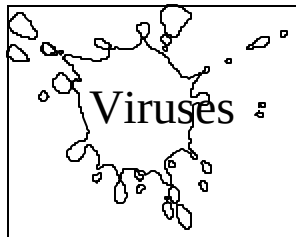
Aarchaeobacteria

- **Comprise three groups that grow in environments that are hostile to other forms of life**
  - ***Halophiles*- salt lovers**
  - ***Thermoacidophiles* (heat and acid lovers)**
  - ***Methane-producing***



Eubacteria

- **Eubacteria (true bacteria)**
  - **plasma membrane is a phospholipids bilayer. Sterols are rarely found in eubacteria but commonly found in eukaryotic cell membranes**
  - **cell wall composed of peptidoglycan**
  - **protein synthesis inhibited by agents that effect 70S ribosomes not 80S**
  - **most are non-photosynthetic**



Viruses

- **DNA or RNA- never both**
- **ss or ds DNA and RNA or fragmented NA**
- **Protein capsid**
- **lipid envelope**
- **obligate intracellular**
- **no cellular organelles**
- **Receptors- for cell attachment**

Microbial Diversity & Antimicrobial Therapy

- **Selective toxicity- must identify differences between eukaryotic and prokaryotic cells and attack differences (i.e., peptidoglycan, ribosomes, reverse transcriptase, etc...)**
- **must have low toxicity for eukaryotes**

Tools for Investigating the Microbial World

- **Microscopes**
  - **Compound- contain at least 2 lenses (objective & ocular)**
    - **resolving power vs magnification (human eye has resolving power of 0.2mm). Shorter the wavelength of beam the greater the resolving power.**
  - **Brightfield microscope -greatest magnification is about 1000X and resolving power about 0.27um**
  - **(see page 66 for summary of microscopes)**

Microscopes (cont.)

- **Darkfield-use of a special condenser that uses a darkfield ring to block light from entering the objective lens directly from the light source. The light that passes through the specimen is scattered and some is deflected into the objective lens so the specimen appears bright on a dark background. Used for viewing living, unstained cells. (Dental Study)**

Microscopes (cont.)

- **Phase-Contrast- View living cells. The lenses can detect differences in transmitted light and translate them into patterns of shadows and light so that different organelles in cell can be observed.**
- **Fluorescence- uses UV light as source. Some microbes fluoresce, but can use specific antibodies with attached Fl dye**

Stains

- **Most bacteria transparent. Must kill bacteria prior to staining by heat fixation or by chemical fixation.**
- **Simple stains- employ a single dye (methylene blue, crystal violet). Can tell shape**
- **Differential stains- Use of more than one dye & react with different structures (flagella, capsule, spores, etc...)**

GRAM STAIN

- **1. Crystal violet (1º stain). Colors cytoplasm of all cells**
- **2. Iodine- used as "mordant" binds to dye and helps resist decolorization**
- **3. Decolorizing agent (alcohol)- G+ cells are not decolorized, G- cells become colorless (decrease pores and trap dye)**
- **4. Counter stain- Safranin (red)**

Gram Stain (cont)

- **Important for ID & characterizing bacteria**
  - **source- g+ cocci=shedding of normal flora, g+ endospore =environmental contamination**
- **Gram stain determines how to treat**
- **Must also isolate bacteria and determine biochemical characteristics and antibiotic susceptibility**

Other Stains: Acid-Fast

- **used to identify members of genus Mycobacterium (causative agents of tuberculosis and leprosy). Contain high levels of lipid and must use hot carbol fuchsin that penetrates bacteria. Difficult to decolorize, and most organisms will be decolorized by acid-alcohol, but NOT Mycobacteria. (sputum sample)**

Other Stains

- **Endospore stain- hard to penetrate spore (*Bacillus* and *Clostridium*)**
- **capsule stain- (*Klebsiella* and *Pneumococcus*)**
- **Flagellar stain - (*Salmonella* and *Proteus*)**

Culture Techniques

- **Aseptic- allow handling of materials without the introduction of contaminants for growth of pure cultures (Koch's Postulates)**
  - **minimize contact with room air and non-sterile surfaces. Keep petri dishes & test tubes closed. Sterilize forceps, loops, wires, etc...**
- **Agar- solidifying agent (not nutrient). Melts at 100° and solidifies at 45°C.**

Isolation Techniques

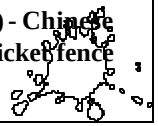
- **Streak plates**
- **Pour plates and spread plates**
- **Selective and Differential agars**
- **Biochemical tests- phenol red (pH indicator), sugar fermentation, growth vs non-growth, salt, heat, etc...**
- **Immunologic tests- specific serotypes**

Prokaryotes

- **Morphology**
  - **Cell size - about 2um in length and 0.5um in diameter. Rod bacteria may have a length up to 60 um.**
  - **shape -**
    - **Cocci**
    - **Bacilli**
    - **Spirillum- spirochetes**
    - **pleomorphic-filamentous (streptomyces [antibiotic]**

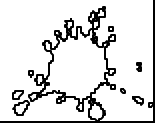
Morphology

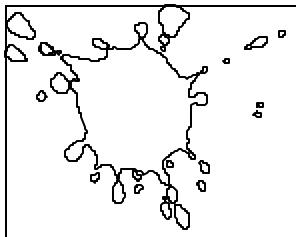
- a few bacteria lack rigid cell walls (Mycoplasmas)
- **Cell arrangement-**
  - divide along a single plane (Neisseria) to produce diplococci
  - form clusters (Staphylococci)
  - form long chains (Streptococci)
  - bend at point of division (Corynebacterium) - Chinese letter morphology/palisade arrangement/ picket fence



Cell Structures: Prokaryotic

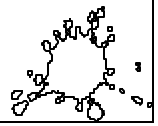
- **Plasma membrane -selective, respiration, cell wall synthesis, monitoring environment**
  - lipid bilayer
  - selectively permeable
    - simple diffusion
    - osmosis
    - facilitated diffusion-bind to permeases which increase rate of diffusion. No energy required
    - active transport- ATP

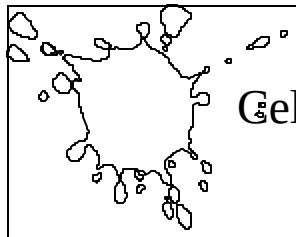




Cell Membrane (cont.)

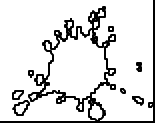
- **secretion of cell products- cell wall components, extracellular digestion products, toxins**
- **respiration and photosynthesis- in eukaryotes respiration done in mitochondria, but this is done on cell membrane in procaryotes**
- **reproduction- specific proteins in membrane attach to DNA and help to separate newly formed chromosomes from each other**





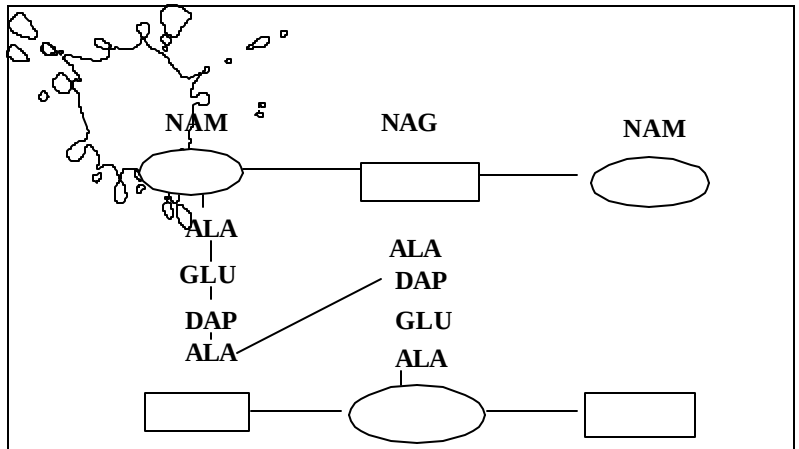
Cell Wall (Peptidoglycan)

- **surrounds the cell membrane (space between=periplasm)[if remove cell wall will have protoplast). Shape of cell wall gives bacteria characteristic shape. Protoplasts are round.**
- **protects cell (osmotic lysis)**
- **peptidoglycan=murein-found only in prokaryotes**

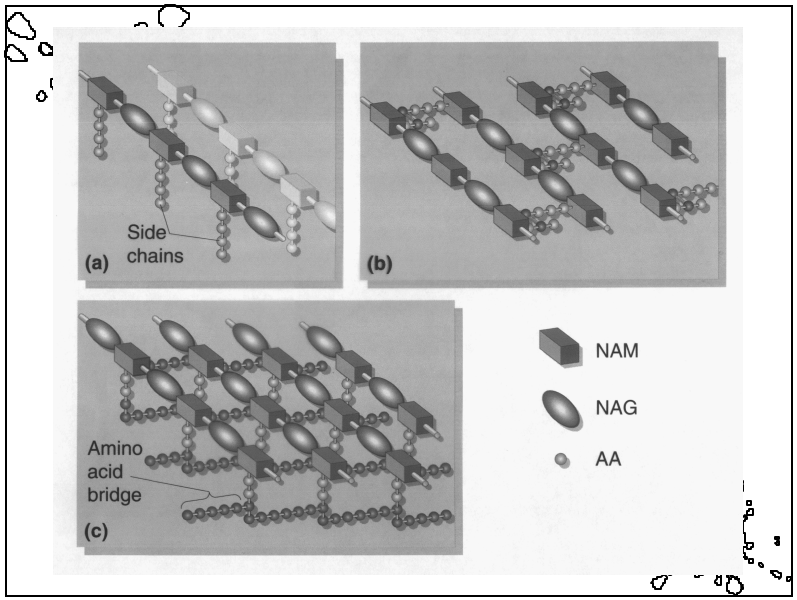


Peptidoglycan

- **composed of AA and sugars**
  - **N-acetylglucosamine (NAG) and N-acetyl-muramic acid (NAM)**
    - **attached to NAM are short side chains that are 4 or 5 AA's long. AA's are Alanine (1st, attached to NAM), Glutamic acid, Diaminopimelic acid (DAP) and Alanine.**
    - **DAP (AA found only in eubacteria) of one tetra peptide attached to 4th AA, alanine, on another tetra peptide.**

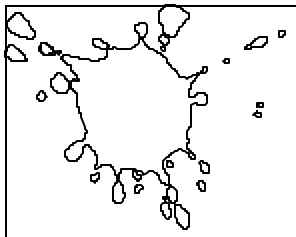


Peptidoglycan cross-linking of tetra peptides



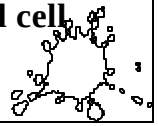
Peptidoglycan Properties

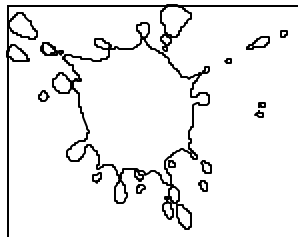
- **Each layer of peptidoglycan increases strength of cell wall. Strength of individual layers determined by the amount of cross-linking between NAG-NAM backbones.**
  - **Penicillin's and cephalosporins prevent peptidoglycan synthesis (lysozyme in tears)**
  - **Most Gram + bacteria also contain teichoic acids (repeating sugars and phosphates). Give membrane negative charge.**



Gram - Bacteria

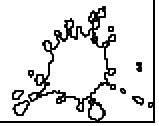
- **cell wall more fragile than G +**
- **Peptidoglycan thinner**
- **Outer membrane surrounds cell wall (lipopolysaccharide-LPS)-endotoxin**
- **Proteins may be trapped in periplasm-space between outer membrane and cell wall**

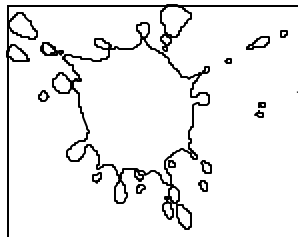




Lipopolysaccharide

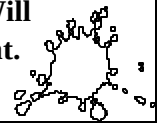
- **lipoprotein is layer closest to cell wall. One end attached directly to peptidoglycan**
- **outer surface composed of "Lipid A"**
- **outer membrane is selectively permeable and prevents passage of most hydrophobic molecules and large hydrophilic ones. (i.e. resistance to many antibiotics)**
- **fever, diarrhea, shock**

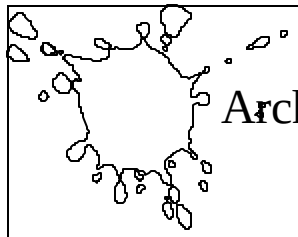




Wall-deficient variants

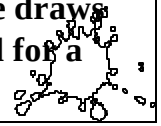
- **Mycoplasmas - exist naturally without cell wall. Sterols in membrane provide some protection from osmotic lysis.**
- **Mycoplasmas live as parasites inside eukaryotic hosts cells**
- **L-Forms -- bacteria normally have cell wall but due to antibiotic tx have lost it. Will survive in proper osmotic environment.**





Archaeobacterial cell walls

- **lack peptidoglycan**
- **NAM replaced by another sugar, and D-AA's are absent from tetra peptide**
- **Halobacteria have cell wall composed of polysaccharide and protein (glycoprotein). Osmotic imbalance draws water out of cell so there is no need for a rigid cell wall**



Other Prokaryotic Organelles

- **Nucleoid- circular DNA (only one copy of chromosome). No nuclear membrane!!**
- **Plasmids- small circular pieces of DNA that can replicate independently of chromosome. Antibiotic resistance/fertility factors**
- **Ribosomes- 70S (streptomycin selective for prokaryotic ribosomes)**
- **No mitochondria, no ER**

Flagella

- **Differs from eukaryotic: rather than a flexible whip, it spins like corkscrew: composed of basal body, hook, filament**
  - ***monotrichous*-single**
  - ***lophotrichous*- possessing many flagella arranged in tufts or clusters at one end**
  - ***amphitrichous*- flagella at both ends, singly or in clusters (polar flagella)**
  - ***peritrichous*- flagella distributed around the entire cell surface**

Flagella & Pili

- **Movement is intermittent: alternate between short runs and tumbles (run=straight line, tumble=turn randomly). Chemotaxis- directed movement**
- **Pili- protein tubes that extend from cell. Found only in certain species of G - bacteria**
  - **conjugation-transfer of genetic material**
  - **attachment to surfaces (intestine, urethra)**

Bacteria- Systematics and Nomenclature

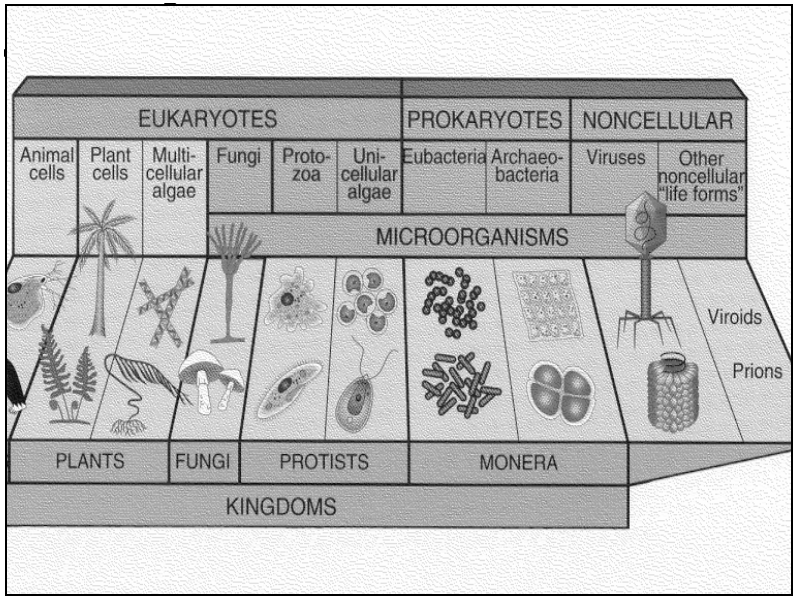
- **Taxonomy: classification scheme**
  - **binomial nomenclature - each organism is assigned two word classification- Genus and species.**
    - **Organisms that comprise a species are thought to be more closely related. Groups of species with common characteristics are pooled to a Genus.**
    - **All names latinized and Genus always capitalized.**
    - **Both names italicized or underlined**

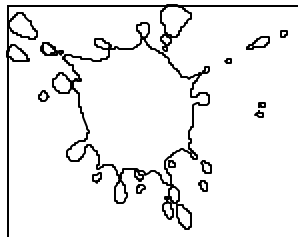
Nomenclature: Prokaryotes

- **How is it determined if a bacterium is genetically similar enough to the members of a particular species to justify being considered the same type of organism?**
- **no single official scheme!!**
  - **chromosome relatedness**
    - **DNA base composition: % G + C, homology**
    - **RNA analysis**
    - **morphology (size, shape, gram stain)**
    - **biochemistry**
    - **Physical-heat, pH, salt, O_2, etc.....**

Nomenclature: Bergey's Manual

- **Bergey's Guide for Determinative Bacteriology: Since 1923**
  - **Separates prokaryotes into 35 groups (30 are eubacteria and 5 are Archaeobacterial)**
  - **Based on Gram stain, morphology, endospores, metabolism, motility and reproduction**
  - **Problems when ID based solely on observable traits since members may vary up to 40% in DNA homology, & others traits.**





Fungi

- **One of major enemies causing some of most persistent diseases (Mycology)**
  - **Microscopic fungi exist as either molds or yeast**
    - **molds form large , long branching filaments called hyphae. Yeasts do not**
    - **Reproductive structure of yeasts are spores, produced by mitosis.**
    - **Non-photosynthetic**

Protists- Protozoa, Algae and Slime Molds

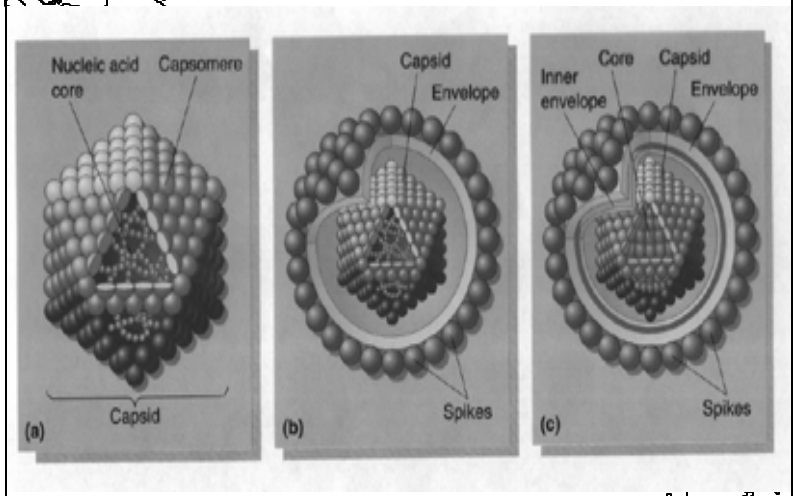
- **Protozoa- unicellular, heterotrophic, no cell wall, usually motile, asexual reproduction by binary fission**
- **Algae- Unicellular, usually photosynthetic, possess cell walls. Only 2 human illnesses caused by algae: paralytic shellfish poisoning and protothecosis**
- **Slime molds- complex life cycle**

Viruses

- **Obligate intracellular parasites**
  - **range from 20 nm to about 400 nm**
  - **filterable**
  - **composed of....**
    - **protein capsid**
      - **protomers and capsomeres**
    - **lipid envelope**
    - **Nuclear material-- DNA or RNA, retroviruses**
      - **ss, ds, linear, circular, fragmented**

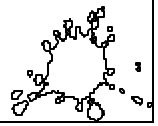
Viruses: Morphology

- **complex (bacteriophages- infect bacteria)**
  - **capsid, contractile sheath (neck), tail fibers**
- **helical (rabies, tobacco mosaic)**
- **icosahedral**
  - **20 triangular faces**
- **Specific for cells that each infect**
  - **receptors**



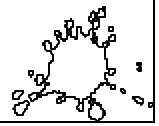
Viruses: Classification

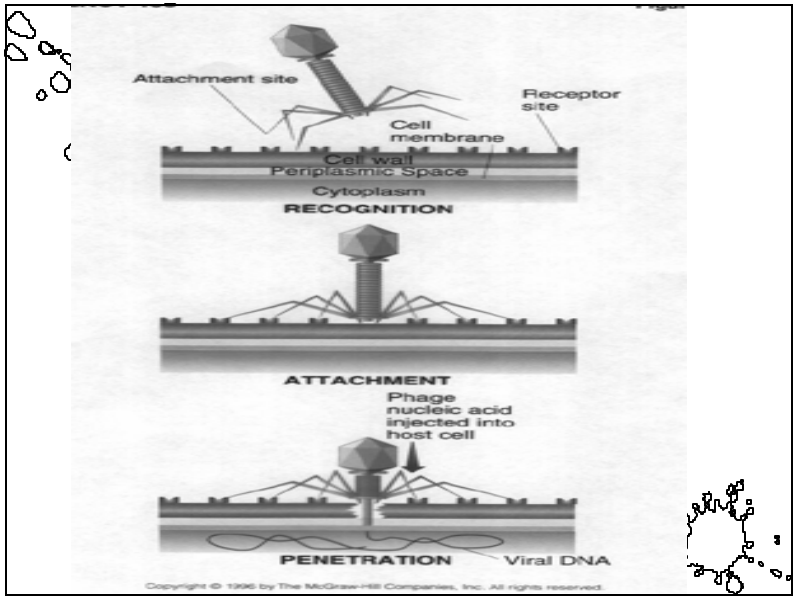
- **Based on....**
  - **type of nucleic acid**
  - **single or double strandedness**
    - **-RNA**
    - **+RNA**
  - **capsid morphology**
  - **presence or absence of envelope**
  - **Host range**



Viruses: Replication

- **Lytic replication**
  - **Attachment**
  - **Penetration**
  - **Uncoating**
  - **Synthesis of viral components**
  - **Assembly**
  - **Release**




Attachment site
Receptor site
Cell membrane
Cell wall
Periplasmic Space
Cytoplasm
RECOGNITION
ATTACHMENT
Phage nucleic acid injected into host cell
PENETRATION
Viral DNA
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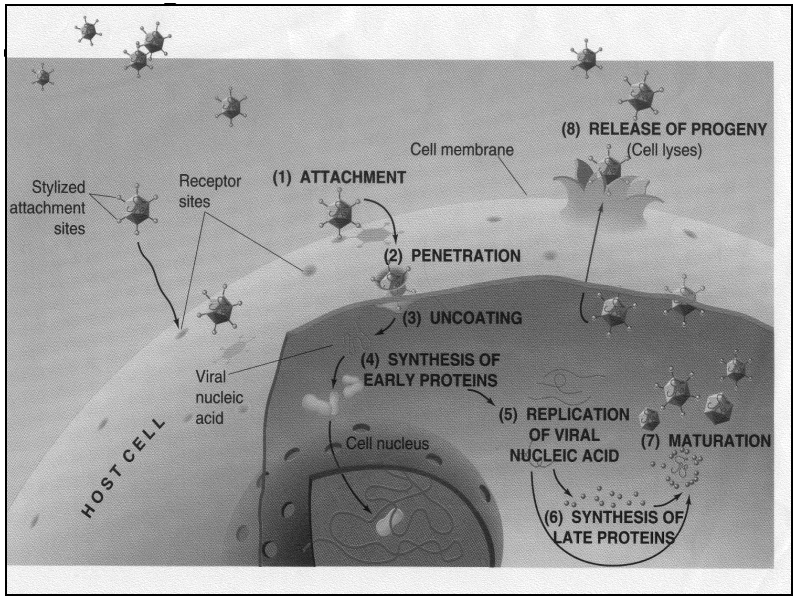
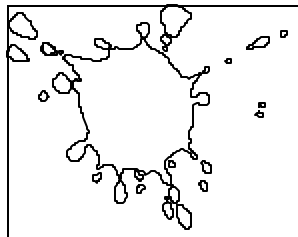
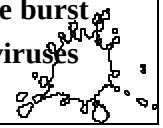
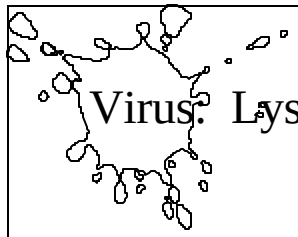

(8) RELEASE OF PROGENY
(Cell lyses)
Cell membrane
Stylized attachment sites
Receptor sites
(1) ATTACHMENT
(2) PENETRATION
(3) UNCOATING
(4) SYNTHESIS OF EARLY PROTEINS
Viral nucleic acid
(5) REPLICATION OF VIRAL NUCLEIC ACID
Cell nucleus
(7) MATURATION
(6) SYNTHESIS OF LATE PROTEINS
HOST CELL



Virus Replication

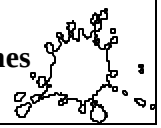
- **One-Step Growth Curve**
  - **simultaneously infecting every host cell**
    - **Eclipse phase- after entering host virus becomes non-infectious. Continues until there are infectious particles (intact virion)**
  - **Production of intracellular virus continues until cell lyses, releasing all particles in a single burst**
  - **rise period is time for all cells to release viruses**





Virus: Lysogenic Cycle

- **Lysogeny: Temperate viruses are bacteriophages capable of becoming lysogenic**
  - **viral chromosome replication turned off by viral specific proteins**
  - **repressed viral NA integrated into host chromosome (= prophage)**
  - **viral NA replicates with host chromosomes**
  - **induction**



Viruses: Oncogenic/Viroid/Prion

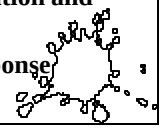
- **Oncogenic- cancer producing.**
  - **caused by "lysogenic" viruses**
    - **oncogenes**
  - **few human cancers caused by viruses (more animal)**
- **Viroids - usually associated with plant diseases.**
  - **ssRNA, circle, unprotected by capsid**
- **Prions - consist solely of protein??**
  - **no viral particles, no nucleic acid**
  - **Kuru, Creutzfeld-Jakob syndrome, slow viral diseases**

Viruses: Detection and Cultivation

- **Can't be seen by microscope (use of EM)**
- **growth on bacteria (plaque production) if bacteriophage**
- **Growth in cell culture (cytopathic effects=CPE)**
- **Viral growth in fertilized chick eggs**
- **serological (antibody) reactions in serum**
- **hemagglutination**
- **PCR=polymerase chain reaction (AIDS)**

Viruses: Resistance & Recovery

- **Interferon- antiviral factor produced by all cells. At least 3 different types: alpha, beta and gamma**
  - **ds RNA induces IFN production**
    - **IFN must exit cell then bind to IFN-receptor on cell surface**
    - **induces proteins that inhibit viral replication and destroy viral RNA's**
    - **IFN enhances hosts immune system response**

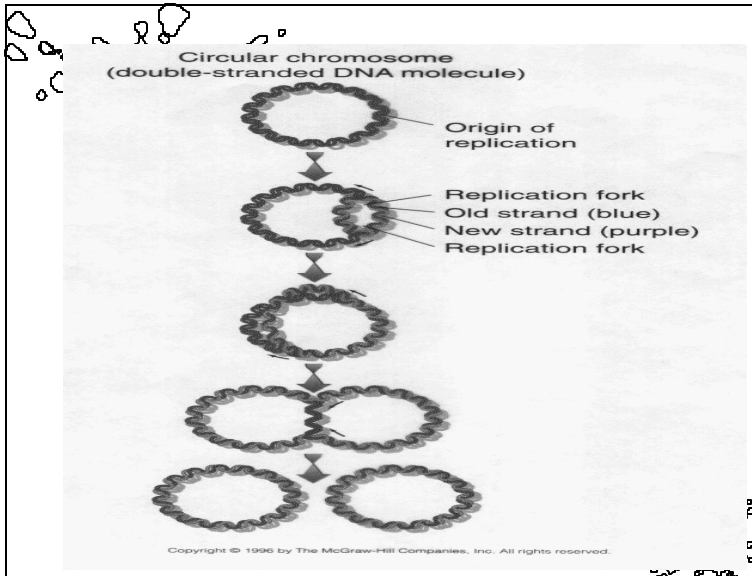


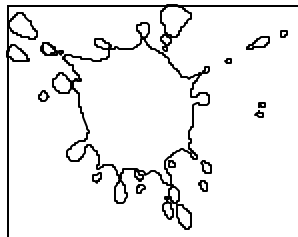
Viruses: Control of viral disease

- **Vaccines**
  - **Active vs passive**
  - **dead, attenuated (weakened but living), or viral pieces (antigenic proteins)**
- **Chemotherapy**
  - **antibiotics NOT effective**
  - **Nucleotide analogs**
    - **Acyclovir-herpes: disrupts DNA syn**
    - **AZT-HIV: inhibits reverse transcriptase**

Bacterial Growth

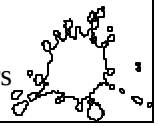
- **Bacterial Growth**
  - **Reproduction: not enlargement of cells**
  - **majority of bacterial reproduce by "binary fission.**
    - **synthesizes and assembles the constituents of its cell wall and cell membrane, volume increases and organelles made. Growth in size and then a septum forms to separate into two identical cells**

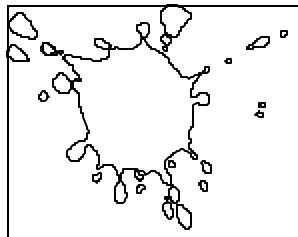




Exponential Growth

- Doubling: generation growth becomes logarithmic, and increases in an exponential manner.
  - Growth rate stays the same but exponential expansion increases.
  - Generation time is constant for each organism (most bacterial about 20 minutes)
  - *Mycobacterium* once every 13 days, and *Treponema pallidum* about every 30 hours





Doubling time

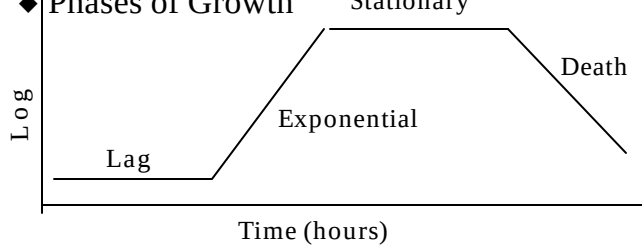
- A microbe with a 30 minute doubling time has 2 generations/ hour and has a capacity to produce 2^{48}-- or 281,474,976,710,656 progeny in 24 hours.
- Closed <u>vs</u> Open System controls capacity of total growth

Growth Curve

- Lag Phase
  - do not begin multiplying immediately but go through a period of adjustment. No detectable increase in cell number, but are metabolically active
- Logarithmic Phase- exponential growth
- Stationary Phase- death=growth
- Death Phase-depletion of nutrients, etc

Bacterial Growth Curve

- Phases of Growth



Nutritional Requirements

- Water/Energy Source/Carbon Source
  - Water- helps to transport substances
    - *N. gonorrhoeae* & *T. pallidum* die w/i 20 sec of drying
  - Source of Energy & Carbon
    - Phototrophs - energy from sun (photosyn)
    - Chemotrophs - energy from chemical bonds
      - organotrophs- use organic compounds for energy
      - lithotrophs- obtain energy from inorganic sources
    - autotrophs- use inorganic C (CO_2)as sole source of C
    - heterotrophs- require organic molecules as C source

Molecules of Life

- **4 Elements**
  - **pure substance that consists of a single atom**
  - **92 naturally occurring**
  - **4 elements that comprise over 98% of all living material**
    - **carbon (C)**
    - **hydrogen (H)**
    - **oxygen (O)**
    - **nitrogen (N)** ***and also***
    - **phosphorus (P) and sulfur (S) (another 1%)**

Molecules of Life

- **Atoms composed of:**
  - **electrons**
  - **protons**
  - **neutrons**
  - **number of protons equal to number of electrons**
  - **each element identified by atomic number (number of protons) and atomic weight (number of protons plus number of neutrons)**
    - **oxygen has atomic number "8" and atomic weight of "16"**

Molecules of Life

- **Molecule- two or more atoms held together by chemical bonds**
  - **the molecular weight of a molecule is the total number of protons and neutrons of all elements**
- **Chemical bonds**
  - **covalent-**
    - **sharing of electrons try to fill outer e^- shell.**
    - **Strong**
    - **single or double**
    - **require energy to make and energy released by breaking of covalent bonds by enzymes**

Molecules of Life

- **Ionic bonds**
  - **electrons completely leave one atom and move to the second without sharing. Leaves one atom with a positive charge and the other with a negative charge**
  - **most common type in inorganic molecules**
  - **in aqueous solutions ionic bonds are weak and break easily (I.e., NaCl----> Na^+ and Cl^-)**
- **Hydrogen bonds**
  - **attraction of +H to different - charged atom.**
  - **weak**

Molecules of Life

- **Organic Molecules**
  - **contain at least one carbon atom covalently bonded to another carbon or hydrogen atom**
- **Distinctive Groups**
  - **amino ($-NH_2$)**
  - **carboxyl (-COOH)**
  - **keto (C=O)**
  - **methyl ($-CH_3$)**
  - **alcohol (-OH)**

Molecules of Life

- **Major Organic Molecules**
  - **Proteins: amino acids to form peptides with peptide bonds. Make up proteins (structural) and enzymes (catalysts)**
  - **Polysaccharides: sugar subunits (mono- and polysaccharides). Primary energy source**
  - **Lipids: glycerol (3 C) and fatty acids (n-24 C)**
  - **Nucleic Acids: purine and pyrimidine bases**

Essential Elements

- In addition to Carbon, all cells need
  - hydrogen- all organic molecules
  - oxygen- all organic molecules
  - nitrogen- AA's, NA's
  - phosphorus- Lipids, NA's
  - sulfur- AA's
  - Metals- K, Mg, Fe, (trace=Cu, Zn, Co) as co-enzyme factors

Physical Requirements

- pH
  - (acidophiles, neutrophiles, alkalophiles)
  - 0--->14 scale
- Temperature
  - Thermophiles- above 40^{o}C
  - Mesophiles- between 20^{o}-40^{o}C
  - Psychrophiles- best below 20^{o}C
    - hydrogen ion concentration
      - negative exponent of H^{+} ion concentration

Molecular Oxygen

- Aerobes
- Facultative Anaerobes
- Microaerophiles
- Strict (obligate) Anaerobes- lack SOD
- CO_2- grow best at elevated levels
- Osmotic pressure- halophiles

Culture Media

- Chemically defined- components known
- Complex media - components unknown
- Specialized media
  - transport media: temporary storage
  - enriched media: support growth of a number of bacteria
  - selective media: grow some, kill others
  - differential media: indicators present

Measuring Microbial Numbers

- Direct counts
  - known volume put on slide and number of organisms counted using brightfield micro
  - electronic particle counter
- Indirect counts
  - plate counts (CFU)- 30-300 colonies
  - turbidity (spectrophotometer)

Metabolism

- Metabolism- sum of all cell-directed chemical reactions
  - anabolism
  - catabolism
    - potential energy can be released into Kinetic energy. Potential stored as chemical energy in bonds (even photosynthetic orgs must use sunlight to convert energy into chemical energy

Oxidation & Reduction

- Oxidation- loss of electrons
- Reduction- gain of electrons
  - For every oxidation reaction there is a reduction reaction
  - highly reduced compounds are more energy-rich than highly oxidized compounds
- ATP (adenosine triphosphate)- high energy compound. Composed of adenine, ribose and 3 phosphates

Electron Carriers

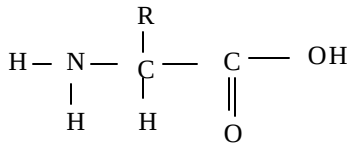
- Energy transfers are oxidation-reduction reactions and require the exchange of electrons as well as of energy. Electron carriers transport e- from one molecule to another. There are 3 carriers:
  - NAD=nicotinamide adenine dinucleotide
  - NADP=nicotinamide adenine dinucleotide phosphate
  - FAD=flavin adenine dinucleotide
- each of these can accept a pair of e-

Electron Transfer Processes

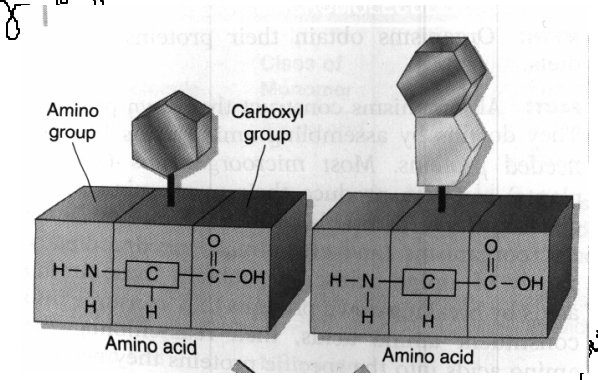
- electrons released from donor molecule in the form of hydrogen atoms (consists of a proton and electron).
  - Oxidized NAD is positively charged and when reduced by accepting a pair of e- become negatively charged (NADH or NADPH or $FADH_2$)
    - potential energy of e- is used to make ATP
    - can transfer e- to compounds being synthesized

Organic Molecules

- Proteins- composed of AA's (20)-linked together by peptide bonds to make protein
  - primary-AA sequence
  - secondary- folding
  - tertiary- folding on folds
  - quanternary- proteins folded onto other proteins



Peptide Structure

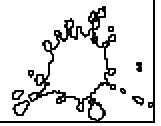


Organic Molecules: Nucleic Acids

- Nucleic Acids- 5 carbon sugar, N-base (single ring=pyrimidine, double ring=purine) , Phosphate
  - adenine, cytosine, guanine, thymine, uracil
  - DNA and RNA differences
  - deoxy-ribose & ribose
  - “four” letter strand and 3 letter codon
  - replication, transcription and translation

Organic Molecules: Polysaccharides

- carbohydrates- contain C, H, O
  - contain monomeric subunits of sugars
    - disaccharides
      - sucrose= glucose + fructose
      - maltose= glucose + glucose
      - lactose= glucose + galactose
  - Major energy source (potential energy)
    - ATP- adenosine triphosphate

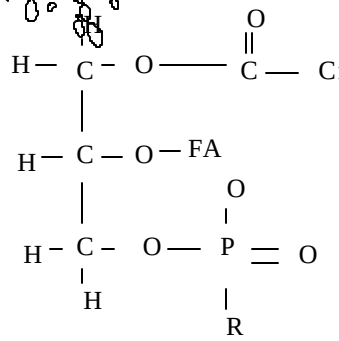


Lipids

- Fats, oils, waxes, steroids- insoluble in water, but can be dissolved in hydrophobic organic solvents: ether, chloroform, etc...
  - glycerol backbone with long-chain fatty acids
  - may have phosphate group on one carbon of glycerol
  - hydrophilic and hydrophobic ends (hydrophilic=phosphate, hydrophobic= long chain fatty acids)

Lipid structure

- Glycerol+Fatty acids +PO^-

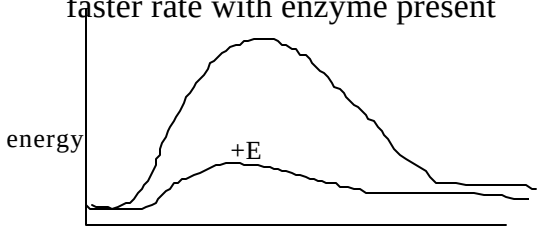


Enzyme Action

- Catalyst- speeds up the rate of chemical reaction by lowering energy of activation
- is not used up in reaction and can be re-used
- E+S----->ES complex---->E + P
  - Active site binds to substrate (mutation??)
  - coenzymes- metals that function as co-factors
- effect of pH, temp., concentration

Enzyme activity Reaction

- Reaction needs less energy and occurs at faster rate with enzyme present



Properties of Enzymes

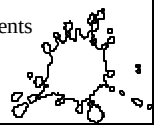
- Composed of protein
- specificity for substrate
  - lock & key fit
- unchanged by reaction
- may require co-enzymes for activity
- they are heat sensitive & pH sensitive
  - denatured
  - heat stable enzymes protected

Enzyme nomenclature

- suffix "-ase"
  - protease: breaks down protein to AA
  - decarboxylase: removes COOH (only CO_2)
  - lipase: fats
  - amylase: starch
  - caseinase: milk protein
  - hydrolase: break bond by addition of water
    - "lytic" enzymes= break, split
      - lipolytic, hydrolytic, etc...

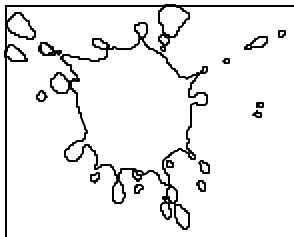
Chemical Inhibitors of Enzyme Activity

- competitive vs non-competitive inhibition
  - competitive: competes for active site of enzyme. Depends on concentration of enzyme and substrate and is reversible.
  - non-competitive: binds covalently to active site and cannot be removed. Irreversible inhibition
- Enzyme inactivation
  - heavy metals: work by altering shape of enzyme of binding to site in which coenzyme attaches and prevents activation. (examples= mercury, lead)



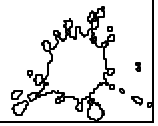
Metabolism

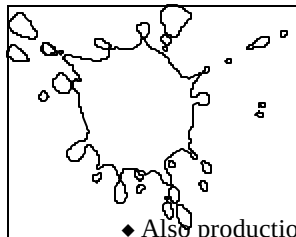
- Energy acquisition
  - chemo-heterotrophs convert food molecules to glucose and then the potential energy in glucose is released by oxidizing the molecule
  - complete oxidation of glucose to CO_2 and H_2O is called *respiration* (oxygen present)
  - when other by-products produced (alcohol, lactic acid, etc...) process may be *fermentation* (O_2 absent of enzymes absent)
- Glycolysis (Embden-Meyerhof Pathway)- is anaerobic. Glucose---> Pyruvic or Lactic acids



Glycolysis

- six carbon sugar split into two molecules of pyruvic acid (3-C) with production of ATP and NADH
- 10 metabolic reactions, each catalyzed by a specific enzyme
  - substrate level phosphorylation = phosphate transferred from organic substrate to ADP. In glycolysis there is production of 4 ATP from each glucose molecule by this method, but 2 ATP were used at 1st step to form a fructose-diphosphate, so there is a net yield of 2 ATP's





Glycolysis

- Also production of 2 NADH's (which will ultimately yield 6 ATP's if O_2 present.
- NAD is "reduced" (gain of electrons) while organic molecules are oxidized
- NAD is essential for oxidation to take place and must be regenerated either by going through the respiratory chain or by the process of Fermentation
- Fermentation- organic compound is both e- donor and final e- acceptor

Fermentation

- Pyruvic acid is one of the final e- acceptors and will form ethyl alcohol, lactic acid, acetic acid or others
- Fermentation requires no molecular O_2
- For Facultative anaerobes fermentation is the only means of obtaining energy in absence of oxygen
  - ability to ferment a specific sugar is diagnostic of the organism

Respiration

- Organic molecule is the donor of e- and inorganic molecule is the final e- acceptor
- Krebs cycle
- Respiratory chain- NADH (3 ATP's each)and $FADH_2$ (2 ATP's each). Many oxidation/reduction reactions

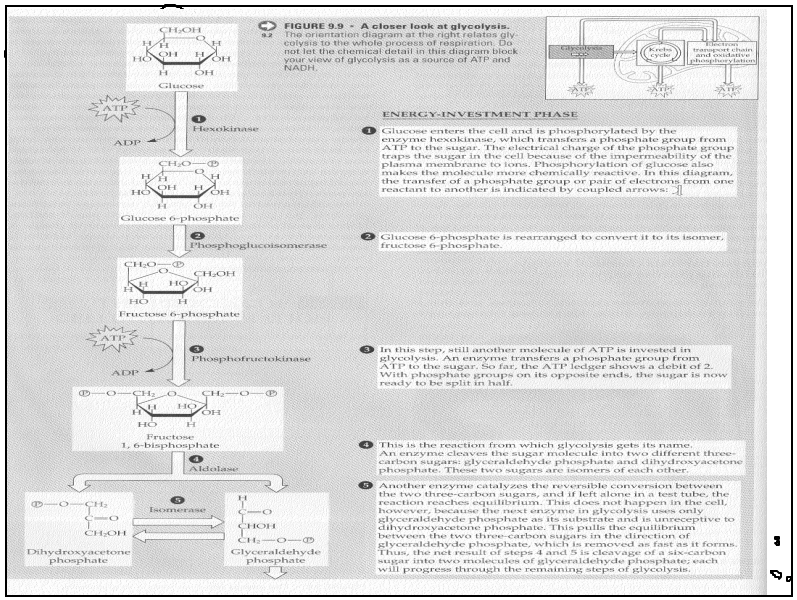
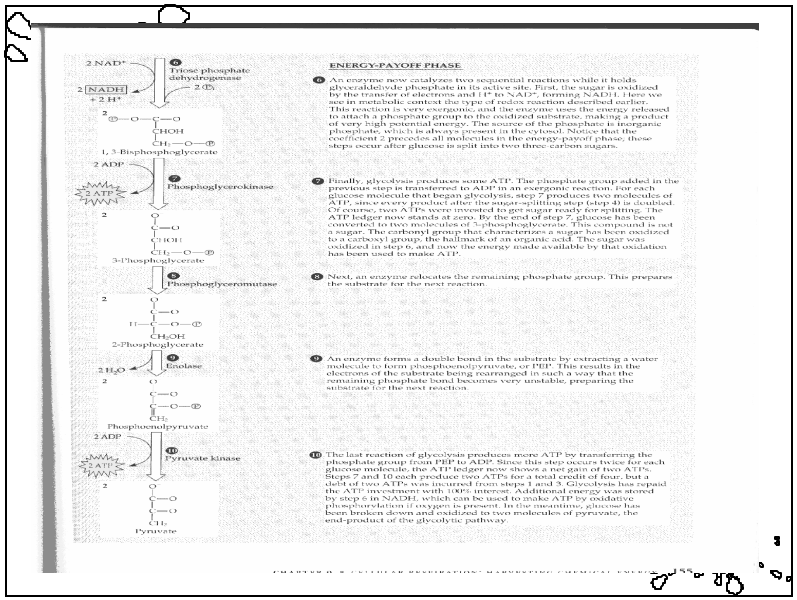


FIGURE 9.9 • A closer look at glycolysis.
The orientation diagram at the right relates glycolysis to the whole process of respiration. Do not let the chemical detail in this diagram block your view of glycolysis as a source of ATP and NADH.
Glycolysis
Pyruvate
Krebs cycle
Electron transport chain and oxidative phosphorylation
ATP
ATP
ATP
Glucose
ATP
ADP
1 Hexokinase
Glucose 6-phosphate
2 Phosphoglucoisomerase
Fructose 6-phosphate
ATP
ADP
3 Phosphofructokinase
Fructose 1, 6-bisphosphate
4 Aldolase
5 Isomerase
Dihydroxyacetone phosphate
Glyceraldehyde phosphate
ENERGY-INVESTMENT PHASE
1 Glucose enters the cell and is phosphorylated by the enzyme hexokinase, which transfers a phosphate group from ATP to the sugar. The electrical charge of the phosphate group traps the sugar in the cell because of the impermeability of the plasma membrane to ions. Phosphorylation of glucose also makes the molecule more chemically reactive. In this diagram, the transfer of a phosphate group or pair of electrons from one reactant to another is indicated by coupled arrows:
2 Glucose 6-phosphate is rearranged to convert it to its isomer, fructose 6-phosphate.
3 In this step, still another molecule of ATP is invested in glycolysis. An enzyme transfers a phosphate group from ATP to the sugar. So far, the ATP ledger shows a debit of 2. With phosphate groups on its opposite ends, the sugar is now ready to be split in half.
4 This is the reaction from which glycolysis gets its name. An enzyme cleaves the sugar molecule into two different three-carbon sugars: glyceraldehyde phosphate and dihydroxyacetone phosphate. These two sugars are isomers of each other.
5 Another enzyme catalyzes the reversible conversion between the two three-carbon sugars, and if left alone in a test tube, the reaction reaches equilibrium. This does not happen in the cell, however, because the next enzyme in glycolysis uses only glyceraldehyde phosphate as its substrate and is unreceptive to dihydroxyacetone phosphate. This pulls the equilibrium between the two three-carbon sugars in the direction of glyceraldehyde phosphate, which is removed as fast as it forms. Thus, the net result of steps 4 and 5 is cleavage of a six-carbon sugar into two molecules of glyceraldehyde phosphate; each will progress through the remaining steps of glycolysis.


2 NAD+
2 NADH + 2 H+
6 Triose phosphate dehydrogenase
2 ADP
7 Phosphoglycerokinase
2 ATP
1, 3-Bisphosphoglycerate
3-Phosphoglycerate
8 Phosphoglyceromutase
2-Phosphoglycerate
2 H2O
9 Enolase
Phosphoenolpyruvate
10 Pyruvate kinase
Pyruvate
ENERGY-PAYOFF PHASE
6 An enzyme now catalyzes two sequential reactions while it holds glyceraldehyde phosphate in its active site. First, the sugar is oxidized by the transfer of electrons and H+ to NAD+, forming NADH. Here we see in metabolic context the type of redox reaction described earlier. This reaction is very exergonic, and the enzyme uses the energy released to attach a phosphate group to the oxidized substrate, making a product of very high potential energy. The source of the phosphate is inorganic phosphate, which is always present in the cytosol. Notice that the coefficient 2 precedes all molecules in the energy-payoff phase, these steps occur after glucose is split into two three-carbon sugars.
7 Finally, glycolysis produces some ATP. The phosphate group added in the previous step is transferred to ADP in an exergonic reaction. For each glucose molecule that began glycolysis, step 7 produces two molecules of ATP, since every product after the sugar-splitting step (step 4) is doubled. Of course, two ATPs were invested to get sugar ready for splitting. The ATP ledger now stands at zero. By the end of step 7, glucose has been converted to two molecules of 3-phosphoglycerate. This compound is not a sugar. The carbonyl group that characterizes a sugar has been oxidized to a carboxyl group, the hallmark of an organic acid. The sugar was oxidized in step 6, and now the energy made available by that oxidation has been used to make ATP.
8 Next, an enzyme relocates the remaining phosphate group. This prepares the substrate for the next reaction.
9 An enzyme forms a double bond in the substrate by extracting a water molecule to form phosphoenolpyruvate, or PEP. This results in the electrons of the substrate being rearranged in such a way that the remaining phosphate bond becomes very unstable, preparing the substrate for the next reaction.
10 The last reaction of glycolysis produces more ATP by transferring the phosphate group from PEP to ADP. Since this step occurs twice for each glucose molecule, the ATP ledger now shows a net gain of two ATPs. Steps 7 and 10 each produce two ATPs for a total credit of four, but a debit of two ATPs was incurred from steps 1 and 3. Glycolysis has repaid the ATP investment with 100% interest. Additional energy was stored by step 6 in NADH, which can be used to make ATP by oxidative phosphorylation if oxygen is present. In the meantime, glucose has been broken down and oxidized to two molecules of pyruvate, the end-product of the glycolytic pathway.

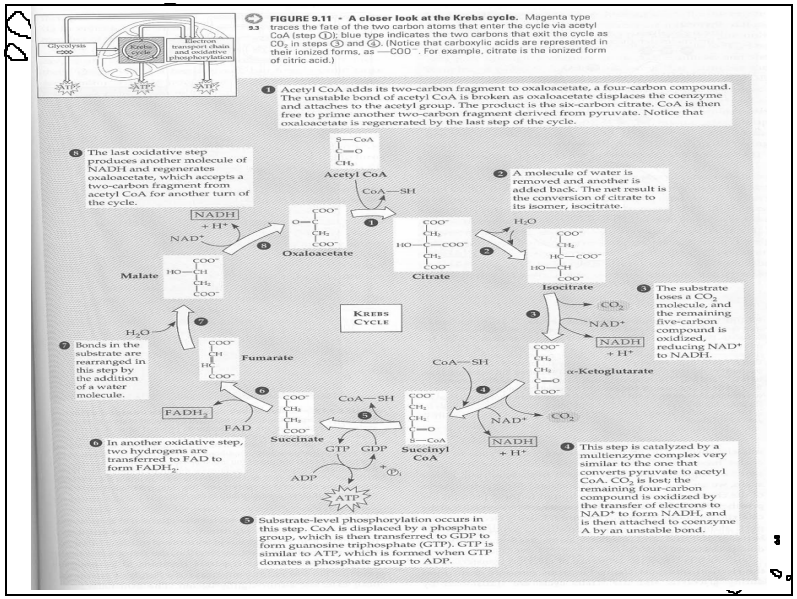


FIGURE 9.11 • A closer look at the Krebs cycle. Magenta type traces the fate of the two carbon atoms that enter the cycle via acetyl CoA (step 1); blue type indicates the two carbons that exit the cycle as CO_2 in steps 3 and 4. (Notice that carboxylic acids are represented in their ionized forms, as $—COO^-$. For example, citrate is the ionized form of citric acid.)

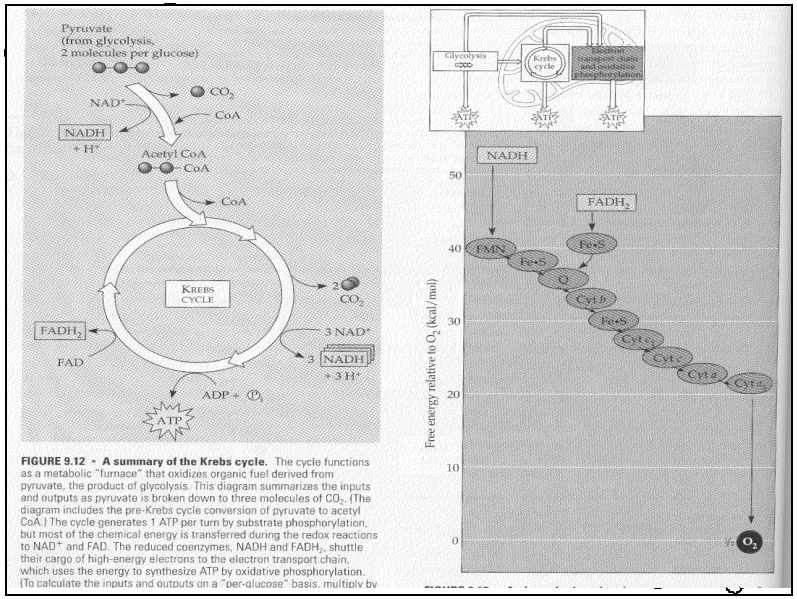
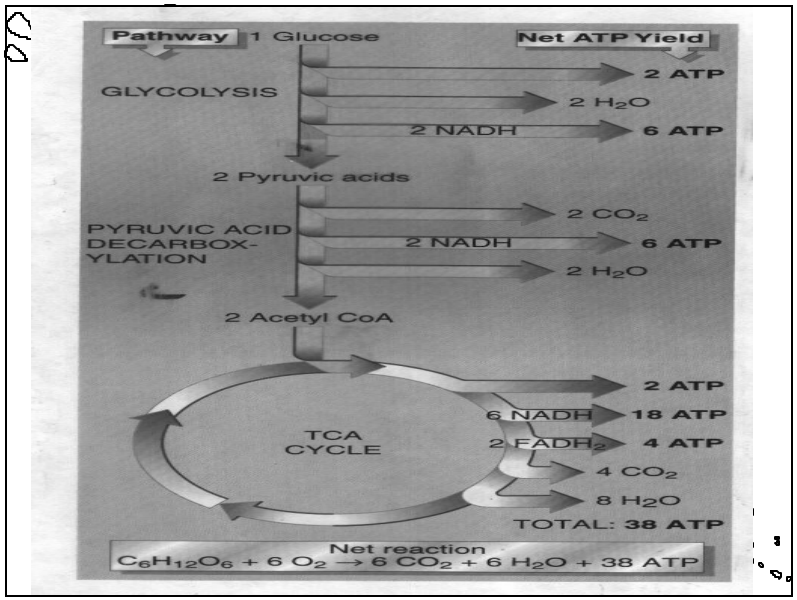
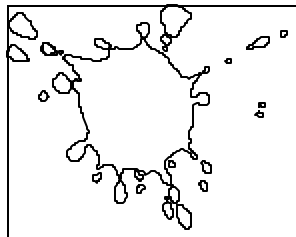


FIGURE 9.12 • A summary of the Krebs cycle. The cycle functions as a metabolic "furnace" that oxidizes organic fuel derived from pyruvate, the product of glycolysis. This diagram summarizes the inputs and outputs as pyruvate is broken down to three molecules of CO_2. (The diagram includes the pre-Krebs cycle conversion of pyruvate to acetyl CoA.) The cycle generates 1 ATP per turn by substrate phosphorylation, but most of the chemical energy is transferred during the redox reactions to NAD^+ and FAD. The reduced coenzymes, NADH and $FADH_2$, shuttle their cargo of high-energy electrons to the electron transport chain, which uses the energy to synthesize ATP by oxidative phosphorylation. (To calculate the inputs and outputs on a "per-glucose" basis, multiply by



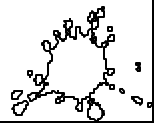
Chemiosmosis

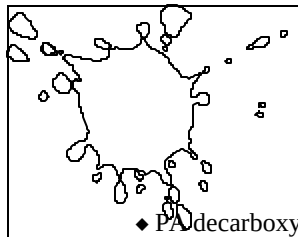
- mechanism for producing ATP
  - energy is released from e- transport and is used to move protons (H+) across a membrane so they accumulate on one side. This generates a proton gradient that is a reservoir of energy= ***protonmotive force .***
  - When e- flow across membrane they release potential energy to make ATP
  - channels have ATP synthetase to phosphorylate ADP-->ATP



Aerobic vs Anaerobic

- **End product of glycolysis is 2 pyruvic acids**
  - **aerobic organisms can get 6 APT's from the 2 NADH produced in glycolysis in the cytochrome system + 2 ATP's directly formed**
  - **anaerobic org's get two ATP's directly: NADH's produced are used to reduce pyruvic acid to lactic acid**
  - **the pyruvic acids in aerobic respiration can go into the Krebs Citric Acid Cycle to produce more energy molecules**





Aerobic respiration

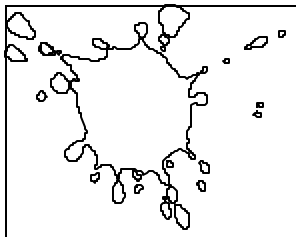
- PA decarboxylated to acetyl Coenzyme A (a 2 carbon molecule) which enters into Krebs Cycle. This reaction yields an NADH for each PA molecule.
- Drebs cycle goes around 2 X for each glucose
- During each cycle, 3 NADH's formed and one $FADH_2$. (6 NADH and 2 $FADH_2$'s total!!). Also, 2 ATP's formed directly.
- 18 ATP's from 6 NADH, 4 ATP's from 2 $FADH_2$, and 2 ATP's direct

Summary of Aerobic ATP'S

- Glycolysis
  - 2 ATP's direct + 6 ATP's from 2 NADH's
- Pyruvic acid ----> Acetyl CoA
  - 6 ATP's from 2 NADH's
- Krebs Cycle
  - 2 ATP's direct + 18 ATP's from 6 NADH's + 4 ATP's from FADH2's
- Total of 38 ATP's from aerobic process

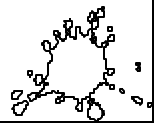
Anaerobic Respiration

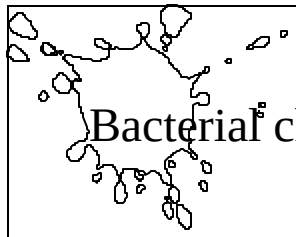
- terminal e- acceptor is an inorganic molecule other than oxygen
  - SO_4^{2-}------> H_2S
  - NO_3------> NO_2^-
- Less efficient than aerobic respiration



Bacterial Genetics

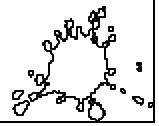
- Genes: linearly arranged along the bacterial chromosome. *E. coli* has single chromosome that has 5000 genes (human has 46 chromosomes that have 100,000 genes.
- Genes strictly regulated (temp, feedback loops, etc...)





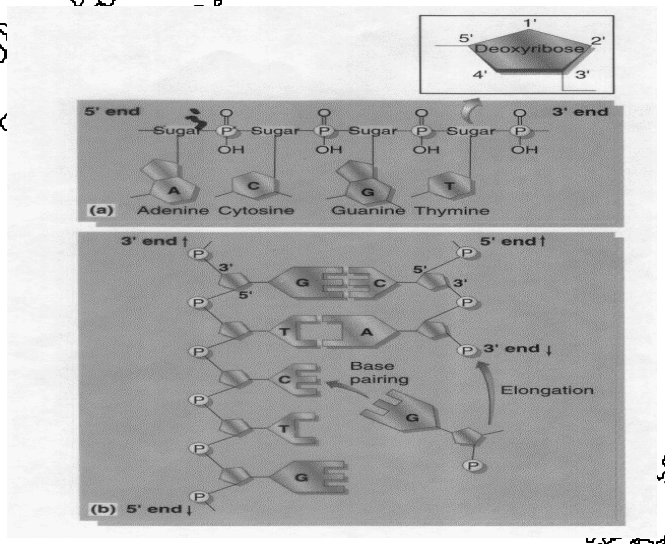
Bacterial chromosome/genes

- circular chromosomes contain haploid cell
  - recessive genes expressed since no 2nd allele present
  - 90% of genes make proteins, the rest code for transfer and ribosomal RNA
- contain plasmids
  - antibiotic resistance
  - fertility factors
  - metabolic options



DNA

- storage of genetic information
- inheritance
- expression of genetic message
  - 1. storage
  - subunits/ 5 bases: adenine, thymine (only in DNA), cytosine, guanine, uracil (only in RNA)
  - A paired with T and C paired with G on two strands
  - 5’ and 3’ ends are antiparallel strands.
  - Codons



Genetic Language

- **depends on order of nucleotides**
  - **codons= code for specific amino acids**
    - **code is redundant (i.e., different codons may code for the same AA)**
    - **64 different combinations**
    - **start (1) and stop (3) codons**
  - **originally thought that there was one gene for one protein, now known that multiple genes may code for one protein**
  - **mutations - base substitution or frameshift**

Mutations

- Base substitution-
  - Substitute a purine for a purine or a pyrimidine for a pyrimidine base
    - Purine = adenine & guanine (NO "Y"): double ring structure
    - Pyrimidine = cytosine & thymine (has "Y"): single ring structure
- Effect- may or may not affect protein function: depends on where in sequence (catalytic site or folding??)

Mutations

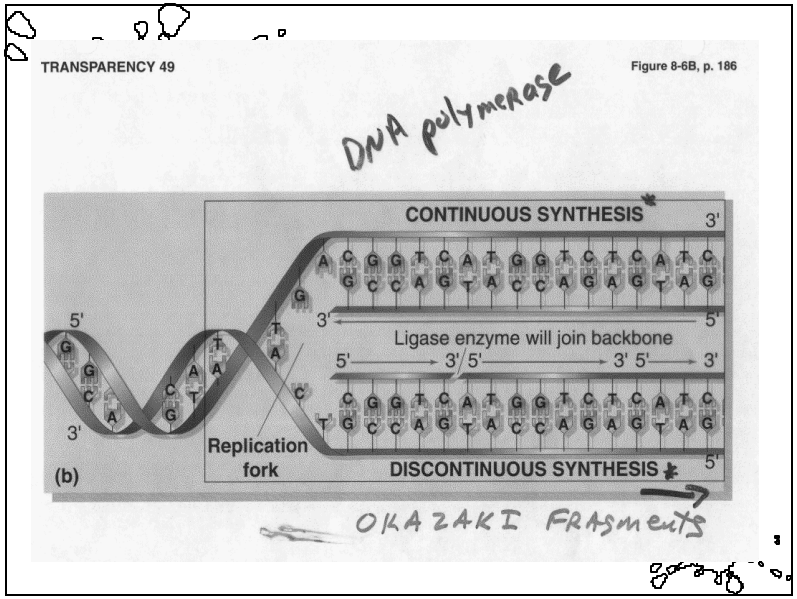
- Frameshift- base addition or deletion
  - CHANGES READING FRAME !! From point of mutation and therefore changes translational product (protein)
    - Stop codons inserted (truncated protein)
    - Non-sense sequence of bases (no functional catalytic site)
    - Wrong folding pattern
    - **Lethal to cell**

DNA Replication

- **make identical copies of DNA for cell division**
- **two strands must unwind and separate and each has a complementary strand made (semi-conservative replication)**
- **replication fork, DNA polymerase**
  - **DNA replication proceeds only in 5'-->3' direction (old strand read in 3'-->5' direction)**

DNA Replication (cont)

- one strand has continuous synthesis (made in 5'-->3' direction) while other strand is made in pieces (Okazaki fragments), discontinuous synthesis.
  - DNA polymerase and DNA ligase
- bi-directional replication
- exons (protein coding) vs introns (non-protein coding) regions

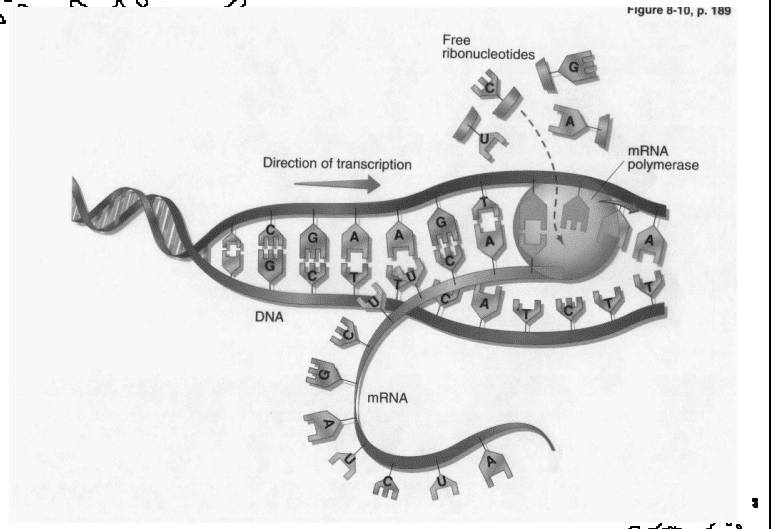


RNA Types

- **messenger RNA (mRNA)- attaches to ribosomes and carries code from DNA**
- **ribosomal RNA- ribosome structure**
- **transfer RNA- 64 types that are specific for each different AA. one tRNA carries one AA**
- **RNA is *transcribed* from DNA**

RNA Transcription

- **copied from only one strand of DNA, not both**
- **same strand is not always copied for all genes**
- **RNA polymerase binds to gene at promoter region which designates start point of transcription**
- **some genes may overlap**

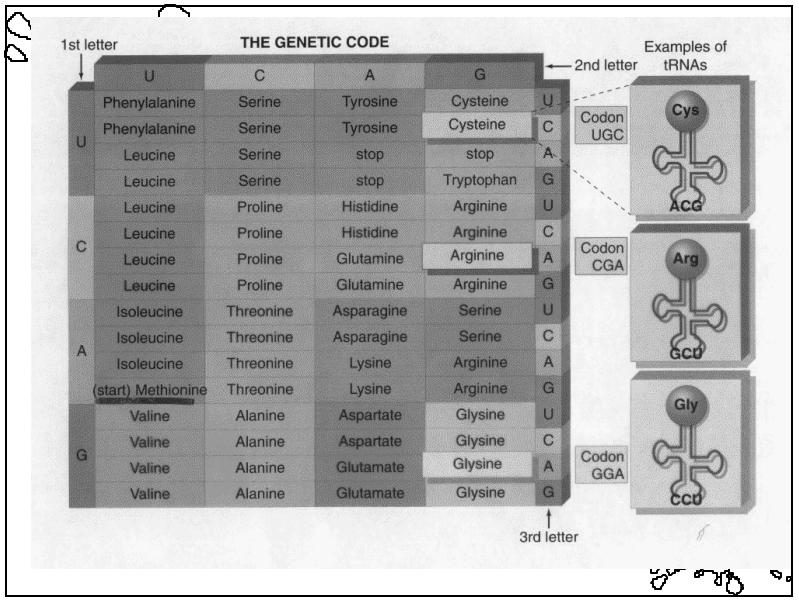


RNA Properties

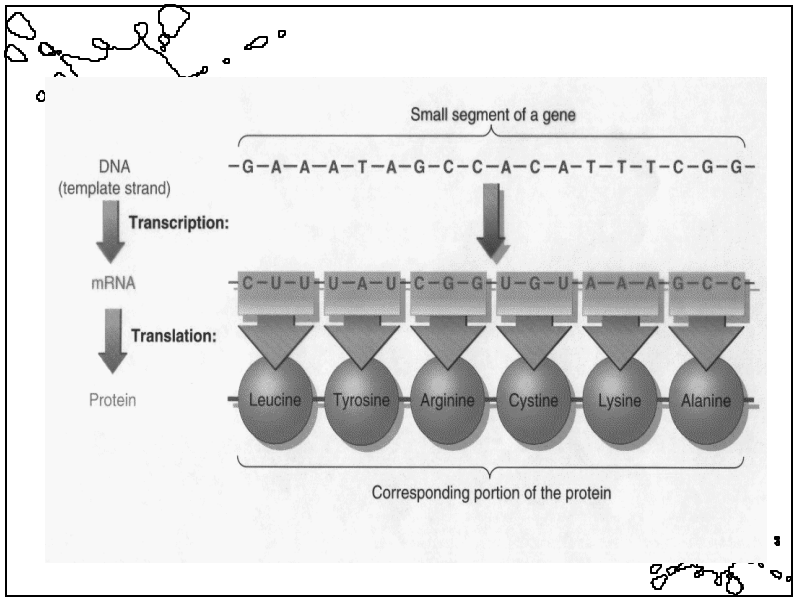
- **RNA made in 5'-->3' direction (therefore DNA transcribed (read) in 3'-->5' direction)**
- **mRNA may be polygenic or monogenic**
- **mRNA is modified after transcription**
  - **5'-methyl cap (stability)**
  - **poly A tail (stability)**
- **mRNA made off of *anti-sense* strand of DNA**

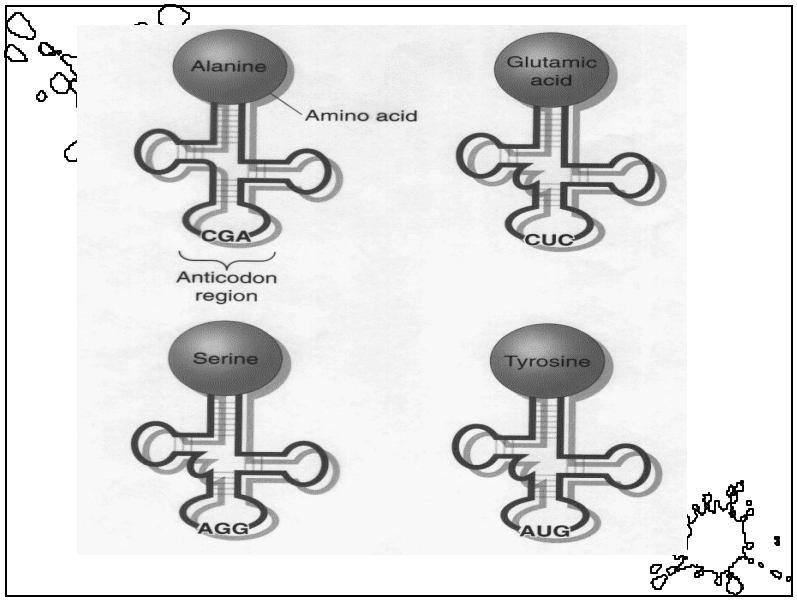
Protein TRANSLATION

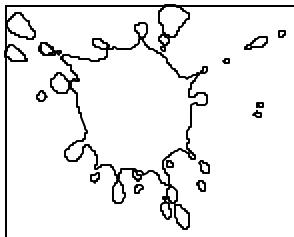
- codons on mRNA and anti-codons on tRNA
- table of codons and what each codes for on page 215)
  - start codon always methionine (AUG)
  - stop codons (nonsense): UAA, UAG, UGA
- each tRNA anticodon matches up with codon to place AA in appropriate spot



1st letter	U	C	A	G	3rd letter
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
	Phenylalanine	Serine	Tyrosine	Cysteine	C
	Leucine	Serine	stop	stop	A
	Leucine	Serine	stop	Tryptophan	G
C	Leucine	Proline	Histidine	Arginine	U
	Leucine	Proline	Histidine	Arginine	C
	Leucine	Proline	Glutamine	Arginine	A
	Leucine	Proline	Glutamine	Arginine	G
A	Isoleucine	Threonine	Asparagine	Serine	U
	Isoleucine	Threonine	Asparagine	Serine	C
	Isoleucine	Threonine	Lysine	Arginine	A
	(start) Methionine	Threonine	Lysine	Arginine	G
G	Valine	Alanine	Aspartate	Glysine	U
	Valine	Alanine	Aspartate	Glysine	C
	Valine	Alanine	Glutamate	Glysine	A
	Valine	Alanine	Glutamate	Glysine	G

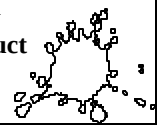

Small segment of a gene
DNA
(template strand)
–G–A–A–A–T–A–G–C–C–A–C–A–T–T–T–C–G–G–
Transcription:
mRNA
C–U–U–U–A–U–C–G–G–U–G–U–A–A–A–G–C–C–
Translation:
Protein
Leucine
Tyrosine
Arginine
Cystine
Lysine
Alanine
Corresponding portion of the protein

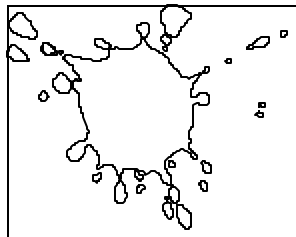

Alanine
Amino acid
Glutamic acid
CGA
CUC
Anticodon region
Serine
Tyrosine
AGG
AUG



Operon Model

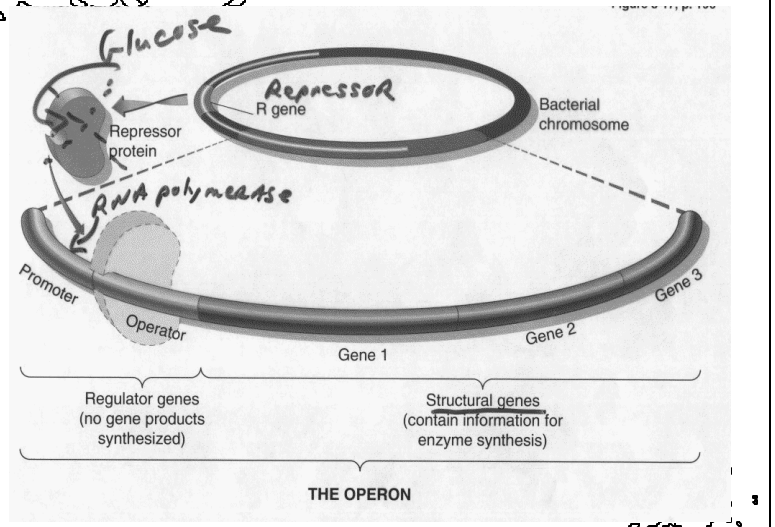
- **A segment of bacterial (eukaryotic?) chromosome the contains genes necessary for the metabolism of one (?) substrate or a series of substrates to an end-product**
  - **can be induced by the presence of a substrate that will induce activation of genes that will produce enzymes necessary to break it down**
  - **can be induced by the need for an end product**





OPERON MODEL

- **Consists of the following genes/regions:**
  - **regulator region- composed of promoter and operator genes. RNA polymerase binds to promoter to initiate transcription. Open operator region necessary to allow for movement of polymerase into structural gene region.**
  - **repressor gene: produces a substance that will inhibit RNA polymerase from moving through the operator region and thereby inhibit RNA synthesis**
  - **structural genes: direct synthesis of proteins necessary for metabolism of substrate**



Regulation of OPERON

- ***Substrate induction*: When substrate present can bind to repressor substance to prevent it from binding to and blocking operator region, thereby allowing RNA transcription to take place.**
- ***End product inhibition*: when End Product is in high concentration it will bind to repressor and activate it so that it inhibits transcription. When End Product is depleted cell want to make more and EP is released from repressor protein to inactivate repressor which allows for transcription.**

Genetic Transfer in Bacteria

- **Transformation-**
  - **When bacteria are lysed they release their DNA. this can be taken up by "competent" bacteria and can give recipient bacteria new genetic traits.**
    - **Griffith: experiments with dead encapsulated *S. pneumoniae* and living, nonpathogenic, non-encapsulated *S. pneumoniae.***
    - **encapsulated bacteria transformed non-encapsulated bacteria into ones that had capsules and made them pathogenic**

Genetic Transfer in Bacteria

- Transduction
  - **virus mediated transfer of genetic material. Bacterial genes may become enclosed within a bacteriophage and transferred to an new infected bacteria to give that bacteria new properties.**

Genetic Transfer in Bacteria

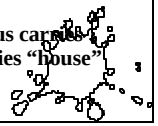
- **Conjugation- (in Gram negative bacteria)**
  - **some bacteria have the capability of attaching to other bacteria and transferring segments of DNA (drug resistance)**
  - **mediated by certain plasmids (F-plasmids) that carry fertility genes that code for the formation of sex pili**
  - **Plasmid genes can be transferred through pili to another bacteria and when they are they transfer ability to produce pili and thus make F^- bacteria F^+**
- **Hfr donors - F plasmid integrates into host chromosome and allows for transfer of host DNA and only rarely plasmid DNA so recipient cell does not become F^+**

Microbial Interactions

- Symbiosis- life together. Inhabitants of same environment and interactions may be beneficial, harmful or neutral
  - Commensalism: one organism benefits and the other is unaffected (human intestinal tract- obligate anaerobes most abundant type. They could not survive w/o facultative anaerobes because they consume all of the oxygen creating the anaerobic environment. Facultative organism gains nothing and obligate anaerobe does

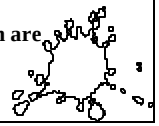
Symbiosis

- Mutualism: both partners benefit and in some cases this is obligatory
  - cross-feeding of two bacterial strains. Each makes a nutrient that the other needs.
    - ***Streptococcus faecalis* & *Lactobacillus arabinosus:* neither can survive in glucose media, but each makes nutrient needed for both to survive**
    - **Lichens - fungus and algae - reside in areas poor in water and nutrients**
    - **Lysogeny - virus residing in bacterium. Virus carries disease gene (scarlet fever) & bacteria supplies "house"**
    - **Biofilms - imp't in dental labs**



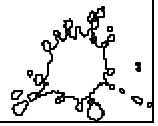
Symbiosis: Mutualism

- Gut microbe examples:
  - **ruminant herbivores**
    - **microbes digest cellulose and ferment these into fatty acid end products**
    - **30 species of bacteria and protozoa**
  - **produce vitamins and compete with pathogens preventing disease**
  - **Blood leeches**
    - **without a species of *Pseudomonas* in the gut, which are needed to lyse blood cells, the leech would starve**



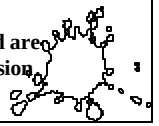
Symbiosis: Antagonism

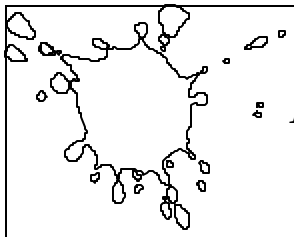
- **when one organism harms another**
  - **disappearance of one organism may benefit another by reducing competition**
  - **amensalism:**
    - **when the harmful organism neither suffers nor benefits from the harm it does**
  - **parasitism:**
    - **a small organism invades a larger one**



Symbiosis: Antagonism examples

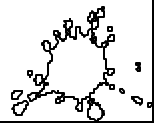
- **between microbes- metabolic by-products help to give one organism survival advantage over another. Release of antibiotics or bacteriocins (anti-microbial proteins whose activity is against only against closely related species**
  - **may also change pH or oxygen concentration**
  - **Rickettsias: small gram negative rods that are obligate intracellular parasites. Found in arthropods (vector) and animals.**
  - **Chlamydias: lack enzymes for energy transfer and are intracellular parasites. Do not divide by binary fission**

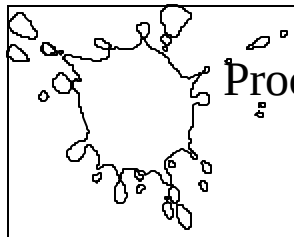




Antimicrobial Methods

- **Microbicidal and Microbistatic Agents**
  - **microbicidal= kill. Have permanent effect**
  - **microbistatic= standstill. Do not kill or remove organisms but stop multiplication and growth. Organisms will begin to grow once agent removed.**
  - **Germicidal= refers to the destruction of microorganisms**



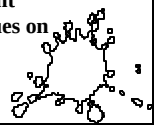


Processes of Antimicrobial Agents

- **Sterilization**
  - **eliminates all forms of life, including vegetative cells, spores, and viruses as well as viroids. No such thing as "almost sterile". Endotoxins and other bacterial products may remain and cause problems. Do not use on body surfaces.**
- **Disinfection**
  - **eliminates vegetative forms of most pathogenic organisms but does not ensure the elimination of all pathogenic organisms (spores resistant). Use only on inanimate objects and never on body surfaces.**

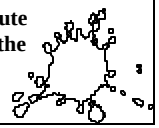
Processes (cont)

- **Sanitization**
  - **supplements disinfection with cleaning. This insures the elimination of dirt and organic debris as well as infectious microbes. Used in food preparation and for reusable instruments in hospitals**
- **Antisepsis**
  - **inhibition or destruction of microbes on the surface of living tissue in an attempt to prevent infection. Antiseptics must not harm the tissues on which they are used**



Factors affecting antimicrobial activity

- **microbial susceptibility**
  - **vegetative bacteria, fungi and enveloped viruses most susceptible**
  - **mycobacteria have waxy coat that makes them more resistant**
  - **spores are resistant**
- **Number of microorganisms**
  - **a fixed % of organisms will die during each minute of exposure, thus, the greater the concentration the less the effectiveness**



Factors affecting antimicrobial activity

- **Concentration (dose) of agent**
  - **at lower concentrations agent may become microbistatic rather than microbicidal**
  - **intensity of exposure of heat or radiation**
- **Length of exposure**
  - **the longer the exposure the greater the number of microbes will be killed**
  - **microbistatic agents must be used during entire time inhibition is to be maintained**

Factors affecting antimicrobial activity

- **Environmental conditions**
  - **temperature, pH, moisture**
  - **absorption by blood, mucus, feces, tissue may reduce activity and eliminate them**
  - **must be concerned with 1/2 life in system**
  - **removal by liver function**
  - **age of patient has effects from all of above**
- **treatment of biofilms hard**
  - **dental water lines**

Physical Agents for controlling Microbes

- **Moist heat**
  - **steam effectively kills by coagulating proteins. In absence of water heat does not coagulate proteins. Dry heat kills, but at higher temperature**
- **pasteurization**
  - **test organism is *Coxiella burnetti***
  - ***low temperature holding pasteurization (LTH)*: exposure of product for 30 min at 144°F (62.8°C)**
  - ***flash high temperature, short time (HTST)*: milk heated for 15 seconds at 71.6°C (161°F) followed by rapid cooling**
  - **ultra high temp (UHT) - 3 sec exposure at 311°F**

Physical Agents for controlling Microbes

- **Boiling**
  - **water temp. 100°C no matter how long you boil**
  - **fractional sterilization (Tyndallization)- requires 3 days and is useful only for material that support microbial growth. 1st day steam heat for 30 min to destroy all vegetative cells, then incubate. Heat-resistant spores germinate and give same treatment 2nd day, then incubate to germinate remaining spores. Heat for 30 min 3rd day**
- **Autoclaving**
  - **steam heat under pressure: exhaust slowly**
  - **biological wastes/ prions survive**

Physical Agents for controlling Microbes

- **Dry Heat**
  - **flaming, incineration and baking**
- **Radiation**
  - **gamma rays, S -rays, UV light: shortest wavelength has greatest energy and effect. Use for heat sensitive materials**
  - **UV light causes thymine dimers which interfere with replication and transcription and expression of genes. There are repair mechanisms. UV rays cannot penetrate glass, plastic or turbid solutions**
- **Filtration- bacteria but not viruses (laminar flow hoods)**

Physical Agents for controlling Microbes

- **Mechanical scrub**
  - **hand washing- before and after contact**
    - **most transient organisms can be removed by 30 seconds of proper scrubbing with soap and water, but microbes that reside in sweat ducts and hair follicles cannot be removed easily. Threat to patients with immune suppression. Use of antiseptic soaps to cleanse**

Chemical Agents for controlling microbes

- **Chemicals that sterilize**
  - **Ethylene oxide (ETO)- used for sterilization of plastics, rubber goods and delicate instruments that are damaged by heat.**
    - **reacts with nucleic acids disrupting protein synthesis**
  - **Vaporized hydrogen peroxide- sterilize surfaces of enclosed areas such as incubators and clean rooms**
  - **Glutaraldehyde and formaldehyde- soaking for 10-12 hours and then thorough rinsing with sterile water**

Chemical Disinfectants & Antiseptics

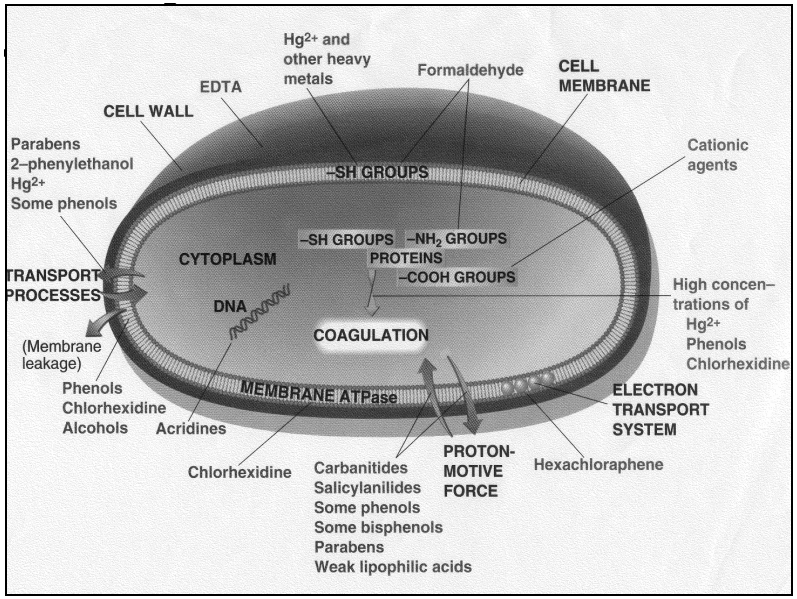
- **High level germicides= ethylene oxide, glutaraldehyde and formaldehyde have capacity to kill all microbes and spores**
- **Intermediate level germicides= kill vegetative cells and most viruses but not endospores**
- **low level germicides= killing limited to a few types of bacteria, fungi and enveloped viruses**

Low-level Germicides

- Comprises most antiseptic agents
  - phenolics- kill by inactivating essential cell enzymes
    - cresols (lysol) (enveloped viruses, ie HIV)
    - hexachlorphene (*S. aureus*)
  - Alcohols- coagulating essential proteins
    - ethanol and isopropanol- use to reduce # of microbes on skin, thermometers and small instruments
  - Chlorine and Iodine- chlorine oxidizes and inactivates enzymes. Iodine irreversibly binds to proteins and inactivates them

Low-level Germicides

- Chlorhexidine:
  - active against both Gram + and Gram - organisms by interfering with plasma membrane permeability. Also active against enveloped viruses
- Heavy metals:
  - bind to and inactivate proteins (not selective for microbes and must be used in dilute concentrations. Mercurochrome and silver nitrate are examples.
- Ozone:
  - O3: gas used in wastewater tx. kills bacteria, viruses, fungi and protozoa



Antibiotics & Chemotherapy

- **Paul Ehrlich- Father of modern chemotherapy. Searched for "magic bullet" a compound that would kill the pathogen without harming the patient (1912). Lead to discovery of Sulfa Drugs in 1932.**
- **Fleming- (1929) discovers 1st antibiotic (chemicals produced by microorganisms that in low concentrations selectively kill or inhibit growth of other microbes**
  - **ideal drugs must have selective toxicity for prokaryotes, last a long time in body, effective at low concentrations, broad spectrum, no toxicity, little interaction with other drugs, good distribution**

Antimicrobial Mechanisms

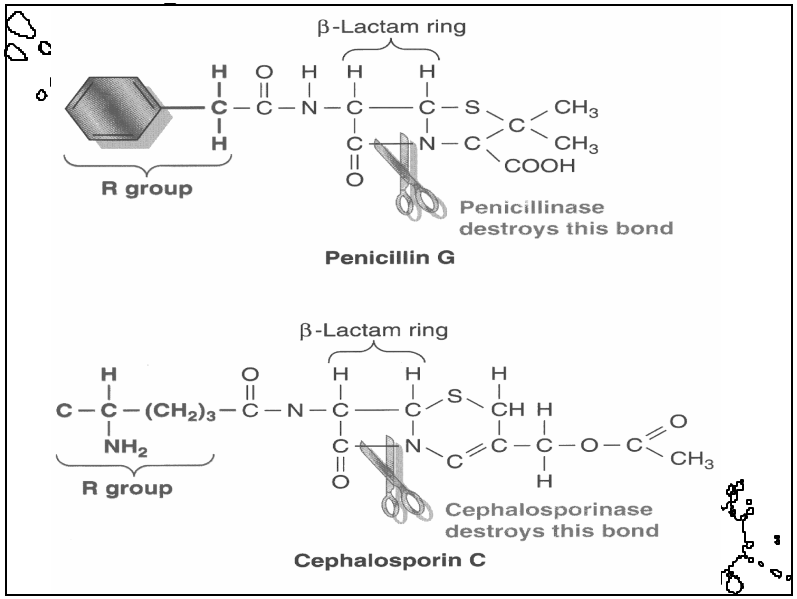
- Bacterial cell walls
  - beta-lactam antibiotics (penicillin & cephalosporins)
    - each contains a ring structure called B-lactam, and some bacteria produce enzymes that break the lactam ring (B-lactamases).
    - depending upon the binding protein in the peptidoglycan or periplasmic space, these drugs may inhibit peptidoglycan synthesis, activate autolytic enzymes, or cause bacteriostasis

Penicillin G

- Alexander Fleming
- antibiotic of choice for Staphylococcal and Streptococcal infections
- metabolic by-product of blue-green mold *Penicillium chrysogenum*
- can improve on natural antibiotic by altering structure slightly
- acid resistance-Penicillin G destroyed by stomach acid. Ampicillin and oxacillin are not when given orally
- penicillinase resistance- methicillin, oxacillin (less active than penicillin though)
- Broad spectrum- even against Gram - organisms (ampicillin and amoxicillin penetrate LPS)

Cephalosporins

- **natural product not used in clinical setting. Semisynthetic derivatives are used widely.**
  - **more than 70 different cephalosporins created**
  - **not destroyed by penicillinases (there are cephalosporin destroying enzymes)**
  - **because of structural similarities, a person allergic to penicillin is not treated with cephalosporin**



Bacitracin/Vancomycin

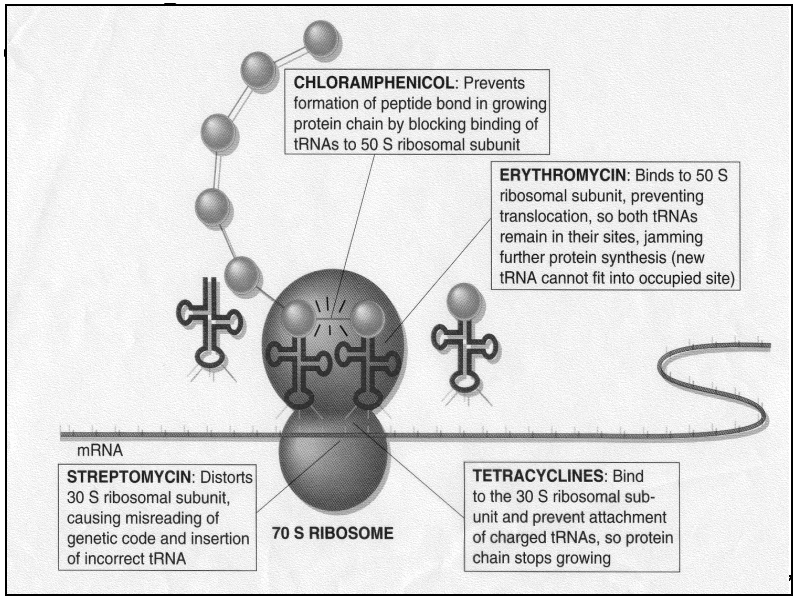
- kills gram positive bacteria because of its effect on inhibiting peptidoglycan formation.
  - bacitracin used only topically because of kidney toxicity
  - vancomycin has no kidney toxicity and is used in place of penicillin in allergic people. Vancomycin-resistant bacteria also appearing!

Cell Membrane Targeted Antibiotics

- Polymyxins
  - bind to phospholipids in bacterial membranes and alter their permeability. Causes leakage and cell death. Good as topical agents in gram - infections in burn patients. Have toxic side-effects.
- Polyenes & Nystatin
  - bind to ergosterol, the sterol in fungal membranes, creating pores and causing leakage. Human cells contain cholesterol instead of ergosterol and do not bind polyene as well. Nystatin used vs Candida and used topically topically because of toxicity

Protein Synthesis as Target

- **Cloramphenicol - blocks binding of tRNA to 50S subunit**
- **Erythromycin - binds to 50S preventing movement of mRNA and tRNA**
- **Streptomycin - distorts 30S subunit causing misreading of code and inserts wrong tRNA**
- **Tetracycline - Bind to 30S subunit preventing attachment of tRNA's**

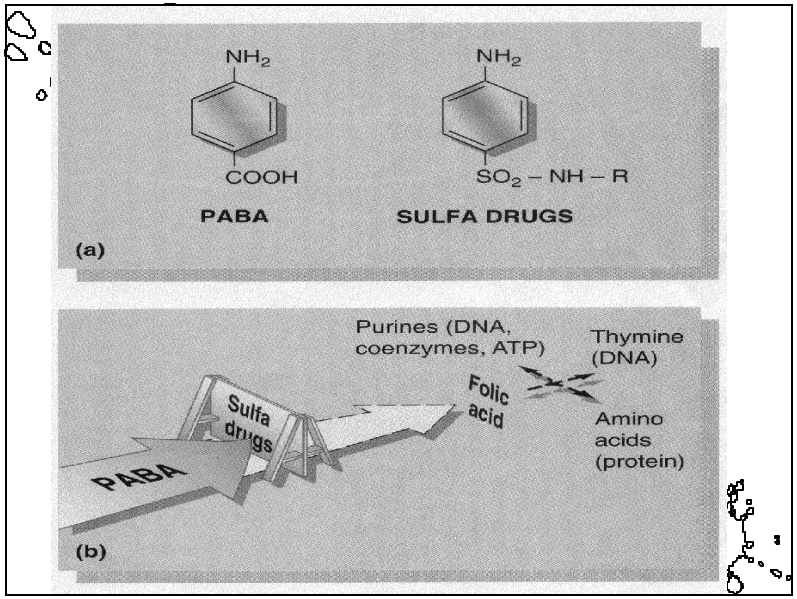


Nucleic Acids as Target

- most are synthetic analogues of natural nucleic acids, and not antibiotics. Analogues are substances with structures that closely resemble a nucleotide base or a substrate for an enzyme.
  - Rifampin: synthetic drug that inhibits transcription of mRNA from DNA by binding to and inactivating bacterial mRNA polymerase. Especially effective against Mycobacterium species (not effective in AIDS infections because of different strain of bacteria).
  - Nalidixic acid (quinolines): inhibit DNA gyrase (unwinding enzyme) imp't in DNA replication. High amounts excreted in urine making it good for UTI's (G-)

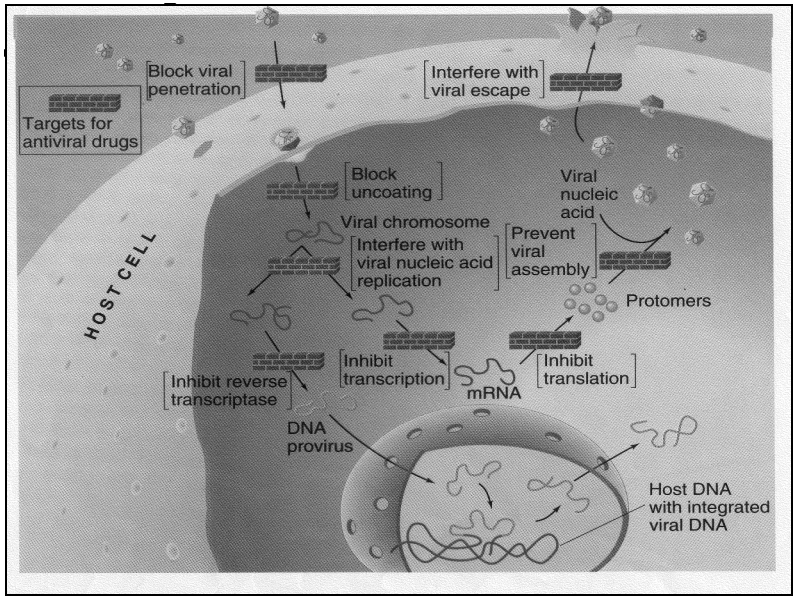
Target: Bacterial Metabolism

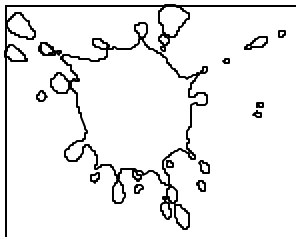
- Use of analogues
  - Sulfa drugs- analogues of para-aminobenzoic acid (PABA)
    - normally PABA converted to folic acid, important in purine synthesis. Sulfa drugs react with the enzyme necessary for this conversion and inhibit reaction. Human cells take up pre-formed folic acid while bacteria make their own. Sulfa drugs are bacteriostatic.
  - Isoniazid (INH)-inhibits mycolic acid synthesis in mycobacteria. Is bactericidal



Target: Bacterial Metabolism

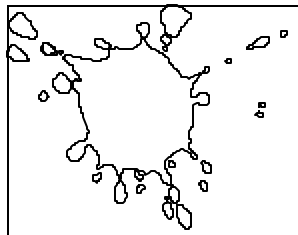
- **Chloroquine- used over 50 years in treating malaria**
  - **interferes with enzymatic digestion of hemoglobin during erythrocytic phase when *Plasmodium* parasites have invaded RBC's.**
- **Antiviral agents -**
  - **control of viral infections due primarily to vaccines**
  - **site of viral sensitivity:**
    - **attachment**
    - **virus specific transcription and translation**
    - **replication**
    - **Assembly**





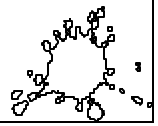
Antiviral Agents

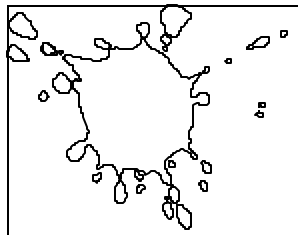
- **Only a few chemotherapeutic chemicals licensed for treatment of viral infections**
  - **Amantadine- blocks uncoating of Type A influenza virus and release of viral RNA into cells.**
    - **No effect on virus once it begins replicating. Can shorten duration if given w/i 2 days.**



Antiviral Agents

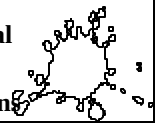
- Acyclovir- inhibits DNA synthesis in herpes viruses
  - analogue of guanine and must be converted to triphosphate form to be active. Phosphorylation is done by an enzyme specific for herpes simplex virus, and varicella-zoster (chicken pox, shingles), and thus occurs only in cells infected with these viruses.
  - used systemically and topically to shorten course of infection
- Zidovudine (AZT=azidothymidine) 1st drug to be approved in US for use against HIV.
  - nucleoside analogues that inhibit reverse transcriptase
  - do not cure AIDS and has side effects

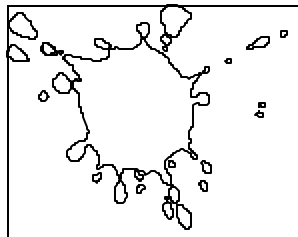




Antiviral Agents

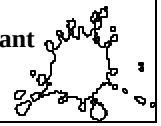
- AZT helps in prophylaxis in health care workers
  - combination drug therapy
  - other drugs not as toxic
    - DDI and DDC
- Protease inhibitors
  - inhibit conversion of large viral protein into smaller fragments necessary for capsid formation
- Ribavirin- analogue of guanine that prevents DNA and RNA synthesis and translation of viral mRNA
- Interferon- induces synthesis of antiviral proteins

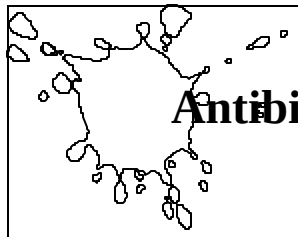




Antibiotic Resistance

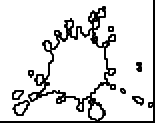
- **Antibiotics do not cause mutations or resistant cells. They do selectively favor the survival and proliferation of drug-resistant strains, which would generally be only a small subpopulation within the majority of sensitive cells.**
- **Resistance is acquired either by mutation in chromosome or by direct transfer of R-factor plasmids.**
- **Overuse of antibiotics selectively favors resistant strains**





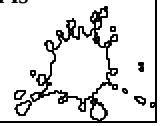
Antibiotics in Animal Feeds

- **antibiotics used extensively in animal feeds to increase meat production. Used mostly at sub-lethal concentrations, too low to kill microbes. Selects for antibiotic resistant forms. Some antibiotic resistant forms can be transferred to human pathogens and normal flora. Some antibiotic resistant pathogens can be directly transferred to humans from livestock, *Listeria* and *Salmonella* (chicken and eggs).**



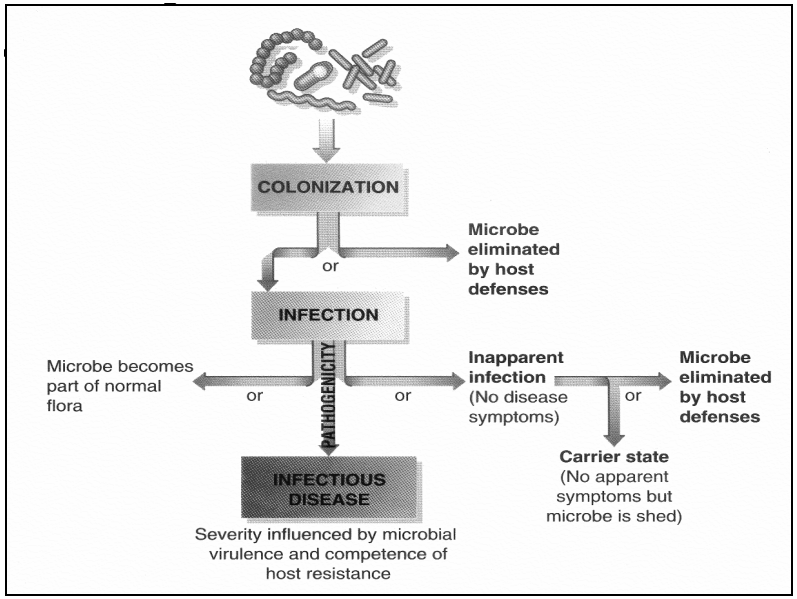
Mechanism of Resistance

- **lack of the target that the antibiotic affects- no peptidoglycan, mutated ribosomal subunit**
- **drug cannot reach site of action- LPS membrane**
- **inactivation of destruction of antibiotic- proteases destroy antibiotic**
- **decreased permeability of membranes**
- **alter metabolism so that site of antibiotic action is by-passed- some bacteria can absorb folic acid**



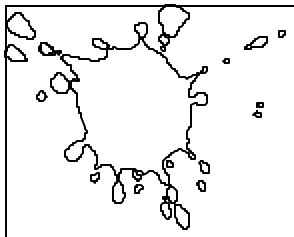
Antibiotic Susceptibility Tests

- Disk Diffusion Method- Kirby-Bauer tech
- Minimum Inhibitory Concentration- (MIC)
  - smallest amount of drug that inhibits the multiplication of the bacteria. This level must be maintained at the site of infection until all pathogens are killed.
    - usually determined by broth dilution method. A std inoculum incubated in tubes containing decreasing concentrations of drug or antibiotic being tested. If it inhibits growth at a certain conc. this is the MIC (the lowest concentration showing no growth).



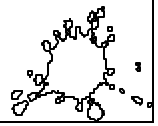
Infectious Processes & Host Responses

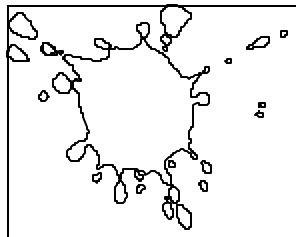
- **Normal Flora vs opportunistic microbes**
- **Infection initiated by**
  - **colonization- may be eliminated by host or become stable and established**
  - **infection phase**
    - **inapparent infection - no symptoms & carrier or elimination**
    - **becomes part of normal flora**
    - **infectious disease**
      - **clinical manifestations**



Infection

- inapparent (subclinical)- carrier state, shedding of microbes
- mild disease- disappears quickly
- severe acute disease- debilitating or fatal
- chronic infectious- persists for long time
- latent infection- dormant following recovery with recurrences





Disease progress

- concentration (infectious dose) of invading organisms
- pathogenicity & virulence of organisms
  - pathogenicity - ability to cause disease
  - virulence - 2 properties
    - infectivity - how easily organism survives normal host defenses
    - severity - extent of damage
- host response to organisms

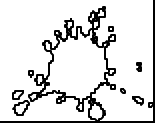
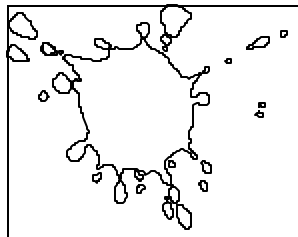


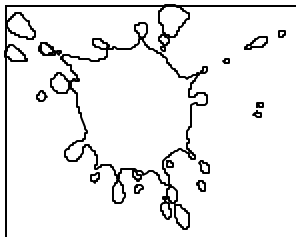
TABLE 17-3 SOME FACTORS THAT INCREASE MICROBIAL VIRULENCE

Virulence Factor	Increases Microbial Ability to	Representative Microbes	Mechanism of Action
Pilus	Establish infection	*Neisseria gonorrhoeae; Escherichia coli*	Facilitates attachment to target tissue
Capsule	Establish infection Survive host defenses	*Cryptococcus neoformans; Streptococcus pneumoniae; Klebsiella pneumoniae*	Facilitates attachment; resists phagocytosis
Neuraminidase	Establish infection	Influenza virus	Facilitates attachment
Exotoxins	Damage the host (advantage to pathogen often obscure)	*Corynebacterium diphtheriae; Clostridium tetani; Staphylococcus aureus*	Interfere with key physiological processes
Endotoxins	Injure host tissue and survive host defenses	Most gram-negative pathogens	Release endogenous pyrogens (induce fever); cause hemorrhage and rash; block capillary contraction, resulting in circulatory collapse, shock, and death
Leukocidin	Survive host defenses	*Staphylococcus aureus*	Kills phagocytic leukocytes
Coagulase	Survive host defenses	*Staphylococcus aureus*	Walls off site of infection in a protective fibrin clot
Collagenase	Spread from initial infection site	*Clostridium perfringens*	Dissolves protein of bone, skin, and cartilage
Lecithinase (α-toxin)	Spread from initial infection site	*Clostridium perfringens*	Destroys host cell membranes
Hyaluronidase	Spread from initial infection site	*Streptococcus pyogenes*	Dissolves hyaluronic acid, the ground substance of connective tissue
Fibrinolysin (streptokinase)	Spread from initial infection site	*Streptococcus pyogenes*	Dissolves fibrin clots



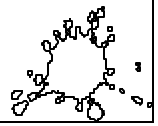
Virulence Factors

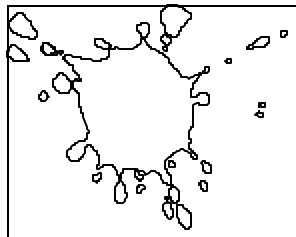
- **mycobacteria has low infectivity rate but has high severity of disease**
- **cold viruses have high infectivity rate but cause little damage (low severity)**
- **most dangerous pathogens have high infectivity and high severity rates (plague and small pox outbreaks)**



Virulence Factors

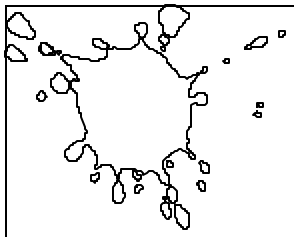
- **Adhesins- anchoring receptors for target cells (pili on gram - bacteria prevent flushing out, capsules or other proteins that help to anchor them). Can acquire ability to anchor by conjugation with virulent donor.**
- **Influenza virus has *neuraminidase* and *hemagglutinin* activities.**





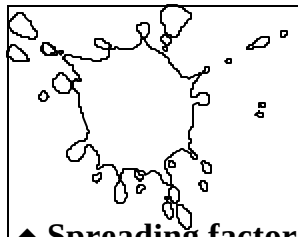
Virulence Factors

- **once established organism must evade host defense**
  - **toxic factors**
    - **exotoxins (diphtheria, scarlet fever, botulism, tetanus, gas gangrene, dysentery, cholera, and whooping couch) & endotoxins (enterotoxins). 1 gram of botulism toxin will kill all people in NY.**
    - **exotoxins elicit immune response and people who survive will be protected**



Virulence Factors

- **antiphagocytic factors**
  - **capsules**
    - ***S. pneumoniae, N. meningitides, K. pneumoniae***
  - **soluble anti-phagocytic factors**
    - **leukocidin**
    - **coagulase**
    - **hemolysin**
    - **anti-chemotactic factors**



Virulence Factors

- **Spreading factors**
  - **collagenase**
  - **lecithinase (alpha toxin)**
  - **hyaluronidase**
  - **fibrinolysin**
- **Others**
  - **proteases that destroy host antibodies (*N. gonorrhoeae & S. pneumoniae*)**

Host Factors

- **mechanical, chemical and microbial defenses on the body's surface**
- **phagocytic cells & non-specific host defenses (macrophages, neutrophiles, complement**
- **Specific host defenses (T and B lymphocytes)**

Compromised Hosts Lead to Opportunistic Infections

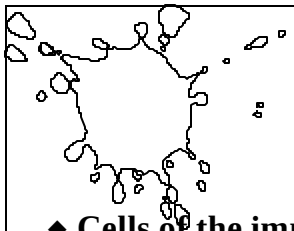
- **diabetes & malnutrition**
- **repeated infections with low virulent organisms**
- **drugs- immune-suppressants, steroids**
- **Genetics**
- **stress**
- **radiation**

Disease Diagnosis

- clinical specimens- collected from where/how?
  - fluids
  - tissues
  - discharges
  - serology
  - cultures

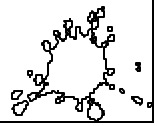
Surface Defenses: Non-Specific Defenses

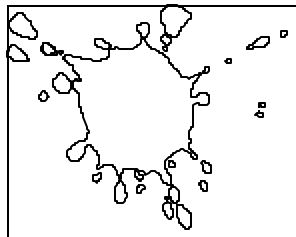
- skin
- ciliated mucosa
  - mucous secretion & cilia action
- flow of urine and tears, saliva
- Chemical defenses- (presence of lysozyme - cell wall destruction, fatty acids in earwax, stomach acid and enzymes, IFN, complement, IL's)
- Inflammation response



Immunology

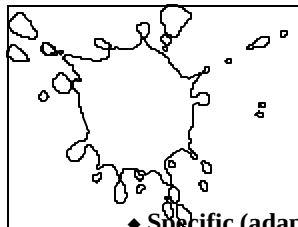
- Cells of the immune system (non-specific)
  - Granulocytes
    - neutrophils
    - eosinophils
    - basophiles
  - Monocytes
    - monocytes- circulating
    - macrophages- tissue fixed
  - Lymphocytes
    - T and B cells, NK cells (specific)





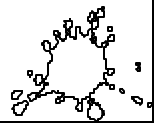
Immunology

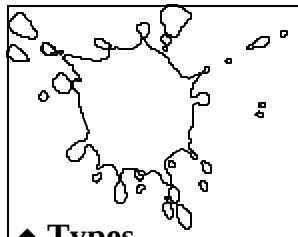
- Types of immunity
  - Non-specific (innate)
    - barriers
      - skin
      - mucous
      - pH
      - enzyme protection
      - phagocytosis
      - complement
      - NK cells



Immunology

- Specific (adaptive)- highly specific receptors
  - Cellular (T lymphocytes) - important in defense against intracellular parasites (intracellular bacteria such as mycobactreia, and against viruses), organ transplants (skin, liver, heart, etc...), contact sensitivity reactions (poison ivy)
  - Humoral (B lymphocytes)- formation of antibodies, important against soluble protein antigens, extracellular parasites (most bacteria, blocking viral receptors, allergic reactions)
- Antigen
  - epitope





T Lymphocytes

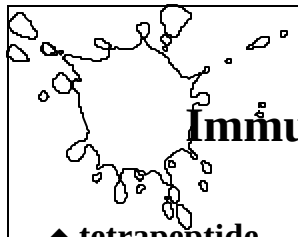
- Types
  - helper (CD4 positive)
    - Thelper 1 (T_H1)- produce IL-2, IFN
    - Thelper 2 (TH2)-produce IL-4, IL-5, IL-10
  - cytotoxic (CD8 positive)
  - suppressor ??? (CD8 positive)
  - are MHC restricted
    - class I (on all nucleated cells) and class II (only on antigen presenting cells (macrophages, B cells, Langerhans cells and Dendritic cells)

MHC antigens

- T lymphocytes must recognize both foreign antigen and self-MHC in order to be activated
- T cells that recognize foreign MHC and/or self antigens are destroyed by programmed cell death (apoptosis) in the thymus before they mature (tolerance to self antigens)
- T cells recognize antigen only after it has been processed by APC's (not native Ag or soluble Ag)

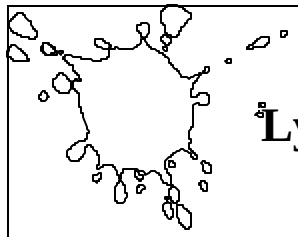
Humoral Immunity

- 5 Ab classes
  - Immunoglobulin (Ig):
    - G- crosses placenta, 2nd response Ab
    - A- dimer, secreted across epithelial barrier
    - M- pentamer, 1st Ab response, activates C'
    - E- allergy antibody, binds to Fc Receptor on Mast cells
    - D- found on mature but not activated B cells, unknown function
  - Function
    - opsonization, complement activation, blocking and neutralization



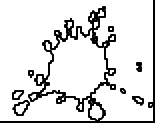
Immunoglobulin Structure

- tetrapeptide
  - 2 Light and 2 Heavy chains
    - composed of variable and constant regions
    - each region coded for by different gene regions
      - Variable genes
      - Diversity genes
      - Joining genes
      - Constant genes
  - Recombinational events lead to large ability to bind to many antigenic epitopes



Lymphocyte Education

- B cells educated in bone marrow
- T cell educated in thymus
  - Self vs non-self recognition
    - tolerance to self antigens
      - clonal deletion
      - suppression
- anamnestic response (memory)
  - primary vs secondary responses



Blood Types/Rh Factor

- **Universal donor and recipient**
  - **A, B, AB, O, and Bombay**
    - **explain in immunological terms**
  - **Rh factor and *erythroblastosis fetalis (blue baby)***
    - **occurs with 2nd baby of Rh- woman when 1st baby was Rh+**
    - **use of Rhogam w/i 72 hours**

Hypersensitivity Reactions

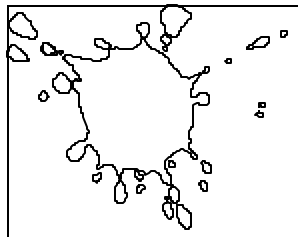
- **Type I- Anaphylaxis (immediate type)**
  - **IgE mediated**
- **Type II- Antibody-mediated Cytotoxicity**
  - **antigens on cell surface**
  - **IgG and IgM, complement mediated**
  - **transfusion rx, erythroblastosis fetalis**
- **Type III- Immune Complex Mediated**
  - **soluble antigens**
  - **IgM and IgG, complement mediated**
    - **serum sickness to horse antigens, SLE**

Respiratory Tract

- kill about 10 X 10^6 people/ year
- nosocomial infections commonly transmitted in this manner
- Upper Respiratory vs Lower Respiratory
  - URT and LRT
  - warm, moist surfaces ideal for growth
  - middle ears connected to URT
  - Table 21-2 has normal flora of URT and LRT

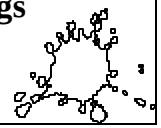
Predisposing factors: RT infections

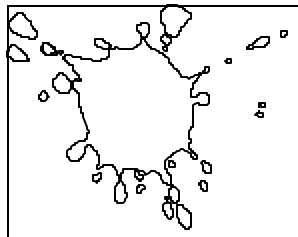
- smoking/age/chronic stress/hormonal imbalance/ diabetes mellitus/decreased phagocytosis
- healthy individuals also get infected with large doses of pathogen:
  - *Yersinia pestis* and measles virus



Human Reservoirs

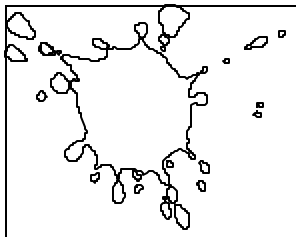
- through coughing, sneezing, talking, singing, spitting-- primarily occurs indoors where it is crowded through **droplet nuclei (formed from evaporation of small respiratory droplets and are more dangerous because desiccation prolongs viability of microbes)**





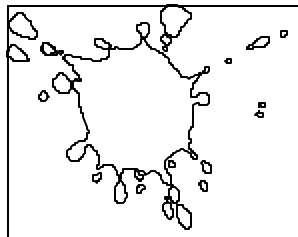
Environmental/Animal Reservoirs

- Aerosols of contaminated water (humidifiers, air-conditioning)
- occupational exposure to animals (zoonoses)- Q fever and anthrax, ornithosis (from birds) a chlamydial infection causing pneumonia



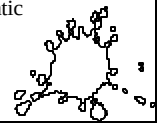
Diseases of URT

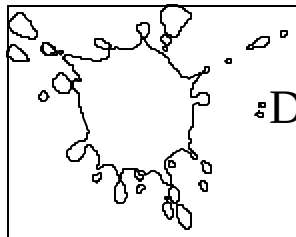
- 90% due to viruses and are resistant to antibiotics
  - common cold (viral)- Rhinoviruses (>113 different viruses)(RNA) replicate at 33ºC and restricted to cooler surfaces of URT. Most spread by direct contact with contaminated hands. Most infectious during first 2 days. Recovery due to production of IgA and IFN. Immunity lasts about 2 years, but only to same identical virus.
  - 80 different non-Rhinoviruses produce colds



Bacterial Colds

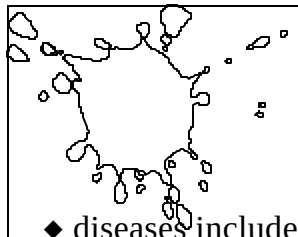
- *Streptococcal pyogenes*- group A Strep: there are 13 groups (A-O [no I or J]) based on antigenicity of C carbohydrate. Also based on protein M
  - Beta-hemolytic- streptolysin O acts poorly in air and streptolysin S is stable in air
  - anti-microbial management of strep is aimed at preventing pneumonia and post-streptococcal sequelae-- rheumatic fever and glomerulonephritis
  - *S. pyogenes* produces erythrogenic toxin that is responsible to scarlet fever





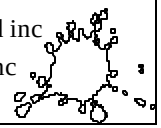
Diphtheria/ Otitis Media

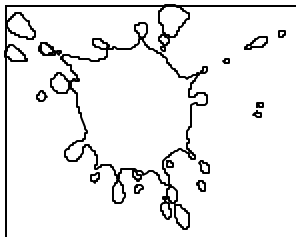
- *Corynebacterium diphtheriae-* contain temperate bacteriophages that carry gene for production of diphtheria toxin. Produces psuedomembrane in throat. Exotoxin inhibits protein synthesis
- Otitis media (middle ear)- in US more than 1/2 children under 5 have an ear infection. If untreated leds to hearing loss.
  - Caused by *S. pneumoniae, H. influenzae*, and *S. pyogenes*



LRT Infections

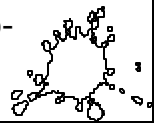
- diseases include pneumonia, influenza, tuberculosis, pertussis, and several mycoses
  - these infections fatal if left untreated
    - *S. pneumoniae*- (83 diff Ag capsules), G+, 1-3 d inc
    - *K. pneumoniae*- G- bacillus, 1-3 d inc
    - *Legionella pneumophila*- G- bacillus, non-communicable, 5-6 d inc
    - *Bordetella pertussis*- G- encapsulated rod, 2-5 d inc
    - Influenza virus- fragmented, env, RNA, 1-3 d inc

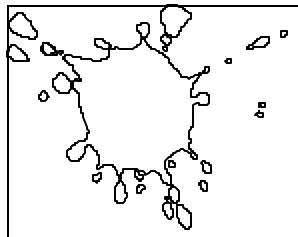




Influenza Virus

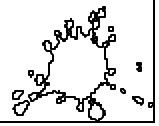
- Orthomyxovirus
  - has spikes: hemagglutinin and neuraminidase
  - Ab's to H block viral infectivity, not Ab's to N
- Antigenic drift- spont. mutations in H Ag
- Antigenic shift- genetic re-assortment of segments when two diff. strains of virus co-infect same cell (i.e., in pigs)





Hantavirus

- 1993- new disease, -RNA virus whose reservoir is rodents
  - disease due to inhalation of aerosolized feces from infected mice.
  - PCR amplification

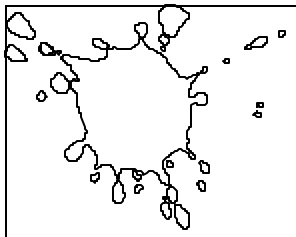


Systemic Infections Acquired through Respiratory Tract

- spread to secondary tissues following URT infections
  - chicken pox/ measles/mumps/rubella/mono-nucleosis
  - usually self-limited and require no therapy

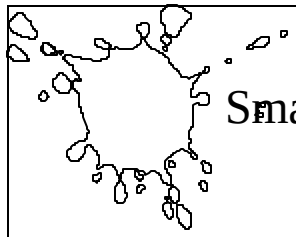
Chicken Pox

- Varicella-zoster: 2-3 wk inc, infects skin & nerves, Shingles and Reye's syndrome (**do not use aspirin in children with chicken pox or influenza**) are compliation or sequelea, more severe in adults
- Measles (rubeola): 11 d inc, lymph and skin, sequelea SSPE=subacute sclerosing panencephalitis 6-8 years after disease. Koplick's spots2-3 d before rash on mucous memb of mouth



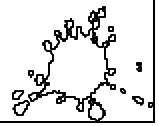
Mumps & Rubella

- Mumps: swollen parotid glands. On one side or both (if on one side then person is still resistant to infection)
- Rubella: (German measles) mild, poorly communicable, congenital may result n heart defects, impaired hearing and vision



Small Pox/ Mononucleosis

- Small pox (Variola): highly contagious, RNA virus, eliminated from world
- Epstein-Barr (EB) virus causes mono. Infection of B cells
  - Burkitt's lymphoma- malaria plays role in tumor development

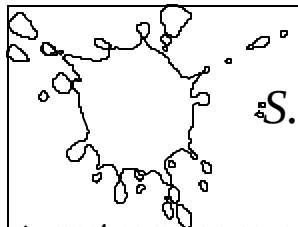


Alimentary Tract

- **Predisposing factors:**
  - **decreased production of acid**
  - **antacid therapy for ulcers**
  - **gastrointestinal obstructions**
  - **tonsillectomy- reduces Ig A**
  - **antibiotic therapy**
  - **malnutrition**

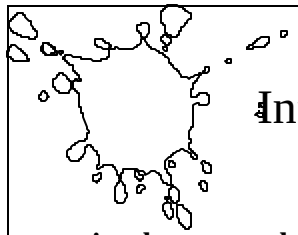
Diseases of Digestive Tract

- intoxications- true food poisonings due to enterotoxins or neurotoxins. Microbial multiplication not required after ingestion and incubation periods short (2-8 h)
  - **Botulism- most potent toxin found. Produced when foods kept under anaerobic conditions (canned), sausage. Due to failure to kill spores. toxin destroyed by heating! Symptoms appear 12-36 hours after consumption. Antitoxin tx**



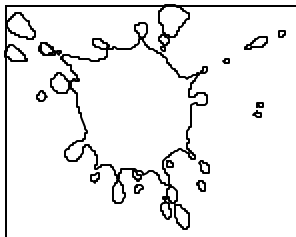
S. aureus food poisoning

- **most common cause of food poisoning in US**
  - **enter food from human source (nasal secretions or infected wounds, boils, abscesses),**
  - **milk from contaminated cows, foods rich in eggs or milk, meat and poultry kept at between 20-35^{o}C supports growth (Turkey dinner)**
  - **2-4 h inc and does not last more than 1-2 d**



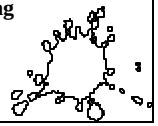
Infections of Oral Cavity

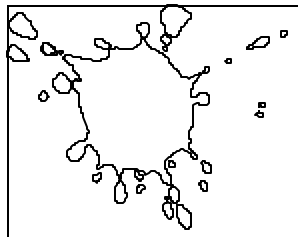
- **microbes on teeth are embedded in a sticky matrix of dextran (polysaccharide produced by *S. mutans*)**
  - **dextran and embedded bacteria= plaque. Plaque formation essential to dev of dental caries. Sucrose needed to produce dextran and "sweet tooth" produces "acid tooth" to inc incidence of dental caries. Fluoride hardens enamel against erosion by acids.**
  - **gingivitis-caused by anaerobes and spirochetes bet gums and teeth**
  - **HSV type I and *Candida albicans (thrush)* also infect mouth**



Infections of GI tract

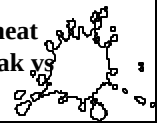
- **Non-invasive**
  - **confined to intestines and cause gastroenteritis by producing enterotoxins and multiplying. Cause water to be released by body into intestines at high rate and cause watery diarrhea. Attach to wall of intestines and not readily removed.**
    - ***Vibrio cholerae*- G-, comma shaped, flagella (polar); contaminated water supplies (food, fingers, flies are vehicles). Stimulates cAMP being made and massive secretion of water (rice water stools)**

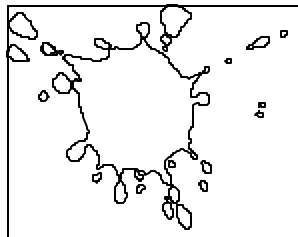




Infections of GI tract

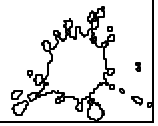
- ***Escherichia coli*:**
  - **need large number of bacteria to initiate infection**
  - **plasmid responsible for toxin production (may also have multiple antibiotic resistance genes). Use pilus for attachment**
- ***E. coli* 0157:H7: results in bloody diarrhea. normal flora in cattle. found in undercooked meat (hamburger). If meet cooked will be killed (steak vs ground beef)**

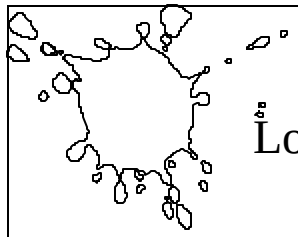




Infections of GI tract

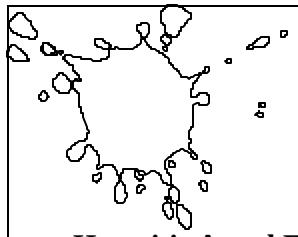
- ***Helicobacter pylori*** **infections - G-, spiral only microbe that thrives in human stomach, survives by producing high levels of ammonia. Causes acute gastritis and lesions that may lead to ulcers and maybe stomach cancer**
- **Giardiasis: flagellated protozoan *Giardia lamblia* causes non-invasive diarrhea**





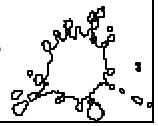
Infections of GI tract
Locally invasive diseases

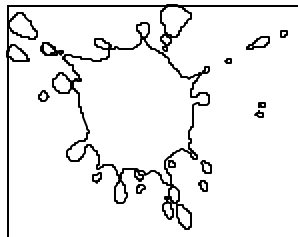
- **Dysentery- presence of blood in stools.**
  - **Shigella and Salmonella**
    - **Shigellosis can be initiated with low infectious dose (10-100 bacteria). Person-to-person spread and food contaminated by flies that have landed on human feces. Sensitive to dry environments and will not survive on many fomites.**
    - **Salmonellosis has more than 2000 serologically distinct organisms. Human disease caused by *Salmonella enteriditis*. Found in poultry, eggs, ducks and turtles and iguanas**



Infections of GI tract
Invasive Microbes

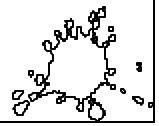
- **Hepatitis A and E:**
  - **transmitted by fecal-oral route in contaminated water and food (raw clams and oysters) or by person-to-person spread**
  - **Hepatitis A has inc period of 2-6 weeks. Multiplies in GI tract then moves into blood and invades liver. Recovery in about 6 wks. No permanent carrier or chronic condition**
  - **Hepatitis E not common in US but is in areas with poor sanitation**

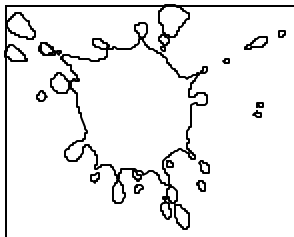




Infections of GI tract
Toxoplasmosis

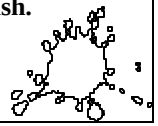
- *Toxoplasma gondii* is a protozoan. Occurs only in cats and is shed in feces which can infect adults who clean cat boxes. Dangerous when transmitted to fetus. Transplacental transmission only occurs when mother gets initial disease, not during reactivation infections. Mother would already have antibodies that will block transmission

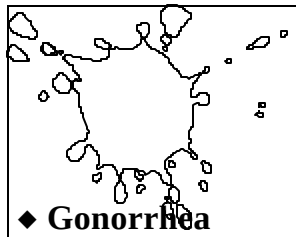




Genitourinary Tract

- **Sexually transmitted diseases (STD's)**
  - **normal flora in urethra, vagina, but in deeper structures there should be no flora (uterus, fallopian tubes, ovaries, etc...). *Candida albicans* and *Trichomonas vaginalis*, reside in vagina, but are normally held in check by normal flora, but if normal flora disrupted then these can flourish.**
  - **hormonal changes affect normal flora**



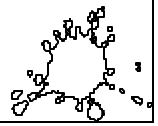


STDs

- **Gonorrhea**
  - **Asymptomatic disease more prevalent in women**
  - **G-, diplococci, *Neisseria gonorrhoeae, attach by pili***
  - **3-5 days males experience symptoms characteristic of urethritis, painful urination and discharge**
  - **In women symptoms may be milder, complications arise at higher rate since more are asymptomatic.**
  - **PID develops in 10-20% of untreated cases**
    - **1% silver nitrate added to eyes at childbirth**

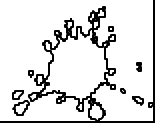
STDs: Chlamydial Infections

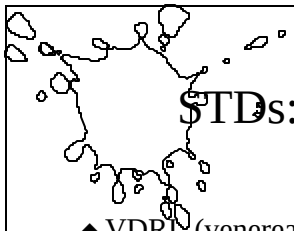
- **obligate intracellular bacteria (*Chlamydia trachomatis*)**
  - **non-gonococcal urethritis (NGU)- similar symptoms to gonorrhea**
  - **ascends reproductive tract and can lead to sterility**
  - **treatment of both partners simultaneously**
    - **treat with erythromycin or tetracycline**



STDs: Syphillus

- ***Treponema pallidum*: sprirochete, dies quickly outside host**
  - **primary stage- characterized by development of a single lesion called chancre at site of entrance (painless)**
  - **secondary stage - untreated leads 3-4 months later have skin rash on trunk and extremities. Predilection for palms of hands and soles of feet. Lesions are highly infectious**
  - **tertiary stage-: 1/3 recover, 1/3 become latent infections, and 1/3 has 3rd stage 3-40 years later (insanity, deafness, blindness (rare)**
  - **death**





STDs: Syphillus (Diagnosis)

- VDRL (venereal disease research laboratory), and RPR (rapid plasma reagin) tests
  - rely on presence of cardiolipin found in normal hearts. cross-reaction is fast and is used to measure precipitation between this antigen and reagin (*T. pallidum* antibody found in serum)
  - Fluorescent treponema antibody (FTA-ABS)- tests for antigen-antibody complex using antibody against human IgG
  - *T. pallidum* immobilization (TPI) test-sera from infected people inhibit motility of *T. pallidum*