Microbiology 201

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I. Objectives of the Course {Mine versus Yours}?
   A. Breadth of Microbiology
   B. Where does it fit into your world?
   C. Everyday interaction with microbes.


III. Grading [handout] - very slight scale

MICROBIOLOGY - “The study of small organisms” - bacteria, yeasts, molds, algae, protozoa and viruses: plus immunity {microbe interactions with macrobe}

SURVEY OF MICROBES

<table>
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<tr>
<th>PROCARYOTES</th>
<th>EUCARYOTES</th>
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<td>bacteria &amp; cyanobacteria</td>
<td>all else</td>
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<tr>
<td></td>
<td>a) nuclear membrane</td>
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<td>b) nucleolises</td>
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<td>c) organelles</td>
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<td>d) mitosis</td>
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<td>e) flagella are complex</td>
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Bacteria - contain a single chromosome, divide by fission, can grow & exist in a wide variety of environments.

Rickettsia, Chlamydia & Mycoplasma - very small unusual

Viruses - Are they alive? DNA or RNA + protein
   replicate only in a host cell = obligate intracellular parasite
   cause numerous diseases {smaller than bacteria}

Protista (Protozoa) - Eucaryotes (animals) - mitosis; blood and tissue diseases

Fungi - Molds & Yeasts - no chlorophyll; fermentation and food, industrial products, antibiotics; decomposers of dead organic matter

Algae - chlorophyll containers (chloroplasts) - diatoms and dinoflagellates

TAXONOMY
Whittaker (1969) Five Kingdoms

I. Procaryotae - bacteria, bluegreen algae & archaebacteria
II. Fungi - Yeasts & Molds
III. Protista - protozoa
IV. Plantae
V. Animalia

Woese (1988) Three Domains

I. Eubacteria
II. Archea (more related to the Eukarya than to the Eubacteria)
III. Eukarya (Kingdoms: Fungi, Protista, Plantae & Animalia)

Genus species (strain)

Bergeys Manual of Determinative Bacteriology (1923) first publication
Division II Bacteria - 19 parts

Identification - a) size; b) shape; c) motion or lack of; d) pathogenic for humans or animals; e) growth requirements; f) staining reactions; g) antigenic nature; h) DNA base ratio or DNA-DNA homology.

Size - \( \mu m = 1 \) millionth of a meter = 1 thousandth of a mm
Protozoa = 100 \( \mu m \) (0.1mm)  Fungi = 10 X 40 \( \mu m \)
Bacteria = 0.3 - 2 \( \mu m \) X 0.5 - 20 \( \mu m \)  Viruses = 20 - 250 nm [0.02-0.25 \( \mu m \)]

Microscopes
Light Microscope - used for discerning: size, shape, staining characteristics and microscopic arrangement

Limit of Resolution = 0.2 - 0.25 \( \mu m \) with an oil immersion objective

Darkfield Microscopy - Inverts the image; good for thin microbes (Treponema pallidum)

Electron Microscope - Transmission Electron Microscope (TEM) & Scanning Electron Microscope (SEM) = Total Magnification about 20 X 10^6 X (observe object 1 nm) Magnets act as lenses for focussing

SEM - gives a three-dimensional image

10^{12} bacteria/g dry weight (2 X 10^{11} bacteria/g wet weight)

Food Microbiology
Bacteria and molds eat the same foods that we do!

Important constituents: organic matter, water content and pH

A. Spoilage [a waste of money and a source of illness]

1. spices - a cover up (also sauces) for unpleasant appearance, odor or taste
2. food intoxications and food infections may not be obvious
3. contaminants: airborne, animal intestines, soil, contaminated water, insects, humans involved in food processing

B. Conditions of the food: (1) water content, minimum of 18-20%; (2) pH down to 5 for bacteria, below 5 for most fungi; (3) physical structure; (4) temperature of storage; (5) chemical composition; (6) O₂ content [environment]

highly perishable - spoils rapidly {poultry, eggs, meat, milk, fish, fruit & vegetables}
semi-perishable - spoils less rapidly {nutmeats, potatoes and apples}
non-perishable - only if kept dry {cereal grains, rice, dried beans, macaroni, sugar, and flour}

C. Chemistry of Spoilage

1. Microbes break down - carbohydrates, proteins and fats
2. Break down Products - H₂S, skatole, amines, gas, acids, fatty acids, slime & pigments

Processed meats and fish spoil much faster [organ meats (such as the liver and kidneys)- already contaminated]

Eggs - shell and membrane are by bacteria penetrated with time
the white of the egg contains lysozyme {hydrolyzes bacterial cell walls}
egg yolk - Proteus - H₂S black rot; Pseudomonas - green rot; Serratia marcescens - red rot

Bakery products - ropy bread [capsule & slime] Bacillus; cream fillings - Salmonella & Staphylococcus (gastroenteritis)

Aflatoxins - "carcinogenic" (induces tumors) Aspergillus flavus in peanut butter

Ergot's Disease - Claviceps purpureae produces and alkaloid when it grows on grains: rye, wheat & barley - causes burning, tingling, vomiting, cramping, weakness and death.
C. Food Preservation

1. Heat Treatment - Appert early 1800s - changes the proteins & kills most microbes
canning - blanching (inactivates certain enzymes)
   *Bacillus* & *Clostridium* spores* - contaminate the cans and cause gas to swell the cans (coliforms & clostridia): flat sour - no gas (Bacillus)

2. Low Temperature - slows down the rate of growth of contaminants

3. Drying - NO WATER ... NO LIFE
   spray driers, heated drum, belt drier, the sun
   {may spoil very rapidly, once it is reconstituted with water}
   LYOPHILIZATION - freeze-drying [also used to preserve bacteria]

4. Osmotic Pressure - salt or sugar (syrups)
   sucks the water out of the microbes.  Used in making jams, jellies, fruit, honey, salted codfish, ham, salted beef, bacon, sauerkraut, pickles

5. Chemical Preservatives
   acids - interfer with the metabolism of microbes
   sorbic acid - syrups, salad dressings, jellies & cakes
   benzoic acid - beverages, catsup, margarine, cider
   propionic acid - breads & bakery products
   lactic & acetic acids - many foods
   smoked foods - formaldehyde, phenols
   SO$_2$ - dried fruits & juices (kills yeasts & molds)
   radiation - UV for meats: gamma rays also: microwaves don't sterilize

D. Foodborne Disease

   Food may serve as a vector & growth medium for "food infection" microbes
   [typhoid fever, salmonellosis, cholera, shigellosis]
   Protozoa - [amoebiasis, balantidiasis, giardiasis]
   Viruses - [hepatitis]
   "food intoxication" - poisons produced by growth of the bacteria in the food
   staphylococcal food poisoning, botulism, *Bacillus cereus* food poisoning

E. Positive Roles for Microbes in Foods
sauerkraut - cabbage & salt are packed tightly and inoculated with *Leuconostoc* and *Lactobacillus* to produce lactic and acetic acids

Pickles - acid (usually acetic, sometimes lactic) softens the cucumbers and other vegetables

Other foods - vinegar, sausages, olives, soy sauce, sour dough breads, alcohol, cheeses, yogurt

Ensilage for animals to feed on during the winter - *Lactobacillus* and *Streptococcus* convert the sugar to acid and this lowers the pH making it hard for other bacteria to grow and cause either spoilage or disease
Dairy Microbiology

Milk is good food for microorganisms too!

Milk Composition: (pH 7)  
87% water  
2.5% casein (protein that has bound Ca++)  
0.5% lactalbumin (another protein)  
5% lactose (milk sugar)  
4% butter fat (on average)  
+ vitamins A, D & E

Spoilage - production of distasteful or noxious end products made by bacterial fermentation of the chemical components of the milk

*Streptococcus* or *Lactobacillus* - produces lactic and acetic acids at lower temperatures yielding sour milk or an acid curd

Sweet Curd - is produced by *Bacillus*, *Proteus*, or *Micrococcus* which alter protein, without much acid production

Stormy Fermentation - clostridia and some coliforms (produce acid from fermentation of lactose clotting the protein, which is then hydrolyzed with release of gas bubbles)

Ropiness - [capsule-producers] *Alcaligenes*, *Klebsiella*, *Enterbacter*; even at low temperatures (4°C) of refrigerators

Putrid of Rancid - [activity of lipolytic enzymes] *Pseudomonas*, or *Achromobacter* butterfat is hydrolyzed to glycerol and fatty acids

Red Rot - *Serratia marcescens* (produces a red pigment)

Blue Rot - *Pseudomonas aeruginosa* (produces a blue or blue-green pigment)

Gray Rot - clostridia

Yeast - different yeasts produce different pigments (pink, yellow or orange)

Molds - tend to produce high amounts of acid, especially in or on cheeses

Milk is usually sterile in the udder - it becomes contaminated afterwards

Milkborne Diseases:
1. Tuberculosis - caused by *Mycobacterium bovis* consumed in contaminated milk, now this is rare in developed countries.

2. Brucellosis - a blood disease caused by *Brucella abortus*, usually transmitted in unpasteurized milk or cheese

3. Q fever - caused by *Coxiella burnetii*, a pneumonic type of disease

4. Other organisms that cause disease associated with milk: *Listeria monocytogenes, Campylobacter, Salmonella, Leptospira and Streptococcus*

   These Problems are Curbed by:
   a) inspection - of the dairy facilities
   b) sanitation - of the dairy facilities and workers
   c) antibiotics - treatment of diseased livestock
   d) immunization - of livestock for specific diseases
   e) pasteurization - of a milk used for human consumption

**Pasteurization** was originally developed by Louis Pasteur to save the wine industry in France in the 1860s; it was later (1890s) modified to be used on milk to curb the spread of tuberculosis. In recent times it is also used to retard spoilage.

Flash Method - HTST (high temperature short time) 161°F (71.6°C) 15-17 sec.
Ultrapasteurization - 82°C for 3 sec. - heavy cream and half-and-half
{the most heat resistant of the pathogens are *Listeria & Coxiella*}

**LABORATORY METHODS**

1. Phosohatase Test - This is a safety test. This is an enzyme always found in milk that has the same sensitivity to the heat of pasteurization as key pathogenic bacteria. Sodium diphenyl-phosphate is added to the milk after it is heat treated, if the milk has been heated enough to kill the pathogens, then the enzyme will have been inactivated and the milk will not turn blue.

2. Standard Plate Count - Dilutions of milk are plated on plate count agar (tryptone glucose yeast extract agar) - count 25 to 250 colonies per plate and determine the total number of bacteria per ml of milk.
   [doesn't normally detect: psychrophiles, anaerobes, viruses, molds or yeasts]

3. Dye Reduction Test - This is a quality test. The determines the relative numbers of bacteria in the milk. Methylene blue (or resazurin) is added to the milk ans the milk is incubated at 36°C, the faster the milk loses its color the poorer the quality of the milk. [2hr = poor milk ... 6hr = good milk]

4. Antibiotic Detection Test - Prevent hypersensitive reactions, especially to penicillin
   Paper disks are soaked in milk and plated on an agar plate seeded with *Bacillus*
subtilis, a bacterium very sensitive to penicillin. After incubation, we look for a zone of inhibition of growth around the disk containing the milk. This is then confirmed by adding penicillinase to a sample of the milk to destroy the penicillin and repeating the test; if the penicillinase-treated milk containing disk shows no inhibition of growth then penicillin was the original cause of growth inhibition.

POSITIVE ROLE OF MICROORGANISMS IN MILK

A) buttermilk - *Streptococcus lactis*, *Streptococcus cremoris* and *Leuconostoc citrovorum* convert the lactose in milk to acetic and lactic acids

B) yogurt - Milk is boiled and powdered milk is added; *Lactobacillus bulgaricus* & *Streptococcus thermophilus* are added turning lactose to lactic acid. Commercially, the product is usually evaporated to thicken it.

C) acidophilus milk - *Lactobacillus acidophilus* is added to the milk to convert the lactose to lactic acid. Most people with a lactose intolerance can drink cultured acidophilus milk.

D) Cheese - *Streptococcus* is used to produce an acid curd (rennin is also added to enhance curd production), then the whey [liquid] is expressed from the curd. This is unripened cheese {up to 20% butterfat}. This is how cottage cheese and ricota cheese are produced.

**ripened cheese** - It is usually salted, inoculated with a specific mold or bacterium and incubated until ripe.

swiss cheese - *Propionibacterium shermanii*
cheddar cheese - geotrichum
Limburger cheese - *Brevibacterium linens*
Muenster cheese - *Brevibacterium linens*
Roquefort, blue & Gorgonzola - *Penicillium* sp.
Water Microbiology

WATER TYPES:

1. Ground Water - Wells & deep springs (bacteria free, by natural filtration)

2. Surface Water - Lakes, streams & shallow wells (contain runoff bacteria)

CONTAMINATED - infectious agent, chemical or biological poison
{polluted - also has unpleasant taste, odor or appearance}

Unpolluted Waters - Contain a few thousand microbes per ml and they are mostly runoff soil microorganisms {they contain low amounts of organic matter}

Polluted Waters - Contain large amounts of organic matter from: sewage, feces or industrial sources. Major bacterial type is the coliform (*Escherichia coli* and *Enterobacter*) Very rapid growth may cause it to go anaerobic and turn into a swamp.

Marine Environment - salt & cold: diatoms are part of the food chain. Dinoflagellates - "red tide" bloom

TYPES OF WATER POLLUTION

A. Physical - particulate matter makes water cloudy, blue-green algae bloom: eutrophication (accumulation of NO₃ & PO₄ allows for much growth of microorganisms) bacterial fermentation products cause stench. also Thermal Pollution

B. Chemical - inorganic and/or organic waste (from mines & pipes) detergents [NO₃ & PO₄], radioactive waste, PCBs mercury, cadmium, petroleum

C. Biological - microbes from sewage, food processing, and medical facilities {only a real problem when water becomes stagnant}

   Biological Oxygen Demand (BOD) - Oxygen depletion over 5 days at 20°C [kills off aerobic microbes and fauna]

Diseases Transmitted by Water

Bacterial: Typhoid Fever, Cholera, anthrax, Leptospirosis, bacillary dysentery, Yersiniosis, Campylobacteriosis, Vibriosis

Viral: hepatitis A, Polio, Adeno, ECHO, Coxsackie, Norwalk, & Rota

Protozoal: Amoebiasis, Giardiasis, & Balantidiasis
TREATMENT OF WATER AND SEWAGE

Water Purification - Prevents pathogenic organisms from reaching body

Sewage Disposal - Removes organisms, kills bacteria (maybe not viruses)

A. WATER PURIFICATION

1. Sedimentation - remove large particles (settling tanks) ± flocculation with alum
2. Filtration - Sand and gravel are used to filter out microorganisms
3. Chlorination - Chlorine gas residual of 0.2-1 PPM (most org. dead in 5 min.)

B. SEWAGE TREATMENT

1. Privy
2. Cesspool - water is allowed to flow away & anaerobic bacteria digest sludge to Nitrates, Sulfates, Carbonates, Carbon Dioxide & Methane
3. Septic Tank - There is a storage tank for sludge and a leach field for broader drainage of the liquid
4. Municipal - Primary treatment: sludge separated from liquid; Secondary treatment: aeration - aerobic digestion of organics; then Filtration; followed by Chlorination {does not kill all viruses}

C. LABORATORY MICROBIOLOGY OF WATER

*Escherichia coli* - human fecal pollution indicator organism

BACTERIAL ANALYSIS

1. Membrane filter Technique

   100 ml sample of water is filtered through a filter with 0.45 µm pores to retain the bacteria. This is usually placed onto selective media for coliforms and incubated, then counted. (total coliform count)

2. Standard Plate Count

   Total bacterial count in a water sample. (>500 per 100 ml requires action)

3. Most Probable Numbers (MPN)
Samples of 10 ml, 1 ml and 0.1 ml are inoculated into lactose broth tubes to check for lactose fermenters (coliforms produce acid and gas from lactose). This a statistical test, run in sets of either 3 or 5 tubes and can estimate the number of coliforms per 100 ml from a chart.

If the MPN tubes are incubated at 44.5°C, only fecal coliforms will grow and produce gas from lactose. \( \geq 1 \) per 100 ml sample requires action

People may also culture for Enterococcus faecalis or Clostridium perfringens.

**POSITIVE ROLES FOR MICROORGANISMS IN SOIL AND WATER**

A. **CARBON CYCLE**

Waste carbon from dead plants and animals collects in the soil - microbes are the primary decomposers - yielding CO₂ which plants convert into carbohydrates.

B. **SULFUR CYCLE**

Methionine and cysteine in proteins have sulfur. When the proteins in dead plants and animals is decomposed by bacteria, the proteins are hydrolyzed to amino acids which are further broken down to release H₂S, which is recycled.

\[
\text{Thiobacillus (aerobe)} \rightarrow \text{SO}_4 \quad \quad \text{Desulfovibrio (anaerobe)} \rightarrow \text{H}_2\text{S}
\]

C. **NITROGEN CYCLE**

The core of amino acids that protein in made of and also urea in urine. Organisms in the soil will decompose to ammonia, nitrate or nitrogen, to be recycled.

\[
\text{Nitrosomonas} \quad \text{NH}_3 \rightarrow \text{NO}_2 \quad \quad \quad \text{Nitrobacter} \quad \text{NO}_2 \rightarrow \text{NO}_3
\]

**NITROGEN FIXATION**

Symbiotic relationship between certain plants and bacteria to fix nitrogen. \{leguminous plants like clover and peas will have nodules on their roots if they are growing in a nitrogen-poor soil. The nodules will be produced by Rhizobia which are capable of
fixing nitrogen, when they are in root nodules and they are supplied with sugar by the plant.

There is also free living nitrogen fixation - *Bacillus, Clostridium, Pseudomonas, Spirillum, Azotobacter* and cyanobacteria.
History of Microbiology

1600s Malpighi and Van Leeuwenhoek - started using microscopes to look at tissues, cells and microbes respectively.

Van Leeuwenhoek (Delft draper, surveyor, wine taster) - ground his own lenses and made his own microscopes [200 X magnification] - observed animalcules.

1676 - first to observe bacteria: rods, cocci, spirochetes and he also described protozoa.

SPONTANEOUS GENERATION

"Life created spontaneously from decaying organic matter" versus "Life as progeny of already existing life".

1667 - Francesco Redi - "Meat without flies produced no maggots"

1748 - Turbevill Needham - animalcules in cooked mutton broth {stoppered with corks}

1799 - Lazzaro Spallanzani - boiled flasks of mutton broth for a longer time and hermetically sealed them: no microbes were produced.

Needham - "Spallanzani had killed the vital spirits"

1805 - Appert - could preserve soups and liquids by extensive heating.

1860s - Pasteur - laid to rest the theory of spontaneous generation with his study using "swan-necked" flasks.

1854 - Schroeder & Von Dusch - cotton stoppers for flasks

1876 - Tyndall - bacterial spores floating in the air.

DISEASE TRANSMISSION

1850 - Ignatz Semmelweis - reported that puerperal fever was being spread by physicians not practicing aseptic technique. {he was ignored}

1854 - John Snow - traced the spread of cholera in London to a particular pump station that was being contaminated with raw sewage. {he was ignored}
THE GOLDEN AGE OF MICROBIOLOGY

Louis Pasteur studied as a chemist - he demonstrated two optical isomers of tartaric acid crystals.

He also demonstrated that wine was produced from grape juice, only in the presence of yeast, and that if the wine soured, bacteria were present.

Robert Koch {Germ Theory of Disease: Proof}

Anthrax - a deadly disease for cattle and sheep, 1876 he demonstrated that *Bacillus anthracis* caused anthrax.

**Koch’s Postulates**

1. That the disease-causing microbe is always isolated from cases of the disease and not from healthy individuals. {problem: healthy carriers}

2. It can be grown in pure culture. {Problem: viruses & intracellular parasitic bacteria can’t be grown outside living cells}

3. A lab animal can be infected with the agent and it suffers the disease. {Problem: host species specificity}

4. The microbe can be reisolated from the lab animal.

Potato slices used for solid media for growing microbes, then gelatin, then silica gel, then Frau Hesse introduced the use of agar (1881); she was working in Koch’s lab at the time, so was Petri who designed the Petri dish.

Pasteur - immunization against anthrax by injecting with attenuated (heat-treated) *Bacillus anthracis*.

Roux and Yersin - Discovered that diphtheria was the effect of an exotoxin produced by *Corynebacterium diphtheriae*.

Emil von Behring - Successfully treated a patient with diphtheria with convalescent antiserum from another person. {awarded the first Nobel prize in Medicine}

1884 Elie Metchnikoff - Discovered phagocytosis, first in daphnia, then in human blood = polymorphonuclear (PMN) leukocytes.

1884 Christian Gram - Developed the Gram stain while working in Dr. Koch’s lab.

Pasteur (1885) - Successfully treated Joseph Meister for rabies with 21 injections of
an attenuated rabies virus.

Robert Koch (1882) - isolated the etiological agent for Tuberculosis. (1883) - isolated the etiological agent for cholera.

Jules Bordet (1906) - isolated *Bordetella pertussis*, the causative agent for whooping cough.

Paul Ehrlich (1908) - Ushered in the era of chemotherapy with the compound #606 arsphenimine to treat trypanosomes, then later to treat syphilis.

Sir David Bruce - cultivated the causative agent of undulant fever, *Brucella abortus*, and also related the tsetse fly to the transmission of sleeping sickness.

Joseph Lister (1867) - Developed aseptic surgical practices by using phenol misters in the surgery.

Almroth Wright - Discovered the opsonic capacity of humoral antibodies associated with enhancing phagocytosis by PMN leukocytes.

1928 Alexander Fleming - Discovered penicillin.

1892 Iwanowski - Demonstrated that tobacco mosaic disease was caused by a virus, a filterable agent, smaller than a bacterium.

**TODAY IS THE GOLDEN AGE OF VIROLOGY**

**NEWER DISCOVERIES IN INFECTIOUS DISEASES**

Legionnaire’s Disease 1976

Toxic Shock Syndrome 1970s

Acquired Immunodeficiency Disease Syndrome (AIDS) 1980s

Lyme Disease (cause - *Borrelia burgdorferi*, vector: ixodid tick, reservoir: white-footed mouse)
Anatomy and Growth of Bacteria

SIZE, SHAPE & ARRANGEMENT
COCCUS, ROD & SPIRAL
CHAINS, CLUSTERS & DIPLO-

FINE STRUCTURE

1. Flagella
   - monotrichous [single flagellum]
   - lophotrichous [a tuft of flagella]
   - peritrichous [flagella all around]

   Flagella are composed of protein and they are used for locomotion and
   attachment to tissue cells and inanimate objects. They are antigenic in nature
   and specific antibodies can be used to identify bacteria by the antigenic nature
   of their flagella.

2. Pili
   - These are also called fimbriae, they are composed of protein, they are antigenic
     and they are used for attachment.

   A special sex pilus is used for initiating a conjugal bridge during transfer of DNA
   from one bacterium to another. {sexual mating}

3. Capsule
   - This is usually composed of polysaccharide (sometimes polypeptide); it serves
     as a buffer to protect the bacterium from being phagocytosed by PMN
     leukocytes, from being dehydrated, and for attachment to solid surfaces.
     This also antigenic and can be used to specifically identify bacteria.

   There are also slimes that are loosely associated polysaccharide material.

4. Cell Wall
   - This a rigid structure in most bacteria that gives shape to the organism. It is
     composed of a unique material called peptidoglycan, constructed of N-acetyl
     glucosamine-N-acetyl muramic acid polymeric backbone with cross-linking
     amino acids for strengthening.

   Penicillin, our first useful antibiotic, works only on bacteria that are actively
   growing and interferes with the cross-linking of the peptidoglycan.

   Gram positive bacteria have a thick (25 nm) layer of peptidoglycan
Gram negative bacteria have a thin (3 nm) layer of peptidoglycan.

A specific enzyme, found in human saliva, tears and granules of phagocytic white blood cells, called lysozyme, specifically hydrolyzes peptidoglycan.

5. Cell Membrane

This is the permeability barrier of the cell. It is composed of a phospholipid bilayer, that is impervious to water, which contains certain proteins. Some of these proteins are involved in transport of nutrients and in anchoring DNA during replication of the bacterium.

6. Cytoplasm

This is composed of water, protein, DNA and RNA. It contains ribosomes, storage granules (glycogen, phosphate, poly-β-hydroxybutyrate), bacteriochlorophyll (±), DNA chromosome, plasmids (±).

7. Endospores

The genera *Bacillus* and *Clostridium* after growth has occurred, a dormant stage may be produced. This endospore has several special layers around it, some containing dipicolinic acid and Ca ++, that cause the endospore to be very resistant to heat and drying.

They create a problem for canning of foods.

Some are associated with human disease:

- *Bacillus anthracis* - anthrax
- *Clostridium tetani* - tetanus
- *Clostridium perfringens* - gas gangrene
- *Clostridium botulinum* - botulism

8. Bacterial Reproduction

Asexual reproduction by a process called binary fission in a logarithmic fashion.

Bacteria vary in their generation time from dividing every ten minutes to dividing only every 33 hours.
NUTRITION

Building blocks and energy - Nutrient broth: gelatin peptone, water, & salt. Common bacteria will grow in nutrient broth, while many more nutritionally fastidious pathogenic bacteria will not grow in nutrient broth.

Many pathogens require additions to basal medium: blood, carbohydrates, serum, hemoglobin, vitamins.

SELECTIVE MEDIA - mannitol salts agar {contains 7.5% NaCl to inhibit most bacteria, plus mannitol and phenol red pH indicator dye to differentiate mannitol fermentors from non-fermentors}.

DEFINED MEDIA - composed of inorganic chemicals (N, P, S, O) plus usually and organic carbon and energy source like glucose.

TROPHIC OR FEEDING STATES

AUTOTROPHS - They require no preformed organic nutrients

HETEROTROPHS - They require preformed organic molecules

SAPROPHYTES - nonpathogens (ie. soil and water microbes)

PARASITES - They live off another organism, some causing disease
SYMBIOSIS - Two organisms living in a very close association with each other. parasitism, mutualism, commensalism, synergism

TEMPERATURE

< 0°C - 20°C  Psychrophiles
20°C - 45°C  Mesophiles
45°C - 105°C  Thermophiles

OXYGEN ATMOSPHERE

AEROBIC - Requires molecular oxygen to metabolize
ANAEROBIC - Requires absence of oxygen to metabolize
FACULTATIVE ANAEROBE - Metabolizes with or without oxygen
MICROAEROPHILIC - Requires reduced amounts of oxygen

ENZYMES NEEDED FOR GROWTH IN OXYGEN ENVIRONMENT

CATALASE - converts hydrogen peroxide to water and CO₂

SUPEROXIDE DISMUTASE - converts superoxide radicals to hydrogen peroxide

HYDROGEN ION CONCENTRATION (pH)

Bacteria generally like the initial pH of their medium to be around neutrality; however, there is a fairly broad range.

*Vibrio cholerae* likes alkaline pH of 8.5 to 9

*Lactobacillus* likes acidic pH of 4.5
Bacterial Metabolism

ANABOLISM - Synthesis (building up)

CATABOLISM - Degradation (destruction, using of fuels)

1. ENZYMES - Protein catalysts (they speed up the chemical reactions)
   
   small amount goes a long way  SUBSTRATE → PRODUCTS

   Inhibitors - heavy metals, lack of cofactors or coenzymes

   NOMENCLATURE - ASE added to the substrate to derive the name
   {lactase, sucrase, ribonuclease}

   CLASSES OF ENZYMES

   a) hydrolases
   b) oxidases
   c) transferases
   d) kinases
   e) ligases
   f) isomerases

   COFACTORS: (cations) Mg++, Ca++, Fe++, K+

   COENZYMES: NAD, FAD, FMN, Coenzyme A (some are vitamins)

   Enzyme function may require some energy - usually ATP or PEP

   ATP → ADP + P_i + 10 Kcal energy

2. Catabolism of Carbohydrates  [glucose = 690 Kcal of potential energy]

   GLUCOSE

   ATP

   GLUCOSE - 6 - PO_4

   FRUCTOSE - 6 - PO_4

   ATP

   FRUCTOSE -1,6 - DIPO_4
GLYCERALDEHYDE - 3 - PO₄  DIHYDROXYACETONE - PO₄

1,3 - DIPHOSPHOGLYCERIC ACID  α - GLYCEROPHOSPHATE

2 - PHOSPHOGLYCERIC ACID  GLYCEROL

PHOSPHOENOL PYRUVATE

PYRUVATE

Yield = 2 ATP + 2 Pyruvates + 2 NADH

Kreb’s Cycle

Pyruvate

CoASH  CO₂

Acetyl CoA  NADH₂

Oxaloacetic Acid  Citrate

NADH₂  α - ketoglutarate

Malic Acid

H₂O  Fumaric Acid  Succinic Acid

FADH₂

KREB’S CYCLE:  PYRUVATE → 3 CO₂ + 4 NADH₂ + FADH + ATP

OXIDATIVE PHOSPHORYLATION (AEROBIC RESPIRATION)

NADH₂  FAD⁺  CoQ  Cyt b  Cyt c  Cyt a  Cyt a₃  ½O₂
From Glycolysis: 2 NADH<sub>2</sub> \[ \Rightarrow \text{2 ATP (anaerobic)} \]

From Kreb's: \[8 \text{ NADH}_2 + 2 \text{ FADH}_2 \Rightarrow 2 \text{ ATP} \]
\[2 \text{ FADH}_2 = 4 \text{ ATP}\]
\[10 \text{ NADH}_2 = 30 \text{ ATP}\] \[\Rightarrow 38 \text{ ATP (aerobic)}\]

ANAEROBIC RESPIRATION:

The absence of molecular oxygen, which is replaced by: NO<sub>3</sub>, NO<sub>2</sub>, SO<sub>4</sub>, CO<sub>2</sub> that are converted to: N<sub>2</sub>, H<sub>2</sub>S, CH<sub>4</sub>. (Generally only 1-2 ATP are generated per e- pair from NADH<sub>2</sub>.

FERMENTATION: The organism must be able to recycle NADH<sub>2</sub> to NAD<sup>+</sup>
aerobically.

This is done by transferring electrons to organic molecules to produce reduced organic molecules, like ethanol, lactic acid, acetyl methyl carbinol, butanol, etc.

CATABOLISM OF PROTEINS AND FATS

FATS \[\rightarrow\] GLYCEROL \[\rightarrow\] DHAP \[\text{[that feeds into glycolysis]}\]
\[\downarrow\]
FATTY ACIDS \[\rightarrow\] β - oxidation \[\rightarrow\] ACETYL CoA \[\text{[feeds into the Kreb's cycle]}\]

PROTEIN \[\rightarrow\] HYDROLYZED TO AMINO ACIDS, THAT ARE DEAMINATED

<table>
<thead>
<tr>
<th>CYS, ALA, TRP</th>
<th>PYRUVATE</th>
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<tbody>
<tr>
<td>SER, GLY, LEU</td>
<td>ACETYL CoA</td>
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<tr>
<td>ASP</td>
<td>OXALOACETIC ACID</td>
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<tr>
<td>TYR</td>
<td>FUMARIC ACID</td>
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<td>GLU</td>
<td>α - KETOGLUTARATE</td>
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<tr>
<td>VAL, MET</td>
<td>SUCCINIC ACID</td>
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ANABOLISM OF CARBOHYDRATES AND FATS

Photosynthesis: \[6 \text{ CO}_2 + 6 \text{ H}_2\text{O} + \text{ATP} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ H}_2\text{O} \ \{\text{plants & cyanobacteria}\}\]

Bacteria use bacteriochlorophyll and ferredoxin
\[ 4 \text{ CO}_2 + 2 \text{ H}_2\text{S} + 4 \text{ H}_2\text{O} \rightarrow 4 (\text{CH}_2\text{O}) + 2 \text{ H}_2\text{SO}_4 \]

Fats  DHAP  →  glycerol  
Acetyl  CoA  →  fatty acids

**PROTEIN SYNTHESIS**

transcrip  transla
DNA (information)  →  RNA  →  Protein

A. Transcription: complementary code of one strand of DNA made of RNA, synthesized by RNA polymerase = mRNA.

B. Translation: the ribosomes are attached to the start of the mRNA and the message is read creating a polypeptide chain of amino acids. **(high energy cost)**

Ribosomes are 70s for procaryotes  and 80s for eucaryotes

Transfer RNA (tRNA) - have an anticodon triplet specific for one of the 20 amino acids and they have to be charged with their specific amino acid.

\[ \text{amino acid} + \text{ATP} + \text{tRNA} \rightarrow \text{tRNA-aa} + \text{ADP} \]

Also for each peptide bond formed, adding a new amino acid to the polypeptide, a GTP is required. Another GTP is required to translocate the ribosome to the next codon on the mRNA; therefore, **3 ATP are required per amino acid added to a polypeptide.**
CONTROL (REGULATION): AT THE OPERON LEVEL

NEGATIVE CONTROL

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<th>R</th>
<th>P</th>
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<th>3</th>
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Repressor Protein

No protein is synthesized

POSITIVE REGULATORY CONTROL

Catabolite Activator Protein (CAP) needs to be bound to the Promoter to efficiently transcribe DNA to mRNA.

Presence of Glucose causes a reduction in cyclic AMP

No Glucose causes an increase in cAMP which binds to CAP, which then sits on the promoter and causes increased protein synthesis.
Bacterial Genetics

Bacterial chromosome - a single piece (circular) of double-stranded DNA free in the cytoplasm of the bacterium. If opened and stretched out = 1 mm length, approx. 1,000 specific loci (several genes = 1 activity) ≅ 4,000 genes.

SEMI-CONSERVATIVE REPLICATION {each new chromosome receives one existing strand of DNA and one new strand}

a) DNA is attached at site on cell membrane

b) initiation is at the Ori C: the helicase (enzyme) opens the DNA

c) on the leading strand (5' → 3') the primase lays down an RNA primer, then DNA polymerase III continuously lays down complementary bases for the new daughter strand.

d) the trailing strand is laid down as Okazaki fragments requiring an RNA primer for each fragment.

e) DNA polymerase I comes in, removes all the RNA primers and replaces them with DNA.

f) DNA ligase (enzyme) binds the new fragmented strands of DNA together.

g) All the while DNA gyrase (swiveling) is working to unfold then refold the DNA for replication.

h) Then Topoisomerase II tightens up the coil of DNA.

BACTERIAL MUTATION

Mutation - a change in the chromosome

Mutagen - a chemical or physical factor that causes a mutation

Mutant - a genetically altered microbe

TYPES OF MUTATIONS

1. Spontaneous (chance) once every $10^7$ - $10^{11}$ base pairs an error is introduced.

2. Induced-mutation - mutagenic agents like: nitrogen mustard gas, ethylene oxide, N-methyl-N-nitrosoguanidine (DNA base pairing is impaired) 
   Effects: Nutritional mutants (enzyme lacking) causes poroduct
accumulation, pigment production lost, temperature-sensitive conditionally-lethal mutation.

BACTERIAL RECOMBINATION

DNA fragments from one bacterium enter another bacterium and recombine to produce progeny with shared characteristics.

A. TRANSFORMATION {Griffith in 1928}

Demonstrated that injecting mice with a mixture of heat-killed virulent *S. pneumoniae* and living avirulent *S. pneumoniae* killed the mice and only virulent, encapsulated *S. pneumoniae* were isolated from the mice. Either strain of bacteria injected alone into mice had no effect.

[What happened, was naked DNA from the heat-killed bacteria entered the living avirulent bacteria and allowed them to start producing capsules]

Transformations: capsule formation, drug resistance, virulence factors, nutritional enzymes

B. CONJUGATION {Transfer of DNA between two living bacteria}

Cell contact is required between a sex pilus-containing and a sex pilus-lacking bacterium of either the same or very similar species.

\[
A^- B^+ \times A^+ B^- \Rightarrow A^+ B^+ \text{ (growing on } A^- B^- \text{ media)}
\]

Male Bacteria are either F+ or Hfr (both containing the sex factor and pilus)

F+ has sex factor free in cytoplasm and always converts F- to F+, but has few recombinants

Hfr has sex factor integrated into the chromosome and never converts F-; it produces recombinants at a high frequency

MAPPING OF THE GENES ON THE CHROMOSOME - By interrupting the mating of at sequential timed intervals, the time of transfer of specific genes was mapped

PLASMIDS: Extrachromosomal pieces of DNA (self-replicating, circular), encode for toxin production, pilus formation, antibiotic resistance, etc.

{Resistance Transfer Factors (RTF) - are plasmids that confer resistance to several antibiotics on a single plasmid}

TRANSDUCTION: A bacteriophage is used to transfer DNA from one bacterium to
another bacterium.

A bacteriophage normally will produce a lytic cycle when it infects a bacterium. This causes the bacterium to produce 500 - 1,000 new bacteriophage and burst open releasing them.

Some infections produce a lysogenic state, where the DNA of the bacteriophage is integrated into the chromosome of the bacterium.

**Generalized Transduction** - causes all the DNA in the bacterium that is lysogenized to be chopped into to pieces that are packaged as bacteriophages and injected into other bacteria = random recombinants.

**Specialized Transduction** - causes only the genes found on either side of the point where the bacteriophage DNA integrates into the chromosome to be packaged as bacteriophages = specific recombinants.

{Diphtheria toxin is coded for by a lysogenic bacteriophage. If the bacterium is not lysogenized, no toxin will be produced and thus no disease.}

**GENETIC ENGINEERING:**

1. Restriction endonucleases (enzymes) chop DNA at specific sites. You can then isolate specific genes. The DNA fragments will have sticky ends.

2. A plasmid is isolated, a piece of DNA is removed from it.

3. Then the specific gene is spliced into the open plasmid with DNA ligase.

4. The plasmid is then added back to a bacterium to allow for production of the desired gene product.

{Human insulin, human interferon, foot and mouth vaccine and human endorphins have been produced by genetic engineering.}

**TRANSPOSONS** - These are small pieces of DNA (≈ 1000 base pairs) that have palindromic sequences so that they can insert themselves into numerous sites on the chromosome. They are called "jumping genes", because when they insert into certain sites they will cause mutations. They also carry different antibiotic resistance markers.

**Infection and Disease**

**INFECTION** - The body is invaded by a pathogenic microorganism.
DISEASE - Alteration from the normal state of health

INFECTIOUS DISEASE - Alteration from a normal state of health caused by a pathogenic microorganism.

PATHOGEN - An organism capable of infection and disease "species dependent"

COMMENSALS - Microorganisms getting benefit from growing in or on the body but not causing any damage.

OPPORTUNISTS - Organisms capable of producing disease under only the right set of circumstances. [usually a suppressed immune system]

VIRULENCE - The degree of pathogenicity. {a quantitative measure}

Progress of Disease

1. Incubation Period - 1 day to 6 years: affected by generation time of microbe, virulence and host resistance

2. Prodromal symptoms - malaise, nausea, headache, fever

3. Period of Acme - body rash, lesions, jaundice, swollen glands {ACUTE}

4. Period of Decline -

5. Convalescence - Return to a normal state of health

Communicable Disease {Transmissable}

1. Indirect Transmission

   Airborne - droplets or dust, water, food, fomites

   Vectors - arthropods (ticks, mites, mosquitoes, flies, lice)

2. Direct Transmission

   Contagious - person-to-person spread (lateral transmission)

   Non-communicable - internal diseases (tetanus, brucellosis)

EPIDEMIOLOGY

ENDEMIC - The disease is always present in the population at low incidence.

EPIDEMIC - A rapid spread of the disease through the population
PANDEMIC - A worldwide epidemic

TYPES OF DISEASES

ACUTE - cholera, typhus

CHRONIC - brucellosis, tuberculosis

PRIMARY - Diseases that attack healthy persons (cholera)

SECONDARY - Diseases that require predisposing conditions (pneumonia)

LOCAL - pustule, abscess

SYSTEMIC - tuberculosis (all organs of the body)

CARRIER - A person having no symptoms of the disease carries the infectious agent and acts as a reservoir. {chronic, transient, incubatory}

ESTABLISHMENT OF DISEASE

1. ENTRY - May have one route or many

2. DOSE - Shigella ID = 100; Salmonella ID = 10^6-7; Yersinia pestis ID = 1

3. TISSUE PENETRATION

4. VIRULENCE FACTORS: AGRESSINS VS. TOXINS

   Staphylococcal Coagulase - boils, causes localization of infection

   Streptokinase - dissolves fibrin clots (spreading factor)

   Lecithinase - dissolves cell membranes "

   Hyaluronidase - " tissue cell cement "

   Hemolysin - lyses red blood cells

   Leukocidin - kills white blood cells

   capsules - helps microbes avoid being eaten by phagocyte

TOXINS

EXOTOXINS

ENDOTOXINS
Gram + & Gram - bacteria

Protein

found in cytoplasm of cell

usually heat labile

highly toxic

good antitoxins

TOXOIDS - alum or formalin treated exotoxins - vaccines [DTP]

NEUROTOXINS - tetanus paralyzes the central nervous system (suppresses synaptic inhibition)

botulism paralyzes peripheral nervous system (blocks acetyl choline release across myoneural junction)

Diphtheria Toxin - Inhibits protein synthesis; produces a pseudomembrane formation

Cholera Toxin - causes a fluid and electrolyte imbalance of intestines - diarrhea

Bordetella pertussis - cause of whooping cough: destruction of ciliated epithelium of the trachea

Endotoxins - pyrogenic [actually trigger the release of pyrogens from PMNs and macrophages → IL-1 & TNF → induce fever, activate complement, reduce platelet counts, increase vascular permeability. May lead to shock and death by Disseminated Intravascular Coagulation (DIC).

NORMAL FLORA - found on skin, in throat, intestines, vagina: normally protective not normally found in blood and urine. most organs generally sterile spleen, kidneys, liver and lungs = filter out organisms

Intact unbroken skin and mucous membranes are the 1st line of defense

Resistance to Infection

NONSPECIFIC RESISTANCE - SPECIES RESISTANCE

only humans get gonorrhea

only hogs get hog colera

only humans get polio

only humans get smallpox
Different populations are more or less susceptible to certain diseases (measles).

**MECHANICAL AND CHEMICAL BARRIERS**

Intact Skin & mucous membranes - very important - generally must have penetration for disease.

Mucus traps → cilia → move on to stomach {acid kills microorganisms}

Bile → in the intestines (salts to dissolve fats) {kill certain bacteria}

Lysozyme → hydrolyzes peptidoglycan, especially in Gram + bacteria

Acid pH of the urine and that found in the vagina

Interferon (produced by lymphocytes) → Active against viruses and intracellular parasitic bacteria

**HUMAN CIRCULATORY SYSTEM**

Plasma (fluid) → clot the blood → Serum pH 7.4

**CELLS:** 1. Erythrocytes (hemoglobin = O₂) [5 billion per cc]
   2. Leukocytes [5-9 million per cc]
      a. granulocytes PMN (2 week lifetime) {phagocytes}
         neutrophils, eosinophils, basophils
      b. monocytes → macrophages
      c. lymphocytes (integral part of the immune system)
         [make immunoglobulins and confer Cell-Mediated-Immunity]

**LYMPH NODES:** Sites of filtering (phagocytes) and lymphocytes

tonsils, peyers patches, adenoids, appendix, spleen
{sites of action during infection}

**PHAGOCYTOSIS (VERY IMPORTANT)**

PMN neutrophils and macrophages (RES)

Chemotaxis → ± Opsonization → Attachment → Engulfment → Phagosome ↓

Egestion ← Destruction ← Phagolysosome
CHEMOTAXINS  OPSONINS
LPS + complement $\rightarrow$ C$_{5a}$, C$_{3a}$ Antibodies
Peptides (emitted by PMN or bacteria) Complement C$_{3b}$

INFLAMMATION (Early defense response to invasion by foreign body)

Dilation of blood vessels, increased capillary permeability, PMN adhere to the site to eliminate the irritant.

Four Signs of Inflammation: Calor, Rubor, Dolor & Tumor

PUS - serum & dead tissue cells plus leukocytes and dead bacteria

ABSCESS - Pus + a fibrin clot capsule

SPECIFIC RESISTANCE (Specificity, Memory, and Recognition of nonself)

ANTIGENS - Substances which elicit a response from the body's immune system. (generally protein or polysaccharide and > 10,000)

HAPTON - A very small molecule that is antigenic when combined with a carrier protein or polysaccharide molecule. (penicillin, poison ivy)

SPECIFIC IMMUNE TOLERANCE - self antigen reacting cells are continually removed from service

TYPES OF ANTIGENS:

AUTOANTIGENS - self tolerance breaks down leads to autoimmune disease

ALLOANTIGENS - blood group and transplantation antigens

HETEROPHILE - shared antigens [rickettsia and proteus OX- series]

IMMUNE SYSTEM STEM CELLS
ERYTHROPOIETIC LYMPHOPOIETIC
<table>
<thead>
<tr>
<th>ERYTHROCYTES</th>
<th>LYMPHOCYTES</th>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>BONE MARROW</td>
<td>THYMUS</td>
</tr>
<tr>
<td>B-CELLS</td>
<td>T-CELLS</td>
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</tbody>
</table>

B-CELLS = They produce specific antibodies (immunoglobulins)

T-CELLS = They are involved in processing of antigens, regulation of the immune response and cell-mediated-immunity (CMI)

These lymphocytes are found in lymph tissue: lymph nodes, spleen, tonsils, etc.

OPERATION OF THE IMMUNE SYSTEM

The maternal antibodies function to protect the newborn for 3-6 months; during this period of time foreign antigens are phagocytosed by PMN, macrophages, monocytes and partially digested and processed, then passed to lymphoid tissue.

**Activated T-cells**
- Produce effector and memory cells

**Activated B-cells**
- Produce effector (plasma) cells and memory cells

**CELL-MEDIATED IMMUNITY**

**HUMORAL IMMUNITY**

Lymphokines: small organic molecules that affect immune system and killer cells

- Produce immunoglobulins

**TYPES OF ANTIBODIES:**

- IgM - the largest molecule, first on the scene (5% of the total)
- IgG - Secondary response, lasting immunity, maternal antibodies (80% of total)
- IgA - Secretory immunoglobulin, GI and respiratory tracts (10% of total)
- IgE - Response to anaphylactic allergies
- IgD - Receptors for antigens on surface of B-cell lymphocytes

**ANAMNESTIC (MEMORY) RESPONSE** - long term humoral immunity and long term cell-mediated immunity (CMI)
CLONAL SELECTION HYPOTHESIS - The initial cadre of B-cells expanded by transposon mutations
Antibodies, Immunity and Serology

ACTIVITIES - neutralization of viruses or toxins, opsonization, complement lysis

COMPLEMENT SYSTEM

11 Proteins function in a cascade fashion

1. Heat sensitive components: inhibit C' by 56°C for 30 minutes

2. Activities:
   a) opsonization
   b) chemotaxis
   c) bacteriolysis
   d) immune adherence
   e) anaphylaxis

3. PATHWAYS OF COMPLEMENT ACTIVATION

   A. CLASSICAL

      Initiated by "antigen-antibody" complex [IgM or IgG] - antibody recognizes
cells surface, C' binds to Fc portion of ab C_{1q} the C_{1r} C_{1s}

      C_4 is altered by C_1 complex → C_{4b} attached to cell membrane

      C_2 is cleaved by C_{1s} → C_{2a} + C_{2b}

      C_{4b} → activate C_3

      Immune Adherence Complex
      C_{4b,2a,3b}  C_{3a} + C_{3b}
      anaphylotoxin
      chemotaxin

      Activates C_5

      C_{5a} + C_{5b}  Cell Membrane

      then C_6C_7C_8C_9 "Membrane Attack complex"

      Cell Lysis

   B. ALTERNATE PATHWAY
MAY BE ACTIVATED BY: endotoxin, zymosan, & capsular polysaccharide
{NO C₁, C₄ or C₂ INVOLVEMENT}

Initiating factor + Activator \( \rightarrow \) C₃ Proactivator

properdin activated \( \rightarrow \) C₃ binds to cell surface

{NO ANTIBODY INVOLVED} \( \rightarrow \) splits C₃

Then works the same as the "classical" pathway

**IMMUNITY**

1. Naturally Acquired Active Immunity - Response to unintentional exposure to antigens, or contract disease (clinical or subclinical case)

2. Artificially Acquired Active Immunity - Vaccine or Toxoid {maybe adjuvants}

3. Naturally Acquired Passive Immunity - Maternal antibodies passed to fetus in utero: mostly IgG (lasts 6 months); IgA in colostrum (nursing mother)

4. Artificially Acquired Passive Immunity - Injection of foreign immunoglobulin (important before the advent of antibiotics); still used for hepatitis, botulism diphtheria and tetanus. {Problem: serum sickness - rash, swollen joints, labored breathing anaphylaxis}

**SEROLOGY:** "Antigen-Antibody Reactions"

Not all react or are all reactions easy to observe. Some require secondary reactions to observe and some may need to be diluted. Haptens and blocking antibodies may cause problems.

1. Radioimmunoassay - Used to measure concentration of low molecular weight antigens. {very highly sensitive}

\[ \text{known amount of } \text{Ag}^* + \text{unknown Ag} + \text{known Ab} \rightarrow \text{AgAb & Ag}^*\text{Ab} \]

\[ \downarrow \]

Measure the remaining soluble \( \text{Ag}^* \) \( \leftarrow \) Precipitated

Standard curve is used to extrapolate the amount of Ag in sample

2. Fluorescent Antibody Test
A. Direct Method

Bacteria are fixed onto the slide, then known specific Ab-Fl* added, slide is rinsed off and if bacteria light up when viewed on a fluorescent microscope, they are identified.

B. Indirect Method

Specific bacteria are fixed to the slide, a patient's serum is added, the slide is rinsed - goat antihuman IgG Ab-Fl* is added, slide is rinsed. If bacteria light up under fluorescent microscope then the patient's serum contains antibodies against the specific bacterium. \{bacterium-patientAb-goatAbFl*\}

3. Neutralization [against toxins and viruses]

Specific antitoxin or antiviral antibodies are added to tissue culture or injected into a control animal to neutralize the virus or toxin. This requires that you have two injected animals, both with toxin, and one with antitoxin. If the one that received the antitoxin survives and the one that only received the toxin died, then you known which specific toxin was in the sample.

4. Precipitation

This reaction involves a soluble antigen and a specific antibody that bind to form a lattice structure that precipitates, if the concentrations of Ab and Ag are just right.

Gel diffusion is easier because you allow the Ab and Ag to diffuse through the gel to reach just the right concentration to precipitate.

5. Agglutination

This reaction involves a particulate Ag and a specific antibody that clump together rapidly. [not concentration dependent]

PASSIVE AGGLUTINATION - Attach soluble Ag to carrier particles (latex spheres or killed bacteria), then they will agglutinate in the presence of specific Ab.

IMMUNE DISORDERS

A. Hypersensitivity - a state of increased sensitivity to an antigen arising from previous
exposure to that antigen. [may involve antibodies and T cells] Immediate vs. DTH

Types I - IV  
I -III = Immediate  
Type IV = DTH

1. Type I = Anaphylactic Hypersensitivity  
   cause vigorous contractions of the smooth muscles  
   allergen (bee venom, pollen, penicillin, serum portein) ≈ 1µg sensitizing dose

   B cells produce IgE which is fixed to mast cells and basophils [containing granules with vasoactive substances]  
   This may require more than one sensitizing dose!

   **Event:** allergen binds to IgE antibody on cell → inhibits adenylate cyclase ↓

   Reactions ← Release granular contents ← cell swells ← Reduction of cAMP

   [Antihistamines activate adenylate cyclase causing an increase in cAMP]

   **Release:** histamine, serotonin, bradykinin, SRS-A  
   (a) skin swelling = edema (fluid accumulation)  
   (b) urticaria = burning itching rash  
   (c) bronchial muscles or GI muscles [contractions]

Desensitization - degranulate mast cells and/or produce IgG blocking ab

   atopic allergies - hay fever, asthma, food allergies

Cause - may be related to a breakdown in T cell(suppressor) control of IgE production

2. Type II Cytotoxic Hypersensitivity

   Ig reacts with cell surface antigens and damages or destroys that cell {Target cell}  
   (complement may be activated and IgM may be involved)

   transfusion reactions - incompatible blood types  
   Erythroblastosis Fetalis - (newborns) [RhoGam - treatment] Immediately

Autoimmune Disorders:  
   Thrombocytopenia (poor blood clotting)  
   Agranulocytosis (few granulocytes)  
   Goodpasture’s Syndrome (ag-ab in kidneys activate C’ cause blood & protein in urine)  
   Myasthenia Gravis (damage to acetyl choline receptors, cancels nerve impulses)  
   Graves Disease (over secretion of thyroxine, raise metabol. rate, goiter)  
   Hashimoto’s Disease (under secretion of thyroxine)
3. Type III Immune Complex Hypersensitivity
   ag-ab complexes accumulate in blood vessels or tissue surfaces, causing 
   activation of $C' \rightarrow C_{3a} + C_{5a} \rightarrow $ Chemoatxis of PMNs $\rightarrow$ Degranulation $\downarrow$
   
   **Cause tissue damage** ← Increase vascular permeability

   Serum Sickness - hives; swollen face, neck and joints; kidney damage

   Systemic Lupus Erythematosis (SLE) - antibodies are made against nucleo-
   proteins of White Blood Cells - ag-ab complexes in skin and body organs
   C' activated - butterfly rash, lesions of heart, kidneys and blood vessels

   Acute Glomeronephritis & Rheumatic Fever - post group A streptococcal
   disease complications

   Rheumatoid Arthritis - joints

   possibly Reye's Syndrome - neuronal involvement

IV. Type IV Cellular Hypersensitivity

   modulated by lymphokines released from T-cells causing a **delayed reaction**
   (24 - 72 hr) $\rightarrow$ **induration** (hardening) and **erythema** (rash)

   a) **infection allergy** - T-cells move to area $\rightarrow$ express lymphokines that cause
   chemotaxis of PMN leading to phagocytosis
   The T-cells stay in the area, and upon repeated exposure to the antigen cause
   heightened inflammation  [tuberculosis, brucellosis, blastomycosis,
   histoplasmosis, candidiasis, smallpox, mumps and LGV]  **TB skin test**

   b) **contact allergy** - allergens in clothing, jewelry, insecticides, coins, cosmetics
   and furs. (also: formaldehyde, copper, dyes, bacterial enzymes, protein fibers)
   drying of the skin, erythema, scaling  {poison ivy}

5. Immune Deficiency Diseases

   a) Bruton's agammaglobulinemia - few plasma cells very low Ig and many
   infections with Staphylococci, Streptococci & Pneumococci {treated with
   artificially acquired passive immunity}

   b) DiGeorge's Syndrome - T-cell maturation is cancelled, highly susceptible to
   fungal, protozoal and some viral diseases. {treated by grafts of thymus tissue}

   c) Chediak-Higashi Syndrome - delayed PMN killing
d) Job’s Syndrome - lazy leukocyte syndrome

e) AIDS - reversal of T4/T8 helper to suppressor ratio

Transplantation Immunology

1870 first skin transplant
autograft - from another part of the same body
isograft - from an identical twin
allograft - same species (rejection rate tempered by relatedness)
xenograft - different species (seldom works)

Rejection
a) skin & tumor grafts - T-cells release lymphokines induce phagocytes which
release lysosomal granules causing cellular necrosis [Type IV hypersensitivity]
b) heart, kidney & other organs - B-cells make antibodies that travel to organs
activate C’ and undergo cytotoxic lysis cause O2 starvation
[T-cell interaction may supplement this] Type II Hypersensitivity

c) Bone Marrow Transplant - Graft vs. Host rejection

Major Histocompatibility Complex (HLA) chromosome #6 = 8 gene clusters with 50
alleles each gene {need tissue typing to match donor to recipient}
[antimitotic drugs - cyclosporin A, steroids, X-irradiation]

Tumor Immunology
surface antigens -
a) oncofoetal antigen: alphafetoprotein (AFP); carcinoembryonic antigen (CEA)
b) chemical carcinogens
c) DNA viruses
d) RNA viruses

Tumors are kept in line by Killer T-cells and Natural Killer (NK) T-cells modulated by
interferon {treatments - BCG vaccination [stimulates]; IL-2 [clones helper T-cells]}

AIRBORNE BACTERIAL DISEASES

Respiratory Tract - especially humans (isolation)

1. Tuberculosis  3 million deaths per year worldwide [3,000 per year in USA]
crowded condiditons, especially poverty spread by droplets - may require
repeated exposure
\textit{Mycobacterium tuberculosis} \rightarrow \text{lungs: chronic cough, high fever, thick sputum
incubation period - 2 to 10 weeks (up to 6 months)}

"tubercle" - layers of cells: lymphocytes, giant cells, PMN with caseation
ecrosis of the center - may calcify or cavitate and spread to organs (lethal)

The bacterial cell surface contains waxy lipids and tuberculoprotein - hard to
stain, acid-fast, and hard to kill be normal cells (requires "Angry-Activated
Macrophage")

TB test: Old tuberculin (OT) or Purified Protein Derivative (PPD) are injected
intradermally \{Mantoux test\} look for erythema in 48-72 hr
(early detection has lowered the number of cases of TB per in the USA by 50 to
60 thousand)

Bacille Calmette Guerin (BCG) = vaccine \{not generally used in USA\)

Treatment - rifampin, isoniazide, streptomycin, ethambutol (prolonged)

MAC - disseminated infections in AIDS patients

2. Diphtheria - \textit{Corynebacterium diphtheriae} (gram positive, club-shaped rod)

metachromatic granules - inside the cell with a special stain

grows in the throat and tonsillar regions - produces toxin - causes cell necrosis
and accumulation of serum and leukocytes \rightarrow \text{"Pseudomembrane"}

The exotoxin specifically ADP-ribosylates the Elongation Factor 2 (EF-2) and
stops protein synthesis by halting peptidole elongation

Lysogenic bacteriophage - codes for the diphtheria toxin
Causes a fatty degeneration of the heart - leads to death
may cause nerve fiber damage - paralysis

Prevention: DTP  Treatment: penicillins

3. Meningococcal Meningitis - gram negative diplococcus

\textit{Neisseria meningitidis}  \hspace{1cm} \text{Droplets} \rightarrow \text{mucous membranes} \rightarrow \text{blood stream} \downarrow
\text{Spinal cord & brain} \leftarrow \text{Meningococcemia} \downarrow
\text{Inflammation} \downarrow \text{Death}
(headache, stiff neck, rash)

Isolate organism - find an oxidase + gram negative diplococcus

Sensitive to: ampicillin, rifampin, sulfonamides {early treatment = stop nerve damage}

Fragile microorganism - human to human transmission

Healthy Nasopharyngeal Carriers are common (this state will immunize them)

4. Streptococcal Sore Throat (pharyngitis) - *Streptococcus pyogenes*

transmitted by droplets or direct contact - cause inflammation of the throat (tonsils)

Erythrogenic toxin - (coded for by lysogenic bacteriophage) causes a rash & fever
only certain strains have this toxin and are capable of causing **Scarlet Fever**

either may lead to **Rheumatic Fever** (immune disease) antibodies to streptococcal antigens attack heart valves - may cause permanent damage

β-hemolytic group A streptococcal sore throat should always be treated with antibiotics

- group A - polysaccharide cell surface antigen
- "M" protein - cell surface (anti-"M" protein is protective, opsonin)
- hemolysins, streptokinase, streptodornase, hyaluronidase, leukocidin
- Diseases - erysipelas, impetigo, glomerulonephritis, puerperal fever

[α-hemolytic streptococci (normal flora) = endocarditis & subacute endocarditis]

5. Pneumococcal pneumonia - (bronchi and lungs)

80% of lobar & bronchial pneumonia due to - *Streptococcus pneumoniae*
gram + diplococci, commonly carried by healthy individuals and acts as a secondary invader to: influenza, common cold, allergy, smoking

Symptoms: high fever, sharp chest pains, consolidated, "rusty sputum"

usually susceptible to: penicillin and erythromycin
80 antigenic types of capsular polysaccharide (only 10-12 types common)

virulence factors: **capsule**, hyaluronidase, pneumolysin

Vaccine: purified capsular polysaccharide (12 types) 2nd generation

α-hemolytic streptococcus that is bile soluble (optochin sensitive)
6. *Klebsiella pneumoniae* gram negative rod with a capsule (carried by 10% population) causes pneumonia and urinary tract infections (UTI) may cause permanent lung tissue damage treat with cephalosporin

7. whooping Cough - *Bordetella pertussis* (gram negative rod)
ciliated epithelium of the trachea disrupted by the bacterium and its toxins
children under 1 year highly susceptible, may be deadly
treatment should be early - penicillin or erythromycin
Vaccine - DPT (merthiolate-killed, crude cell preparation)
1930s - 200,000 cases USA  Now - 2,600 cases

8. Primary Atypical Pneumonia "Walking" Pneumonia - *Mycoplasma pneumoniae*
filterable, pleomorphic organism containing no cell wall {cholesterol is required}
there is low fluid involvement, dry hacking cough, fever, fatigue
sensitive to erythromycin and tetracyclines {not penicillin}

9. Meningitis - *Haemophilus influenzae* (gram negative rod)
common secondary invader to influenza
most common cause of meningitis in infants (ages 0.5 to 2 years)

10. Legionnaires' Disease - *Legionella pneumophila* (gram negative rod)
summer flu, defective cooling towers, only type 1, sensitive to erythromycin
1222 cases in 1991 in USA [Pontiac fever, much milder, more common]

**FOODBORNE AND WATERBORNE BACTERIAL DISEASES**

1. Botulism - *Clostridium botulinum* gram (+ sporeforming rod)
exotoxin - most potent known to mankind [1 oz. kill 30 billion people]
found in intestine of man, fish, cows, birds and horses
manure, sewage, and organic fertilizers
spores cling to vegetables and germinate & grow in anaerobic conditions
toxin encoded by a plasmid

only slight growth needed to produce the toxin - botulism

symptoms - blurred vision, difficulty swallowing, slurred speech, respiratory distress

mech. of action of toxin - no acetylcholine released at the myoneural junction of the peripheral nervous system

heat inactivates the toxin - boiling a few minutes

common contaminated foods - sausage, salami, canned mushrooms, olives, canned salmon, mostly home-canned foods

Respiratory therapy, antitoxin

Infant Botulism - SIDS? infection-intoxication, (no honey under age 2yr.)

2. Staphylococcal food poisoning - *Staphylococcus aureus* (gram + coccus, clusters)

only certain strains produce an exotoxin (mol.wt. = 35kD), heat stable

short incubation - 2 to 6 hours {projectile vomiting, cramps, diarrhea, nausea}

foods - creamed, mayonnaise, potato salads, pastries, custards

reservoir - humans, anterior nares, boils

3. perfringens food infection-intoxication - *Clostridium perfringens*

  toxin produced during sporulation, usually in the intestines

  cause - intestinal cramps, diarrhea

  usually found in protein-rich foods {beans, meats, gravies}

4. Typhoid Fever - *Salmonella typhi* (gram - rod)

  usually transmitted in contaminated water or food

  human carriers (may be chronic or transient)

  found in contaminated shellfish and sewage
symptoms - constipation, then bloody diarrhea, then fever & "Rose" spots on the abdomen (rash)

isolate organisms - from the urine, feces, blood, bone marrow

Widal test - serum agglutination test

26,000 cases/yr in USA in 1931  552 cases/yr in USA in 1990

Salmonellosis (hundreds of serotypes) - food infection  *Salmonella typhimurium*

symptoms - nausea, cramps, diarrhea, vomiting (gastroenteritis)

10 to 20 hr incubation period (no bloody stool)

ice cream, potato salad, poultry products (especially eggs)

**Bacillary Dysentery** - *Shigella* (gram - rod)

several thousand cases per year in USA, mostly *S. sonei*

dose - small several hundred

reservoir - humans (transmitted by water, eggs, shellfish, dairy products)

organisms produce toxin that attacks the colonic cells cause a watery diarrhea, with some blood (may cause dehydration, convulsions & death) many bowel movements, small volumes

Resistance Transfer Factors (RTF) - plasmids carrying DNA coding for resistance to several antibiotics

**Cholera** - *Vibrio cholerae* (gram - rod)

causes vomiting, cramps and diarrhea (dehydration)

untreated mortality approaches 70%

a disease of the upper intestines, the bacteria attach to the jejunul and ileum, produce toxin and it causes loss of fluids and electrolytes

7 Pandemics in history - El Tor biotype

usually requires large inoculum to get past the acid in the stomach
exotoxin - A and B subunits (B binds and A intoxicates) {genome encoded}

A causes adenylate cyclase enzymes to produce high levels of cAMP, which inhibits uptake of Na⁺ ions and increases release of Cl⁻ ions, causing release of NaCl, NaHCO₃ and water {leading to dehydration and acidosis}

"Rice Water" stools - diarrhea with mucus cells, and electrolytes (≈ 25 L/day)

*Escherichia coli* diarrhea - usually less severe than cholera

enterotoxigenic *E. coli* (ETEC) - traveler's diarrhea

enteropathogenic *E. coli* (EPEC) - infantile diarrhea

enteroinvasive *E. coli* (EIEC) - like shigellosis

enterohemorrhagic *E. coli* (EHEC) - bloody diarrhea & HUS

*Brucellosis* - *Brucella abortus, B. melitensis, & B. suis* (small gram - rod)

causes abortions in cattle & sheep {erythritol = tissue localization factor}

symptoms in humans - weakness, night sweats, backache, headache

occupational hazard - inhaled, ingested, conjunctiva, abrasions

facultative intracellular parasite inoculum = 10 to 100 organisms

"zoonosis" treat with tetracyclines or erythromycin

*B. canis* - dogs, especially beagles in kennels

*Campylobacteriosis* - *Campylobacter jejuni* (gram -, curved rod)

causes a bloody diarrhea, abdominal pain, occasional enteric fever

from: chickens, cattle, turkeys, milk

*Vibrio parahaemolyticus* - (gram - curved rod) from eating contaminated shellfish

marine vibrio that causes - cramps, diarrhea and vomiting

*Yersinia enterocolitica* - (gram - rod) usually from contaminated water

gastroenteritis, usually in children or immunosuppressed

**SOILBORNE AND ARTHROPODBORNE BACTERIAL DISEASES**
1. **Antrax** - *Bacillus anthracis* (gram + sporeforming rod)

   - Zoonosis - a disease of cattle and sheep, spread to humans
   - Spores in the soil - cattle & sheep highly susceptible (fulminating septicemia)
   - Poly-D-glumatic acid capsule (virulence factor) invasin
   - Humans - usually resistant  
     - Woolsorters' disease = lungs
     - Gastrointestinal - violent bloody diarrhea
     - Skin - malignant pustule
   - Exotoxins - Lethal Factor (LF), Edema Factor (EF), and Protective Antigen (PA)
     - Any alone = no effect; LF + PA = death; EF + PA = edema
   - Picked up in animal products - leather and animal bristle brushes
   - Treatment - penicillin
   - Vaccine - PA then capsule-free (attenuated *B. anthracis*)

2. **Tetanus** - *Clostridium tetani* (gram + sporeforming anaerobic)

   - Tetanus toxin (exotoxin) - 2.5 ng human lethal dose {1 oz. kill 12 billion}
   - Coded for by 40-70 mD plasmid, toxin labile to heat & light
   - Stryknine-like action - on the central nervous system (suppresses synaptic inhibition) causes total spasms
   - Spores everywhere in the soil, air and water (especially horse manure)
   - Deep puncture wound - anaerobic - slight growth - toxin synthesis
   - 38% fatality rate - this is reduced with symptomatic treatment
   - Respiratory therapy, antitoxin, curare, barbiturates, antibiotics
   - About 100 cases per year in the USA

3. **Gas Gangrene** - *Clostridium perfringens* (Gram + sporeforming anaerobic)

   - Also *C. novyi* & *C. septicum* (mixed contamination)
   - Require much traumatized, devascularized wound to initiate myonecrosis
   - Grow fast, much gas, spreads through healthy tissue
virulence factors - lecithinase, hyaluronidase, hemolysin, collagenase

treatment - penicillin, debridement, amputation, hyperbaric oxygen

4. **Listeriosis** - *Listeria monocytogenes* (gram + motile rod)

from soil animals, and unpasteurized milk
healthy carriers - slaughterhouse workers, food processors

Listeric meningitis - stiff neck headache and coma
uterine form - abortions in humans

some very resistant to heat and/or cold

4. **Bubonic Plague** - *Yersinia pestis* (gram - rod)

The reservoir is the rat, the rat flea is the vector and humans are incidental hosts

When fleas are infected it closes their esophagus and the think they are hungry and bite more

goes to the blood - lymph - lymph nodes (swelling = buboes) may go to the lungs and cause "pneumonic" plague and be contagious spread by aerosol

hemorrhages in the skin - black death

sylvatic plague - desert rodents in the southwest USA

5. **Tularemia** - *Francisella tularensis* (gram - rod)

"rabbit fever" rodents in the USA
cause crater-like ulcer & swollen lymph nodes

hard to diagnose, treat with tetracycline or streptomycin

concentrated in: Missouri, Arkansas, and Oklahoma

6. **Leptospirosis** - *Leptospira interrogans* (gram - spirochete)

transmitted in contaminated soil or water (urine of rodents)

occupational hazard for: dock workers, mine workers, farmers, sewage plant workers

frequently transmitted to domestic animals first then to humans
symptoms: fever, jaundice, blood in vomit, splenomegaly, skin hemorrhage
penicillin - much more effective if given early

7. **Relapsing Fever** - *Borrelia recurrentis* (gram - spirochete)
   transmitted by ticks and lice (filth and poverty)
   symptoms: similar to leptospirosis, but symptoms cycle up to 10 times
   because of surface antigen capping (genetically altering surface ag)
   treat with tetracyclines

8. **Lyme Disease** - *Borrelia burgdorferi* (gram - spirochete)
   transmitted by *Ixodes scapularis* (tick), reservoir white-footed mouse
   and white-tailed deer
   humans are incidental hosts
   symptoms: rash (50%), arthitis (common), neurological signs (less common)
   treat with antibiotics early to to be more effective

**CONTACT AND ENDOGENOUS BACTERIAL DISEASES**

**Gonorrhea** - *Neisseria gonorrhoeae* (gram - diplococcus)
≈ 1 million cases per year reported in the USA
microbe - fragile, nutr. fastidious, temp. sens., sens. to drying
Pelvic Inflammatory Disease (PID) in females, also commonly asymptomatic
males - urethritis, epididymitis, sharp pain, possible sterility (also in females)
conjunctivitis, pharyngitis, ophthalmia neonatorum (AgNO₃ treatment)
treatment - penicillin: PPNG = spectinomycin or tetracycline
diagnosis - pussy penile discharge - PMN with gram - diplococci
oxidase +, catabolize glucose not maltose
Syphilis - *Treponema pallidum* (gram - spirochete)

**obligate extracellular parasite** (was much more deadly earlier)

attacks mucous membranes through abrasions

1. Primary stage - **Chancre** - "hard - raised" and painless (infectious)
   loaded with spirochetes - usually found on the genitals, lips, skin or pharynx
   21 day incubation (cases increasing in the last few years) heals < 1 mo.

2. Secondary Stage - maculopapillary rash (containing spirochetes): the person
   may die or may recover - may go to latent syphilis (high ab titer, no symp.)

3. Tertiary Stage - gummas (lesions of cardiovascular or nervous system or skin)
   paralysis, heart failure, insanity

   darkfield micro exam., TPI or serum FTA-ABS, VDRL. Rapid Plasma Reagin,
   TP hemagglutinin test.

   treatment: penicillin for primary & secondary syphilis

   number of cases increasing (now ≈ 50,000 cases per year in USA)

**Chancroid** - *Haemophilus ducreyi* (gram - rod)

soft, painful chancre, swelling of inguinal lymph nodes

Nongonococcal Urethritis (NGU) - *Chlamydia trachomatis* (obligate intracellular parasite)

most common type of STD in USA, may cause sterility.

**Ureaplasma urealyticum** - (no cell wall, requires cholesterol & urea)

   similar to gonorrhea, abortions, sterility, prostatitis

   all treated with tetracyclines

**Leprosy** - *Mycobacterium leprae* (gram + rod)

   treatment places - Molokai & Carville Louisiana

   10 million people affected around the world

   long incubation period - may be years
treatment - dapsone

Immunity - CMI (lepromin test)

**Yaws** - tropical disease  (Africa, S.A., Asia)

syphilis like organism - Treponema pertenue

bejel & pinta - from the soil through abrasions

an ulcerous lesion - may spread to other areas - may disappear

mostly in children - treat with penicillin

**Actinomycosis** - *Actinomyces israelii* (gram + anaerobic rod)

lumpy jaw in cattle, cervico-facial lesion in humans

swelling draining sinuses, may be in the thoracic or abdominal cavity

an IUD - may lead to infectious abortion or PID

normal flora of the oral cavity; treat with penicillin

**Trench Mouth** - "stress-induced" synergistic disease

Acute Necrotizing Ulcerative Gingivitis (ANUG) - *Leptotrichia buccalis* & *Treponema vincentii* - destruction of the gingiva & papilli (bad odor & bad taste in the mouth)

also *Eikenella corrodens* & *Treponema denticola*

treat with penicillin, hydrogen peroxide or use good oral hygiene

**Toxic Shock Syndrome** (TSS) - 1978 *Staphylococcus aureus* with specific toxin

SPE or SEF causes rash, fever, vomiting, watery diarrhea, desquamation of the palms of the hands and the soles of the feet - shock - death

Rely tampons - fibers caused strains to grow better and produce more toxin

**Urinary Tract Infections** (UTI) - frequently endogenous - *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter faecalis* - cystitis (bladder)

**Bacteroides** - anaerobic abscesses - abdomen, peritoneum, oral cavity
PHYSICAL METHODS FOR CONTROLLING MICROORGANISMS

I. Heat - Thermal Death Time (time at which organism killed at a given temperature) 
time and temperature related death

A. Direct Flame - seconds

B. Hot Air - 160°C for 2 hours (oxidizes protein) - good for: powders, oily substances, 
and glassware [dry heat does not penetrate well]

C. Boiling Water - spores may take up to several hours to kill (C. botulinum >24 h) 
(denaturing protein)

D. Autoclave - 121°C (15 psi) live steam, no air - good for: blankets, bedding, utensils, 
intravenous solutions, and lab media [moist heat]

II. Filtration

A. Seitz - (asbestos) porcelin - not really used any more!

B. Diatomaceous Earth - as a filtering aid, to clarify solutions

C. Membrane Filters - (cellulose acetate and mixed esters) - used for detecting 
contamination and sterilization of solutions that are heat labile

III. UV-light - (265nm wavelength) produces Thymine dimers in the cell's DNA 
used mostly to keep air and surface contamination down 
[UV-light has very weak penetration power]

IV. Ionizing Radiation - (X-rays and γ-rays) these create organoperoxides and other 
ions that break DNA [microwaves?]

V. Cavitation - (ultrasonication) - used in cleaning instruments and breaking open 
microorganisms [does so by creating many tiny vacuums]

CHEMICAL METHODS OF CONTROLLING MICROORGANISMS

Disinfectant versus Antiseptic Bacteriocidal versus Bacteriostatic

Qualities: fast-acting, broad-spectrum, long shelf life, non-toxic to humans, 
water soluble, good penetrating power and not very reactive with 
organic debris [functional temperature and pH important]

Phenol Coefficient = a means of comparing the potency of various agents 
[Phenol is 1 the standard]

I. Halogens
A. Chlorine - 0.2 to 1 ppm (parts per million) residual of free chlorine obtained used NaOCl (Clorox) [produces trihalomethanes] - used to purify water and decontaminate sewage - chloramines more chemically stable \{this agent is not sporocidal and only kills certain viruses\}

B. Iodine - iodophors (I\textsubscript{2} + polyvinyl alcohol) used as an antiseptic: has slow release and loosens microorganisms [iodine works on tyrosine amino acids in proteins] Wescodyne and Welladol

II. Phenols - (coagulates protein) - cresols like orthophenylphenol [Lysol] inactivates protein toxins \{the only disinfectant that does this\} also there are other agents: hexachlorophene and hexyresorcinol

III. Heavy Metals

A. Mercury - HgCl\textsubscript{2}, merthiolate, mercurochrome (works on proteins)

B. Copper - algicides

C. Silver - AgNO\textsubscript{3} 1% solution used in the eyes of newborn babies \(N.\ gonorrhoeae\)

IV. Alcohol - ethyl or isopropyl - 70% is better than 100% (denatures proteins, dissolves lipids and dehydrates) - time required 10 min to 2 hours

V. Alkylating Agents (change chemical structures of nucleic acids and proteins)

A. Formaldehyde - used in vaccines and toxoids

B. Ethylene Oxide - used especially for sterilizing plastics (sporocidal, good penetration)

C. Glutaraldehyde - kills viruses, bacteria and spores (unaffected by organics)

D. β-Propiolactone - used only to disinfect surfaces (less explosive than glutaraldehyde)

VI. Hydrogen Peroxide - \(H_2O_2\) - used to clean utensils and irrigate wounds, especially good against anaerobic bacterial infections

VII. Detergents - quaternary ammonium ion complexes (cationic) - used to disinfect surfaces, food utensils and on skin as an antiseptic

VIII. Dyes - triphenylmethane dyes - crystal violet and malachite green = gram + acridine orange = gram - and + (interferes with DNA & RNA synthesis)
 IX. Acids - benzoic and salicylic used on skin - especially for fungal infections [ringworm]

CHEMOTHERAPEUTIC AGENTS AND ANTIBIOTICS

Chemotherapeutic Agents are Manmade

1. Paul Ehrlich 1909 - Compound #606 (Arsphenamine) Salvaran originally made to inhibit Trypanosomes; later found to work against T. pallidum [the cause of syphilis]

2. Gerhard Domagk 1930s - Prontosil was converted to sulfanilamide in the intestines active against Gram + staphylococci & streptococci [acts as a competitive inhibitor of p-Aminobenzoic Acid (PABA) in folic Acid synthesis: bacteria make their own folic acid]

later - sulfamethoxazole-trimethoprim = Bactrim, was also used because it interferes with the conversion of folic acid to folinic acid, the active form (especially good for treatment of UTI and Haemophilus infections)

Isoniazide (INH) - it is a competitive inhibitor of vitamin B₆ which is important in cell wall synthesis [used to treat tuberculosis]

Metronidazole - treatment of Trichomonas vaginalis & anerobic infections

Chloroquine & Primaquine - to treat malaria, caused by a Plasmodium (proteozoan)

Dapsone - used to treat leprosy

ANTIBIOTICS

1. Penicillin - Fleming 1928 - 1940s Florey & Chain industrially produced enough penicillin for treatment of disease [acts specifically by interfering with the cross-linking of the peptidoglycan cell walls of bacteria. It is only effective in growing bacteria. Penicillin is a β-lactam ring structure that penicillinase inactivates by breaking the β-lactam ring] low cost, broad spectrum, and few side effects (best against gram +) {Synthetic penicillins = Ampicillin, Amoxicillin, Carbenicillin, etc.}

2. Cephalosporins - produced by Cephalosporium, another mold (works by inhibiting bacterial cell wall synthesis) effective against - staphylococci, streptococci & gram - UTI infections

3. Streptomycin - Discovered by Selman Waksman in 1939 (a product of Streptomyces griseus, a soil actinomycete) - Active against the bacteria that tuberculosis, brucellosis, plague and chancroid. Problems = causes middle ear problems and deafness. {interferes with protein synthesis at
the 30s ribosomal subunit}

Other Aminoglycosides: gentamicin (gram -); Neomycin; kanamycin and Amikacin (Pseudomonas & UTI infections) {may cause kidney damage or failure}

4. Chloramphenicol - Discovered 1947, broad spectrum, works on the 50s ribosomal subunit to inhibit protein synthesis (produced by a streptomycete)
Active against: meningitis, typhus fevers & typhoid fever
{may cause aplastic anemia, especially in infants}

5. Tetracyclines - broad spectrum from streptomycyes (acts on 30s ribosomal subunit)
good for treating: rickettsia, chlamydia, NGU, brucellosis, tularemia, cholera, mycoplasma pneumonia, syphilis and gonorrhea. {side effect - may cause colitis, due to Candida (yeast) overgrowth}

6. Others: 
Erythromycin (streptomyces) works on 50s subunit [works on: staph, strep, syphilis, mycoplasma and Legionnaire’s disease {cause colitis}

Vancomycin - IV injection only - severe staphylococcal infections
Used to treat Antibiotic-Associated Colitis (orally) because the drug is poorly absorbed from the stomach and small intestines

Rifampin - for treatment of tuberculosis [inhibits DNA-dependent RNA polymerase]

Clindamycin - treatment of anaerobic bacterial infections (inhibits protein synthesis) {may induce ACC or PMC, because of resistant strains of Clostridium difficile}

Polymyxin B & Bacitracin - (produced by Bacillus) acts by disrupting membranes

Antibiotic Susceptibility Assays

Tube Dilution & Agar Dilution Tests
Minimum Inhibitory Concentration (MIC)
Minimum Bactericidal Concentration (MBC)

Kirby-Bauer (disc diffusion) Assay - Mueller-Hinton Agar (zone size of inhibition is measured) the rate of diffusion will vary with different compounds - the bacterial inoculum is standardized and the medium is standardized and dated

Antibiotics may not work as well in-vivo as they do in-vitro
1. May not be able to reach functional level in the blood
2. The rate of inactivation or clearance from the body may be too rapid
3. Biofilms - (these may be present on indwelling plastic devices) - they frequently require higher levels of antibiotics than planktonic bacteria, because the bacteria on the inside of the biofilm are not as accessible

**Rickettsia and Chlamydia**

These two genera of bacteria are obligate intracellular parasites, which are grown only inside cells (tissue culture cells, embryonated yolk sacks of eggs, animals), they are frequently studied along with the viruses.

**Rickettsia**

They are small gram - coccobacilli

They require: ATP, NAD and coenzyme A

They are transmitted by arthropod vectors, which may also serve as reservoirs ticks (transovarian passage), mites and lice serve as vectors

The organisms are in tick feces and regurgitation and they are scratched in by itching

The diseases usually have a rash and a high fever

There are cross-reacting (heterophile) antigens [Proteus OX-19, OX-K, OX-2] that are used diagnostically

The diseases are treated with tetracyclines or chloramphenicol

1. Rocky Mountain spotted fever (RSMF) more prevalent in the eastern US (less virulent) transmitted by the dog tick (Dermacentor variabilis) - the disease in the western US is less common (more virulent) and transmitted by the wood tick (D. andersoni) - a rash starts out on the palms & soles - then moves to the trunk of the body - caused by Rickettsia rickettsii

2. Epidemic typhus - killed half of Napoleon's troops in their march to Russia - killed millions in World Wars I & II - caused by Rickettsia prowazekii - transmitted by pediculus (body lice)

    promoted by poor hygiene, overcrowding, lack of sanitation

    fever (104-105°F), rash - on the trunk of the body first - then spreading
70% mortality rate - stop epidemics by DDT to kill the vector (lice)

3. Endemic typhus - *Rickettsia typhi*, rodents (reservoir), flea (vector) - mild disease

4. Scrub typhus - *Rickettsia tsutsugamushi*, mites & chiggers (vectors) usually seen in marchy scrub land of Japan & Southeast Asia

   there is an eschar (lesion) at the site of bite

5. Q fever - *Coxiella burnetii* - this is not a true member of the rickettsiae because it does not require a vector - it may be transmitted in contaminated milk [it is one of the most heat resistant bacteria that are transmitted in milk]

   this organism resists the phagolysosomal contents

   it may be spread by ticks or aerosols of their dried feces

   occupational hazard of dairy farmers - influenza-like disease

CHLAMYDIA

These are small gram negative spherical bacteria that are obligate intracellular parasites that have an unusual growth cycle - They are either small (0.3µm), dense, infectious Elementary Bodies or the are larger (0.9µm), less dense, non-infectious, replicating Reticulate Bodies. These bacteria also require ATP from their host cell.

They have a 24 hour growth cycle where they infect the host cell as a Elementary Body - then reorganize into Reticulate Bodies in about 8 hours - they undergo binary fission to generate 500-1,000 new cells - then take several hours to convert these Reticulate Bodies back to the infectious Elementary Bodies before they lyse the infected cell. [This is much like a one-step growth curve of a typical virus]

1. Trachoma - *Chlamydia trachomatis* - found most frequently in hot dry areas of the world (Africa, Asia, S.W. USA) - transmitted by fingers, fomites, aerosols - goes to the conjunctiva & cornea - causes formation of follicles & nodules - may produce scar tissue - which leads to blindness (primary cause of infectious blindness in the world)

   diagnosis can be made from conjunctival scrapings - iodine [inclusion bodies]

   treat with tetracyclines or erythromycin ointments

2. Nongonococcal Urethritis (NGU) - sexually transmitted disease

3. Chlamydia psittaci - cause parrot fever - influenza-like disease - transmitted from birds [bird houses, turkeys, ducks, chickens] to humans
VIRUSES AND VIRAL DISEASES

1. Viral Morphology

   Size: 12nm to 250nm [0.012 to 0.25µm]

   Shape: icosahedral (20 facets), helical, or complex

2. Viral Components

   A. Genome - DNA or RNA (1.5 x 10^6 - 2 x 10^8: 3-300 genes)

   B. Capsid - protein coat

   C. ± envelope phospholipid + protein (may contain spikes) [hemagglutinins & neuraminidase]

3. Viral Replication

   A. Receptor - uncoating of virus or ingestion of virion or NA

   B. Viral NA directed proteins synthesized (hydrolysis of cell protein)

   C. Viral protein & NA synthesized

   D. Viruses may bud out through membrane or cause cell lysis [may go lysogenic]

      the repressor protein is coded for by the viral NA

4. Culturing - tissue culture cells & yolk sack of chick embryos or lab animals

5. Viral Inhibition

   A. Antibody Neutralization - interfere with infectious process & enhance phagocytic killing

   B. Chemicals - thiosemicarbazone, IDU, Amantadine

   C. Interferons - translational inhibitory protein [genetically engineered E. coli - human interferon (INF)] plus an enzyme that cleaves viral m-RNA

   D. Vaccines - inactivated, attenuated, chimeric, recombinant Human Diseases

I. Pneumotropic - incubation 1-3 days
A. Influenza [Orthomyxoviridae] - replicates in the cell nucleus, assembled in the cytoplasm. ss-RNA, helical capsid, envelop + H & N spikes. “antigenic variation” genome “8” segments - reassorting during assembly thus changing antigenic nature [Antigenic Drift & Antigenic Shift (Pandemics)]

Symptoms: abrupt onset, sudden chills, fatigue, headache, fever > 103°F, severe cough, stuffy nose, dry throat

transmission by aerosol droplets

secondary bacterial invasion may occur

detection: inoculate embryonated chick eggs, rising ab titer in paired sera

treatment - may pretreat with Amantadine (6-8 weeks required)

B. Rhinoviruses [picornaviridae]

icosahedral ss-RNA (replicates in cytoplasm, ds-RNA - 2nd strand is template)

causes common head cold - 113 antigenic types (20 common)

transmitted by droplets and fomites

immunity lasts - 1 to 2 years

II. Dermatotrophic

A. Chickenpox [Varicella] a herpes virus

icosahedral ds-DNA virus {replicates in the cell nucleus}

transmitted by droplets and skin contact (incubation period 10-23 days)

Symptoms: anorexia, fever, headache, vessicles (contain infectious fluid) predilection for the skin and nervous tissue

 treat - Ara-A

Herpes-Zoster (same virus) causes shingles in adults - painful, infectious

B. Herpes Simplex (cold sores, eczema, encephalitis, genital herpes)

HSV-I = above the waist [gingivostomatitis in children]

HSV-II = below the waist [genital herpes]
DNA - icosahedral capsid, with envelop and spikes - multiplies in the nucleus of infected cells [produces "inclusions"]

Sexually Transmitted Disease - HSV-II = lesions on penis, vagina and cervix may infect newborn

treatment: IDU, Ara-A, Vira-A, acyclovir [competitive inhibitor]

Also in this category: measles, mumps, smallpox, and german measles (congenital birth defects)

III. Viscerotropic Viral Diseases

A. Yellow fever - [flaviriridae] enveloped ss-RNA Walter Reed "mosquito" vector

occurs naturally in jungle animals, transmitted by mosquitoes (humans are incidental hosts)

Symptoms: high fever, attacks liver (jaundice), 40% mortality rate

B. Infectious Mononucleosis  (Epstein-Barr virus) ds-DNA herpes

transmitted by saliva  [may cause Burkitt's lymphoma, when immunocompromised]

goes to the lymph nodes and spleen - sore throat, fever, jaundice high mononuclear white blood cell count

the host cell for the virus is the B-cell lymphocyte

viruses probably carried for life

C. Cytomegalovirus (fever, malaise, splenomegaly)

herpes ds-DNA virus  [may be a congenital disease] crosses the placenta and damages the fetus

may cause: mental retardation and hearing defects

diagnosis: enlarged cells of the salivary glands, epithelium & liver with Lipshutz bodies (inclusions in the nuclei of infected cells)

D. Coxsackie [picornaviridae]

transmitted by feces contaminating: water, food, and shellfish
group A - "herpangina" - in children vesicles on tonsils & palette - painful swallowing

group B - "Pleurodynia" - chest pains - myocarditis & common cold

E. Hepatitis

1. "Infectious" [picornaviridae] - transmitted in feces and sewage
   contaminated water and shellfish - hepatitis A
   inactivated by chlorine 10-15 ppm 30 min (this is a long exposure)
   boiling will inactivate in 5 min

   2 to 6 week incubation period: anorexia, chills, fever, ± jaundice
   recovery slow with relapses (may treat with immune globulin)

   60 to 150 day incubation period - lower fever, less abrupt onset
   transmission: blood to blood, tattoos, IV drug use, renal dialysis
   1981 - vaccine HB_sAg (having this disease may predispose liver cancer)
   presence of δ-agent usually makes this disease worse

3. NANB - "C" hepatitis - insidious onset, 1/3 of cases are chronic
   transmitted by blood [retrovirus or flavivirus]

IV. Neurotropic Viral Diseases

A. Rabies [rhabdoviridae] - very high mortality rate
   occurs in almost all animals - usually fatal, except in bats
   caused by an enveloped RNA virus
   transmission usually by bite (saliva) or by aerosol (caves - bats droppings)
   incubation period - 6 days to 1 year (location of the bite)
   multiplies in the muscles - moves to the nerves - then to the brain
   starts with a tingling, burning sensation - fever, headache - difficulty swallowing
   salivation then hydrophobia (fear of water)
treatment: antirabies antibodies plus vaccination - duck embryo vaccine - 14 daily injections in the fat pads of the abdomen

Merieux human diploid cell vaccine - 2 to 7 IM injections in the arm

B. polio [picornaviridae] very small virus 27 nm

virus multiplies in the nerve tissue cells

transmitted in contaminated water or food - tonsils - then intestines
99% of the cases are subclinical - incubation 3 to 21 days

Symptoms: nausea, vomiting intestinal cramps, constipation
may go on the the central nervous system and lead to paralysis of arms and legs and respiratory collapse - iron lungs

3 types - type I most common (some paralysis)
  type II sporadic (more paralysis)
  type III rare (rare paralysis)

infections may be: abortive, non-paralytic, and paralytic

vaccines: Salk (1955) - inactivated [injected]; Sabin (1961) - attenuated [oral]

F. AIDS Human Immunodeficiency Virus (HIV) [retroviridae]

About 1979 first recognized in homosexuals

GP120 (spikes) have specific receptors CD-4 on cells (espec. T₄ lymphocytes)

nucleocapsid contains 2 RNA + reverse transcriptase (enzyme that makes DNA from RNA) DNA template integrates into host cell genome causes latent infection

person becomes HIV seropositive in about 2-3 months (initial symptoms "mono" like) - soon disappear - goes to latent stage - 2 to 15 years AIDS

maybe ARC(AIDS-related complex) - fever, swollen glands, fatigue, diarrhea opportunistic infections, weight loss, Kaposi’s sarcoma (cancer)

AIDS - T₄ cells < 500 /mm³ blood (diagnostic)

also diagnostic are: Pneumocystis carinii pneumonia, cryptosporidiosis, candidiasis, and cryptococcosis

STD - lesions promote the spread of AIDS
Diagnosis - latex agglutination test 99% accurate or ELISA, backup and confirm with Western blot for anti-HIV antibodies

Vaccines: surface antigens mutate rapidly (a big problem), latency of infection

CDC HIV infections

Group I  acute (mononucleosis-like)
Group II asymptomatic
Group III persistent generalized lymphadenopathy AIDS Related Complex (ARC)
Group IV Other
   A. Weight loss, fever, diarrhea
   B. Neurological
   C. Secondary Infections
   D. Secondary Cancer [Kaposi's sarcoma]
   E. Other

FUNGI

Eukaryotes = yeasts (single cells) & molds (multicellular, filamentous)

No chlorophyll, chitin and/or cellulose in their cell wall

usually feed on dead or decaying matter

FRUITING BODIES (ASEXUAL REPRODUCTION)

   1) sporangiospores
   2) conidia
   3) arthrospores
   4) chlamydospores
   5) budding (yeasts)

SEXUAL REPRODUCTION

   oospore  water molds
   zygospore  rhizopus
   ascospore  penicilium, aspergillus, saccharomyces
   basidiospore  agaricus [mushrooms]

Oomyces - mildew - grapes & rust on cabbage - potato blight

Zygomycetes - bread mold, rice fermented to sake, production of cortisone
Ascomycetes - mildew on grapes, dutch elm disease, yeast for alcohol
industrial production of: citric acid, soy sauce, cheese, penicillin

Basidiomycetes - rusts and smuts on grains, edible mushrooms, Amanita (poison)

Deuteromycetes - no known sexual cycle

Dimorphism (thermal) - 25°C - mold phase
37°C - yeast phase

Yeasts - unicellular, carry out division by budding: *Saccharomyces, Candida*

Cryptococcosis - *Cryptococcus neoformans* (Basidiomycete) - disease of lungs &
meninges - transmitted by pigeon droppings - through inhalation
this is a tuberculosis-like disease - may move to meninges via the blood

CMI (T-cells) important for fighting the disease
untreated - ± fatal - treat with amphotericin B {may cause: anemia, hypotension
and kidney damage}

Candidiasis - *Candida albicans* (normal inhabitant of the human oral cavity, intestines
and vagina)

may cause vulvovaginitis in young females, or vaginal overgrowth in females
using birth control pills - oral antibiotics may also lead to an overgrowth in the
intestines

oral "thrush" may be seen in new borns and in cancer and AIDS patients

Aspergillosis - *Aspergillus fumigatus* [Ascomycete] in a compromised host

1. inhaled - aspergilloma in the lungs
2. ear wax - otomycosis
3. systemic - rare, may be deadly - treat with amphotericin B

*Aspergillus flavus* & *parasiticus* - aflatoxins (found in grains & peanuts)

may cause liver tumors in livestock [may be transmitted in meat & dairy]

coccidioidomycosis - *Coccidioides immitis* (in the Southwestern USA)

endemic - especially a problem in migrant workers [Mexicans & blacks]
pregnant females are at high risk {estrogens promote growth}
arthrospores are found in the soil - they are inhaled into the lungs and produce
an influenza-like disease [spherules (20-60 µm diameter) produced in lungs]
North American blastomycosis - *Ajellomyces dermatitidis* [biphasic Ascomycete]

transmitted through pigeon dropping and dusty soil - inhaled or in wounds

in the lungs - a tuberculosis-like disease
in the skin - a wart-like lesion

may go progressive - identify by cultivation of the fungus

Histoplasmosis - *Emmonsella capsulata* (carried by starlings)

transmitted by bird droppings - inhaled - a "summer flu" - chest pains, headache, chills & fever most cases are untreated

the disease may become TB-like & attack many organs [endocarditis & meningitis] treat with amphotericin B

dimorphic fungus: 25°C (white cottony) - 37°C (creamy yeast)

DTH - skin test with histoplasmin

Dermatomycosis - (skin, hair and nails) "tinea" - deuteromycetes

*Trichophyton, Microsporum, and Epidermophyton*

humans - human to human transmission is common [anthropophilic]

pets - transmission to humans common [zoophilic]

environment - from the soil [geophilic]

not really very common because of a functional CMI

Sporotrichosis - *Sporothrix schenckii* - a subcutaneous infection

caused by contamination of a wound by a plant (rose thorn or evergreen trees) produces a chronic granulomatous lesion
PROTOZOA

I. Single-celled Eucaryotic animals - the Kingdom is now called Protista

II. Structure and Growth of Protozoa
A. no cell wall, they have a cell membrane and maybe a pellicle
B. Ingest food by phagocytosis, except for a few phototrophs
The feeding form is a trophozoite and the cyst is the dormant form
They reproduce by mitosis - and possibly a sexual cycle

III. Classification
A. Sarcodina - amoeboid cells that move by "pseudopods"
B. Mastigophora - the shape of a vase and move by one or two flagella
C. Ciliophora - covered with hairlike "cilia" in longitudinal spiral rows and some have a feeding groove
D. Sporozoa - parasitic protozoa, adults are nonmotile

IV. Protozoal Diseases Due to Amebas and Flagellates
A. Amebiasis - mostly in tropical areas, especially with low sanitation

Caused by - Entamoeba histolytica (caused by consuming contaminated water or food washed in contaminated water) - a type of dysentery

Treated with metronidazole or paromomycin {which don't work against the cysts}

B. Giardiasis - another intestinal disorder - cramps, foul-smelling diarrhea and flatus

Caused by - Giardia lamblia [transmitted from human or animal feces to water]
cysts may be shed for a long time, even if the patient is treated (may become chronic carriers).

C. Trichomoniasis - frequently a sexually-transmitted disease, but may be transmitted by fomites (towels or clothing) - it thrives in the human vagina, causing vaginitis

Caused by - Trichomonas vaginalis {intense itching and burning during urination, with a creamy white frothy discharge} does not produce cysts

Males may be asymptomatic, but both sexual partners should be treated.

Treatment - metronidazole, tinidazole or miconazole (usually antifungals)

D. African Sleeping Sickness - transmitted by the tsetse fly (Glossina palpalis)
Produces a chancre at the site of the bite - then goes to the bloodstream

Caused by - Trypanosoma brucei var. gambiense {chronic bouts of fever, severe headaches, paralysis and a general wasting away - then the patient slips into a deep coma and dies} Treated with - suramin sodium or pentamidine isethionate.

V. Diseases Due to Ciliates and Sporozoa

A. Balantidiasis - transmitted in contaminated water or pork, usually warmer climates

Caused by - Balantidium coli - cause profuse diarrhea, nausea and weight loss rare in the United States - treated with metronidazole or paromomycin

B. Malaria - transmitted by Anopheles mosquitoes - it is caused by Plasmodium vivax, P. malariae, P. ovale and P. falciparum. 250 million people around the world with malaria.

Two different hosts in the life cycle of the malaria parasite - the mosquito and an animal host.
A blood meal from an infected human passes the gametocytes to the mosquito - they become a zygote, which produces sporozoites - that are transmitted
to a human - which go to the liver and are converted to merozoites that infect the erythrocytes - causes **cycled bouts of fever & chills** when they are released.

Different forms of malaria are associated with the different species of plasmodia.

Treatment - first was quinine, then chloroquine & primaquine. One of the more recent ones is fansadar.

C. Babesiosis - a malaria-like disease caused by *Babesia microti* - lives in *Ixodes* ticks (found in this geographic location). Causes anemia, headache, possibly meningitis.

D. Cryptsporidiosis - a diarrheal disease of animals, that can be transmitted to humans.

Caused by - *Cryptosporidium coccidi* - immunocompetent individuals suffer mild diarrhea for one to two weeks, while immunocompromised individuals experience cholera-like diarrhea and often die of the disease.

E. Pneumocystosis - the major cause of non-bacterial pneumonia in America

Caused by - *Pneumocystis carinii* - has a complex life cycle in the lungs of infected person. Only a real problem in immunosuppressed persons, found in at least 50% of AIDS patients.

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**The Multicellular Parasites**

I. Flatworms - Platyhelminthes (flattened bodies, ribbon-like structure) they contain
bilateral symmetry and some are hermaphroditic

Classes: Turbularia (freeliving)
        Trematoda (flukes)
        Cestoda (tapeworms)

A. Flukes - leaflike parasitic worms with a complex life cycle which may include encysted egg stages and temporary larval forms, may use an intermediate and a definitive host

in humans - flukes may inhabit the blood, liver, lungs and the intestines from the human intestines - eggs are released in the feces - in the water they hatch and develop into ciliated larvae called miricidia - the miricidia penetrate snails and undergo a series of asexual reproductive stages including redia - the redia become tadpolelike cercaria which are released back into the water - the cercaria may produce an encysted form called metacercaria, which make their way back to humans.

1. Blood Fluke Disease - Schistosoma [S. mansoni - Africa & S. America; S. japonicum - Far East; S haematobium - Africa] a liver disease in humans - eggs - feces - water - snails - cercaria attach themselves to bare skin of humans = blood: cause fever and chills - liver = damage; intestines = ulceration and diarrhea (swimmers itch - caused by bird schistosome unable to penetrate the skin)

2. Chinese Liver Fluke Disease (Clonorchis sinensis) - the cercaria penetrate fish muscle - then form encysted form which can be ingested by humans in raw or poorly cooked fish - leads to gall bladder blockage or liver damage

3. Other Fluke Diseases - consumed in water chestnuts and water bamboo (raw), poorly cooked crab and watercress (diseases of the lungs, intestines and the liver)

B. Tapeworms - These worms have a long, flat body (proglottids) with a head region, called a scolex, containing hooks and a suckerlike device - generally live in the intestine

1. Beef and Pork Tapeworm Diseases - (Taenia saginata & T. solium) - proglottids pass in human feces to the soil and are consumed by cattle and pigs, where the parasites encyst in the muscle and are transmitted in poorly cooked meat - may produce mild diarrhea

2. Other Tapeworm Diseases: Diphyllobothrium latum = fish tapeworm - two intermediate hosts, the copepod and the fish which is consumed raw or poorly cooked
   Hymenolepis nana = lives in the intestine and is passed from human to human
through contaminated food or contact with contaminated objects

C. Roundworms (Aschelminthes: nematodes)

1. Pinworm Disease - *Enterobius vermicularis* - common in USA in children and adults - cause diarrhea and itching in the anal region - recontamination by the “anal-oral” route (treatment should be for all members of the family) - examine for the presence of eggs in the feces (sticky tape)

2. Roundworm Disease - *Ascaris lumbricoides* may measure up to a foot, infects the intestines, may cause perforation to blood infect the lungs and cause pneumonia - hundreds of millions affected around the world

3. Trichinosis - *Trichinella spiralis* - a common problem where pork is consumed in high amounts - worms migrate to the blood, penetrate muscles and form cysts - passed to humans by consumption of *poorly cooked pork* - the disease is intestinal first, then spreads to the muscles, may cause hemorrhaging, paralysis and even death

4. Hookworm Disease - *Ancylostoma duodenale* and *Necator americanus* - worms are found in warm moist soil and enter through bare skin to blood vessels to intestines - suck blood and cause anemia

5. Strongyloidiasis (resembles hookworm in appearance) - inhabit the small intestine, especially the duodenum causing nausea, vomiting, diarrhea with constipation. May also cause pneumonia. Treat with thiabendazole. Vietnam war veterans picked this disease up. *Strongyloides stercoralis* is the cause.

6. Filariasis - caused by *Wuchereria bancrofti*. The worm breeds in the human lymphatic system and causes severe inflammation and damage. After years of infestation, the arms, legs and scrotum swell enormously and become distorted with fluid. This condition is known as elephantiasis. This disease is transmitted by mosquitoes.
Industrial Microbiology

Microbes - grow and divide rapidly, genetically stable, work on a myriad of substrates

enrichment culture - encourage growth on a particular substrate

selective medium - agent added to inhibit certain organisms

1. Batch technique - up to 100,000 gallons - sterilized and seeded with heavy inoculum works for days or months - microbes are removed - products are isolated

2. Continuous Flow - (chemostat) - keep microorganisms in logarithmic phase of growth by continuous addition of fresh medium

Production of Organic Compounds

A. Citric acid (soft drinks, candies, inks, dyes, anticoagulants, etc.) - isolated from citrus fruits or synthesized by molds - *Aspergillus niger* - growing on corn meal or molasses with salts & N added - aerobic metabolism - Kreb’s cycle [lacking an enzyme, aconitase] - stops at citric acid

B. Lactic acid - (source of Ca++ and Fe++) - *Lactobacillus bulgaricus* - homolactic acid fermentation at 43°C add Ca(OH)₂ to prolong fermentation (no acid shut off)

C. Vinegar - (3-5% acetic acid) - alcohol is produced from apples or grapes by *Saccharomyces cerevisiae* (anaerobically) - then Acetobacter aceti converts ethanol to acetic acid (aerobically)

D. Amino acids & Vitamins
   glutamate - *Micrococcus, Arthrobacter, Brevibacterium* (food supplement or additive)
   lysine - *E. coli* - then *Enterobacter* (required amino acid)
   [also: methionine, tryptophan and valine]
   vitamin B₁₂ - *Pseudomonas, Propionibacterium, Streptomyces* - with a malt extract or corn steep liquor [used to avoid pernicious anemia]

E. Enzymes
   Amylase - *Aspergillus oryzae* [spot remover, adhesive, removes sizing (starch from textiles)]
   Pectinase - *Clostridium* [used to rett flax and clarify fruit juices for jelly making]
   Proteases - *Bacillus subtilis, Aspergillus oryzae* [batting hides, liquid glues, laundry pre-soaks, meat tenderizers and spot removers]

Steroid Transformations - bile acids to cortisone (37 steps in 1952, cost $200/g) reduced the number of steps - *Aspergillus ochraceus* (cost $0.68/g)
   gibberellins - plant hormones that set blooms produced by fungus - *Gibberella*
Alcoholic Beverages

1. Beer - made from barley, which is malted (this is a process of sprouting and turning on amylase to digest starch) - this is mashed and water is added - hops are added for flavor and as an antibacterial - this is filtered and *Saccharomyces cerevisiae* is added - this is fermented, then aged - either pasteurized or filtered bottled [4% alcohol]

2. Wine - *Saccharomyces elipsoideus* id used to ferment fruit juice, usually grape juice the grapes are crushed and SO₂ is added to get rid of wild yeast - this is called the “must” and the yeast is added for fermentation - the process is aerobic first to produce lots of yeast - than it is capped off to produce ethanol - aged months to years - may be pasteurized, filtered and then bottled [10%+ alcohol]

3. Distilled Alcohol - fermentation of fruit juice for brandy, molasses for rum, barley for scotch, rye for rye and corn for whiskey.  The alcohol is distilled off, water is added back and a flavoring agent added or process is carried out

Antibiotics - *Streptomycyes, Bacillus, Penicillium* (about 100 useful ones out of several thousand) - mostly penicillin and its derivatives

*Bacillus thuringiensis* - crystals kill gypsy moth caterpillars and horn worms

FUTURE - Plasmids and genetic engineering - free DNA fragments in bacteria self-replicating code for 2-250 genes, may be induced to make up to 300 copies/cell and thus increase greatly the amount of material they code for [plasmids may be transferred to harmless bacteria for production of a needed commodity, they may be spliced together with several genes to produce several things]

*Amplification usually yields lower costs!*